Metabolism of 2,2-Dichlorovinyl Dimethyl Phosphate in Relation to Residues in Milk and Mammalian Tissues

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The metabolism and residues of 2,2-dichlorovinyl dimethyl phosphate-P^{32} (DDVP or Vapona) were examined with rats, cows, and a goat. Studies with rats also utilized carbon^{14} labeled DDVP, dichloroacetaldehyde, and dichloroethanol, and phosphorus^{32}-labeled O-methyl 2,2-dichlorovinyl phosphate and O,O-dimethyl phosphate. In addition, several rats and a single cow were treated orally with 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate-P^{32} (Dibrom). These insecticides are rapidly hydrolyzed in mammals to yield no persisting tissue residues and only trace levels in milk. The initial phosphorus-containing metabolites of DDVP, O,O-dimethyl phosphate and O-methyl 2,2-dichlorovinyl phosphate, are low in toxicity and rapidly excreted or further degraded. The 1-carbon of the 2,2-dichlorovinyl group in DDVP is excreted in urine predominantly as a conjugate of dichloroethanol, probably the glucuronide, in the feces as unknown derivatives, and in the expired air as carbon dioxide. Small amounts of dichloroacetic acid may be formed, and some of the C^{14} persists in liver, blood, and other tissues in an unidentified form. Limited metabolism studies of DDVP in plants and of DDVP and Dibrom in bovine rumen fluid are also reported.

THE INSECTICIDE, 2,2-dichlorovinyl dimethyl phosphate (DDVP or Vapona), has insecticidal activity suitable for many control problems where man or animals might be directly exposed to the vapors at the time of treatment or shortly thereafter. Extensive toxicity studies with man and farm animals have indicated that insecticidal concentrations of DDVP can be safely used, even in relatively confined areas (*4*, *5*, *9*, *12*, *15*, *16*). Residue studies based on bioassay procedures have failed to detect DDVP in tissues or milk from treated animals (*16*). Enzymatic studies have established the rapid degradation of DDVP in plasma, liver, kidney, and spleen (*7*). The metabolic pathway has been deduced from these enzymatic studies using a variety of analytical procedures for determining the fragments of the DDVP molecule (*7*). Dibrom, 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate, formed on bromination of DDVP, is also biologically unstable (*10*) and of interest as a relatively safe but highly effective insecticide.

The distribution and fate in the animal body of DDVP was investigated by radiotracer techniques. Limited studies were also conducted with Dibrom-P^{32}.

Methods and Materials

Chemicals. DDVP was used as the nonradioactive material prepared as an analytical standard, as the P^{32}-tagged compound, and with C^{14} in the alpha or 1 position of the dichlorovinyl group. These samples were all prepared by reaction of trimethyl phosphite with chloral (*18*). A sample of Dibrom-P^{32} prepared by bromination of DDVP-P^{32} was also utilized. Specific activities for these materials were 7.5 to 13 mc. per gram for DDVP-P^{32}, 4.3 mc. per gram for Dibrom-P^{32}, and 10.6 mc. per gram for DDVP-1-C^{14}. Prior to use, the radiolabeled insecticides were purified on silica gel [120 grams of silicic acid plus 86 ml. of water (*17*)] columns with hexane-chloroform mixtures. Chromatography of the DDVP-P^{32} and Dibrom-P^{32} resolved three to four components; chromatography of the DDVP-C^{14} separated seven components. The identity of the main component as the insecticide was confirmed in each case by infrared spectrograms and partitioning characteristics in several pairs of immiscible solvents. DDVP and Dibrom readily eluted with hexane, while the impurities which were more polar eluted with the hexane-chloroform mixtures. The chemical nature of the impurities was not determined. The radiochemically pure insecticides were thus recovered from reaction mixtures of 67 to 78% purity for DDVP-P^{32}, 61% purity for Dibrom-P^{32}, and 78% purity for DDVP-C^{14}. Once purified, all compounds were held in dilute solution in anhydrous hexane at −20° C. until used.

Radiolabeled sodium *O*-methyl 2,2-dichlorovinyl phosphate (des-methyl DDVP) and dimethyl phosphate were prepared from DDVP-P^{32}, and dichloroacetaldehyde-1-C^{14} and dichloroethanol-1-C^{14} were prepared from DDVP-1-C^{14}. Their structures were ascertained by chromatography and/or derivative formation (*7*). The β-glucuronidase was purified from bovine liver (California Foundation for Biochemical Research, Los Angeles). Sources for other chemicals utilized are indicated in a previous report (*7*).

Animals and Administration of Chemicals. Four Guernsey cows and a goat were treated with P^{32}-labeled compounds for milk residue studies as follows: intravenous administration in a propylene glycol–saline mixture of 1.00 mg. per kg. of DDVP to a 612-kg. cow; subcutaneous administration in a propylene glycol–saline mixture of 1.00 mg. per kg. of DDVP to a 518-kg. cow; oral administration on bran in gelatin capsules of 1.00 mg. per kg. of DDVP to a 484-kg. cow, followed after 7 days by a 20.0 mg. per kg. oral treatment of DDVP to the same cow; oral adminsitration on bran in gelatin capsules of 20.0 mg. per kg. of Dibrom to a 530-kg. cow; and subcutaneous administration in a propylene glycol–saline mixture of 1.52 mg. per kg. of DDVP to a 60-kg. goat. A tranquilizer [125 mg. per animal of Diquel or 3-ethyl-10-(3-dimethylamino-2-methylpropyl) phenothiazine, Jensen-Salsbery Laboratories, Inc., Kansas City, Mo.] was used for the cows injected with DDVP-P^{32}. The animals were stanchioned and catheterized to allow separate and total collection of urine and feces.

Albino rats for metabolism studies and mice for toxicity studies were obtained from Dan Rolfsmeyer Co. (Madison, Wis.). The compounds were administered in water, corn oil, or propylene

glycol as indicated with the individual experiment. Female mice weighing 24 to 26 grams were used for the acute toxicity determinations. Limited studies also utilized guinea pigs.

