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.

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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.)

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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*).

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Table I. Recovery of Kelthane Added to Whole Milk, Butterfat, and Body Fat from Dairy Cows

Grams Analyzed	Absorbance	P.P.M. Kelthane Added	P.P.M. Kelthane Found	% Recovery
		Butterfat[a]		
13.7	0.020	0	0.32	...
14.5	0.053	0.57	0.48	84.2
13.4	0.060	0.71	0.67	94.4
12.0	0.063	0.71	0.83	116.9
20.0	0.126	1.42	1.09	76.8
15.3	0.177	2.84	2.23	78.5
		Butterfat[b]		
17.2	0.002	0	0.03[c]	...
	0.016	0	0.20	...
	0.043	0.58	0.43	74.1
	0.058	0.58	0.56	96.6
	0.098	1.45	1.13	77.9
	0.111	1.45	1.30	89.7
		Body Fat		
3.0	0.007	0	0.51	...
	0.009	0	0.66	...
	0.018	1.67	0.73	43.7
	0.020	1.67	1.02	61.1
	0.030	3.33	1.83	55.0
	0.028	3.33	1.46	43.8

[a] Kelthane added to whole milk.
[b] Kelthane added to melted butterfat.
[c] Use 0.12 p.p.m. as background for net Kelthane.

Table II. Residues of Kelthane in Butterfat from Cows Fed 1 and 2 P.P.M. Kelthane

Sample	P.P.M. Kelthane[a] Gross	Net
	1 P.P.M.	
Control	0.26 (±0.057)	...
Test Cow I	0.35 (±0.088)	0
Test Cow II	0.39 (±0.121)	0
Test Cow III	0.32 (±0.064)	0
	2 P.P.M.	
Control	0.19 (±0.081)	...
Test Cow I	0.58 (±0.193)	0.39
Test Cow II	0.59 (±0.231)	0.40
Test Cow III	0.42 (±0.078)	0.23

[a] Confidence limits; 95% probability.

Cream from 1 liter of raw milk is separated in the cold overnight and churned into butter on a Burrell wrist-action shaker the next morning. The butter is washed with distilled water, melted, and the solid and protein matter are removed by decanting and filtering through Whatman No. 1 paper. Body fat tissue is filtered in a 70° C. oven to remove protein and solid materials.

About 20 grams of filtered butterfat or 3 grams of body fat is placed in the reaction tube (*3*). In the analysis of body fat, an equal volume of xylene added to the reaction tube will prevent excessive bubbling. To ensure initial acidity, one drop of 1*N* HCl is added to the reaction tube prior to the addition of the fat. With the acid- and pyridine-traps disconnected, the reaction tube and contents are heated by means of a boiling water bath while the fat is swept for 45 minutes with a stream of nitrogen adjusted to a flow rate of about 80 ml. per minute. Degassing at this stage will also help to remove any HCl which will interfere later with the Fujiwara reaction causing the formation of a yellow acid-complex.

Five milliliters of 85% sulfuric acid is added to the acid-trap. Nine milliliters of pyridine, 0.6 ml. of water, and 0.4 ml. of 50% sodium hydroxide are placed in the pyridine-trap, which is then shaken gently for one-half minute to equilibrate the contents. After the initial 45-minute nitrogen sweep, the acid- and pyridine-traps are connected in the apparatus, and water is then circulated through the condenser.

Decomposition of Kelthane. The reaction tube is unseated, and 3 ml. of 1*N* tetraethylammonium hydroxide is added to the fat. The reaction tube is immediately reseated against the joint and the nitrogen flow rate adjusted to about 80 ml. per minute. Heating is continued while the fat is swept with nitrogen for an additional 45 minutes.

Color Development. After the 45-minute nitrogen sweep, the pyridine-trap is removed from the apparatus and placed in a boiling water bath for 5 minutes. The trap is then transferred to an ice water bath to cool for about 2 minutes, after which the absorbance is read at 535 mμ against a pyridine blank.

Recoveries. Standard curves were prepared from known concentrations of pure Kelthane (Rohm & Haas Co.) in xylene. It was found necessary to prepare a standard curve for each new bottle of tetraethylammonium hydroxide. Known concentrations of Kelthane in xylene should be analyzed at weekly intervals and the results checked against the standard curve currently in use. The slopes of standard curves at first ranged in value from 220 to 300 μg. of Kelthane per absorbance unit. The reason for these variations has been discussed by Gordon and Haines (*3*). However, a freshly prepared solution of the base consistently gave slope values close to 220 μg. per absorbance unit.

Recovery studies were made on whole milk by adding 20 to 100 μg. of Kelthane directly to 1-liter samples; recoveries ranged from 76 to 117% (Table I). Recovery studies were conducted on body fat of dairy cows by adding 1.67 and 3.33 p.p.m. of Kelthane (Table I). Possible reasons for low recoveries are discussed below. Recoveries were also made on butterfat by adding 0.58 and 1.45 p.p.m. of Kelthane and ranged from 74 to 97% (Table I).

Feeding Experiment. Five Holstein cows were selected and placed on a ration of about 0.5 kg. grain concentrate plus alfalfa hay and grazing to make up a total daily average consumption of approximately 20 kg. per day. Feeds were analyzed for insecticides by gas chromatography with a microcoulometric detector and total chloride titration. The feeds contained less than 0.05 p.p.m. of chlorinated insecticides as determined by the total chloride method. During a 1-week preliminary feeding period, milk samples were found to contain insignificant amounts of apparent Kelthane. Any value less than the upper confidence limit of the control (95% probability) was considered zero. Sensitivity and accuracy of the method are discussed below. Fat analyses by Babcock test on the milk during this preliminary period showed that the average fat content ranged from 3.5 to 4%.

