only are the toxicities of the allylic halides related to their reactivities, but also those of the saturated halide, n-butyl bromide, and the two  $\alpha$ -substituted halides, chloroacetonitrile and ethyl chloroacetate.

A similar plot can be obtained with the eggs of this insect, which suggests that the mode of action of these halides is the same with the two different stages in the life cycle of the oriental fruit fly and also with the two different organisms.

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### **INSECTICIDE RESIDUES IN MILK**

## **Effects of Feeding Low Levels of In**secticide Residues on Hay to Dairy Cattle on Flavor and Residues in Milk

 ${
m E}$  xcellent control of forage insects is possible with insecticide treatments which leave no more than 1 or 2 p.p.m. of residue at harvest (2, 10, 12, 13, 21). While these low residues were believed not to affect the health of the dairy cattle or to produce detectable off-flavors and odors or residues in milk, the lack of exact data had prevented the recommendation of many of the new organic insecticides for use on forages. In 1950, a 3-year study was initiated on the various effects of feeding insecticidetreated hay to dairy cattle. Two early progress reports of very limited distribu-

<sup>1</sup> Deceased.

tion were issued (11, 15). This is the final, complete report of that work.

#### Methods and Materials in 1950-51 Tests

Four insecticides, DDT, lindane, parathion, and aldrin were selected for forage insect control. These materials were formulated as dusts and applied to a second-cutting stand of alfalfa of the variety Ontario Variegated at the rate of 1 pound of actual toxicant per acre. Thirty-six days after treatment, the plots were harvested, mow-dried, and stored. Samples of hay were taken for residue analysis at the time of treatment, harvest,

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start of feeding, 3 weeks after feeding, and at the end of the feeding period (Table I).

#### Breeds of Cattle and **Experimental Design in 1950 Tests**

Five Holstein and five Brown Swiss cows were chosen from the regular dairy herd and one cow of each breed was assigned to each insecticide and the untreated check. The cows were then assigned in a completely randomized order to the stalls they were to occupy during the experiment. Each stall was so built that no cow could steal feed from her neighbor. This arrangement made it possible



Low levels of aldrin, lindane, DDT, parathion, and methoxychlor residues on hay up to 10 p.p.m. were fed to dairy cows for periods up to 3 months. No parathion or methoxychlor were found in any of the milk samples. No lindane was found in the milk in the 1950–51 experiment, but in 1951–52 the mean lindane residue content for all sampling dates was significantly greater than that of the check. However, the lindane present at any single sampling date was not significantly different from the check. Small but significant amounts of DDT and aldrin were present in the milk when cows were fed 10 p.p.m. of each for 1 month. At 2 to 4 p.p.m., no aldrin was detected and no effect was noted on the cows' well-being or vital organs of the slaughtered cows. There were no off-flavors or odors attributable to the feeding of any of the insecticides.

#### Table I. Insecticide Residues on Alfalfa after Treatment with Dust Formulations<sup>a</sup>

	R			
Frequency of Sampling	DDT	Lin- dane	Al- drin	Para- thion
Time of treat-				
ment	16.0	3.5	0.8	24.9
Harvest	0.0	0.6	0.0	0.05
Start of feeding	0.8	0.0	0.1	0.40
Three weeks				
later	1.4	0.0	0.0	0.07
End of feeding	1.5	0.0	0.0	0.07
4 One pound i	ner act	e		

One pound per acre.

to ascertain accurately the amount of food eaten by each cow and prevented contamination.

#### Feeding Schedule

On October 18, 1950, all animals were placed in the stalls and for the next 3 weeks, or pretest period, they were fed the normal daily rations: 15 pounds of untreated alfalfa hay, 40 pounds of corn silage and grain of a regular herd mix were fed at the rate of 1 pound of grain per day for every 4 pounds of milk produced. The portions of these feeds were divided in half and fed twice daily. Before each new feeding, any hay, grain, or silage that had been left was removed from the manger, weighed, and recorded. The feeding schedule was the same for the test and control cows as to time and method.