Fate of DDVP-P^{32} and Derivatives and Dibrom-P^{32}. Tissue Extraction and Counting. Tissues for analysis were cut into small pieces immediately after autopsy of the animal for determining total P^{32} content. One-gram subsamples were then homogenized in 20 ml. of acetone in a glass homogenizer and centrifuged until the protein was well packed; the acetone was then decanted. The acetone was added to 5 ml. of water and distilled off on a steam bath, and the residual aqueous solution was made up to 10 ml. in a volumetric flask. Chloroform-water partitioning of the P^{32} was used to determine the proportion of unhydrolyzed DDVP in this acetone-soluble fraction. The residue from the acetone was homogenized in 4.0 ml. of water, centrifuged, and the water was decanted. The residue was then remixed with an additional 3.0 ml. of water and centrifuged. This water was decanted, added to the first, and made up to 10 ml. in a volumetric flask. Total counts and chloroform-soluble counts were used to determine the DDVP content of this fraction which was initially protein bound, but could be recovered by water washing. Recoveries of unhydrolyzed DDVP from fortified samples varied from 57 to 65% for brain, heart, kidney, liver, and muscle. With this procedure, DDVP recovered was 94% in the acetone-soluble fraction, 5.4% in the protein-bound fraction that could be recovered by washing with water, and 0.6% that remained with the protein. About 10 to 12% hydrolysis of DDVP occurred by this procedure so that the remaining losses occurred during evaporation of the acetone into the water.

Analyses of other tissues, feces, and blood from DDVP-P^{32}- and Dibrom-P^{32}-treated rats were made in a similar manner. Urine was used directly for counting and chloroform-water partitioning.

Milk Sampling and Analyses. Animals were milked 5 hours prior to treatment with the radioactive compounds; this pretreatment milk sample was used as a control. Following treatment, the milk from one quarter was used for the 0.25-hour sample, from a second quarter for the 0.5-hour sample, from a third quarter for the 1.0-hour sample, and from the fourth quarter for the 2.0-hour sample. Oxytocin was given just prior to the 4.0-hour milk sample, and all four quarters were milked at this time and for each subsequent sampling time when oxytocin was not used. Representative samples of the total milk were used for analysis.

Analysis of the milk consisted of determining total radioactivity in the whole milk, which would represent the combined DDVP or Dibrom and metabolites, and extracting the milk to ascertain the level of organosoluble radioactivity. Samples of raw milk freshly fortified with these compounds usually yielded 90 to 100% recovery in the organosoluble fraction in preliminary studies. Experimental milk samples were refrigerated and extracted within a few hours from the time of milking. A series of fortified samples at nine levels of insecticide were run along with the experimental samples for each cow. All data reported are the average of duplicate analyses. The per cent recovery in the fortified samples during the time of analysis of the experimental milk samples from treated cows averaged 87% for the 1.0 mg. per kg. DDVP dose, 92% for the 20 mg. per kg. DDVP dose, and 79% for the 20 mg. per kg. Dibrom dose. Residue results reported take these recoveries into consideration.

The following method was used to recover the organosoluble materials from the milk:

Twenty milliliters of fresh milk and 40 ml. of chloroform were homogenized in a Lourdes blender for 2 minutes. Centrifugation separated chloroform, protein, and aqueous layers. The protein layer was removed, extracted similarly in a Lourdes blender with 50 ml. of acetone, and the mixture was passed through a Büchner funnel to recover the acetone. The chloroform and acetone were combined, dried with sodium sulfate, filtered, and evaporated to a small volume under reduced pressure with a rotary evaporator. The residue was transferred to planchets, the remaining solvent evaporated at room temperature, and total radioactive counts were made on the organosoluble material.

By this extraction method, 2.0% of the radioactivity was recovered in the aqueous phase, 37.9% in the chloroform, 59.8% in the acetone, and 0.2% in the residue. Partitioning between chloroform and water to differentiate between DDVP and hydrolysis products indicated that 76% of the radioactivity in the aqueous phase, 99% in the chloroform, and 97% in the acetone was due to DDVP. The organosolubles (combined chloroform and acetone) accounted for 90 to 100% of the radioactivity in milk fortified with DDVP and less than 0.1% when radiolabeled dimethyl phosphate was used to fortify the milk. Fresh, raw milk fortified with about 5 p.p.m. of radiolabeled DDVP was incubated at 37° C., and milk samples were analyzed for DDVP content after various intervals. Recoveries were 97% at zero time, 97% at 30 minutes, 87% at 2 hours, 81% at 8 hours, and 54% at 24 hours.

Rumen Juice. The stability of DDVP-P^{32} and Dibrom-P^{32} in stagnating bovine rumen fluid was determined according to a described procedure (*1*). Hydrolysis was ascertained by partitioning, between chloroform and water, aliquots taken after varous incubation times up to 4 hours at 37° C. Fresh rumen fluid was always compared with boiled rumen juice. Studies were made with initial pH values for the rumen fluid adjusted to 6.0 and 7.0 and with organophosphate concentration varying between 5 and 50 p.p.m. The pH of the rumen fluid increased during incubation by about 0.7 units for the unboiled and 1.1 units for the boiled rumen fluid.

Plants. DDVP-P^{32} was administered to corn (8 to 10 inches tall, hybrid field corn, Wisconsin Experimental W423A variety), cotton (5 inches tall, Delta Pine 15 variety), and pea (4 to 5 inches tall, Perfection variety) seedlings. Short term studies were necessitated by the volatility and biological instability of the compound. Cotton seedlings were treated, by spreading 100 μl. of a 0.1% DDVP-P^{32} aqueous solution, as uniformly as possible over the upper leaf surface of one first true leaf per plant. Treated leaves from duplicate plants were removed for analysis at 0, 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 4, 8, 16, 24, 36, 48, and 72 hours after DDVP application. Each leaf was washed twice with 7 ml. of water to recover the surface residue, and then the leaves were homogenized in 7 ml. of water to recover the absorbed residue. In the root absorption study, seedlings of each of the plant species were placed with the roots in a 250-p.p.m. DDVP-P^{32} aqueous solution for 8 hours, the roots then thoroughly rinsed with distilled water, and the plants transferred to distilled water for 40 more hours. Plants were removed at 0, 8, 16, 24, 32, and 40 hours and divided into leaves and stems plus roots which were separately homogenized in water. Analysis in all cases consisted of determining the total DDVP-P^{32} equivalents by direct counting and the organosoluble DDVP-P^{32} equivalents by chloroform-water partitioning. All plants were held in a glass house at 30° C.

