

TOXAPHENE

Transmission Studies of Milk of Dairy Cows Fed Toxaphene-Treated Hay

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This work is a part of a larger research project which relates to the toxicity of the newer insecticides to farm animals and the accumulation of such insecticides in products consumed by man. Toxaphene has been recommended by federal and state agencies for control of insects which damage alfalfa. Because of differences in climate, insects and farm practices it is desirable to test the toxicity of insecticides in parts of the country. The results obtained in this experiment indicate that alfalfa treated with 2 and 4 pounds of toxaphene per acre showed good economic control of insects which damage alfalfa under Utah (or arid) conditions. Feeding of toxaphene-treated hay to dairy cows for 112 days had no measurable influence on hay or grain consumption or milk and butterfat production, nor did it alter the liver and kidney tissues of the animals. No toxaphene was found in the milk of cows receiving untreated hay, but it was found in the milk of cows receiving treated hays, the amounts being 2.5 and 2.3, 4.3 and 3.9, and 18.2 and 8.3 ppm. for the 1-, 2-, and 4-pound per acre treatments, respectively.

THE RESIDUE REMAINING on alfalfa hay treated with economic levels of DDT and methoxychlor, and the amount of insecticide found in the milk and tissues of dairy cows (1, 2), the tissues of lambs (7), and the tissues and eggs of hens (3, 5), after they consumed such treated hay, have been reported. Laakso and Johnson (9) determined the toxaphene residue on alfalfa hay after various methods and levels of application, and Diephuis and Dunn (4) reported the residue present in the tissues of steers and sheep that consumed the treated hay. Ely, *et al.* (6) stated that less than 1 p.p.m. of toxaphene was found in milk of cows fed alfalfa hay treated with 1.5 pounds of toxaphene per acre. Leighton, Kuiken, and Smith (12) reported that toxaphene sprayed on

cows as emulsifiable oil concentrates was excreted in the milk for 5 to 7 days after spraying.

The present data indicate the levels of toxaphene appearing in milk of dairy cows fed alfalfa hay that had been treated with toxaphene for the control of insects.

Materials and Methods

A 30-acre field of first crop dry-land (nonirrigated) alfalfa was divided into four blocks of four plots each with intervening buffer strips, and one plot chosen at random from each block received the following treatments: no treatment, 1, 2, and 4 pounds of toxaphene per acre, respectively. Thus, each treatment was repeated four times in the field.

The toxaphene was applied as a water emulsion spray from standard spraying equipment at the rate of 7.5 gallons per acre.

Just prior to application of the toxaphene and on the third and seventh days after spraying, the alfalfa weevil population was determined in each plot. On the eighth day, the alfalfa was cut, raked into windrows, allowed to sun-cure, and baled, after which it was stored under cover until the feeding trials began. No rain fell between the application of the toxaphene and storing of the hay.

At the time of the experiment, it was not possible to select from the station experimental herd eight Holstein cows that were in the same stage of lactation or producing a uniform amount of milk. Of the eight cows selected, one of the

four producing the highest amount of milk and one of the four producing the least amount were allotted to each of the four hay treatments. Feeding began January 9, 1952, and continued until May 1, 1952, a 112-day period. Milk samples from each cow were taken before feeding the experimental hays and at approximately weekly intervals throughout the period of feeding and analyzed for toxaphene.

Hay samples were taken from each bale fed to each cow during the periods January 16 to 29 and April 22 to May 1 and analyzed for their toxaphene residue.

The hay, milk, and tissue samples were analyzed for toxaphene by the total chloride method of Umhoefer (15), and titrated amperometrically by the method of Laitinen and Kolthoff (10, 11). The method of amperometric titration is described in detail in a toxaphene manual (8).

The samples were prepared for analysis as follows: Fat and hay samples were extracted with benzene. Milk was extracted with Skellysolve B according to the method of Schechter *et al.* (14). The remainder of the tissues were ground with a meat grinder, further macerated with water and ethyl alcohol (1 to 1) in a Waring Blendor, and extracted with Skellysolve B in the same manner as milk.

Results and Discussion

Good economic control of alfalfa weevil larvae, the principal insect present on the alfalfa at the time of spraying, was obtained at the 2- and 4-pound-per-acre levels of toxaphene application, the percentage control being 88 and 94, respectively, on the seventh day after application.

The toxaphene residue present on the hay during the feeding period is given in Table I. There was some variation in the amount of residue present on hay from different plots receiving the same treatment, but the variation (with the exception of plots 3 and 4) would not seem excessive when the technical difficulties associated with application of the toxaphene, harvesting, sampling of the hay, and chemical analysis are considered. The residues at the 4-pound level are somewhat greater than those reported by Laakso and Johnson (9) following application of the same amount of toxaphene under Montana conditions.

