checked for isothiocyanate. No color was produced at an estimated sensitivity of 1 μg. of isothiocyanate ion.

The possibility of Lethane degrading in the milk while stored was investigated. Table II shows the constancy of Lethane recovery from fortified milk stored under refrigeration over various periods. Two types of fortification were employed: Lethane added from a hexane solution and Lethane added via ethanol. Recoveries in each case proved constant over various periods.

Table III indicates over-all recovery on 100-gram milk samples fortified with varying amounts of Lethane. Recovery is based on the theoretical amount of cyanide produced from hydrolysis of Lethane. In the experimental cow spray study, the absorbance of the milk controls averaged 0.060 ± 0.007 measured in 2-cm. path length cells. With a 100-gram milk control, a fortification of 1 μg. of Lethane gives an absorbance of 0.115 ± 0.005 in 2-cm. cells.

As a measure of reproducibility, Table IV shows data for duplicate determinations on milk samples from Lethane-sprayed cows. Control values are expressed in apparent parts per million denoting amount of interference of controls as compared to the pyridine reagent used in the reference absorption cell.

Results

Detailed results of the cow spray experiment with respect to topical application, animal treatment, and excretion of the insecticide have been reported (*6*). Figure 3 summarizes results obtained during the four treatment stages of the investigation. No detectable residues of Lethane were present in any pre- or post-treatment samples or in any samples taken following application of the first three treatment formulations. Lethane was detected in milk the first day following application with a 6% 1-ounce spraying twice daily. This is a spray level equal to four times that which is recommended. This period showed a residue level ranging between 0.012 and 0.016 p.p.m. The residue disappeared the day following withdrawal from treatment.

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INSECTICIDE RESIDUES IN MILK

DDT Residues in Milk from Dairy Cows Fed Low Levels of DDT in Their Daily Rations

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A feeding experiment with 12 dairy cows was conducted in which pairs of animals were fed 0 to 5.0 p.p.m. of DDT added to their daily rations for 31 days. In another experiment, six cows were fed 1 p.p.m. of added DDT over a period of 8 weeks. Milk was analyzed periodically for fat and DDT content. A maximum level of 0.5 p.p.m. of added DDT in the feed did not produce a residue of 0.01 p.p.m. or greater in the milk. At levels of 1, 2, 3, and 5 p.p.m. of added DDT, detectable residues were found in the milk of all animals. DDT concentration in milk was proportional to DDT level in feed. There was a correlation between DDT residue and fat concentration in milk at each feeding level but not between total DDT excretion and total fat production.

The excretion pattern of various chlorinated pesticides in the milk of dairy cows has been established by Gannon, Link, and Decker (*3*). The animals were placed on rations to which these pesticides were added daily at different levels. Ten parts per million was the lowest level for DDT which produced a detectable residue 7 days after feeding was started. The residue in the milk, however, did not seem to reach a plateau even after 14 to 16 weeks of feeding at this level of DDT in the feed. The maximum dose of DDT in daily rations that would not result in a detectable residue in the milk was not established by these workers (*3*).

Present work was undertaken to attempt to define a "safe" level of DDT in feeds that would produce milk with undetectable amounts of this insecticide. Twelve dairy cows, including producers of both high- and low-fat milks, were fed daily rations containing 0 to 5 p.p.m. of DDT, based on their feed intake. Milk samples were analyzed for DDT by colorimetric and paper chromatographic methods.

After the maximum safe level of DDT in the feed was determined, results were corroborated by placing another group of 10 animals on a ration

Table I. Example of Calculation of DDT Residue Determinations Adjusted to Milk with 4% of Fat

Date	Sample	Absorbance, 596 Mμ	Net Absorbance
8/29/60	Holstein (control)	0.027	
8/29/60	Jersey (control)	0.022	
	Av.	0.025	
8/29/60	Jersey (3 p.p.m. DDT-19th day)	0.198	0.173

From standard curve, an absorbance of 0.173 is equivalent to 19.0 μg. of DDT. Fat content of milk 5.5%. DDT in milk corrected to 4% fat basis: $\frac{19.0}{5.5} \times \frac{4}{100}$ = 0.14 p.p.m.

containing zero and twice the safe level of DDT.