Fate of DDVP-1-C^{14} and Derivatives in Rats. Female white rats weighing 180 to 220 grams were administered aqueous solutions of DDVP-1-C^{14}, dichloroacetaldehyde-1-C^{14}, and dichloroethanol-1-C^{14}. For ascertaining the percentage of the dose expired as $C^{14}O_2$, the compounds were each given by the intraperitoneal route at 1.00 μmole per rat, and this dose of DDVP-1-C^{14} was also administered orally. Treated rats were placed in individual chambers designed for complete trapping of expired carbon dioxide by bubbling the air exhausted from the animal chamber through an ethanolamine–ethylene glycol monomethyl ether mixture. Car-

bon-14 was then determined by counting in ethanolamine–ethylene glycol monomethyl ether–toluene with 2,5-diphenyloxazole in a Tri-Carb liquid scintillation spectrometer Model 314-A (Packard Instrument Co., La Grange, Ill.) according to a described procedure (*8*). Studies were conducted for 24 hours after treatment, during which time rats administered sodium acetate-1-C^{14} expired 74% of the administered dose as $C^{14}O_2$. All experiments were run in duplicate.

Similar rats were treated at 4.00 mg. per kg. with DDVP-1-C^{14} in aqueous solution via the intraperitoneal and oral routes. These rats were atropinized just prior to treatment so that cholinergic symptoms persisted no more than 60 minutes. Treated rats were held 7 days in cages designed for quantitative and separate collection of urine and feces. Tissue samples were removed after 7 days for determination of total C^{14} content. Three pairs of rats were used for the urine and feces studies and four rats for the tissue studies with each administration route. Urine samples were prepared by adding 0.20 ml. of urine to 0.5 ml. of 1.5% gelatin in 0.015*N* sodium hydroxide in aluminum planchets and air drying overnight. The alkali immediately converted DDVP to a nonvolatile derivative which could be directly compared with the nonvolatile derivatives excreted by suitable correction for self-absorption effects. Heparinized blood samples were diluted with alkali to a final concentration of 33% blood in 0.005*N* sodium hydroxide before pipetting 1.0-ml. aliquots and air drying. Tissue and feces samples were homogenized at 5% w./v. in 0.015*N* sodium hydroxide; 0.5-ml. samples were removed and air dried overnight. All samples were counted on a Nuclear-Chicago Model 183B Scaler with a gas flow counter, utilizing a Micromil window, and were corrected for self-absorption effects.

The nature of the C^{14}-containing DDVP metabolites in the total urine samples was investigated by derivative formation. The procedure for determining dichloroacetaldehyde released in acid at 100° C. for 30 minutes, and for free dichloroacetic acid and dichloroethanol has been reported (*7*). Total dichloroethanol was determined by refluxing the urine sample in 8.0*N* hydrochloric acid for 6 hours prior to extraction of the free dichloroethanol for phenylurethane formation. Refluxing in 2.0*N* hydrochloric acid gave similar results, but 0.5*N* acid was not adequate for complete hydrolysis of the conjugate. The conjugate was also partially split by adding 5 mg. of β-glucuronidase to 0.2 ml. of rat urine and 0.8 ml. of 0.05*M* pH 4.5 sodium acetate and incubating for 12 hours at 37° C., prior to extraction of the free dichloroethanol.

Other Methods. Ion exchange chromatography, colorimetric procedures, and cholinesterase assays were utilized as in previous studies on organophosphate esters (*3, 7, 11, 13*).

Results

Fate of DDVP-P^{32} and Derivatives in Rats and Cows. Tissue Distribution and Hydrolysis in Rats. DDVP-P^{32} administered orally to rats at 10 mg. per kg. is rapidly absorbed, distributed among the tissues and hydrolyzed (Table I). Similar results were obtained with male and female rats. The tissue distribution of P^{32} is that expected of a compound which is rapidly hydrolyzed and excreted except for inorganic phosphate which enters the normal phosphate pool of the organism. Further studies confirming the rapid in vivo hydrolysis involved intraperitoneal administration of several doses to rats and mice. Female rats treated every half hour with 4 mg. per kg. of DDVP-P^{32} for a 2-hour period and then autopsied one half hour after the last treatment showed largely hydrolysis products in the tissues. Mice treated with 10 mg. per kg. every 15 minutes for three treatments followed by another treatment 15 minutes before autopsy showed that more than 95% of the DDVP had been hydrolyzed in the liver, kidney, and small intestine.

Fate of Dimethyl Phosphate and Des-methyl DDVP in Rats. Dimethyl phosphate-P^{32} and des-methyl DDVP-P^{32} were administered orally at 500 mg. per kg. in water to male rats. Autopsy, 90 hours after treatment, indicated that the rat administered dimethyl phosphate-P^{32} had eliminated almost the entire dose. The urine, containing only unmetabolized dimethyl phosphate-P^{32}, accounted for about half of the radioactivity; the tissues were almost devoid of P^{32}-containing materials. The rat administered des-methyl DDVP eliminated about 14% of the dose via urine in 90 hours, and the tissues were similar in distribution of P^{32} to a rat 90 hours after DDVP administration (Table I). The very high proportionate radioactivity in the bone was indicative of rapid degradation to phosphoric acid, as was chromatographic analysis of the cumulative 0 to 90 hour urine sample which showed 86% of the metabolites as phosphoric acid and 14% as des-methyl DDVP.

Excretion of P^{32}-Containing Metabolites. From 67 to 100% of the administered radioactivity was recovered in the combined urine and feces within a week following treatment of animals (Table II). The percentage excretion did not vary greatly with dosage based on studies with both male and female rats and doses between 0.1 and 80 mg. per kg. Excretion of P^{32} in the feces accounted for only 11 to 15% of the administered dose, except with cows treated orally where about half the P^{32} was excreted via this route. Two examples of the excretion rates are shown in Figure 1.