At the end of the pretest period, the ration of hay was increased to 20 pounds of hay per cow per day as several cows appeared to lose weight on the smaller ration.

On November 8, 1950, feeding of the insecticide-treated hay was initiated. Its feeding was divided into three stages, each consisting of feeding hay with a higher level of insecticide. The first stage consisted of feeding field-treated hay for a period from November 8 to December 13, 1950. This hay possessed insecticide residues of less than 1 p.p.m. On December 13, the insecticide content of the hav fed was increased to 2 p.p.m. by spraying daily the untreated hay with a technical grade of the test insecticides

dissolved in acetone a few hours before feeding. This new level was maintained until December 26 when all the feeding of treated hay was discontinued, except to one cow each on the DDT (Cow 4) and aldrin (Cow 7) treatments. On December 27, the insecticide content of the hay being fed these two cows was increased to 5 p.p.m. and the feeding, at this level, to these two cows was continued until January 5, 1951, when all test feeding was stopped.

#### Methods of Sampling Milk for Residue

The cows were milked each day at 5:00 A.M. and at 4:00 P.M. Milk samples in duplicate of 100 or 200 ml. were taken immediately after milking to assure a well-mixed sample. Half of each milk sample came from the morning's milking and half from the evening's milking. The halves were composited the day they were taken and placed in a milk cooler, immediately after collection. The samples from each cow were maintained separately in cold storage until analyzed for residue.

Milk samples for residue analysis were taken every third day for the first 2 weeks in which the treated hay was fed, thence once weekly while the level of the insecticide remained the same. Whenever the level of the insecticide fed was increased artificially or stopped, the samples were again taken every third day. Samples were also taken prior to any feeding of the insecticide-treated hay and again after it was discontinued.

#### Methods Used for Flavor and Odor Tests

One pint of well-mixed milk was taken from each cow once weekly in the pretest period and was subjected to the standard flavor, odor, and butterfat tests by flavor specialists of the Department of Dairy Industry. The early samples were used to standardize the samples taken during the actual test period. These latter samples were taken at the same time as those for residue analyses and were refrigerated immediately. Testing on the raw milk samples was conducted on the following day.

Butterfat, odor, and flavor tests were

conducted separately, on both the morning and afternoon samples.

#### Physical Examination of the Test Animals

During the entire experiment, including the pretest period, all the cows were examined by the veterinarian. The examinations included the following observations: temperature, respiration, pulse rate, salivation rate, pilo excretion, and pupillary size.

The weights of the animals were also checked at biweekly intervals.

Blood samples were taken once during the pretest period and every other week during the test period to determine the hemoglobin concentration. If any significant deviations from normal occurred, blood counts of the erythrocytes and leucocytes were made.

#### Other Samples during the Experiment

A fecal sample was taken from each animal 3 weeks after the start of the feeding of the treated hay. The samples were analyzed for residues by the same methods as used for the analysis of the milk.

On December 11, 1950, a fat sample for residue analysis was removed by biopsy from Cow 7 on the DDT treatment and from Cow 9 on the aldrin treatment. Two cows, No. 8 on the parathion treatment and No. 9 on aldrin, were slaughtered within 3 weeks of the termination of the experiment and portions of the liver, kidney, spleen, pancreas, brains, and omental fat were removed for residue analysis and examination by the veterinarian.

#### Methods Used in Chemical Analysis of Milk Samples

For DDT. The butterfat of the milk samples was extracted with hexane, after breaking of the emulsion with acetic acid as suggested by Mann and Carter (14). The butterfat and other interfering materials were removed with a celite-sulfuric acid column using the procedure outlined by Davidow (3). The final determination was made by a modification by Downing and Norton (5) of the method for DDT analysis of

Schechter *et al.* (18). The results were corrected for losses during extraction and from other sources by a factor derived from the average recovery of a series of check samples to which different amounts of DDT were added before extraction.

