Table VI. Recoveries of Heptachlor (H) and Heptachlor Epoxide (HO) Residues from Carrington Silt Loam Soils and Crops Grown during 1960, 1961, and 1962 on Heptachlor-Treated Plots

	Heptachlor Applied to Soil, Lb./5-Inch Acre								
	1[a]			2[a]			3[a]		
	1960	1961	1962	1960	1961	1962	1960	1961	1962
	Recovered from Soils at Harvest Time, P.P.M.[b]								
H + HO	0.19	0.32	0.41	0.39	0.67	0.90	0.93	1.25	1.33
% HO[c]	16	19	27	13	16	23	8	11	20
	Recovered from Crops at Harvest, P.P.M.								
Carrots									
H + HO	0.14[d]	0.18[d]	0.15[e]	0.31[d]	0.36[d]	0.44[e]	0.51[d]	1.12[d]	0.69[e]
% HO	21	22	35	15	17	22	14	12	19
Potatoes									
H + HO	0.05[bd]	0.05[b]	0.10[e]	0.10[bd]	0.13[b]	0.14[e]	0.17[bd]	0.27[b]	0.27[e]
% HO	100	100	67	70	77	82	77	63	72
Radishes									
H + HO	0.03[bd]	0.03[bd]	0.07[e]	0.07[bd]	0.07[bd]	0.12[e]	0.09[bd]	0.10[bd]	0.21[e]
% HO	100	100	71	100	70	65	100	80	62
Beets									
H + HO		0.01[bd]	0.05[e]		0.05[bd]	0.10[e]		0.08[bd]	0.29[e]
% HO		100	70		100	70		87	65

[a] Heptachlor applied at 1, 2, or 3 lb./5-inch acre in May of each year (1960, 1961, and 1962). Total application: 3, 6, or 9 lb./acre over 3-year period.
[b] By colorimetric analyses.
[c] Heptachlor epoxide in per cent of total residues recovered (H + HO).
[d] By bioassay.
[e] By gas-liquid chromatography.

higher (Table VI). Most of the crops grown on soils treated with aldrin at 3 pounds per 5-inch acre did not contain relatively higher insecticidal residues.

These results were obtained with a silt loam under Wisconsin conditions (average yearly rainfall, 30.16 inches or 766.07 mm.; average yearly temperature, 45.3° F. or 7.3° C.; average temperature for the period May through October, 62.4° F. or 16.9° C.). In areas of higher temperatures and higher humidities, insecticidal soil residues would dissipate faster (*1, 7, 8*) and possibly present a reduced hazard in terms of translocation of toxicants from soils into crops.

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INSECTICIDE STORAGE IN FAT

Storage of Heptachlor Epoxide in the Body Fat and Its Excretion in Milk of Dairy Cows Fed Heptachlor in Their Diets

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CHLORINATED hydrocarbon insecticides ingested or absorbed by dairy animals may be stored in the animals' body fat and excreted in milk (*3–5, 8, 14, 15, 17, 19*). Animals grazed on pastures treated with heptachlor (*6, 7, 12, 13*) and chlordan (*18*) excreted heptachlor epoxide for a considerable time after treatment and removal from the treated pastures. Dairy cattle fed alfalfa hay grown on soil treated with heptachlor excreted significant quantities of heptachlor epoxide (*9*). Some heptachlor epoxide was found in the milk up to 45 days after intake was discontinued.

Such compounds vary appreciably in their propensity for storage and excretion (*8*). Some, such as aldrin and heptachlor, are not stored as such but are converted to and are stored as their epoxides, dieldrin and heptachlor epoxide. In the case of aldrin, it was apparent that an animal receiving *X* amount of aldrin daily would store more dieldrin than a comparable animal receiving the same amount of dieldrin

