Residues in Milk from Dairy Cows Fed Low Levels of Toxaphene in Their Daily Ration

GUNTER ZWEIG, EARL L. PYE,[1] RANJIT SITLANI, and S. A. PEOPLES

Pesticide Residue Research and Department of Physiological Sciences, University of California, Davis, Calif.

Sixteen lactating dairy cows were placed on daily rations containing 0 to 20 p.p.m. of the insecticide toxaphene for 77 days. Their milk was sampled periodically and analyzed for toxaphene by a total chloride procedure. By extrapolation, it was estimated that toxaphene concentrations of less than 1.0 p.p.m. in the daily ration of these cows resulted in less than 0.03 p.p.m. of toxaphene in the milk. Uncontaminated milk was produced by all but one animal within 14 days after the animals were taken off their respective toxaphene diet.

TOXAPHENE has been shown to be an effective insecticide, but the extent of residues of toxaphene present in milk of cows fed rations containing low levels of this insecticide has not been established. Claborn and coworkers (*1*) have studied the excretion of toxaphene in the milk of cows fed high levels of toxaphene (20 to 140 p.p.m.) in their daily feed. The present work was undertaken to attempt to establish a level of toxaphene in the feed which would not result in detectable residues in the milk. Sixteen dairy cows were fed daily rations containing 0 to 20 p.p.m. toxaphene in the daily feed for 77 days. Milk samples were taken once or twice weekly and analyzed by a total chloride method.

Experimental

Feeding Procedure. Fifteen Holsteins and one Guernsey were placed on a ration consisting of 0.5 kg. of grain concentrate plus alfalfa hay and grazing to make up a total daily average consumption of about 20 kg. Prior to the feeding experiment, feed and milk were analyzed by total chloride, as described below for insecticides. The feed was found to contain less than 0.05 p.p.m. chlorinated insecticide, and the milk was found to contain less than 0.01 p.p.m. total organic chloride. The cows were divided into six groups which were fed daily rations containing none (control group), 2.5, 5, 10, 15, and 20 p.p.m. toxaphene based on 20 kg. daily feed intake. Each group originally had three cows; however, two cows had to be sacrificed shortly after the experiment commenced, resulting in two cows for the groups fed at the levels 2.5 and 10 p.p.m. toxaphene. At each afternoon's feeding time, the appropriate volume of 2% toxaphene in 10% acetone–90% ethanol was pipetted onto the grain concentrate. The cows were observed until the grain concentrate had been consumed before they were given alfalfa hay. Samples of milk were taken from the afternoon milking and analyzed once or twice weekly. Feeding of toxaphene at the 2.5 p.p.m. level lasted for 1 month and at the other levels for $2^1/_2$ months. The cows were then fed a toxaphene-free ration, and analyses were continued until the apparent-toxaphene level had fallen to that of the control animals.

Method of Analysis. The cream from a 1-liter sample of milk was separated after keeping the milk in 1-liter separatory funnels overnight in the cold. The cream was churned to butter on a Burrell wrist-action shaker. The butter was washed with ice water, melted, and, while still hot, centrifuged (1500 r.p.m.) to remove proteinaceous material. The butterfat was then decanted, filtered through Whatman No. 1 paper, and 10 ml. of melted butterfat was mixed with 250 ml. of hexane in a 500-ml. separatory funnel. A 20-ml. portion of a 2:1 concentrated–fuming H_2SO_4 mixture was added to the separatory funnel and shaken gently. The layers were permitted to separate, and the lower, acid-fat phase was discarded. Successive treatments with 20-ml. portions of the acid mixture were applied until the lower phase remained colorless. Generally, four such treatments were sufficient. The hexane solution was then washed with deionized-distilled water until free of acid. Four washes with 20-ml. portions of water were usually sufficient. The hexane was dried over anhydrous sodium sulfate and evaporated on a steam bath in a Kuderna-Danish evaporative concentrator equipped with a three-ball Snyder column. The residue was taken up in 0.1 ml. of hexane and transferred to a paper sample carrier which was ignited in a Schöniger combustion apparatus (*5*, *6*). The Schöniger oxygen flask was rinsed with successive 1-ml. portions of titration solution (10% acetic acid–0.1*N* HNO_3) until 2.5 ml. of solution had been collected. This solution was then titrated for inorganic chloride by means of a Cotlove-type (*2*) chloridometer. The concentration of chloride, a function of the time of titration with Ag^+, was converted to p.p.m. toxaphene, based on 68% chlorine content.

