fat after an 8-week feeding period were 8.4 p.p.m. for the 60 p.p.m. feeding, 14.3 p.p.m. for the 100 p.p.m. feeding, and 24.3 p.p.m. for the 140 p.p.m. feeding.

There was no evidence that the cows found the toxaphene-treated hay or concentrate unpalatable, and there were no clinical signs of poisoning in any of the animals.

The use of toxaphene or Strobane as 0.5% emulsion or suspension sprays or as a 2% oil spray on dairy cows gave residues in milk up to a maximum of 0.8 p.p.m. Since no residue tolerances are currently permitted in milk, these insecticides cannot be recommended for use on dairy cows.

Literature Cited

(1) Bateman, G. Q., Biddulph, C., Harris, J. R., Greenwood, D. A., Harris, L. E., J. AGR. FOOD CHEM. **1,** 322 (1953).
(2) Brundrett, H. M., Richards, R., Smith, C. L., *J. Econ. Entomol.* **48,** 224 (1955).
(3) Carter, R. H., Hubanks, P. E., Poos, F. W., Moore, L. A., Ely, R. E., *J. Dairy Sci.* **36,** 1172 (1953).
(4) Hudy, J. A., Dunn, C. L., J. AGR. FOOD CHEM. **5,** 351 (1957).
(5) Roark, R. C., *U. S. Dept. Agr. Bur. Entomol. Plant Quarantine* **E-802,** June 1950.
(6) Smith, C. L., Richards, R., *J. Econ. Entomol.* **48,** 566 (1955).

Received for review August 3, 1962. Accepted October 22, 1962.

INSECTICIDE RESIDUES

Residues in Fatty Tissues and Meat of Cattle Grazing on Pastures Treated with Granular Heptachlor

L. L. RUSOFF, R. S. TEMPLE,[1] R. G. MYERS, L. D. NEWSOM, and E. C. BURNS

Louisiana Agricultural Experiment Station, Baton Rouge, La.

W. F. BARTHEL, CALVIN CORLEY,[2] and AVA ALLSMAN[3]

U. S. Department of Agriculture, Gulfport, Miss.

Small amounts of heptachlor epoxide residue were found in fatty tissue of cattle grazing on pastures treated with 0.25 pound of heptachlor per acre as a granular formation. Five dairy steers were placed on a treated pasture at intervals of 1, 8, 15, 29, and 43 days following application of heptachlor with a Buffalo turbine. Biopsy samples of omental fat, taken 30 days following commencement of grazing, showed gradually decreasing levels of residue (maximum 2.5 p.p.m.) in successive samples and no residue in the sample from the 43-day animal. In a second experiment, omental fat samples taken at varying intervals from four beef calves, four yearlings, and three cows which were permitted to graze at the time of aerial application also contained small decreasing amounts (maximum 3.45 p.p.m.) of residue through 125 days of grazing. The residue gradually decreased to 1.7 p.p.m. as the interval from treatment increased. Less than 1 p.p.m. of residue was found in the raw and cooked meat of animals slaughtered after 125 days of grazing. No residue was found in the brain, liver, or kidney of these animals.

FOR THE CONTROL of the imported fire ant (*Solenopsis saevissima richteri* Forel), grasshoppers, and other insects on pasture and rangeland, heptachlor has been used effectively. However, milk and fatty tissues from cattle grazing on such treated pastures have been found to contain significant amounts of heptachlor epoxide residue, the metabolic product of heptachlor (*1, 2, 6*). The Entomology Research Division (*1*) reported up to 4 p.p.m. of heptachlor epoxide in fatty tissues of steers 93 to 103 days after continuous grazing on pastures aerially treated with 2 ounces of heptachlor per acre. This sole study, including direct grazing on heptachlor-treated pastures by beef animals, involved the use of spray applications of heptachlor in solution of diesel oil.

The present recommendation for eradication of the imported fire ant 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 (*7*). The instructions issued for use of heptachlor in this manner require that beef animals being finished for slaughter not be allowed to graze on treated pasture for 60 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 meat of animals grazing on such pastures. Since no reports have been found in the literature to verify this assumption, this study was undertaken.