The feeding study was begun by placing three cows on daily rations containing 2.0 p.p.m. Kelthane, based on 20 kg. feed per day. Two other cows were kept as controls throughout the experiment. One milliliter of a Kelthane solution in acetone (40 mg. per ml.) was pipetted on the concentrate, and the solvent was allowed to evaporate. This feeding rate was continued for 76 days. The Kelthane was then withdrawn for 25 days, after which feeding was resumed at the rate of 1.0 p.p.m. (0.5 ml. of Kelthane solution) and continued for an additional 42 days. Butterfat analyses were made until the level of Kelthane dropped to zero. Approximately 25 grams of omental fat was removed from each cow on the third day after the feeding at the 1.0 p.p.m. rate had started.

One-liter milk samples were taken twice weekly from the afternoon milking and stored in the cold room until analyzed. Control samples gave an apparent p.p.m. Kelthane background which is shown in Table II.

Results and Discussion

Analytical Method. The method presented is a sensitive procedure for analysis of Kelthane in fats with the advantage that the substrate materials need not be separated from the insecticide prior to analysis. Recoveries from milk and butterfat are somewhat better than those recoveries from body fat. No explanation for low Kelthane recoveries from body fat can be offered since only a limited number and relatively small size of samples were available. Thus, a statistical evaluation of these results was not possible. It is readily understood that the reliability of this procedure is directly proportional to the size of the sample being analyzed.

Freshly prepared tetraethylammonium hydroxide solutions should be used but may also be repurified by evaporating to dryness on a steam bath with a stream of dry air and dissolving the recovered solid in sufficient water to make a 1*N* solution.

The absorbance of 0.005 may be arbitrarily chosen as the limit of readability of the Beckman DU spectrophotometer which was used. This is equivalent to 1.10 μg. of Kelthane or an ultimate sensitivity of 0.06 p.p.m. based on 20 grams of butterfat. Apparent Kelthane residues for controls were 0.19 and 0.26 p.p.m., so that these blanks must be considered in the practical limit of detection. Standard deviation and confidence limits of each set of data from the 1 and 2 p.p.m. feeding trial were calculated and are shown in Table II.

Feeding Experiment. Results of the Kelthane feeding study are shown in Table II. Plateau values seemed to be reached within 9 days, but could not be verified statistically. The three test animals produced butterfat which had a gross Kelthane residue of 0.58 (±0.193) p.p.m., 0.59 (±0.231) p.p.m., and 0.42 (±0.078) p.p.m. compared with 0.19 (±0.081) p.p.m. for control animals. In terms of 4% milk, these residues are $^1/_{25}$ of the calculated values. Such low residues have been considered nondetectable for DDT (*10*); however, they must be considered significantly above control for the studies presented here. At the 1 p.p.m. Kelthane feeding level, residues in the butterfat were not significantly different from the controls.

Analyses of body fat at the onset of the 1 p.p.m. feeding experiment indicated that some Kelthane had been retained by the animal from the previous feeding experiment, as shown by results of 2.70 and 1.07 p.p.m. Kelthane in body fat of the experimental cows.

It appears, therefore, that an initial feeding rate of 1.0 p.p.m. Kelthane in the daily feed might not result in detectable residues of Kelthane in the milk.

Data from the Kelthane studies may be compared with those of a DDT-feeding experiment (*10*). The amount of Kelthane excreted in the milk of Holstein cows was much lower than that for a corresponding DDT-feed intake. This might be expected since Kelthane is a more polar, thus more water-soluble, compound. The fact that any Kelthane was detected at all at the 2 p.p.m. level of daily Kelthane intake was due to the greater sensitivity of the method than that for DDT. This greater sensitivity was achieved by being able to analyze up to 20 grams of butterfat sample, whereas a 5-gram sample for DDT analysis by the official method (*8, 9*) represented the practical limit.

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INSECTICIDE RESIDUES IN MEAT

Residues in Body Tissues of Livestock Sprayed with Sevin or Given Sevin in the Diet

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ENTOMOLOGICAL STUDIES have shown Sevin (1-naphthyl *N*-methylcarbamate) to be a promising insecticide for the control of ticks (*1*), horn flies [*Haematobia irritans* (L.)] (*4*), and lice (*5*) on livestock, and Gyrisco *et al.* reported that the compound was effective against the gypsy moth caterpillar [*Porthetria dispar* (L.)] (*2*). These possible uses of Sevin on livestock and forage make residue studies necessary on meat and milk. Roberts and coworkers (*6*) described a study on Sevin in which residues were determined in milk from dairy cows fed Sevin in the diet, and in milk from dairy cows and in fat of beef cattle sprayed with the insecticide. Gyrisco and coworkers (*2*) also reported on the residues in milk after feeding high levels of Sevin to dairy cattle. No residues of Sevin were reported by either group. To obtain more information on residues in other tissues of cattle and in the tissues of other farm animals after spraying with or feeding Sevin, a study was planned by the U. S. Department of Agriculture in cooperation with Union Carbide Chemicals Co. In this study, residue determinations were made on seven kinds of tissue from cattle, sheep, goats, and hogs sprayed with Sevin, and on the same tissues from beef cattle fed Sevin in the diet. All tissues were analyzed for Sevin and for 1-naphthol, a likely metabolite. Liver and kidney tissues were also analyzed for conjugates of 1-naphthol.

Experimental Techniques

Treatment of Animals. Spray treatments were applied to one group each