Table I. Calculation for Toxaphene in Milk on One Sampling Date (May 5, 1961)

Sample	P.P.M. Toxaphene Apparent	Net[a]
Cow No. 6	0.107	0.056
Cow No. 7	0.083	0.032
Cow No. 8	0.125	0.074

[a] Net p.p.m. toxaphene = p.p.m. toxaphene − 0.051. Reported p.p.m. toxaphene = $\frac{0.056 + 0.032 + 0.074}{3}$ = 0.05.

Toxaphene residues in milk samples from experimental animals were corrected by subtracting the apparent p.p.m. toxaphene from the controls. The apparent p.p.m. toxaphene for the control animals remained essentially constant throughout the experiment. The correction made in each case was the average of all (100) control samples, 0.051 (std. dev. = 0.030). The reported value of p.p.m. toxaphene present in milk is the average of the values found for all animals in one group on a particular sampling date. Table I is an illustration of the method of calculation. The sensitivity of the method is about 0.02 p.p.m. toxaphene based on milk, and any value below this was considered nondetectable. All calculations were based on a 4% butterfat content in milk. Recoveries were studied by fortifying whole milk from the control cows with toxaphene and analyzing the milk as

[1] Present address: Chemistry Department, California State Polytechnic College, Pomona, Calif.

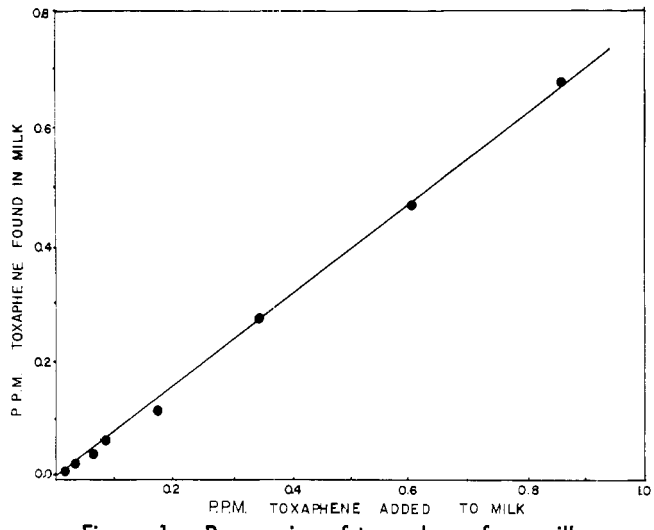

Figure 1. Recoveries of toxaphene from milk

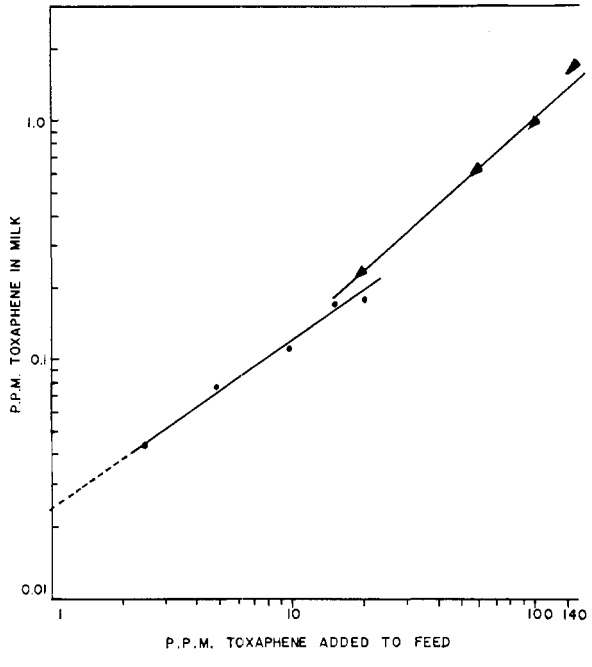

Figure 2. Log.-log. plot of plateau levels of toxaphene in milk vs. concentration of toxaphene in feed

● data from these studies, ▲ data from Claborn *et al.* (*1*)

Table II. Toxaphene Concentration (P.P.M.) in Milk from Cows Fed Daily Rations Containing Varying Amounts of Added Toxaphene