When heptachlor epoxide was fed to Shorthorn dairy cows at levels of 0.2, 0.5, 1.5, 10, and 50 p.p.m., at the end of 12 weeks it appeared in the butterfat of milk at levels of 4.25, 11.25, 21.7, 119.7, and 460 p.p.m., and in body fat at levels of ?, 7.1, 14.7, 83.5, and 293.4 p.p.m., respectively. In a subsequent feed-off period, the heptachlor epoxide residue in body fat declined slowly, with detectable residues present 23 months later in the fat and butterfat of the most contaminated animals.

daily. Gannon, Link, and Decker (*8*) showed that heptachlor had a much lower propensity for storage than aldrin. It was assumed that the same relationship might prevail in the case of heptachlor and heptachlor epoxide, but as we soon found and as was later reported by Claborn and Bushland (*2*), heptachlor epoxide was stored in the body fat at approximately 10 times the storage rate of heptachlor. Bache *et al.* (*1*) reported that feeding heptachlor epoxide for 2 weeks at 0.5 and 1.0 p.p.m. of the roughage intake of dairy cows resulted in excretion of 0.38 and 1.94 p.p.m., respectively, in the butterfat.

Many of the studies cited above were of short-term duration, whereas the studies reported herein were undertaken to establish, if possible, a mathematical relationship between parts per million of heptachlor epoxide in the diet and in the body fat or butterfat when fed for 12 consecutive weeks and to determine its persistence therein up to approximately 2 years after intake of heptachlor epoxide was discontinued.

Experimental

Toxicants and Animals under Test. Heptachlor epoxide was fed to rather fat cows of the Shorthorn breed, which were chosen to provide milk and fat for biopsy studies. The technical grade of heptachlor epoxide used contained 85 to 90% active material. It was formulated at various concentrations in acetone, so that 1 ml. of solution would treat 1 pound of dry feed at the desired parts per million concentration of heptachlor epoxide. Two cows were used for each of the five levels of heptachlor epoxide intake—50, 10, 1.5, 0.5, and 0.2 p.p.m. in the animal's daily ration. As a check against previous studies, two cows were fed 50 p.p.m. of dieldrin and two cows were fed 100 p.p.m. of DDT in their daily diets. Two cows fed insecticide-free rations were maintained as checks to provide a base line for analysis of butterfat and body fat.

Rations and Feeding Procedure. The concentrate mixture fed consisted of 1/3 oats and 2/3 ear corn ground in a hammer mill. The hay was No. 1 grade, first-cutting alfalfa grown on land which had not been treated with any insecticide and the crop was not treated with any chemical material during its period of growth. The corn and oats were raised on land near Rossville, Ill., and neither crop was treated with any insecticide during the growing season. The cows were fed concentrate from individual feed boxes throughout the experiment; each cow was fed from the same box throughout the trial. During the time the cows were eating the concentrate and alfalfa they were confined in stanchions.

Heptachlor epoxide was added to the concentrate and hay immediately before being offered to the animals. Any feed that was not consumed was weighed and recorded. Before the experiment began, efforts were made to estimate the approximate minimum feed intake for each cow. During this experiment the amount of feed provided for each cow was slightly less than the maximum amount the individual cows had been found to consume. This procedure reduced to a minimum the amount of unconsumed feed and accounted for the very few occasions when any feed was refused by the cows.

Sampling Procedure. On days when milk samples were to be collected, the entire production of each cow at 6:00 A.M. was placed in a separate sterile tinned can, and the same procedure was followed at 6:00 P.M. The milk in each can was thoroughly mixed, and representative A.M. and P.M. samples were placed in waxed paper milk cartons lined with a Pliofilm bag. The samples were then properly labeled and placed in a cold room at 20° F.

Omental fat samples from the lactating cows were taken by biopsy, placed in a Pliofilm bag, labeled, and frozen until time of analysis.

General Discussion of Analysis and Methods. The Davidow (*4*) colorimetric method was used for the determination of heptachlor epoxide in milk and animal fat. Confirmation analyses were made by Mills' method of paper chromatography (*11*) and gas chromatography with electron capture detection, as described by Lovelock and Lipsky (*10*).