Nature of P^{32}-Products in Urine and Feces. DDVP was hydrolyzed

Table I. Tissue Distribution of Total DDVP-P^{32} Equivalents in Male and Female Rats Following 10.0 Mg. Per Kg. Oral Dose

Tissue or Organ	Total DDVP-P^{32}, P.P.M. Equivalents at Time Indicated													
	0.25 Hour		1.0 Hour		4.0 Hours		14 Hours		1 Day		4 Days		7 Days	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Blood	7.0[b]	4.3[c]	6.0[b]	4.5[c]	2.7	2.5	1.3	1.1	1.0	0.4	0.3	0.5	0.2	0.1
Bone	1.2	1.0	6.3	4.0	9.0	8.2	11.5	11.2	11.3	12.9	13.5	7.4	11.4	8.1
Brain	1.6[c]	2.1[d]	2.8[a]	4.8[d]	1.9	3.5	1.3	1.7	1.3	2.0	1.3	1.9	1.1	1.7
Fat	1.1[c]	1.4[c]	2.0[a]	1.9[d]	1.4	0.8	0.5	0.8	0.7	0.6	0.4	0.4	0.1	0.3
Heart	3.0[e]	2.6[d]	5.6[c]	5.4[c]	5.1	5.9	3.8	4.1	3.2	3.5	1.4	1.8	0.8	1.3
Kidney	8.5[b]	15.1[a]	25.7[b]	23.3[b]	12.2	22.5	7.2	10.0	4.9	10.2	2.9	2.8	2.0	2.5
Liver	37.6[a]	31.6[b]	59.1[c]	34.2[c]	22.7[c]	22.3[c]	16.5[c]	11.6[b]	8.3[a]	12.5	3.2[a]	3.3	1.4	2.7
Muscle	0.6[c]	0.5[a]	1.7[a]	1.4[a]	1.3	1.4	0.9	1.5	0.7	1.8	1.0	1.0	0.9	1.3
Stomach	123.1[f]	124.1[f]	129.7[d]	69.3[e]	22.2[d]	22.0[e]	...	1.7[c]	1.2[b]	1.2[c]	0.8[c]	1.8[c]	0.5[a]	0.9[a]
Small intestine	2.0[c]	8.3[b]	87.5[b]	69.7[a]	2.8	6.4	7.7	4.0	2.3	3.0	0.0	5.5	0.0	1.3
Large intestine	0.9[c]	0.6[c]	13.1[d]	3.8[b]	1.8	5.8	6.8	3.8	2.0	5.3	1.2	0.6	0.3	1.5

[a-f] The organosoluble-P^{32} (presumably DDVP) was subdivided into the following ranges as percentage of the total P^{32} without correction for extraction efficiency; *a*, less than 1%; *b*, 1 to 5%; *c*, 6 to 20%; *d*, 21 to 40%; *e*, 41 to 60%; and *f*, 61 to 72%.

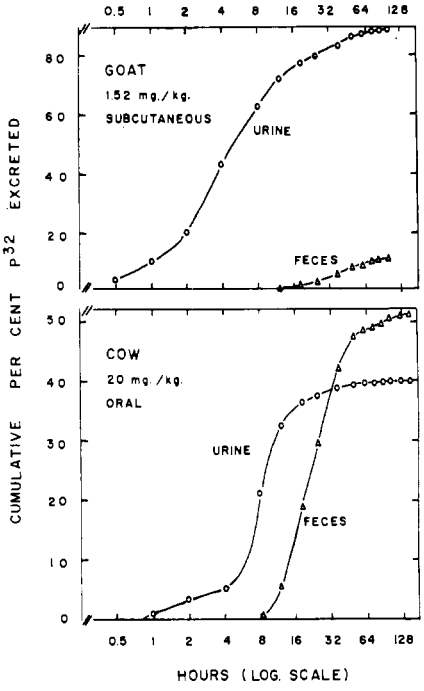

Figure 1. Rate of excretion of DDVP-P^{32} equivalents (DDVP plus P^{32}-containing metabolites) by a cow and goat

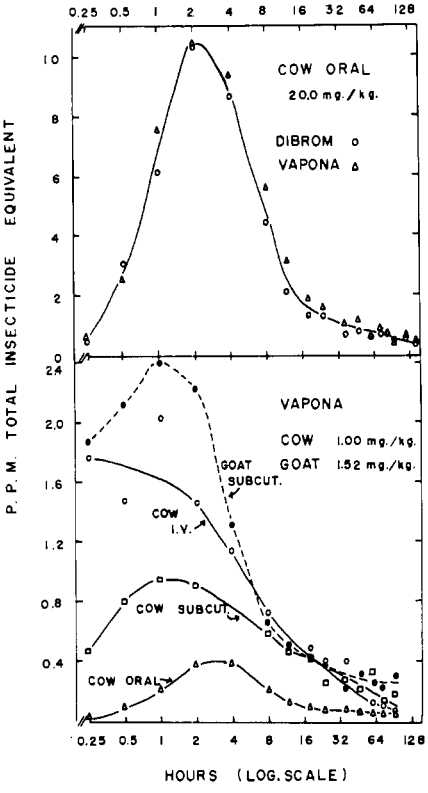

Figure 2. Blood levels of total insecticide-P^{32} equivalents (insecticide plus P^{32}-containing metabolites) following administration of DDVP-P^{32} and Dibrom-P^{32} to cows and a goat

prior to excretion in the urine, since 99 to over 99.9% of the radioactivity in the urine remained in the aqueous phase on partitioning with chloroform. The excreted metabolites appeared to be des-methyl DDVP, dimethyl phosphate, and inorganic phosphate based on cochromatography with authentic compounds on ion exchange columns. Results for monomethyl phosphate are reported along with the dimethyl phosphate because of the incomplete resolution of these materials. The proportion of des-methyl DDVP decreased rapidly in the rat, cow, goat (Table III), mouse, and guinea pig. Inorganic phosphate increased in proportion as the administered DDVP was more completely degraded in the body. Cows treated via the oral, subcutaneous, and intraperitoneal routes with 1.0 mg. per kg. of DDVP yielded similar chromatograms for the urine. No difference was noted between male and female rats.

The P^{32} in the feces of treated cows was present predominantly (greater than 95%), if not entirely, as ionic DDVP derivatives. No further characterization was attempted on these products in feces.