Procedure

For the first experiment six Holstein, four Jersey, and two Guernsey cows were selected and placed on a ration of 19.5 kg. (average) of dry alfalfa hay plus about 0.5 kg. of grain concentrate. During a preliminary 2-week feeding period, samples of feed and milk, respectively, contained 0.2 and 0.1 p.p.m. of DDT or less. These levels were considered satisfactory for the feeding trial to begin. One Holstein and one Guernsey or Jersey were paired and placed on a ration containing 0, 0.5, 1.0, 2.0, 3.0, or 5.0 p.p.m. of DDT based on 20 kg. of feed per day. At each afternoon's feeding time, the appropriate volume of 1% DDT in acetone was pipetted on the grain concentrate. Care was taken that the animals consumed all of the concentrate before the alfalfa. Two or three times a week, milk produced by individual cows during a 24-hour period was pooled, and 100-gram portions were processed for analysis. DDT feeding was continued for 31 days. Milk analyses were continued thereafter until the level of DDT dropped to that of the controls. Fat percentages were determined by the Babcock method, and DDT residues in milk were expressed in terms of milk containing 4% of fat.

For the second study, five Holsteins and five Jerseys were placed on a measured ration of hay pellets and grain. As in the first study, feed and milk samples were first found to have satisfactorily low levels of DDT prior to the trial. Then, six of the animals (three Holsteins and three Jerseys) were fed daily rations containing 1 p.p.m. of DDT, while the remaining four were kept as controls. Milk

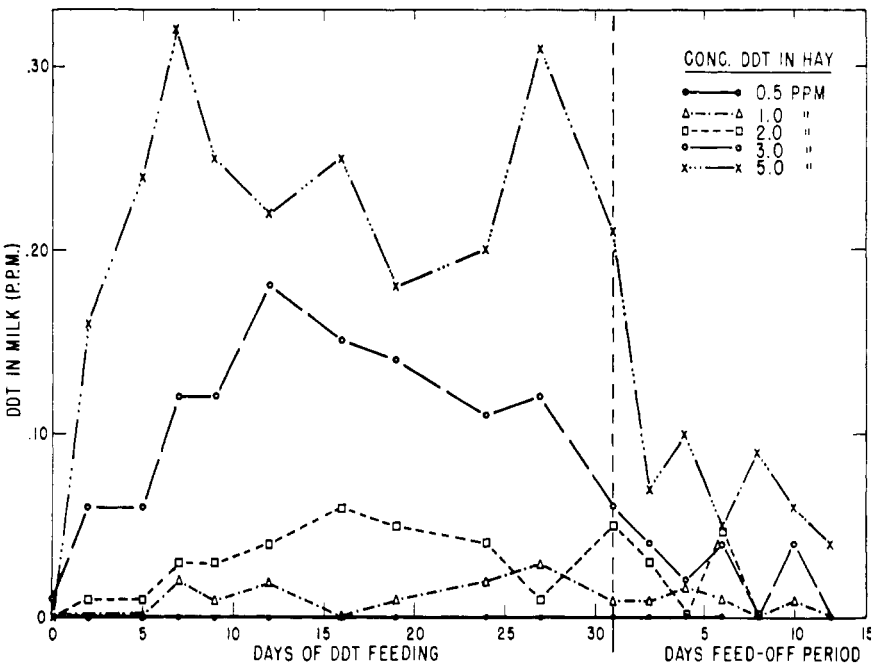

Figure 1. Concentration of DDT in milk of Jersey and Guernsey cows fed various levels of DDT for 31 days

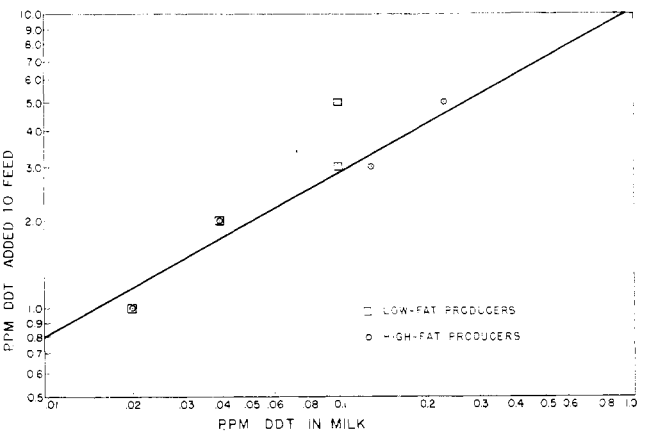

Figure 2. Log-log plot of DDT concentration in milk (highest plateau) vs. concentration of DDT added to daily rations of dairy cows (Experiment I)

samples were collected once a week for DDT analysis. Feedings were continued for 8 weeks.