For Lindane. The lindane and butterfat were extracted from a 200gram sample of milk with hexane, after breaking the emulsion with alkali, according to the technique of Tufts et al. (23). The hexane was evaporated off and the butterfat was weighed. The butterfat was taken up in carbon tetrachloride and the fat and other interfering materials were removed from the lindane by passage through a Celite-sulfuric acid column, by the procedure of Davidow (3). Most of the solvent was removed, and the lindane was determined by the method of Schechter and Hornstein (17). The quantity of lindane determined on the final sample was corrected on the basis of the yield of butterfat obtained by extraction as compared with the butterfat determined by the Babcock test. Known quantities of lindane added to milk were recovered almost quantitatively by this procedure.

For Parathion. The 200-gram samples were extracted with hexane by the procedure outlined by Tufts et al. (23). After evaporation, of the hexane, the butterfat was dissolved in alcohol and the parathion was determined by the method of Averell and Norris (1) with the addition of three washes with hexane, immediately after the reduction step, to remove fats and other acid-insoluble materials. The results were corrected for incomplete extraction and for other sources of loss by a factor derived from the recovery of known amounts of para-'thion added to the check milk samples before extraction.

For Methoxychlor. Methoxychlor was extracted by the method of Tufts et al. (23). The hydrolysis and separation of the saponification products of the fats were carried out by the procedure of Prickett et al. (16). The resulting solution was chromatographed by the method of Doble and Thornburg (4). The fraction which should contain the methoxychlor was tested by the method of Fairing and Warrington (9). For uniformity in the chromatographic procedure, 4 grams of the extracted fat for each sample were run through the last part of the procedure, and the results were corrected for the total amount of extracted fat.

For Aldrin. In 1951–52, the aldrin milk samples were extracted with a 95% ethyl alcohol-Skellysolve B solvent. The Skellysolve B, which now contained the butterfat, was washed several times with water to remove the ethyl alcohol, dried over anhydrous sodium sulfate, and bottled. Because of the poor sensitivity and poor reproducibility of the phenylazide method used in 1950–51, a better method seemed de-

Tuble II. Residues III MIIK IIOM COWS Fed Aldrin-Tredied Hay	Table II.	Residues in Milk from Cows Fed Aldrin-Treate	d Hay
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Sampling Date	Field Treated Hay, Nov. to Jan. 1950–51 Aldrin, Level Fed, P.P.M.		in Milk, P.P.M. Cow 9	Sampling Date	Barn- Treated Hay, Nov. to Feb., 1951– 52 Aldrin Level Fed, P.P.M.	Net Residue ir Cow 4	Milk, P.P.M. Cow 14
Nov. 9 12 15 18 21 28 Dec. 5 12		$(-)0.05 \\ 0.03 \\ (-)0.03 \\ 0.02 \\ 0.00 \\ (-)0.10 \\ 0.06 \\ (-)0.6 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0.10 \\ 0.06 \\ (-)0$	$(-)0.19 \\ 0.11 \\ 0.07 \\ 0.05 \\ 0.02 \\ 0.11 \\ (-)0.37 \\ 0.01$	Nov. 20 25 27 Dec. 4 11 18 21 26	0 2 2 2 2 2 2 5 5 5 5 5 5 5	$\begin{array}{c} 0.00\\ \\ 0.01\\ \\ 0.03\\ (-)0.01\\ \\ 0.02\\ \\ 0.02\\ \\ 0.01\end{array}$	(-)0.01 0.00 0.01 0.02 0.01 0.03
15 18 26 30 Jan. 5 12	2.0  2.0  2.0  5.0  5.0  0	$\begin{array}{c} 0.01 \\ 0.028 \\ 0.01 \\ (-)0.20 \\ (-)0.05 \\ (-)0.01 \end{array}$	$\begin{array}{c} 0.19\\ 0.34\\ 0.00\\ (-0.07\\ 0.05\\ 0.07\end{array}$	Jan. 1 8 15 18 22 29 Feb. 5 12 19	5 5 10 10 10 10 10 10 0	$(-)0.01 \\ 0.00 \\ 0.04 \\ 0.06 \\ 0.02$	0.02 0.01 0.02 0.02