The samples used were saponified with KOH, extracted, and cleaned up on

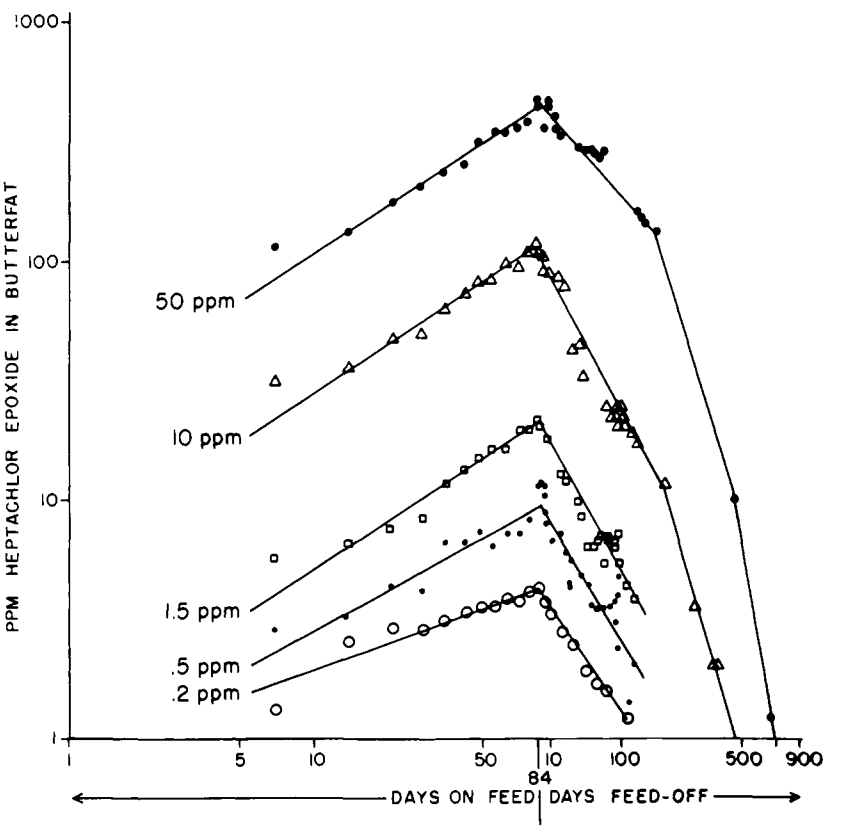

Figure 1. Heptachlor epoxide in butterfat at indicated intervals during and after feeding

Animals fed 0.2, 0.5, 1.5, 10, and 50 p.p.m. heptachlor epoxide in daily ration for 84 days (log-log relationship

MgO and Florisil. The same cleanup procedure was used where paper chromatography or electron capture detection was employed to analyze the samples.

With every set of samples analyzed, fortified butterfat recovery samples and standards were analyzed in order to keep constant check on the reliability of the analyses and to provide the basis for calculating the heptachlor epoxide residues. Butterfat and body fat from untreated cows served as analytical blanks and all assays reported herein are corrected for any inherent interference coming from the product analyzed.

All analyses were based upon grams of extracted butterfat or body fat. Butterfat was extracted by the method described by Storherr and Mills (*16*). Aliquots of butterfat or animal fat used in the analysis ranged from 1 to 20 grams, depending upon the amount of heptachlor epoxide present. The minimal detectable amount of heptachlor epoxide by the Davidow method was about 2 μg. Results from fortified recoveries using the Davidow colorimetric method may vary as much as 70 to 110%, with 90% of the recoveries within the range of 90 to 100%. The majority of the butterfat check samples gave a negative base line value as computed from the absorbances at 390, 415, and 440 mμ. One-gram butterfat samples were used in paper chromatography and only 100-mg. butterfat aliquots were used for electron capture analysis.

Experimental Data and Discussion

The general trends of the results obtained in this investigation are in accord with the results of similar studies with other compounds, but whereas heptachlor has a rather low propensity for storage, heptachlor epoxide appears to have the highest propensity for storage of any of the chlorinated hydrocarbon insecticides studied. As shown in Figure 1, by the end of one week sizable residues had appeared in the butterfat at all levels of intake and, in magnitude, were somewhat in proportion to the rates of intake. However, it was apparent even then that the lower the concentration in the diet, the higher the percentage of that intake in the butterfat. By the end of the twelfth week these ratios ranged from 1 to 22 at an intake of 0.5 p.p.m. to 1 to 9 at 50 p.p.m. (Figure 1).