Methods of Analysis

One hundred grams of milk was treated according to the method of Mills and Storherr (*4*, *7*) by hexane–ethyl ether–ethanol extraction, followed by acetonitrile treatment and passage through a Florisil column. The final eluate was analyzed for DDT by the colorimetric procedure of Schechter *et al.* (*5*, *6*), except that the nitration period was shortened to 15 minutes. Some samples were examined semiquantitatively by the paper chromatographic technique of Mills (*4*). Color absorbance was measured with Bausch and Lomb Spectronic 20 and Beckman DU spectrophotometers. Spectral curves for several samples developed by the Schechter-Haller procedure were further examined with a Beckman DK recording spectrophotometer. Standard curves were prepared from known concentrations of pure *p,p'*-DDT (Geigy Co.) in hexane.

Absorbance readings for milk samples from treated animals were corrected by subtracting "apparent absorbance" for corresponding samples from control animals. These corrections were averages of two replicates for each day's controls, which varied from 0.015 to 0.067 absorbance. The absorbance curves showed that this apparent DDT color was nonspecific background without the characteristic 596-mμ maximum for the blue color produced by the re-

action of nitrated DDT with sodium methylate. Similar observations were made by Schechter, Pogorelskin, and Haller (*5*), but no satisfactory explanation has been given for this color. Recovery experiments on whole milk were conducted by adding 20 μg. of DDT to 100-gram samples, and recoveries averaged 92.5%. All DDT residues were corrected to a 4% fat milk, as illustrated in Table I. Any value less than 0.01 p.p.m. of DDT in milk was considered "zero" or undetectable. Feeds were analyzed for DDT by extracting samples with 10 volumes of chloroform, freezing out waxes in cold acetonitrile, passing the final solution through a Florisil column, and developing the color as above (*1*).

Results and Discussion

Results of the first feeding trial are tabulated in Figure 1 and Table II. At the feeding level of 0.5 p.p.m. of DDT, DDT was detectable in milk from one animal on only two dates (27 and 31 days) after the trial began. A slight residue, 4 and 6 days after DDT feeding of this same animal had been discontinued, suggested that these traces of DDT residue were insignificant and might represent experimental variations. It is emphasized again that even the milk from control animals produced a variable amount of color in the colorimetric analysis. Furthermore, no chlorinated compounds could be detected by paper chromatography, which in the hands of an experienced analyst may have a sensitivity of 0.004 p.p.m. of DDT, based on milk. Since the feeds contained 0.2 p.p.m. of DDT, it may be reasoned that the test animals were on rations containing 0.2 p.p.m. of DDT plus the amounts actually added. Therefore, it may be concluded that cows in Experiment I that received feed with 0.5 p.p.m. of added DDT for 31 days produced milk with undetectable amounts of DDT.

At a DDT level of 1.0 p.p.m. added to the daily ration, slight DDT residues in the range from 0.01 to 0.03 p.p.m. were consistently present in milk from both cows after 19 days (Table II). This was confirmed by the second trial, in which six cows kept on a 1-p.p.m. DDT level were excreting traces of DDT in their milk by the 19th day (Table III). Spectral curves of DDT color on the 12th day of the second experiment failed to yield the characteristic DDT 596-mμ absorption maximum, indicating that no DDT detectable by the colorimetric procedure was present at that time. By the 33rd and 53rd days, all animals, including even two controls, were excreting traces of DDT and its metabolites in their milk. These amounts ranged from traces to approximately 0.06 p.p.m., as estimated from paper chromatograms.

