Excretion of Toxaphene and Strobane in the Milk of Dairy Cows

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Emulsions, suspensions, and oil solutions of toxaphene or Strobane sprayed on dairy cows at entomologically effective concentrations caused residues of the insecticides in the milk. When toxaphene was fed to dairy cows in feed at levels of 20, 60, 100, and 140 p.p.m., the insecticide was excreted in the milk at all the dosage levels. There was a rapid decrease in the residue to a level of 0.1 to 0.3 p.p.m. the first week after feeding ceased; further decreases were at a slower rate for animals fed more than 20 p.p.m. Residues of toxaphene in omental fat in cows given treated feed daily ranged from 8.4 to 24.3 p.p.m. for the three highest dosage levels at the end of the 8-week feeding period.

ENTOMOLOGICAL STUDIES during the period 1948–55 showed toxaphene (chlorinated camphene containing 67 to 69% chlorine) and Strobane (a mixture of chlorinated terpene isomers containing 65% chlorine) to be very valuable insecticides for the control of external parasites on beef and dairy cattle. In 1950, Roark (*5*) published a digest of information on toxaphene, and in 1955, Brundrett *et al.* (*2*) and Smith and Richards (*6*) reported tests with Strobane for the control of livestock pests. The possible uses of these insecticides on dairy cattle prompted the USDA to conduct tests at Kerrville, Texas, in 1954 and 1955 to determine how much of the materials was excreted in the milk of dairy cows sprayed with entomologically effective concentrations. Further, it was of interest to know whether there was any difference in the amounts excreted of these closely related insecticides.

Toxaphene was shown to be a valuable insecticide in the control of insects on forage and feed crops (*5*) which could be used as livestock feed, and therefore the USDA became interested in investigating the residues in milk possibly resulting from the feeding of these treated crops to dairy cows. In 1953, studies were made at Beltsville by Carter *et al.* (*3*) and in Utah by Bateman *et al.* (*1*) to determine the residues of toxaphene appearing in milk when dairy cows were fed alfalfa hay that had received normal toxaphene treatments for insect control. These studies showed that toxaphene residues did appear in the milk. In 1954, the USDA initiated a residue study at Kerrville, Texas, to acquire further information on the amounts of toxaphene excreted in the milk of dairy cows when known amounts were added to the diet for definite feeding periods.

The studies on toxaphene residues were carried out in cooperation with the Hercules Powder Co., and the residue study on Strobane in cooperation with the B. F. Goodrich Chemical Co. The levels of toxaphene that would give the desired information in the feeding tests were selected by representatives of the Hercules Powder Co., the USDA, and the Food and Drug Administration.

Procedure

Animal Sprays of Toxaphene or Strobane. For the spray tests, three formulations of each insecticide were used. An emulsifiable concentrate of each insecticide was prepared in our laboratory to contain 50% insecticide, 40% xylene, and 10% emulsifier (CTX-54). Each cooperating company furnished a 50% wettable-powder formulation of its product. Oil sprays of each insecticide were made in our laboratories by dissolving 2.0% of the technical grade of each insecticide in deodorized kerosine.

In the toxaphene test, eight Jersey cows were used—six were sprayed and two were used for controls. Two of the cows were sprayed twice at 3-week intervals with a 0.5% emulsion of toxaphene, two were sprayed at the same intervals with a 0.5% suspension of the wettable powder, and two were sprayed twice daily for 21 days with 1 ounce of the 2% oil spray.

In the tests with Strobane, the toxaphene test described above was duplicated, with six Jersey cows being treated with Strobane sprays instead of toxaphene. The two control cows served for both tests.

The cows used in both tests were of mixed ages, and in good health. They were in early to mid-lactation and were producing an average of 25 pounds of milk daily. Milk samples were taken from the cows sprayed with the emulsions and the suspensions before the first spraying and 1, 2, 3, 5, 7, 14, and 21 days after each of the two sprayings. Samples were taken at the same intervals from the control cows and from the cows sprayed daily with the oil sprays.

Feeding of Toxaphene in the Diet. For the feeding test, 14 Jersey cows of mixed ages were used; these animals were in early to mid-lactation and producing an average of 25 pounds of milk daily. They were in good health, and at the initiation of the test were free of mastitis. However, the disease soon appeared, and affected several cows during the test but did not appear to affect results. The cows were maintained on the experimental feeds, without insecticide, for 2 weeks prior to the addition of toxaphene to the diet. Control milk samples were taken from all cows during this period and analyzed to ensure that the milk was free of chlorinated insecticides before the start of the test. The cows were grouped as uniformly as possible according to production; two cows were used as controls and three for each of four levels of toxaphene feeding—20, 60, 100, and 140 p.p.m. in all items of the diet.