Blood Levels with Cows and a Goat. The parts per million of total insecticide equivalent (insecticide plus hydrolysis products) based on P^{32} are shown in Figure 2. Subcutaneous administration to either a goat or cow gave peak blood levels at 1 to 2 hours. When cows were administered 1.0 mg. per kg. of DDVP, the peak blood levels were higher and were attained sooner with the intravenous than with the subcutaneous route, which in turn

Table II. Excretion of DDVP-P^{32} and Dibrom-P^{32} Derivatives in Urine and Feces

Route of Administration	Dose, Mg./Kg.	Hours	Administered Dose Excreted, % Urine	Feces	Total
		DDVP-Goat			
Subcutaneous	1.52	96	89	11	100
		DDVP-Cow			
Intravenous	1.00	96	68	13	81
Subcutaneous	1.00	96	79	15	94
Oral	1.00	168	15	53	68
Oral	20.0	144	40	51	91
		DDVP-Female Rats			
Oral	0.1, 1.0, 10, 20, 40, 80	148	70	11	81
		DDVP-Male Rats			
Oral	0.1, 1.0, 10, 20, 40, 80	148	59	12	71
		Dibrom-Cow			
Oral	20.0	144	9	34	43

Table III. Nature of P^{32}-Containing Hydrolysis Products in Urine

Animal	Dose, Mg./Kg., and Route	Hours after Administration	% Excreted P^{32} Chromatographing in Position of: Inorganic phosphate	Mono- and dimethyl phosphates	Des-methyl DDVP
		DDVP			
Male rat	10 oral	0–3	3.2	83.3	13.5
		3–6	4.4	88.5	7.1
		6–12	6.6	90.3	3.1
		12–24	15.6	82.2	2.2
		24–48	8.6	91.4	0.0
Male and female rats	20 and 80 oral	0–12	4.3	83.8	11.9
Goat	1.52 subcutaneous	0–2	<0.2	81.7	18.2
		2–12	<0.2	97.8	2.1
		12–36	2.4	94.8	2.8
Cow	20 oral	0.5–1	1.3	68.9	29.8
		2–4	<0.2	97.8	2.2
		8–12	8.8	91.2	<0.2
		Dibrom			
Cow	20 oral	0.5–1	<0.2	71.0	29.0
		2–4	<0.2	95.2	4.8

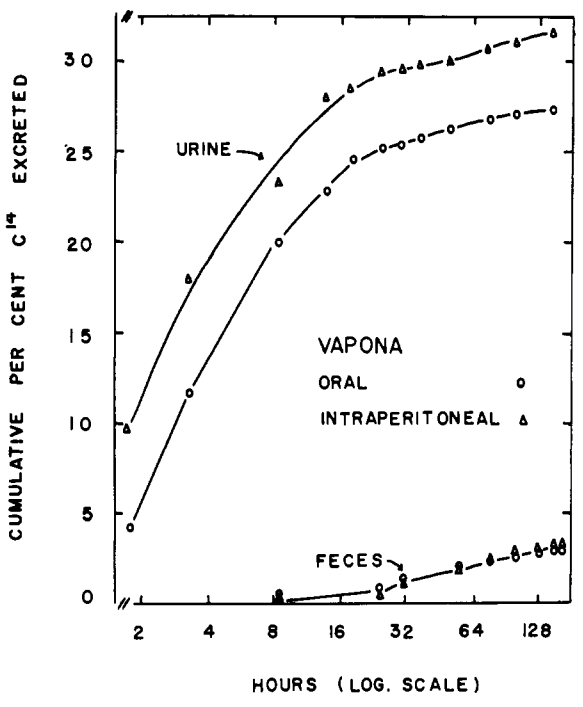

Figure 3. Rate of excretion of DDVP-C^{14} equivalents (DDVP plus C^{14}-containing metabolites) following oral and intraperitoneal administration of DDVP-1-C^{14} to female rats

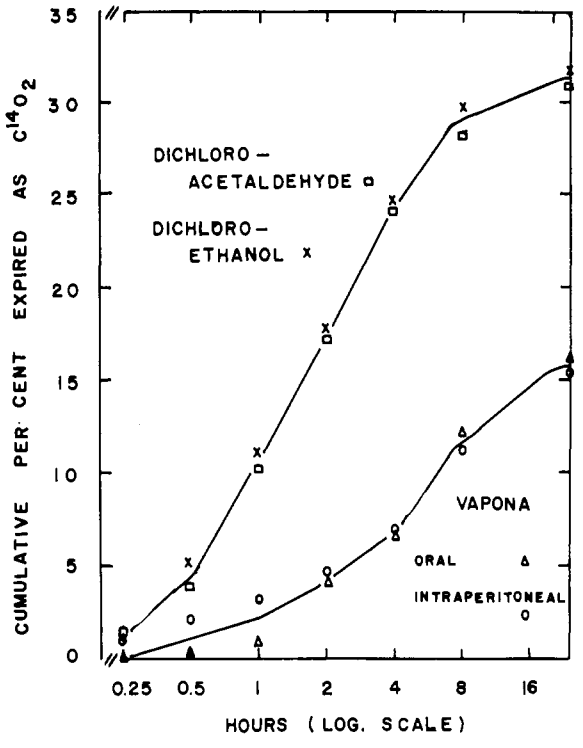

Figure 4. Rate of expiration of $C^{14}O_2$ by female rats following intraperitoneal administration of DDVP-1-C^{14} dichloroacetaldehyde-1-C^{14} and dichloroethanol-1-C^{14} and oral administration of DDVP-1-C^{14}

Table IV. Total Insecticide-P^{32} Equivalents (Insecticide plus Hydrolysis Products) and Residues of Organosoluble-P^{32}-Derivatives Following Administration of DDVP and Dibrom to Cows and a Goat

	DDVP-Cow				DDVP-Goat, Dibrom-Cow,	
Hours after Treatment	Intravenous, 1.00 mg./kg.	Subcutaneous, 1.00 mg./kg.	Oral, 1.00 mg./kg.	Oral, 20.0 mg./kg.	Subcutaneous, 1.52 Mg./Kg.	Oral, 20.0 Mg./Kg.
			Total Insecticide-P^{32}, P.P.M.			
0.25	<0.70[a]	<0.30	<0.004	0.84[b]	0.22	<0.10
0.5	<0.70	<0.30	<0.004	1.11[b]	0.13	<0.10
1	<0.70	<0.30	0.0045	0.99[b]	0.11	<0.10
2	0.93	0.30	0.0067	0.79[b]	0.92	0.13
4	0.77	1.24	0.13	7.6	1.82	2.8
8	1.95	1.83	0.36	10.5	1.31	2.5
12	2.51	2.15	0.61	11.1	0.84	7.5
18	0.97	0.83	0.58	9.3	0.50	7.8
24	0.80	0.75	0.60	7.9	0.42	7.7
36	<0.70	0.52	0.50	7.6	0.30	6.0
48	<0.70	0.52	0.38	6.2	0.30	5.1
60	<0.70	0.32	0.31	4.5	0.27	4.5
72	<0.70	<0.30	0.27	3.7	0.21	3.5
84	...	...	0.25	3.0	0.25	3.3
96	<0.70	<0.30	0.18	3.0	0.20	2.7
120	...	...	0.15	2.3	...	2.1
144	...	...	0.12	1.3	...	2.1
			Residues, Organosoluble-P^{32}, P.P.B.			
0.25	80	<15	1.5	16	12	24
0.5	38	<15	2.3	67	8	37
1	<10	<15	0.4	77	2	77
2	<10	<15	1.3	31	<0.4	78
4	<10	<15	<1.0	3	<0.4	9
8–96 av.	<10	<15	<1.0	<10	<0.4	<8