sirable. Therefore the extracted samples were stored along with some check samples to which different known amounts of aldrin had been added. Final analyses were not completed until 2 years later.

Further work at the Shell Chemical Co. resulted in a modified phenylazide method of high sensitivity and good reproducibility (19).

Because the samples had been extracted by methods already in use in 1952, in 1954, saponification of the whole milk sample before extraction was omitted. Instead, the butterfat extracts were saponified directly and the analysis was carried out as indicated by the remainder of the Shell method. Average recoveries for the modified phenylazide method were about 85%. However, the recoveries obtained from the samples fortified 2 years earlier indicated an average recovery of only about 69%. The data reported for 1951–52 in Table II are not corrected for recoveries.

# Methods and Materials in 1951–52 Tests

The same insecticides, with the addition of methoxychlor, used in 1950 were used again in the 1951 experiments.

The experiment in 1950 had demonstrated that it was impossible to control the levels of insecticide residues from applications made in the field. Therefore, all of the test insecticides were applied (in a manner previously described) to alfalfa hay at the time of feeding at the rates of 2, 4, and 10 p.p.m. Each level of insecticide was fed consecutively for 1 month in an ascending order of residue level. Each insecticide was fed to two dairy cows chosen at random from a group of 14. Although, the cows were again Holstein and Brown Swiss, no attempt was made to assign the cows on the basis of breed as the previous year's experiment had not indicated any difference in the reaction of the cows to the treated hay on the basis of breed.

In a separate experiment which was conducted simultaneously, field DDTtreated hay was used in comparison with hay treated in the barn with DDT to determine if there were any differences in the residues in milk between the two methods of treatment. Two acres of a second-cutting stand of timothy and red clover were sprayed with DDT at the rate of 2 pounds of actual toxicant per acre. The hay was cut 3 hours later on the same day, field-dried, baled, and stored until needed. Before feeding the field-DDT-treated hay which was compared with the barn-DDT-treated hay with a residue of equal magnitude, samples of hay for analysis were taken from the bales. These samples were taken at random, two from each of three bales of the DDT-treated hay. Of the two samples, one was taken from the inside and the other from the outside of the bales.

The cows were fed, cared for, and the milk was sampled for flavor and residues in the manner already described. The dates and frequency of sampling are indicated in the various tables under 1951–52.

A fecal sample was taken from each of the test animals 85 days after the start of the feeding of the treated hay. The samples were stripped immediately after collection. One hundred grams of green manure were blended in a Waring Blendor with 200 ml. of redistilled hexane and 200 ml. of 95% ethyl alcohol. The filtrate of the samples was analyzed for