Experimental Procedure

This work was conducted in two phases:—Phase A, dairy steers were grazed on pasture treated with heptachlor granules applied with a Buffalo turbine mounted on a jeep; and Phase B, beef animals were grazed on pasture treated with heptachlor granules applied with air craft.

Application of Insecticide. PHASE A. Approximately 15 acres of pasture, seeded to Johnston grass, were divided after treatment by wire fence into five plots approximately equal in size. Two

[1] Present address: Animal Science Dept., University of Tennessee, Knoxville, Tenn.
[2] Present address: Pesticide Chemicals Research Branch, A.R.S., U. S. Department of Agriculture, Beltsville, Md.
[3] Resigned.

Table I. Heptachlor Epoxide Residue in Omental Fat of Steers Grazing for 30 Days at Different Intervals on Pasture Treated with 0.25 Pound of Heptachlor Granules per Acre

Steer No.	Day Steer Placed on Plot after Treatment of Pasture	Day Fatty Tissue Obtained from Steers	Heptachlor Epoxide, P.P.M.
1	1	31	2.5
2	8	38	2.4
3	15	45	1.0
4	29	59	0.8
5	43	73	0

Table II. Heptachlor Epoxide Residue in Omental Fat of Beef Animals Grazing on Pasture Aerially Treated with 0.25 Pound of Heptachlor Granules per Acre

Experimental Animals[a]	Heptachlor Epoxide Residue, P.P.M. Time Animals Grazed after Treatment of Pasture	
	29–30 Days	99 Days
Calf 1703	3.1	2.7
Calf 1798	4.0	2.7
Yearling 1462	3.1	1.3
Yearling 1498	3.2	2.7
Cow 771[b]	3.7	1.3
Av.	3.45	2.14
	58–62 Days	124–125 Days
Calf 1713	4.5	2.2
Calf 1894	2.5	2.9
Yearling 1531	1.7	1.3
Yearling 1546	1.8	1.3
Cow 21	2.5	0.7
Cow 702	1.9	...
Av.	2.5	1.7

[a] Calves 1908 and 1956, yearlings 1501 and 1642, and cows 31 and 700 were on untreated pasture and were used as controls. These animals showed no residue of heptachlor epoxide at any time.
[b] Insufficient sample of cow 106 on treated pasture was obtained.

Table III. Residues of Heptachlor Epoxide in Meat of Animals Grazing on Pasture Treated with 0.25 Pound of Heptachlor Granules per Acre

Animal	Heptachlor Epoxide Residue, P.P.M. Raw meat	Cooked meat
	FAT	
1498	2.4	...
1703	2.5	...
1798	2.5	...
Av.	2.47	
	STEAK	
1498	0.8	0.6
1703	0.4	0.9
1798	0.5	0.8
Av.	0.57	0.77
	ROAST	
1498	0.4	0.8
1703	0.5	0.8
1798	0.5	0.9
Av.	0.47	0.83

per cent heptachlor granules were applied to the five pasture plots on May 13, 1960, by a jeep equipped with a Buffalo turbine. Swath widths were 30 feet, and the machine was calibrated to deliver approximately 12.5 pounds of insecticide per acre.

PHASE B. Approximately 500 acres of good Dallis and Bermuda grass pasture were treated by airplane with approximately 12.5 pounds of 2% heptachlor granules per acre on June 2, 1960.

Distribution of Insecticide. The distribution of insecticide applied to the pastures was determined by placing dishpans, each 1 sq. foot in area, at random over the pastures to collect the insecticide as it was applied. Following treatment, the insecticide collected in the pans was transferred to shell vials, and taken to the laboratory where any foreign matter was removed, and the sample weighed. Amount of insecticide actually collected per pan and calculated as pounds per acre was determined. Ten dishpans were placed on each plot in phase A, and 34 dishpans in the pasture in phase B.

Animals. Phase A. Five dairy steers weighing 350 to 400 pounds from the Dairy Science Department, Louisiana State University, were used. After application of the insecticide to the pasture, one dairy steer was placed on a separate treated plot for continuous grazing at 1, 8, 15, 29, and 43 days following application of the insecticide. Omental fatty tissues of the steers were obtained by a veterinarian prior to and 30 days following placement on treated plots according to the technique of Radeleff (*5*). Samples were stored in plastic bags and frozen until analyzed for heptachlor residue.