[a] Values indicated as "<" were below the level of significance in counting.
[b] High early values resulted from a previous treatment of this cow with 1.00 mg. per kg. of DDVP 7 days prior to the 20.0 mg. per kg. treatment with a preparation of lower specific activity.

reached higher levels and sooner than with oral administration.

Cholinesterase assays on the whole blood showed no inhibition due to treatment with 1.0 mg. per kg. of DDVP either by the oral or subcutaneous routes. Intravenous administration yielded 29% inhibition in 1 to 2 hours with rapid recovery thereafter. Subcutaneous treatment of the goat yielded 44% inhibition at 0.25 hours, 46% at 0.5 hours, 41% at 1 hour, 35% at 2 hours, and rapid recovery thereafter. The higher level of 20.0 mg. per kg. administered orally to a cow yielded inhibition reaching 25% at 1 hour, 32% at 2 hours, and 21% at 4 hours, followed by rapid recovery.

Milk Levels with Cows and a Goat. The levels of total and organosoluble equivalents of DDVP-P^{32} as calculated from the specific activity of the original insecticide administered are shown in Table IV. The majority of the radioactivity in the milk was due to hydrolysis products. Only within the first 2 hours was the level of organosoluble radioactivity significantly above background. With the sampling method utilized, the recovery of organosoluble radioactivity occurred only in the first four samples in which milk was secreted continuously from the time of administration. Thus, the samples were actually 0- to 0.25-, 0- to 0.5-, 0- to 1-, and 0- to 2-hour samples, whereas the next sample, because of the use of oxytocin, contained

Table V. Tissue Distribution of Total DDVP-C^{14} Equivalents in Female Rats 7 Days after Administration of 4.00 Mg. Per Kg. Dose

Tissue or Organ	Total DDVP-C^{14} Equivalents, P.P.M. Oral	Intraperitoneal
Blood	0.99	0.92
Brain	0.29	0.39
Fat	0.24	0.29
Heart	0.36	0.28
Kidney	0.49	0.59
Liver	3.10	2.85
Muscle	0.25	0.26
Stomach	0.25	0.21
Small intestine	0.42	0.35
Large intestine	0.35	0.37

a small amount of milk secreted within the first 2 hours but was predominantly a 2- to 4-hour sample. After the 4 hour time, the samples corresponded solely to the milk secreted in the interval since the last sample.

Biopsies of subcutaneous back fat taken 1.5, 3, and 6 hours after treatment showed 0.20, 0.28, and 0.20 p.p.m. of total DDVP-P^{32} equivalents, of which about 30% was not acetone soluble.

Fate of DDVP-1-C^{14} and Derivatives in Rats. DDVP-1-C^{14} is eliminated from rats at a similar rate following either oral or intraperitoneal administration. Based on 7-day studies, excretion in the urine accounted for 27 to 32% and in the feces for 3% of the administered dose (Figure 3). $C^{14}O_2$ expired within 24 hours after treatment accounted for 16% of the administered dose with DDVP and 32% with dichloroacetaldehyde and dichloroethanol (Figure 4). Considerable radioactivity remained in the tissues, particularly the blood and liver, even after 7 days when little further excretion was occurring (Table V). No attempt was made to define the chemical nature of the radiolabeled derivatives remaining in the tissues.

More than 90% of the radioactivity in the urine of rats treated with DDVP-1-C^{14} appeared as a conjugate of dichloroethanol. This conjugate was completely split by refluxing the urine in acid, and partially split by β-glucuronidase. The principal conjugate was therefore probably dichloroethyl glucuronide, in agreement with a study where dichloroethanol was administered to rabbits (*14*). Little, if any free dichloroethanol appeared in the rat urine. Some dichloroacetaldehyde-1-C^{14} was recovered on acid hydrolysis of the urine. This radioactivity was due to either dichloroacetaldehyde or des-methyl DDVP, since DDVP per se is not excreted in the urine. Since DDVP-P^{32} studies have shown des-methyl DDVP excretion in urine, the dichloroacetaldehyde recovered on treatment with acid probably resulted from partial hydrolysis of this material. The acid hydrolysis was not drastic enough for complete degradation of des-methyl DDVP to dichloroacetaldehyde (*7*). Averaging the results from all urine samples fractionated gave 95.6% as dichloroethanol conjugates, 1.4% as derivatives which hydrolyzed in acid to yield dichloroacetaldehyde, and 3.0% as acids which crystallized along with dichloroacetic acid on formation of a dicyclohexylamine salt. These percentage values represent the derivatives recovered during carrier crystallization and do not take into account incomplete hydrolysis which was probably a factor with dichloroacetaldehyde.

Fate of Dibrom-P^{32} in Rats and a Cow. Adult male rats were treated with 25 mg. per kg. of Dibrom-P^{32} in corn oil by stomach tube. Tissue samples taken 0.5, 4, 24, and 72 hours after treatment were analyzed by the same procedure used for DDVP. The radioactivity in all the tissues other than the stomach appeared predominantly, if not entirely, as hydrolysis products. The high specific activity occurring in the bone within a day after treatment indicated at least partial hydrolysis of Dibrom to products other than dimethyl phosphate, which were further degraded to inorganic phosphate. Following intraperitoneal administration of 10 mg. per kg. of Dibrom-P^{32}, the fat contained a high proportionate level of radioactivity within 15 minutes, but this material quickly partitioned back out of the fat to leave no persisting residue.