Table III. Residues in Milk from Cows Fed DDT-Treated Hay

Barn-Treated Hay, Nov. to Feb., 1951–52				Field-Treated Hay, Nov. to Jan., 1950–51			
Net Resid P.	DDT, level fed.	Samplina			DDT, level fed,	Sampling	
Cow 11	p.p.m.	date	Cow 7	Cow 4	p.p.m.	date	
	0	Nov. 20	0.00	0.03	<1.0	Nov. 9	
						12	
0.00	2			(-)0.01		15	
0.01				(-)0.02		18	
0.02			0.02	(-)0.02		21	
0.06		18	0.01	(-)0.04	<1.0	28	
0.09	4	21	0.00	(-)0.02	<1.0	Dec. 5	
0.05	4	26	(-)0.05	(-)0.01	<1.0	12	
0.05	4	Jan. 1	0.02	0.03	2.0	15	
0.03	4	8	(-)0.01	0.02	2.0	18	
0.17	4	15	0.01	(-)0.04	2.0	26	
0.13	10	18	0.00	0.00	5.0	30	
0.18	10	22		0.02	5.0	Jan. 5	
0.14	10	29		(-)0.01	0	12	
0.18				, ,			
	Õ	19					
	Net Resic P. Cow 11 0.00 0.00 0.01 0.02 0.06 0.09 0.05 0.05 0.05 0.05 0.03 0.17 0.13 0.14 0.14 0.20	DDT, level         Net Resic           fed,         P.           p.p.m.         Cow 11           0            2         0.00           2         0.01           2         0.02           2         0.02           2         0.02           4         0.05           4         0.05           4         0.03           10         0.13           10         0.18           10         0.14           10         0.20	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	

Table IV. A Comparison of Residues from Field DDT-Treated Hay at 70 P.P.M. with Equivalent Amount of Barn-Treated Hay

Sampling Date 1951–52	Field-treated hay, Cow 6	Barn-treated hay, Cow 9
Dec. 11	-0.01	0.00
18	0.01	0.00
21	0.23	0.64
26	0.36	0.56
Jan. 1	0.66	1.30
8	0.77	3.10
15	0.61	2.90
18	0.93	3.40
22	0.86	2.90
29	0.88	1.40
Feb. 5	0.84	4.60
12	0.75	2.00

insecticide residues by the same methods as used for the analysis of the milk and tissue samples.

At the end of 2 months on January 16, 1952, before the residues on the hay were raised to 10 p.p.m., a fat sample was removed by biopsy from a lindane-fed cow (Cow 2) and analyzed.

After the termination of the experiment on February 13, 1952, two of the cows were slaughtered (Cow 11-DDT, and Cow 10-lindane) and portions of omental fat and kidney were analyzed for DDT and lindane, respectively. An examination was made by a veterinarian.

#### **Discussion of the Results**

**Results with DDT-Fed Cows.** In 1950–51 tests, no residue of DDT was found in the milk, although 2 p.p.m. of DDT or more were fed for 23 days and less than 2 p.p.m. for an additional 36 days. Similarly, no DDT was found in the omental fat after feeding hay having less than 2 p.p.m. of DDT for 36 days (Table III).

In the 1951-52 experiments, the residue level required for significance, at any single date, was estimated to be about 0.10 p.p.m. Hence, it was not until January 15, or about 56 days after starting the feeding of DDT, that significant amounts of DDT were secreted. Seven days after the feeding of DDT had stopped, the amount of DDT secreted was not significant (Table III). These data are not completely in agreement with those of other workers who found a prompt appearance of DDT in the milk which persisted for some time after the feeding of DDT was discontinued (20, 22). In most of these cases the daily levels of intake were greater, and these differences may account for some of the discrepancies.

The residues in the milk (Table IV) from the cow fed barn-DDT-treated hay

was consistently and significantly higher than that from the cow fed field-treated hay. This may be a real difference, or it it may be an overestimation of the DDT content of the field-treated hay. Four samples of the field-treated hay were run, two when the feeding was started and two later in the feeding period. The difficulties of sampling and/or lack of uniformity of the deposit expected from a crop harvested immediately after the application, is reflected in the range of 39 to 128 p.p.m. found in the different samples. Assuming that the 70 p.p.m. applied to the barn-DDT-treated hay was a good average for the residue on the field-treated hay, one can conclude that artificial treatment of hay prior to feeding does not favor lower milk residues, because of such treatments. On the contrary, it appears to be a conservative method for residue studies (Table IV).

The flavor and odor of the milk in the DDT-fed cows were satisfactory except in the cases of Cow 11 (1951) and Cow 7 (1950) which were in the very late stages of lactation, when the milk flavor normally is not satisfactory.