PHASE B. Eighteen beef animals consisting of six Angus cows, their six crossbred calves, and six crossbred yearlings from the Animal Science Department Louisiana State University, were used in this phase. Twelve animals—four cows, four calves, and four yearlings—were on the pasture during aerial application of the insecticide, and continuously thereafter, for a period of 124 days. Six animals—two cows, two calves, and two yearlings—were used as the control group and were on an untreated pasture.

Omental fatty tissues of all animals were obtained prior to treatment of the pasture. Following treatment of the pasture, omental fatty tissue was obtained from half of the number of animals of each age from both groups, after approximately 30 days and 100 days, and from the remaining half after 60 days and 124 days of grazing.

Analysis for Residue. The frozen sample of tissue was cut with a knife into very small pieces and macerated with twice its weight of sodium sulfate. The tissue was then placed in a quart Mason jar with the sodium sulfate and 500 ml. of colorimetric-grade pentane, and the sample was extracted by allowing it to stand overnight. Further standing did not increase the extracted material. The weight of sample used varied from 10 grams for actual fat to 50 grams for steak or roast.

All analyses for heptachlor epoxide in the fat were made by the technique of Meyer *et al.* (*3*), as modified by Murphy and Barthel (*4*). The modification involved the use of 7% fuming sulfuric acid for removing the fat from the pentane solution and use of a three-part chromatographic column, consisting of Florisil, Florisil–carbon black, and Florisil in three successive layers, for the separation of the heptachlor epoxide from the interfering substance. Absorbance readings of samples were made with a Bausch and Lomb Spectronic 20 spectrophotometer. In practically all cases, there were two pretreatment blank samples for each animal. The average absorbance of these samples was used as a blank for subsequent determinations on residue for that animal. The optical density readings for blanks as determined on the Spectronic 20 varied from 0.025 to 0.050, or a difference of 100% which permits definition of residues as low as 0.5 p.p.m.

Results and Discussion

As expected, there was considerable variation in the amount of heptachlor applied on the pastures. The over-all application, as estimated from the amount collected in the dishpan, was at the rate of 13.97 pounds per acre of the insecticide formulation in phase A, and 12.3 pounds per acre in phase B, instead of the intended application of 12.5 pounds of 2% heptachlor granules.

Residues in Omental Fat. All dairy steers and beef animals showed no heptachlor or heptachlor epoxide residue in their fatty tissues in samples obtained prior to their placement on treated pasture. Although the heptachlor fraction was determined routinely with every sample obtained prior to and during grazing of treated pasture, no detectable residue of heptachlor was found.

The figures presented for heptachlor epoxide in the fat samples are the average of at least two replicates. The agreement between replicates was only fair as they were not run at the same time but rather several months apart. The second replicate was almost always slightly higher in value than the first, due to the loss of moisture from the samples during storage which resulted in concentration of the residue in the sample. Since the values were within the limits imposed by the blank variation, it was not considered necessary to

lend much significance to this slight variation.

Table I shows the amount of heptachlor epoxide residue (p.p.m.) in the omental fat of the dairy steers grazing 30 days after each was placed on treated plots. The earlier an animal was placed on a plot after treatment with heptachlor, the higher the level of residue in the omental fat. In general, the results followed the same residue pattern as was obtained in the butterfat of lactating dairy cows grazing the same plots (*6*).

Table II shows the heptachlor epoxide residues in the omental fat of beef animals grazing on aerially treated pasture for various lengths of time. The highest levels of residues were present in the 30-day samples, regardless of the age of the animals. After 125 days of grazing, heptachlor epoxide residues were found in all fat samples.

The levels of heptachlor epoxide residues in the omental fat of the animals grazing on aerially treated pasture for 30 days were much higher than the level found in the dairy steer grazing on surface-treated pasture. Since the beef animals were on pasture during the aerial application of heptachlor, as is usually practiced, apparently some of the insecticide may have been inhaled through the respiratory tract or absorbed through the skin in addition to ingestion of treated forage. Also, the fact that animals were grazing the same day of treatment may account for the higher level in the omental fat of these animals.