A cow was administered 20.0 mg. per kg. of Dibrom-P^{32} orally for direct comparison with the same dose of DDVP-P^{32}. Blood levels (Figure 2) and milk results (Table IV) were quite similar for Dibrom and DDVP. No more than 10% blood cholinesterase inhibition resulted at any time after treatment with the 20.0 mg. per kg. Dibrom dose. The proportion of administered radioactivity recovered in urine and feces was lower for Dibrom than for DDVP (Table II), with the small proportion appearing in the urine being particularly outstanding. As with DDVP, the P^{32} excreted in urine and feces was at least 95% and probably greater than 99% as hydrolysis products. Chromatography of the urine yielded P^{32}-metabolites in the positions of inorganic phosphate, mono- and/or dimethyl phosphate, and des-methyl DDVP. No further studies were made on the chemical nature of the Dibrom hydrolysis product which cochromatographs on Dowex-I with des-methyl DDVP. Biopsies of subcutaneous back fat taken 1.5, 3, and 6 hours after treatment showed 0.23, 0.30, and 0.08 p.p.m. of total Dibrom-P^{32} equivalents, of which about 80% was not acetone soluble.

Other Studies. Rumen Fluid. DDVP and Dibrom did not appear to be significantly hydrolyzed by microbial or enzymatic attack in the stagnating rumen fluid. Although 20% hydrolysis of DDVP and 30% of Dibrom occurred within 4 hours in rumen fluid initially adjusted to pH 7.0, the difference in activity between the boiled and unboiled samples could be accounted for on the basis of the pH differences in these preparations on incubation. This lack of marked hydrolytic activity of the bovine rumen fluid is consistent with studies on similar organophosphate substrates (*1*) and indicates that with DDVP and Dibrom, the hydrolysis in the rumen is not a major factor in the metabolism of the compound in the bovine organism.

Stability of DDVP-P^{32} in Plants. About half of a 100-μg. dose of DDVP-P^{32} applied in 100 μl. of water to the upper surface of the first true leaves of cotton seedlings volatilized within 5 minutes. An additional 45% was absorbed within 20 minutes so that only 5% remained on the surface 20 minutes after application. The surface residue was nonhydrolyzed DDVP, while 70 to 80% of the absorbed material appeared as organosoluble-P^{32}, presumably nonhydrolyzed DDVP, during the first 30 minutes after treatment (Figure 5). Disregarding the DDVP lost by volatilization during the first 5 minutes after treatment, the half life of the DDVP was 1.2 hours with the primary loss occurring by hydrolysis. In addition to the volatilization of DDVP, which resulted in the total DDVP equivalents dropping from 100 μg. per leaf at 0 time to 35 μg. per leaf at 30 minutes, the hydrolysis products were also lost from the treated leaves, since only 14 μg. per leaf remained after 24 hours and 7 μg. per leaf after 72 hours despite the fact that no DDVP or other organosoluble-P^{23} derivatives were detected after 8 hours. The loss of hydrolysis products from treated leaves may have occurred, at least in part, from translocation away from the leaf sampled.

The parts per million of total DDVP-P^{32} equivalents taken up from a 250-p.p.m. solution in 8 hours were 27 for corn, 105 for cotton, and 48 for peas. At the end of this 8-hour absorption period, 77% of the absorbed P^{32} appeared as hydrolysis products in peas, and 33% in corn and cotton. Once the plants were removed from the insecticide source, the hydrolysis of the absorbed DDVP followed first order reaction kinetics with half-life values for both corn and cotton of 9 hours and for pea of 3 hours. The hydrolysis rate with all plants was the same for the combined root and stem and for the separately analyzed leaves. The total DDVP equivalents did not drop appreciably during the 40-hour post-absorption pe-

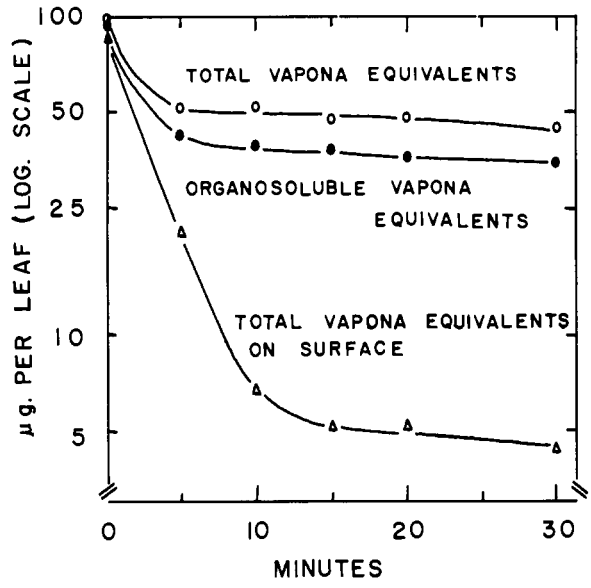

Figure 5. Volatilization, penetration, and hydrolysis of DDVP-P^{32} applied to cotton leaves

riod allowed for metabolism. This indicates that the excretion or volatilization of P^{32}-hydrolysis products is not rapid following this route of administration, although the possibility remains of some DDVP loss by volatilization prior to hydrolysis. Once within the plant, the P^{32} was translocated from the root and stem into the leaves more rapidly with cotton than with corn, which in turn was more rapid than with peas.

These findings on the instability of DDVP applied to or absorbed by plants are in agreement with earlier reports (*2, 6*).

Acute Toxicity of DDVP Derivatives. Studies with mice on the acute toxicity of DDVP and potential hydrolysis products showed relatively high toxicity associated only with the unhydrolyzed compound (Table VI). The symptoms associated with the "shock dose" of DDVP (*16*) do not appear to be associated with the hydrolysis products, since the level for narcosis with dichloroethanol and dichloroacetaldehyde is considerably higher than would be present as DDVP hydrolysis products at this "shock dose."

Discussion

DDVP and Dibrom are readily hydrolyzed by mammalian enzymes (*7*). The metabolites of DDVP include dimethyl phosphate, monomethyl phosphate, *O*-methyl 2,2-dichlorovinyl phosphate, inorganic phosphate, dichloroacetaldehyde, dichloroethanol, dichloroethyl glucuronide, and possibly dichloroacetic acid (*7*). The metabolic products from Dibrom include DDVP, dichlorobromoacetaldehyde, dichloroacetaldehyde, dimethyl phosphate, and certain complex amino acid conjugates of the degraded products (*10*). In addition to enzymatic degradation, Dibrom is reported to react readily with sulfhydryls including well-known amino acids to rapidly destroy residues on crops (*10*). Studies with rats and a cow indicate that the initial site of hydrolytic attack on a portion of the dose is at the P-O-methyl group which would yield additional metabolites from Dibrom. It also appears probable that the aldehyde mixture from Dibrom metabolism is reduced at least in part in mammals and conjugated prior to excretion. The acute toxicity of the DDVP metabolites is low relative to that of DDVP itself. The phosphorus-containing metabolites of DDVP other than inorganic phosphate are quickly eliminated from the body. A portion of the C^{14} from DDVP-1-C^{14} persists in the body for a least 1 week in the liver, blood, and to a lesser extent in other tissues. The chemical nature and toxicological significance of these metabolites are not known. Subacute and chronic feeding studies with DDVP (*4, 9, 16,*) give no indication of involvements other than those attributable to cholinesterase inhibition by the original compound.