In Table II are found the number of days the cows were in lactation at the beginning of the test, the average pounds of 4% fat-corrected milk produced per cow during the 10 days previous to the test and during the test period, body weight at the beginning, and the gain in weight per cow per day. A study of the average milk production of each cow before and during the test indicates that

the cows fed toxaphene-treated hay maintained their production as well as the controls. The gains in body weights of the cows are in line with the stage of lactation. Cows furthest along in lactation made the larger gains, while those recently fresh and producing at the highest levels gained the least. This is expected in normal lactation and indicates no effect of toxaphene on gains in weight during the 112-day feeding period.

No toxaphene was present in the milk of any of the cows at the time feeding of the hays was begun (Table III). The first milk samples taken thereafter showed toxaphene in the milk of all the cows consuming toxaphene-treated hay but none in the milk of those consuming untreated hay. There was some variation in the amount of toxaphene from week to week in a given cow, but generally the amount present in the milk

Table I. Toxaphene Residue on Alfalfa Hay Fed to Dairy Cows

Toxaphene, Lb./Acre	Plot		Cow No.	Toxaphene ^a Residue, P.P.M.	Plot		Cow No.	Toxaphene ^b Residue, P.P.M.
	No.	Days fed			No.	Days fed		
0	4	0-63	E 221	0	1	64-112	E 215, E 221	0
	3	0-92	E 215	0	2	93-112	E 215, E 221	0
1	2	0-52	Hu 132	57.4	1	53-112	Hu 132	81.3
	4	0-87	E 220	64.6	3	88-112	E 220	49.4
2	1	0-83	Hu 187	128.2	2	84-112	Hu 187	161.6
	4	0-74	A 145	110.6	3	75-112	A 145	130.8
4	4	0-87	Hu 188	409.8	3	88-112	Hu 188	238.2
	2	0-112	W 254	226.6	2	0-112	W 254	278.1

^a Average of duplicate determinations on samples collected Jan. 16 to 29.

^b Average of duplicate determinations on samples collected April 22 to May 1.

Table II. Stage of Lactation, Milk Production, Body Weights, and Average Daily Gain of Cows

Toxaphene Applied, Lb./Acre	Cow No.	Stage of Lactation at Beginning of Test Day	Milk Production 4% FCM ^a , Lb.		Body Weight at Start, Lb.	Av. Gain, Lb./Day
			Av. 10 days preceding test	Av. 112-day test period		
0	E 221	59	46.7	238.0	1403	0.46
	E 215	216	29.7	3.2	1259	0.77
1	E 220	113	33.4	23.3	1304	0.50
	Hu 132	193	28.6	19.6	1433	1.00
2	Hu 187	130	40.9	36.1	1300	0.45
	A 145	114	29.3	26.0	1252	0.79
4	Hu 188	85	46.7	41.2	1215	0.36
	W 254	277	22.4	19.7	1166	0.66

^a Fat-corrected milk.

Table III. Toxaphene in Milk of Dairy Cows Fed Alfalfa Hay Treated with Toxaphene

Day Milk Sampled	Toxaphene Applied, Pounds per Acre							
	0 Cow		1 Cow		2 Cow		4 Cow	
	E 221	E 215	E 220	Hu 132	Hu 187	A 145	Hu 188	W 254
	Toxaphene Found, P.P.M.							
0 ^a	0	0	0	0	0	0	0	0
5	0	0	1.3	0.8	1.3	2.2	5.6	4.6
8	0	0	1.5	0.0	2.3	0.2	...	0.0
13	0	0	11.6	4.5	11.6	13.3	11.3	16.2
19	0	0	0.3	1.0	2.8	3.7	10.1	27.5
22	0	0	1.8	3.7	5.3	2.7
29	0	0	7.3	1.2	1.8	3.5	18.4	5.0
35	0	0	0.3	0.7	7.1	1.7	20.6	5.0
42	0	0	3.7	1.8	2.8	3.0	21.7	6.0
50	0	0	1.8	2.2	1.8	2.8	21.2	6.5
56	0	0	0.8	1.8	3.3	8.1	26.7	8.1
63	0	0	1.8	1.8	3.5	3.4	20.9	4.2
70	0	0	2.1	2.6	2.9	3.4	23.7	8.1
77	0	0	1.1	4.1	4.5	2.9	29.2	9.7
84	0	0	0.8	2.3	8.4	3.7	27.0	5.4
91	0	0	1.6	1.3	5.7	3.4	14.1	9.9
98	0	0	3.1	1.5	2.6	3.2	12.3	6.2
105	0	0	2.6	3.1	4.4	...	17.1	6.3
112	0	0	1.1	4.4	4.7	3.9	11.7	8.4
Av.	0	0	2.5	2.3	4.3	3.9	18.2	8.3

^a Feeding of hay began. Milk samples were taken from cows E 220, Hu 187, and Hu 188 34 days after being taken off the treated hay; no toxaphene was present in any of these samples.