Table II also shows that the 2-, 3-, and 5-p.p.m. DDT feeding levels produced significant amounts of insecticide in milk. At the 5-p.p.m. level, 2 days after feeding began, an appreciable residue was observed in milk from the Guernsey. In general, the Holsteins used for these studies gave consistently lower concentrations of DDT residues in milk than did the Jerseys and Guernseys. Also, it took longer for the Holsteins to establish their highest levels.

Data for DDT excretion in milk from one representative date (24th day) during the highest level plateau were compared with total fat production (Table IV). Animals that produced milk with a higher fat percentage showed

Table II. DDT Concentration in Milk from Cows Fed Daily Rations Containing Varying Amounts of Added DDT

(Experiment I)

	DDT in Milk, P.P.M.									
Time, Days	0.5[a]		1.0[a]		2.0[a]		3.0[a]		5.0[a]	
	J[b]	H	J	H[b]	G[b]	H	J	H	G	H
0	0	0	0	0	0	0.01	0.01	0	0	0
2	0	0	0	0.01	0.01	0.05	0.06	0.04	0.16	0.02
5	0	0	0	0.01	0.03	0.03	0.06	0.03	0.24	0.02
7	0	0	0.02	0.02	0.03	0.01	0.12	0.08	0.32	0.08
9	0	0	0.01	0	0.04	0.01	0.12	0.05	0.25	0.06
12	0	0	0.02	0.01	0.06	0.03	0.18	0.07	0.22	0.07
16	0	0	0	0.02	0.06	0.04	0.15	0.09	0.25	0.09
19	0	0	0.01	0.02	0.05	0.02	0.14	0.08	0.18	0.09
24	0	0	0.02	0.01	0.04	0.02	0.11	0.09	0.20	0.10
27	0	0.02	0.03	0.02	0.10	0.05	0.12	0.10	0.31	0.10
31	0	0.01	0.01	0.03	0.05	0.05	0.06	0.09	0.21	0.10
Days after DDT Feeding Discontinued										
2	0	0	0.01	0.01	0.03	0.02	0.04	0.04	0.07	0.02
4	0	0.01	0.02	0.01	0	0.01	0.02	0.04	0.10	0.03
6	0	0.01	0.01	0	0.05	0.06	0.04	0.01	0.05	0
8	0	0	0	0	0	0	0	0.03	0.09	0
10	0	0	0.01	0.01	0	0	0.04	0.04	0.06	0.03
12	0	0	0	0.01	0	0.01	0	0.03	0.04	0

[a] DDT added to ration in parts per million.
[b] J. Jersey cow. G. Guernsey cow. H. Holstein cow.

Table III. DDT Concentration in Milk from Cows Fed Daily Rations Containing 1 P.P.M. of Added DDT

(Experiment II)

	DDT in Milk, P.P.M. Days after Beginning of Trial				
Animal No.	5	12	19	33	53
Jersey 1	0[a]	0	0.04 (+)[b]	(+)	(+)
Jersey 2	0.01	0	0.02 (+)	(+)	(+)
Jersey 3	0.01	0	0 (+)	(+)	(++)
Holstein 1	0	0	0.04	(+)	(+++)
Holstein 2	0.01	0	0.01	(+)	(++)
Holstein 3	0	0	0.04	(+)	(+)

[a] Colorimetric data obtained with Beckman DU or DK spectrophotometer.
[b] (+) Indicates total DDT, DDE, and DDT estimated by maximum spot density method from paper chromatograms (*2*).

Table IV. Comparison of DDT Excretion in Milk with Per Cent Fat in Milk and Total Fat Production on 24th Day of Trial I

Animal	Concn. DDT Added to Feed, P.P.M.	Fat in Milk, %	Concn. DDT Found in Fat, P.P.M.	Total Fat Production, Grams	Daily DDT in Milk		
					Intake, mg.	Excretion	
						Mg.	%
Holstein	0	4.0	0	382	0	0	
Jersey	0	7.2	0	336	0	0	
Holstein	0.5	3.5	0	227	10	0	0
Jersey	0.5	5.2	0	195	10	0	0
Holstein	1.0	3.6	0.24	623	20	0.15	0.75
Jersey	1.0	7.9	0.43	295	20	0.13	0.65
Holstein	2.0	4.3	0.53	259	40	0.13	0.33
Guernsey	2.0	5.4	1.01	209	40	0.21	0.53
Holstein	3.0	3.8	2.30	568	60	1.30	2.17
Jersey	3.0	5.7	2.61	100	60	0.26	0.43
Holstein	5.0	3.4	2.50	372	100	0.93	0.93
Guernsey	5.0	4.9	5.05	327	100	1.65	1.65