	P.P.M. Toxaphene Added to Ration				
Days	*2.5*	*5*	*10*	*15*	*20*
2	0.04	...	...	...	...
5	0	...	...	...	...
7	...	0.08	0.13	0.26	0.16
9	0.05	...	...	...	...
11	0.06	...	...	...	...
14	0.03	0.10	0.14	0.18	0.08
18	0.03	...	...	...	...
21	0.02	0.05	0.06	0.11	0.18
25	0.07	...	...	...	...
28	...	0.10	0.19	0.14	0.24
32	0.07[a]	0.07	0.08	0.14	0.20
35	...	0.20	0.10	0.15	0.22
42	...	0.09	0.05	0.13	0.14
49	...	0.05	0.15	0.34	0.26
56	...	0.03	0.11	0.31	0.18
63	...	0.01	0.05	0.08	0.11
70	...	0.06	0.12	0.16	0.19
74	...	0.07	0.10	0.09	0.18
77	...	0.08	0.16	0.16	0.18

[a] The 2.5 p.p.m. level was discontinued after the 32nd day.

Table III. Toxaphene Concentration (P.P.M.) in Milk from Cows during Feed-Off Period Following Feeding of Rations Containing Varying Amounts of Added Toxaphene

Days after Toxaphene Feeding Was Discontinued	*P.P.M. Toxaphene Added to Ration*				
	2.5	*5*	*10*	*15*	*20*
1	0.07	...	...	...	...
4	0.03	0.01	0.07	0.09	0.09
7	...	0.01	0.01	0.05	0.02
8	0	...	...	...	...
11	0	0.01	0.03	0.08[a]	0.06
14	...	0	0	0.02	0.02
18	...	0	0	0	0.02
21	...	0	0	0	0.02
25	...	0	0	0	0.01
27	...	0	0	0	0
30	...	...	...	0	0.01
34	...	...	...	0	0
41	...	...	...	...	0

[a] These results for the 11th day and all subsequent dates exclude results of one cow of this group which had become virtually dry.

outlined above. As may be seen from Figure 1, recoveries averaged 78.2%.

Results and Discussion

Although a colorimetric procedure for the residue determination of toxaphene was available (*4*), the total chloride method was preferred in these studies involving milk. The latter method afforded greater sensitivity (0.02 p.p.m.) and ease of manipulation. Thus, a trained technician could process and analyze 12 milk samples in an 8-hour working day. Background chloride from solvents or control milk samples posed no problems and gave an average value of 0.051 p.p.m. apparent toxaphene based on over 100 different samples.

The toxaphene feeding studies of dairy cows have confirmed results from earlier studies with other chlorinated pesticides, e.g., aldrin, dieldrin, DDT, and heptachlor (*3*, *8*). Detectable amounts of toxaphene were found in the milk at all feeding levels (2.5 to 20 p.p.m.) 7 to 9 days after the feeding trial had commenced (Table II). Plateau levels of toxaphene residues in the milk seemed to be established approximately after the 28th day, except at the 2.5 p.p.m. feeding level, at which the plateau was reached after the ninth day. The plateau values for toxaphene in the milk at the 2.5, 5, 10, 15, and 20 p.p.m. toxaphene feeding level were 0.043 (±0.020), 0.076 (±0.046), 0.110 (±0.043), 0.173 (±0.080), and 0.179 (±0.051), respectively. A log-log plot of these data results in a straight line confirming previous observations by Gannon *et al.* (*3*) and Zweig *et al.* (*8*) (Figure 2).

The authors have again confirmed the findings of Claborn and coworkers (*1*) that a rapid decline in pesticide residues in milk was observed 4 days after the toxaphene feeding had been stopped. Fourteen days of feed-off produced milk from all groups of animals with nondetectable amounts of toxaphene (less than 0.02 p.p.m.) (Table III).

Exception to the rapid decrease of toxaphene residues in milk was our observation with one animal (Cow No. 17) which became virtually dry at the start of the feed-off period. To secure sufficient milk for analysis during this period, the cow was milked only on the sample days. Results of analyses of milk

Table IV. Toxaphene Concentration in Milk from Cow No. 17 (Which Became Dry at End of Feeding Experiment) during the Feed-Off Period, Following Feeding at 15 P.P.M. Toxaphene Added to Rations

Days After Toxaphene Discontinued	*P.P.M. Toxaphene in Milk*
4	0.08
7	0.08
11	0.12
14	0.06
21	0.07
27	0.17
41	0.08

from this cow during the feed-off period are tabulated in Table IV. After 41 days, the milk still showed 0.08 p.p.m. toxaphene, indicating that excretion in milk is a principal route for elimination of toxaphene.