Alfalfa hay and a feed concentrate mixture were fed in an approximate ratio of 2.25 to 1. The hay was a good grade, and the concentrate was composed of corn chops, crushed oats, cottonseed meal, steamed bone meal, and salt. Each cow was fed as much hay as she would readily consume and an amount of concentrate based on her milk production.

Each lot of hay and concentrate was analyzed and determined to be free of chlorinated hydrocarbons before being used. Two acetone solutions of toxaphene were prepared for each feeding level. The concentration of one solution was such that 1 ml. of solution provided the desired amount of toxaphene for 1 pound of concentrate, and the concentration of the other solution was such that 2 ml. provided the desired amount for 1 pound of hay. Hay and concentrate were fed twice daily. Each cow's feed was weighed and treated with the

appropriate amount of toxaphene, well distributed, just before feeding.

Milk samples were taken from each cow 13, 11, 7, and 5 days before feeding of toxaphene began, at weekly intervals during the 8-week feeding period, and 2, 7, 14, and 21 days after the feeding of toxaphene ceased.

To compare the toxaphene residues in the butterfat of the milk with the residues in the omental fat, one cow from each of the three higher feeding levels was subjected to omental biopsy on the day following the final feeding of toxaphene to obtain fat samples for analysis.

Sampling Methods. All milk samples were obtained by milking each cow with an individual mechanical milker assembly with individual stainless steel containers for each cow's milk. Special precautions were taken to prepare the cows' udders and prevent mechanical contamination. The milker assemblies were opened in the laboratory and samples taken for analysis.

The cows were milked twice daily on a 6-18 hour schedule instead of the usual 12-12 hour schedule so that the experiments could be conducted during the 8-hour work day. In the spray tests, 3-liter samples were taken from the morning milking; in the toxaphene feeding test, each sample consisted of 2 liters from the morning milk and 1 liter from the afternoon milk.

Methods of Analysis

The analytical method used was based on the assumption that the insecticides were present in the butterfat of the milk. Since toxaphene and Strobane are only slightly soluble in water and extremely soluble in fat, there can be little doubt that the insecticide residues are virtually completely partitioned into the butterfat. The procedure for separating and washing the butterfat for removal of inorganic halides was not quantitative; rather, the analysis was based upon a weighed sample of washed butterfat, which was analyzed for organically bound chlorine, and the residue was calculated in parts per million of insecticide that would appear in milk containing 4% butterfat.

Three-liter samples of milk were left in separatory funnels for 24 hours at approximately 5° C. for the cream to separate. The lower layer was drawn off and discarded, which left about 500 to 600 ml. of cream. The cream was churned in a Waring Blendor. The butter that separated was drained and then washed by macerating with three portions of distilled water. The butter samples were placed in 250-ml. beakers and heated in a water bath until the fat separated. The oily layer was decanted into a 200-ml. centrifuge bottle, 10 grams of anhydrous sodium sulfate was added, the mixture was shaken and then centrifuged while it was still hot for 5 minutes at 2500 r.p.m. The oil was decanted into a clean centrifuge bottle and was washed by shaking three times with boiling distilled water; each time the oil was centrifuged to separate the water, which was removed with a siphon. The washed butterfat was poured into a wide-mouthed bottle and stored in the refrigerator. Ten-gram samples of the chilled butterfat were weighed out and analyzed by burning in a combustion furnace of the type developed by Hudy and Dunn (*4*). The chloride was titrated amperometrically with 0.0025*N* silver nitrate. The parts per million of chloride was multiplied by a factor of 1.46 or 1.50 to convert to parts per million of toxaphene or Strobane in the butterfat. These values were then divided by 25 to obtain the parts per million of toxaphene or Strobane that would appear in milk containing 4% butterfat.

Recoveries of toxaphene and Strobane added to butterfat and determined by the method described above are shown in Table I.

When total chlorine methods are used for estimating insecticides in butterfat, analysis of uncontaminated samples always gives a titration indicating the presence of halogen-containing compounds in the normal fat. This titration is usually termed the blank and must be subtracted from test samples. To test the suitability of the individual cows for the toxaphene feeding experiment and the dependability of the method for giving low and constant blanks, four control samples taken from each cow over a period of 2 weeks were analyzed before the feeding of toxaphene began. The results of these analyses are shown in Table II. The average of these four control samples was used as the blank for each cow, and this value was subtracted from the test samples.