Body fat samples were obtained from the animals at intervals of 3, 7, 10, 14, 42, 49, and 84 days, with a feed-off period of 102 weeks (714 days). The heptachlor epoxide, dieldrin, and DDT content of body fat encountered at these intervals is shown in Figures 2, 4, and 5, and the relationship between the residue content of butterfat and body fat is shown in Figures 3, 4, and 5.

Plotting the twelfth week data as log concentration in diet *vs.* log concentration in butterfat (Figure 3), a straight-line relationship is established. From

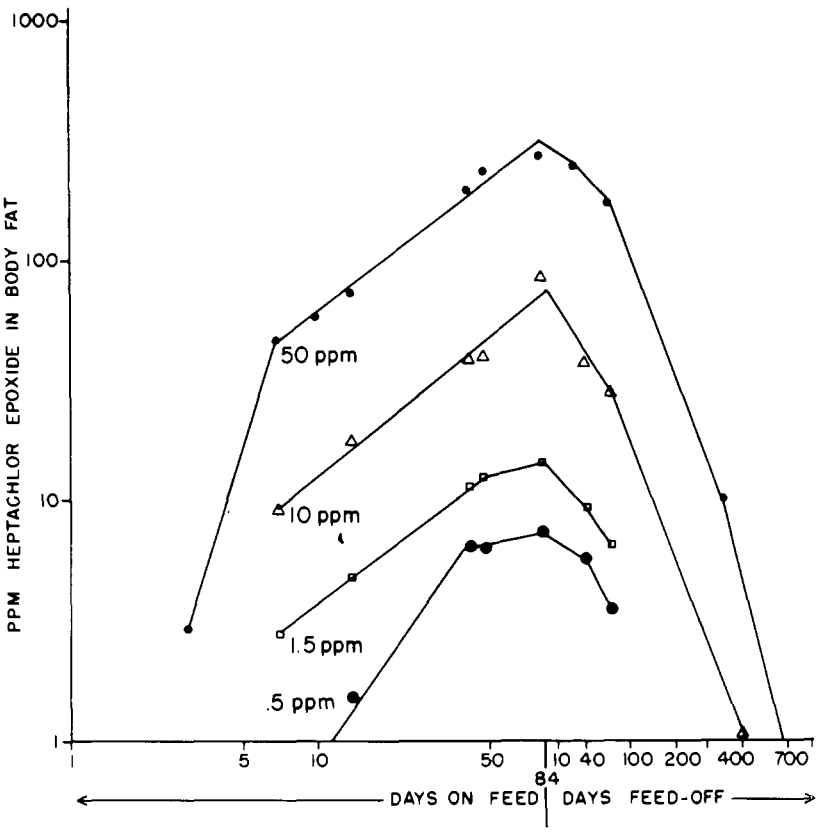

Figure 2. Heptachlor epoxide in body fat at indicated intervals during and after feeding

Animals fed 0.5, 1.5, 10, and 50 p.p.m. heptachlor epoxide in daily ration for 84 days (log-log relationship)