The consumption of feed and weight gains were normal and the treatments with DDT had no adverse effects on the health of the cattle that could be determined by periodic examination of the cows or by blood sampling.

Cow 11 was slaughtered on February 13, 1952, at the end of the experiment and 0.6 p.p.m. of DDT was recovered from a sample of kidney and 6.6 p.p.m. of DDT was present in a sample of the omental fat.

In the manure sample, the residues found were proportional to the amount of DDT fed. These were 0.18 and 0.13 p.p.m. for Cows 11 and 13 and 0.48 and 1.55 p.p.m. for Cows 6 and 9, respectively.

Results with Lindane-Fed Cows. In the 1950-51 tests, no lindane residue was detected in the milk which could be reported as a real difference from the checks (Table V). In the 1951-52 experiments (Table V) the mean lindane content of the milk from the treated cows (0.05 p.p.m.) over the entire test period is significantly greater than the check at the 1% level. However, the level required for a real difference at a single date is estimated to be about 0.18 p.p.m. Hence, few of the variations noted in the lindane content of the milk can be definitely attributed to real effects, rather that to chance fluctuations. These data agree with those of Ely et al. (8) in work done about the same time.

No off-flavors or odors that could be attributed to the lindane were detected, even when as much as 10 p.p.m. of lindane were fed for 1 month. These data agree with those of Ely *et al.* ( $\vec{6}$ ) which were presented at a later date at the meeting of the Entomological Society of America at Philadelphia on December 16, 1952.

The consumption of feed, weight gains, and the health of the cattle were normal. The fat sample taken from Cow 2 by biopsy on January 16, 1952, when analyzed for residue, showed 0.42 p.p.m. of lindane, while the fat and kidney taken from Cow 10 at the end of the experiment showed 0.25 and 0.08 p.p.m. of residue, respectively. No residue was found in the manure. The vital organs of the slaughtered cow were normal in all respects.

**Results with Parathion-Fed Cows.** None of the cows over the 3 years of feeding showed any residue in the milk. The small residues recorded were insignificant and within the range of error of the analytical method (Table V).

The flavors and odors of milk from cows fed parathion, up to 10 p.p.m., were satisfactory and normal.

All cows except No. 8 remained in normal health during the experimental period. Cow 8 became ill during the