Significant milk residues of DDVP and Dibrom resulting from insecticidal concentrations sprayed, rubbed, or painted on animals, appear to be highly unlikely. Calculations made from oral administration of relatively high levels to Guernsey cows illustrate this point. The organosoluble insecticide equivalents in the milk expressed as parts per billion were calculated for the first and second 12-hour periods after feeding. These levels were 0.46 and 0.39 for 1.00 mg. per kg. of DDVP, 21.1 and 7.3 for 20.0 mg. per kg. of DDVP, and 24.2 and less than 5 parts per billion for 20.0 mg. per kg. of Dibrom. Other routes of administration of DDVP to cows and subcutaneous administration to a goat were consistent with these very low residue levels.

High insecticidal activity combined with relatively low acute toxicity and rapid metabolism in mammals should make possible the utilization of DDVP and Dibrom where man or animals are exposed directly to small amounts of the insecticide.

Table VI. Toxicity of DDVP and Metabolites to Mice by Intraperitoneal Route

Compound	Vehicle	LD_{50}, Mg./Kg., 72 Hour
DDVP (technical)	Corn oil	28
Des-methyl DDVP, sodium salt	Water	1500
Dichloroacetaldehyde[a]	Corn oil	440
Dichloroethanol[a]	Corn oil	890
Dichloroacetic acid	Corn oil or water	250
Sodium dichloroacetate	Water	>3000
Mono- and dimethyl phosphoric acid mixture	Water	1500
Sodium mono- and dimethyl phosphate mixture	Water	>3000

[a] Threshold dose for narcosis shortly after injection was 450 to 550 mg./kg. for both dichloroacetaldehyde and dichloroethanol.

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INSECTICIDE RESIDUES IN BUTTERFAT

Residues of Heptachlor Epoxide in Butterfat of Dairy Cows Grazing Pastures Treated with Granular Heptachlor

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Six groups of two dairy cows each (a high and low producer) were placed on a pasture at various intervals after application of approximately 0.25 pound of granular heptachlor per acre. The butterfat from these animals showed continuous low level residues (maximum 3 p.p.m.) of heptachlor epoxide when animals were allowed to graze the treated pasture continuously after 1, 8, 15, 29, and 43 days following application. Residue was present in trace (indeterminate) amounts in the butterfat of the animals placed on the pasture 57 days after treatment. Animals continued to excrete residue in their butterfat after removal from the treated pasture (80 days after application) and placed in a dry lot for 40 days.

HEPTACHLOR applied as a spray has been used effectively on pastures and range land to control many insects. Milk and fatty tissues of dairy cattle fed heptachlor-treated hay or grazing heptachlor-treated pastures have been found to contain heptachlor epoxide, the metabolite of heptachlor (*2–5, 8*).

A maximum level of 13.3 p.p.m. of heptachlor epoxide was found in the butterfat of four dairy cows grazing pasture sprayed with 2 ounces of heptachlor in Diesel oil for grasshopper control. This peak was reached after 28 days and, thereafter, consistently and slowly declined with levels reaching 5.9 and 1 p.p.m. after 84 and 165 days, respectively (*3*). In the one other study involving direct grazing of heptachlor-treated pasture (0.5 pound in emulsion per acre) by cattle, Gannon and Decker (*4*) reported a maximum concentration of 0.2 p.p.m. of heptachlor epoxide in the milk, or approximately 5.0 p.p.m. in the butterfat, within 3 to 7 days, which declined thereafter.

These previous studies with heptachlor were based on spray applications. The present recommendation for eradication of the imported fire ant, *Solenopsis saevissima richteri*, is the application of heptachlor granules at the rate of 0.25 pound (12.5 pounds of 2% heptachlor granules) per acre in each of two treatments at 3- to 6-month intervals (*9*). The label issued for use of heptachlor in this manner requires that dairy cattle not be allowed to graze treated pasture for 30 days after application. Because of the physical properties of granular heptachlor, it was assumed that there would be little contamination of forage, and consequently little danger of residue in milk. Since no published reports were found in the literature to verify this assumption, this study was undertaken. In addition, it was desirable to determine how much time should elapse after application of heptachlor before it would be safe to place cattle on treated pasture.

Experimental Procedure

Experimental Plots. FORAGE AND SOIL SAMPLES. Approximately 15 acres of dairy pasture with no previous history of insecticide application was mowed to a height of approximately 4 to 6 inches in March, prior to initiation of the experiment, and 200 pounds per acre of nitrate of soda were applied. It was disked lightly and seeded to Johnson grass, *Sorghum holepense* (L.). The 15 acres were divided, with wire fence, into five plots approximately equal in size. Samples of forage and soil from each plot were collected prior to and after insecticide treatment for determination of heptachlor and heptachlor epoxide. For forage, ten randomly distributed subsamples, approximately 1 foot square, were collected from each plot, placed in a 5-gallon lard can, and stored at 40° F. until ready for processing. Twenty-five randomly distributed soil samples were collected from each plot with a soil auger. Each soil sample was 2 inches in diameter and was trimmed to include only the top inch of soil, as described by Murphy and Barthel (*7*). Soil samples were stored in the same manner as forage samples.

Two days prior to application of insecticide, the plots were clipped to a height of 4 to 6 inches. Similar clipping commensurate with good pasture management was performed during the course of the experiment. After application of the insecticide, forage samples were again collected as described above at 2-, 4-, 6-, and 8-week intervals. Soil samples were also collected 6 and 30 days after application, as previously described.

APPLICATION. Two per cent heptachlor granules were applied to the five pasture plots between 12 noon and 2 P.M. on May 13, 1960, by a jeep equipped