The fat samples taken by biopsy were extracted with benzene. After removal of the benzene, the extracted fat was washed with hot distilled water with the same procedure used for washing the butterfat. Fifteen-gram samples of the washed fat were weighed out and analyzed in the same way as the butterfat.

Results and Discussion

The results of the analyses of the milk samples taken during the experiment from the control cows in the spray tests are shown in Table III. These results are provided to show that the blank was not excessive, to give an indication of the variation from day to day, and to show that there was no increase in the organically bound chlorine content of the milk from the control cows during the test period.

The results of the analyses of the milk samples from the cows sprayed with the emulsion and suspension sprays are given in Table IV. The values in the tables are averages of the residues found in the milk samples taken from each

Table I. Recovery of Strobane and Toxaphene Added to 10 Grams of Butterfat

(Equivalent to 250 ml. of milk containing 4% butterfat)

Amount Added		Amount Recovered	
μG.	P.p.m. in milk	μG.	%
	STROBANE		
400	1.6	383	96
300	1.2	290	97
200	0.8	187	93
	TOXAPHENE		
100	0.4	96	96
75	0.3	75.5	101
50	0.2	46	92
25	0.1	24.7	99

Table II. Organically Bound Chlorine in Control Milk Samples from Toxaphene Feeding Test

(Each sample consisted of 2 liters from morning milk and 1 liter from afternoon milk)

Cow No.	P.P.M. at Indicated Days before Toxaphene Feeding[a]				
	13	11	7	5	Av.
1	0.02	0.06	0.06	0.05	0.05
2	0.04	0.06	0.06	0.05	0.05
3	0.06	0.06	0.05	0.03	0.05
4	0.06	0.06	0.07	0.06	0.06
5	0.04	0.03	0.06	0.03	0.04
6	0.04	0.04	0.06	0.05	0.05
7	0.02	0.03	0.02	0.05	0.03
8	0.08	0.06	0.05	0.05	0.06
9	0.07	0.04	0.04	0.04	0.05
10	0.05	0.05	0.06	0.07	0.06
11	0.05	0.05	0.04	0.04	0.04
12	0.05	0.06	0.06	0.04	0.05
13	0.06	0.05	0.04	0.03	0.04
14	0.04	0.06	0.05	0.04	0.05

[a] The values given have been corrected for the reagent blank which was equivalent to 0.04 p.p.m.

Table III. Analysis of Milk Samples Taken from Unsprayed Control Cows at Intervals Paralleling the Treated Animals

Sampling Date	P.P.M. of Organically Bound Chlorine in Milk Containing 4% Butterfat from:	
	Cow No. 1	Cow No. 2
Before start of experiment	0.08	0.13
Days after start of experiment:		
2	0.07	0.18
7	0.08	0.16
14	0.07	0.16
21	0.07	0.13
28	0.10	0.14
35	0.11	0.12
42	0.08	0.12
Av.	0.08	0.14

group of two cows given the same treatment. The results shown are corrected for the blank.

Residues resulted from both types of water sprays of both toxaphene and Strobane. The maximum residues occurred 1 or 2 days after spraying and decreased to very low values at 21 days. No appreciable difference in amounts of residue was detected between the two types of formulations or between the two insecticides.

The residues resulting from the daily oil sprays are shown in Table V. The residues of both toxaphene and Strobane reached a maximum about the third day of spraying. Increases during the remaining 18 days of spraying were very slight. There was no significant difference in the amount of residues of the two insecticides.

Toxaphene residues in the milk from the toxaphene feeding test are shown in Table VI. Residues of toxaphene were excreted in the milk at all dosage levels. At all levels of feeding, the residues in milk increased rapidly and reached a maximum within 4 weeks after feeding started. Slight increases were noted in the two lower dosage groups through the first 6 weeks of feeding, after which the residues decreased.

There was a rapid decrease in residues immediately after the feeding of toxaphene ceased. The rate of decrease was the same in all groups during the first week, and residues fell to the 0.1 to 0.3 p.p.m. level. Further decreases were at a slower rate for animals fed more than 20 p.p.m. Three weeks after feeding terminated, no residue was detected in the milk of cows fed 20 p.p.m., and the milk from those fed 60 to 140 p.p.m. contained 0.07 to 0.2 p.p.m. The increase of organic chlorine in the control group during the weeks 4, 5, and 6 indicate a slight contamination of the feed at this period, although the authors have no explanation of how this could have occurred.