Table V. Residues in Milk from Cows Fed Lindane- and Parathion-Treated Hay

			,	Barn-Treate	d Hay,	Nov. to Feb.,	1951-52
Sampling	Level fed,		in Milk, P.P.M.	Sampling	Level fed,		n Milk, P.P.M.
date	p.p.m.	Cow 2	Cow 10	date	p.p.m.	Cow 2	Cow 10
Nov. 9 12 15 18 21 28 Dec. 5 12 15 18 26 30 Jan. 12	<1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 1.0 < 2.0 2.0 2.0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	(-)0.17(-)0.14(-)0.090.000.030.00(-)0.030.060.060.06	$\begin{array}{c} \text{Lindan} \\ 0.00 \\ (-)0.47 \\ (-)0.06 \\ 0.02 \\ (-)0.09 \\ 0.02 \\ 0.02 \\ 0.03 \\ 0.06 \\ 0.03 \\ 0.06 \\ 0.03 \\ 0.06 \end{array}$	Nov. 20 25 27 Dec. 4 11 18 21 26 Jan. 1 8 15 18 22 29 Feb. 5	0 2 2 2 2 2 2 2 4 4 4 4 4 4 4 10 10 10	(-)0.10  0.00 0.08 0.26 0.06 0.09 0.04 0.16 0.20 0.17 (-)0.04 0.06 0.04	$(-)0.10 (-)0.02 0.07 0.02 0.08 0.10 0.05 0.21 0.08 0.10 (-)0.07 (-)0.12 (-)0.04 0.04 0.04 \\0.04$
			_	12 19	$\begin{array}{c} 10\\0\end{array}$	$\begin{array}{c} 0.14 \\ 0.02 \end{array}$	$\begin{array}{c} 0.05 \\ 0.02 \end{array}$
N 0	<1.0	( )0.02	PARATHI		0	( )0.02	0.02
Nov. 9 12 15 18 21 28	<1.0 <1.0 <1.0 <1.0 <1.0 <1.0 <1.0	$(-)0.03 \\ 0.05 \\ 0.02 \\ 0.02 \\ 0.01 \\ (-)0.01$	(-)0.060.000.00(-)0.01(-)0.01(-)0.01	Nov. 20 25 25 Dec. 4 11 18	0 2 2 2 2 2 5 5 5	$(-)0.02 \\ 0.02 \\ (-)0.02 \\ 0.00 \\ 0$	$\begin{array}{c} 0.02 \\ 0.02 \\ 0.00 \\ (-)0.01 \\ 0.00 \\ 0.03 \end{array}$
Dec. 5 12 15 18 26 30	$ \begin{array}{c} <1.0 \\ <1.0 \\ 2.0 \\ 2.0 \\ 2.0 \\ 2.0 \\ 0 \end{array} $	(-)0.06 0.00 0.01 0.00 (-)0.06	(-)0.010.040.00(-)0.020.000.03	21 26 Jan. 1 8 15 18	5 5 5 5 5 10	$(-)0.02 \\ 0.01 \\ 0.00 \\ 0.02 \\ 0.00 \\ 0.01$	(-)0.02 0.01 0.01 0.00 0.02 0.00
Jan. 12	0	(-)0.03	(-)0.02	22 29 Feb. 5 12 19	10 10 10 10 0	0.00 0.00 (-)0.02	(-)0.02 0.03 0.02 (-)0.02 (-)0.02

experiment. A blood sample was taken from her to check the erythrocyte cholinesterase activity against that of a cow not fed parathion. Cow 8 gave normal values which showed no evidence of parathion poisoning. The cow was slaughtered on January 23, 1951, but none of the pathological findings were attributable to the feeding of parathion. The other three parathion-fed cows remained normal.

Neither parathion nor *p*-nitrophenol were detected in the manure.

Results with Methoxychlor-Fed Cows. No methoxychlor was found in any of the milk samples. The early analyses of samples were run before the analytical technique was perfected. Some interference was present. No evidence of methoxychlor was found, but the confidence limits were broader than those analyzed at a later date. After December 11, the interference was at such a low level that the indicated methoxychlor would be less than 0.01 p.p.m. even without deduction of the check. Both low interference and high sensitivity of the analysis permitted the conclusion that the methoxychlor residues were below 0.01 p.p.m. throughout the experiment, even though residues as high as 10 p.p.m. were fed for 1 month. These data were confirmed by Ely et al. (7) who fed higher levels at a later date

without detecting any residues in the milk.

The flavor and odor of the milk was satisfactory during the entire test period. No methoxychlor was recovered in the

manure.

Results with Aldrin-Fed Cows. In the 1950-51 experiment, the small amounts of aldrin recovered were not significant and were within the range of error expected for the rather poor analytical method available at that time. However in the 1951-52 experiment, small but significant (at 5% level) amounts of aldrin were recovered in the milk for the last three sampling dates, January 29, February 5, and February 12, when 10 p.p.m. of aldrin were being fed. The samples were not analyzed for dieldrin.

Except for Cow 14 whose milk ranged from slightly salty to very salty for most of the testing period, none of the other three cows gave off-flavors or odors. None could be attributed to the aldrin fed.

Analysis of samples of pancreas, kidney, liver, spleen, brain, and omental fat taken from Cow 9 (1950) and Cow 14 (1951) revealed no residue present in any of these tissues. A trace of aldrin was present in the manure.

Post-mortem examination showed all vital organs to be normal.

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