Table IV. Average Daily Feed Consumption and Milk and Butterfat Production of Dairy Cows for 112-Day Feeding Period on Toxaphene-Treated Hay

Toxaphene Applied, Lb./Acre	Cow No.	Hay Consumed, Lb.	Grain Consumed, Lb.	Milk Produced, Lb.	Butterfat	
					Produced, lb.	In milk, %
0	E 221	46.1	8.6	42.4	1.38	3.3
	E 215	36.5	5.1	24.6	0.90	3.6
	Av.	41.3	6.9	33.5	1.14	3.4
1	E 220	39.7	5.6	25.5	0.86	3.4
	Hu 132	40.4	3.9	21.2	0.73	3.5
	Av.	40.0	4.8	23.4	0.79	3.5
2	Hu 187	35.6	7.7	38.4	1.35	3.6
	A 145	41.1	5.5	28.5	0.98	3.4
	Av.	38.4	6.6	33.5	1.17	3.5
4	Hu 188	36.0	9.4	40.1	1.69	4.2
	W 254	30.9	6.0	19.9	0.78	3.9
	Av.	33.5	7.7	30.0	1.24	4.0

followed the amount applied to the alfalfa, as is indicated by the figures at the bottom of Table III, which give the average amount of toxaphene present in the milk of each cow during the feeding period. Cow Hu 188 had an average of 18.2 p.p.m., whereas cow W 254, consuming hay treated with the same level of toxaphene, had an average of 8.3 p.p.m. The hay consumed by cow Hu 188 during the first half of the feeding period had a residue of 409.8 p.p.m. of toxaphene, nearly double the residue present on the hay consumed by cow W 254, which may account partially for the difference in the toxaphene content of the milk of the two cows. Another factor may be the variation in cows in ability to eliminate ingested toxaphene via the milk.

On day 13 of the feeding trial the toxaphene values for all of the milk samples of the experimental cows were out of line with the values immediately before and after that date (except cow Hu 188). These differences cannot be explained; as far as could be ascertained, there was nothing about the chemical analysis or treatment of the cows that would account for the difference.

Milk samples from cows E 220, Hu 187, and Hu 188 were analyzed on the 34th day after feeding of the treated hays was discontinued, and no toxaphene was found, indicating that this insecticide disappears from the milk soon after feeding of treated hay is discontinued.

The average daily feed consumption and milk and butterfat production per cow during the period of feeding are given in Table IV. There was a high- and a low-producing cow on each level of toxaphene, except the 1-pound-per-acre treatment, where the production of both cows was about equal. The average production of the latter treatment is consequently well below that of the other three groups. No significance is attached to this difference, because the low average of these two cows is related more to the stage of lactation than to any other factor. It would seem that the ingestion of toxaphene-treated hay under the conditions of these experiments had no clear effect on the milk or butterfat pro-

duction, or on hay or grain consumption.

Three cows were slaughtered on the last day of the feeding period. Analysis of liver, muscle, kidney, and kidney fat showed that toxaphene was present in the fat of the cows that received hay treated with 2 and 4 pounds of toxaphene per acre (Table V). The fat of the control cow and the liver, muscle, and kidney tissues from all three cows did not contain any toxaphene. The amount of toxaphene in the fat was correlated with the amount present on the hay (Table I) at the time of slaughter, there being approximately twice as much insecticide on the hay and in the fat following the 4-pound treatment as the 2-pound.

Examination of histological sections of liver and kidney showed no changes that could be attributed to the ingestion of toxaphene by the cows. Neither were symptoms or abnormalities observed in any of the cows at any time. The symptoms and pathology of toxaphene poisoning have been adequately described by Radeleff (13).

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Table V. Toxaphene in Tissues of Cows Consuming Toxaphene-Treated Hay

Toxaphene Applied, Lb./Acre	Cow No.	Toxaphene Content, P.P.M.			
		Liver	Muscle	Kidney	Kidney fat
0	E 221	0	0	0	0
2	A 145	0	0	0	88
4	W 254	0	0	0	197