Residues of toxaphene in the omental

Table IV. Insecticides (P.P.M.) in Milk from Cows Sprayed Twice at 3-Week Intervals with 0.5% Sprays of Toxaphene or Strobane[a]

	Emulsion Spray		Suspension Spray	
Sampling Date	Toxaphene	Strobane	Toxaphene	Strobane
Before spraying	0	0	0	0
Days after first spraying:				
1	0.60	0.74	0.64	0.59
2	0.61	0.61	0.74	0.61
3	0.44	0.41	0.58	0.46
5	0.23	0.21	0.26	0.21
7	0.16	0.07	0.20	0.10
14	0.06	0.06	0.12	0.04
21	0.08	0.05	0.12	0.05
Days after second spraying:				
1	0.52	0.46	0.71	0.56
2	0.55	0.55	0.81	0.68
3	0.41	0.37	0.57	0.52
5	0.24	0.18	0.31	0.39
7	0.16	0.06	0.18	0.16
14	0.10	0.01	0.08	0.04
21	0.06	0.02	0.02	0.01

[a] The insecticides were calculated in p.p.m. of milk containing 4% butterfat. The values given are averages from the analysis of two milk samples. Each sample consisted of 10 grams of butterfat. The titration of the control sample from each cow was used as the blank and was subtracted from subsequent samples.

Table V. Insecticides (P.P.M.) Excreted in Milk of Dairy Cows Sprayed Twice Daily for 21 Days with 1 Ounce of 2.0% Oil Solution of Toxaphene or Strobane[a]

Sampling Date	Toxaphene	Strobane
Before spraying	0	0
Days after spraying started:		
1	0.12	...
3	0.41	0.28
7	0.33	0.33
14	0.26	0.32
21	0.30	0.31
Days after spraying ceased:		
7	0.09	0.07
14	0.10	0.01
21	0.06	...

[a] Calculated from organic chlorine determinations for milk containing 4% butterfat. The values given are averages from the analyses of two milk samples of 3 liters each.

Table VI. Toxaphene (P.P.M.) in Milk from Cows Fed Different Levels of Toxaphene in the Diet

(Each sample consisted of 2 liters from morning milk and 1 liter from afternoon milk)

		Weeks Fed								Weeks after Shift to Untreated Feed		
Dosage, P.P.M.	Cow No.	1	2	3	4	5	6	7	8	1	2	3
20	3	0.17	0.24	0.24	0.31	0.29	0.33	0.25	0.21	0.10	0.01	...
	4	0.26	0.31	0.31	0.41	0.24	0.42	0.31	0.25	0.06	0.04	...
	5	0.16	0.24	0.24	0.35	0.35	0.35	0.26	0.24	0.06	0.02	...
	Av.	0.20	0.26	0.26	0.36	0.33	0.37	0.27	0.23	0.07	0.02	...
60	6	0.61	0.65	0.74	0.70	0.67	0.68	0.47	0.44	0.08	0.05	0.04
	7	0.61	0.69	0.87	0.66	0.69	0.77	0.53	0.52	0.14	0.11	0.09
	8	0.47	0.50	0.65	0.67	0.53	0.69	0.48	0.48	0.16	0.13	0.09
	Av.	0.56	0.61	0.75	0.68	0.63	0.71	0.49	0.48	0.13	0.10	0.07
100	9	0.90	0.99	0.92	1.06	0.87	0.96	0.93	0.90	0.11	0.05	0.08
	10	0.87	1.00	1.08	1.19	1.13	1.04	0.97	0.96	0.18	0.16	0.15
	11	0.85	1.05	1.04	1.19	0.92	0.89	0.68	0.88	0.17	0.18	...
	Av.	0.87	1.01	1.01	1.15	0.97	0.96	0.86	0.91	0.15	0.13	0.12
140	12	1.46	1.56	1.68	1.75	1.31	1.39	1.36	1.52	0.19	0.17	0.12
	13	1.13	1.09	1.40	1.45	1.23	1.23	1.53	1.44	0.30	0.22	0.21
	14	1.74	2.36	2.32	2.47	1.96	2.31	2.24	2.51	0.46	0.80	0.26
	Av.	1.44	1.67	1.80	1.89	1.50	1.64	1.71	1.82	0.32	0.40	0.20
Control	1	0.00	0.02	0.00	0.07	0.20	0.17	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.04	0.11	0.14	0.00	0.00	0.00	0.00	0.00
	Av.	0.00	0.01	0.00	0.06	0.16	0.16	0.00	0.00	0.00	0.00	0.00

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).

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INSECTICIDE RESIDUES

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

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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

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