Residues in Organs of Beef Animals. Samples of brain, kidney, liver, and meat were obtained from three animals after grazing on treated pasture for 125 days to find out whether heptachlor epoxide might concentrate in these organs.

No residue was found in any of the organs of these animals although their fatty tissues showed approximately 2.5 p.p.m. of heptachlor epoxide. Since the residue concentrates in fatty tissue, any variation in amounts found in organs and tissues reflects a variation in fat content. Less than 1 p.p.m. of residue was present in raw and cooked meat (Table III). The increase in concentration of the heptachlor epoxide residue in cooked meat over raw meat is no doubt due to the dehydration of meat after cooking.

Literature Cited

(1) Entomology Research Division, U. S. Dept. Agr., *J. Econ. Entomol.* **52,** 1206 (1959).
(2) Gannon, N., Decker, G. C., *Ibid.*, **53,** 411 (1960).
(3) Meyer, C. F., Malina, M. A., Polen, P. B., J. AGR. FOOD CHEM. **4,** 694 (1956).
(4) Murphy, R. T., Barthel, W. F., *Ibid.*, **8,** 442 (1960).
(5) Radeleff, R. D., *Vet. Med.* **45,** 125 (1950).
(6) Rusoff, L. L., Waters, W. H., Gholson, J. H., Frye, J. B., Jr., Newsom, L. D., Burns, E. C., Barthel, W. F., Murphy, R. T., J. AGR. FOOD CHEM. **10,** 377 (1962).
(7) *U. S. Dept. Agr., Plant Pest Control Div., Control Manual* **807-25.6100,** (1960).

Received for review May 31, 1962. Accepted September 4, 1962.

INSECTICIDE RESIDUES

Method for Phosphamidon Residue Analysis

RUDOLF ANLIKER
Ciba, Ltd., Basle, Switzerland
R. E. MENZER[1]
Department of Entomology, University of Maryland, College Park, Md.

A paper chromatographic method has been developed for the determination of phosphamidon in plant tissue. After chromatography with a selective solvent system, phosphamidon and its metabolites were detected with a specific blue tetrazolium dye. After elution into test tubes with methanol, a total phosphate analysis was carried out by the phosphomolybdenum blue method. The sensitivity of the method is 0.2 to 0.4 p.p.m. depending on the dilutions and aliquots used.

PHOSPHAMIDON (1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate) and its metabolites (*2*) were extracted from plant material by maceration in a Waring Blendor with methylene chloride. An aliquot of the extract was evaporated to dryness, taken up in a small measured quantity of methylene chloride, and applied to Whatman No. 1 filter paper. The extracts were chromatographed by a solvent system developed by Bush (*4*). After chromatography, the papers were developed and eluted with methanol into test tubes. A blue solution, the absorbance of which could be read on a spectrophotometer, was developed through the use of the phosphomolybdenum blue method, based on the modifications made by Bartlett (*3*).

[1] Present address: Department of Entomology, University of Wisconsin, Madison 6, Wis.

Reagents

Methylene chloride, technical, redistilled.

Chromatography solvent system, made by mixing by volume 5 parts petroleum ether (b.p. 90°-100° C.), 5 parts toluene, 7 parts methanol, and 3 parts water, and separating the two phases. All solvents were redistilled.

Blue tetrazolium solution [3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-bis(2,5-diphenyl-2H-tetrazolium chloride)], (Matheson, Coleman and Bell No. 7596), 0.1%, stored in a dark bottle.

Hydrogen peroxide, 30%, analytical grade.

Potassium permanganate solution, 0.01*N*, made up fresh weekly.

Sodium oxalate solution, 0.01*N*, made up fresh weekly.

Ammonium molybdate solution, 5%, made up fresh weekly.

1-Amino-2-naphthol-4-sulfonic acid, 0.2%, containing 12% sodium metabisulfite, and 1.2% anhydrous sodium sulfite, made up fresh daily.

Apparatus

Chromatography cabinet, Research Specialities Co., No. B-550, or equivalent. Chromatography jars were found to give unsatisfactory results because the at-