a higher concentration of DDT in butterfat at feeding levels of 1.0 to 5.0 p.p.m. of added DDT. Total amount of DDT excreted in the milk of each pair of animals seemed to have no correlation to total fat production. Therefore, these data suggest that one might predict DDT excretion patterns of different animals on the same feed by comparing the fat percentage of their milk rather than their total fat production. Percentage of DDT excreted in the milk based on total daily intake, ranged between only 0.3 and 0.8% at the 1- and 2-p.p.m. DDT feeding levels, and between 1.0 and 2.2% at the 3- and 5-p.p.m. levels.

Data from Table II were plotted to calculate probable safe levels of DDT in feeds that would result in undetectable levels of DDT in milk (Figure 2). The logarithm of the highest DDT excretion level for each animal was plotted against the logarithm of the corresponding DDT concentration added to feed. The data fell along a straight line that, on extrapolation to a residue of 0.01 p.p.m. of DDT in milk, corresponded to a feeding level of 0.8 of p.p.m. DDT in feed. This value of 0.8 p.p.m. of DDT in feed is in good agreement with the observation that 0.5 p.p.m. of added DDT resulted in an undetectable residue (Table II). Extrapolating this curve to 10 p.p.m. of added DDT, the value obtained is 0.85 p.p.m. of DDT in milk, which is in good agreement with Gannon's data (*3*).

Work is in progress in our laboratories on the excretion pattern of a number of other chlorinated pesticides fed at low levels to dairy cows.

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INSECTICIDE EFFECTS ON ANIMALS

Response of Experimental Animals to Phosdrin Insecticide In Their Daily Diets

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The level of Phosdrin insecticide in the diet that induced fatal intoxication in rats was 400 p.p.m. over a period of 13 weeks; in dogs, 200 p.p.m. over a period of 14 weeks. Nonspecific toxic degeneration and necrosis of the liver and renal tubular epithelium were noted. Characteristic alterations in exocrine glands of the animals were observed and could be correlated with the quantity of the insecticide in the diet. At dietary levels below 25 p.p.m., there was no gross effect on rats or dogs. At levels of 2 to 5 p.p.m. in the diets of rats, there was moderate depression of the cholinesterase in the erythrocytes, slight depression of the cholinesterase in the plasma, and no effect on the cholinesterase of the brain. Slight inhibition of the cholinesterase activity of erythrocytes and plasma of dogs was noted at dietary levels of 2.5 and 5 p.p.m.

THE development of Phosdrin insecticide, *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate, as an effective new insecticide having worldwide application in the control of insects harmful to agricultural crops, indicated the need to determine the response of animals to the compound and to investigate its effects on the physiology of animals. Such information would make possible the formulation of procedures for the safe handling and use of the material.

Phosdrin is soluble in water and fat and can be absorbed through the skin, the lungs, or the gastroenteric tract. It is highly toxic to warm-blooded animals—for example, the LD_{50} for rats is approximately 6 mg. per kg. (*10*). The toxicity and physiological action of Phosdrin were reported to be similar to those of other well known organophosphorus insecticides, such as tetraethyl pyrophosphate (*6*).

Experimental animals were exposed to lethal and sublethal quantities to determine the type of injury to the internal organs associated with acute intoxication and to measure the effects on growth. The second phase of the experimental program was to study the inhibition of the cholinesterase of the brain and of erythrocytes and plasma in the peripheral blood.

The toxicity of parathion, diethyl *p*-nitrophenyl thiophosphate, has been studied extensively and is of the same order of magnitude as Phosdrin (*8*). For this reason parathion was used as a positive control in studies of Phosdrin.

Materials

Phosdrin. The formula for Phosdrin,

$$(CH_3O)_2POC(CH_3) = CHCOCH_3,$$

shows the possibility of cis-trans isom-

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