To establish a safe level of toxaphene in the feed of lactating dairy cows, plateau values of p.p.m. toxaphene in milk were plotted against p.p.m. toxaphene added to the daily ration on log.-log. paper (Figure 2). Extrapolating this straight line to 0.02 p.p.m. toxaphene in milk, which was assumed to be virtually zero, a safe level of about 1 p.p.m. toxaphene in the feed was obtained. This value compares to 0.8 p.p.m. for DDT and 1 p.p.m. for Kelthane in feeds (*7, 8*). This straight line obtained by the authors also fits the data obtained by Claborn *et al.* (*1*) for higher levels of toxaphene (Figure 2).

The conclusions drawn from these observations are that safe levels of chlorinated pesticide residues in daily rations of dairy cows may be established. This is predicated upon the assumption that no detectable residues are found in the milk and that these results are based on an accepted and sensitive method of analysis. Nondetectability should further be extrapolated to a value of 0.01 p.p.m. in milk which may be considered a practical zero.

Acknowledgment

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INSECTICIDE RESIDUES IN MILK AND MEAT

Residues in Butterfat and Body Fat of Dairy Cows Fed at Two Levels of Kelthane (1.0 and 2.0 P.P.M.)

GUNTER ZWEIG, EARL L. PYE,[1] and S. A. PEOPLES

Pesticide Residue Research, Laboratory and Department of Physiological Sciences, University of California, Davis, Calif.

A microanalytical method for analysis of Kelthane in butterfat or body fat of dairy cows, without the removal of the substrate material, was developed. Five cows were fed daily rations containing 2.0 p.p.m. Kelthane for 71 days and 1.0 p.p.m. for 39 days. Milk and body fat were analyzed for residues of Kelthane. A feeding level of 2.0 p.p.m. Kelthane in the daily feed produced an average of 0.23 to 0.40 p.p.m. Kelthane in the butterfat, while 1.0 p.p.m. Kelthane added to the cows' daily feed produced insignificant residues of Kelthane in the milk. Body fat, analyzed during the latter part of the experiment, contained 1.07 to 2.70 p.p.m. Kelthane.

RESIDUES of a chlorinated pesticide, DDT, in milk from cows placed on low levels of DDT in their daily feed over an extended period have been studied recently by Zweig *et al.* (*10*). It was of interest, therefore, to study the excretion pattern of another chlorinated pesticide, Kelthane [4,4′-dichloro-α-(trichloromethyl)benzhydrol](produced by Rohm & Haas Co.) which has been shown to be an effective acaricide and insecticide (*1, 4, 5*). To what extent the residue of this material present in dairy feed contaminates the milk and body fat of cows has not been established.

An initial study (*6*) indicated that no detectable Kelthane was present in milk of a cow fed at the rate of 0.1 p.p.m. Kelthane added to that cow's daily ration for 15 days, followed by the rate of 1.0 p.p.m. for 13 days. A cow fed at the rate of 5.0 p.p.m. for a period of 17 days showed a maximum amount of 0.22 p.p.m. Kelthane in the milk and reached plateau values in 6 days. A cow fed at the rate of 30 p.p.m. for 3 days showed no Kelthane in any tissue except kidney fat which contained 0.68 p.p.m.

The present work includes an extension of this initial study. Five dairy cows were fed daily rations of 1.0 and 2.0 p.p.m. Kelthane, based on their daily feed intake, for 39 and 71 days, respectively. Butterfat was sampled periodically and analyzed colorimetrically. Some tissue fat samples were also analyzed for Kelthane during feeding studies.

Experimental

Analytical Procedure. The analytical method of Rosenthal *et al.* (*7*) with modifications by Gordon and coworkers (*3*) is based on the alkaline decomposition of Kelthane to chloroform which is determined colorimetrically by the Fujiwara reaction (*2*).

[1] Present address: Chemistry Department, California State Polytechnic College, Pomona, Calif.