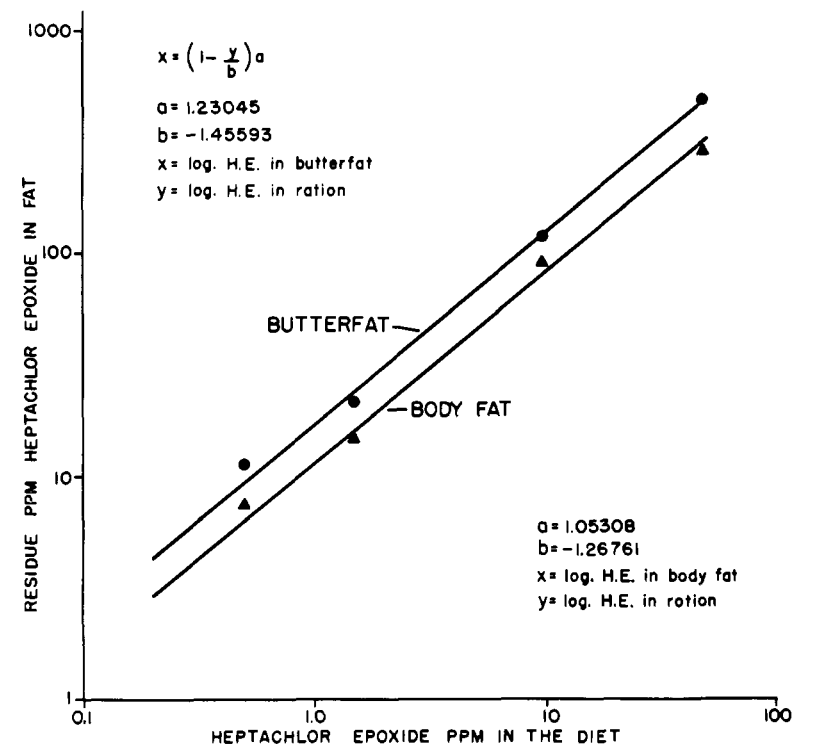

Figure 3. Heptachlor epoxide in butterfat and body fat from cows on continuous heptachlor epoxide intake for 12 weeks

0.2, 0.5, 1.5, 10, and 50 p.p.m. heptachlor epoxide in daily ration. Amount of heptachlor epoxide in butterfat (log-log relationship) or body fat at end of 12-week feeding period can be calculated for any intake level from mathemotical formula $x = (1 - y/b)a$

this a mathematical expression was derived: $x = (1 - y/b)a$.

x = log heptachlor epoxide in butterfat
y = log heptachlor epoxide in diet
a = 1.23045
b = −1.45593

This formula can be used to calculate parts per million of heptachlor epoxide in butterfat at 12 weeks for any dietary feeding level of heptachlor epoxide.

Likewise, the amount of heptachlor epoxide in body fat after 12 weeks of feeding can be calculated for various intake levels by the formula: $x = (1 - y/b)a$, as indicated in Figure 3.

x = log heptachlor epoxide in body fat
y = log heptachlor epoxide in diet
a = 1.05308
b = −1.26761

At the end of the 12-week feeding period the dieldrin and DDT content of the butterfat from cows receiving 50 p.p.m. of dieldrin and 100 p.p.m. of DDT was somewhat lower than the heptachlor epoxide content of butterfat from cows receiving 50 p.p.m. of heptachlor epoxide (Figures 4 and 5). The values obtained here are practically identical with those obtained in an earlier study (*8*): dieldrin 264 (262) and DDT 120 (119).

As shown in Figures 1, 4, and 5, heptachlor epoxide, dieldrin, and DDT, once stored in body fat during a feeding period, continue to contaminate butterfat long after chemical intake has been discontinued. There appears to be a rather rapid decline in the chemical content of butterfat for a short period (absence of the contribution due to daily intake) and then the rate of loss becomes slow and gradual. In all cases sizable residues were still present several months after intake was discontinued. Where continued lactation permitted the continuance of observations, in all cases detectable residues were present in butterfat for several months, and for nearly 2 years where the intake had been at 50 p.p.m. in the diet.

There are minor inconsistencies in analytical data of the various figures and tables, but recovery data based upon analysis of fortified samples are considered satisfactory if they fall within 80 to 120% of expected, and good if within the range of 90 to 110 %. Thus, if one reading is high and the next low, it erroneously appears that a plateau has been reached or even passed. Some variation might be attributable to periodic or other variations in the physiology or metabolism of the cow. There is some evidence (Table I) that morning milk samples contain higher residues than samples taken in the evening.

Comparison of Analytical Methods. Since any single analytical method may, for reasons not apparent, give biased

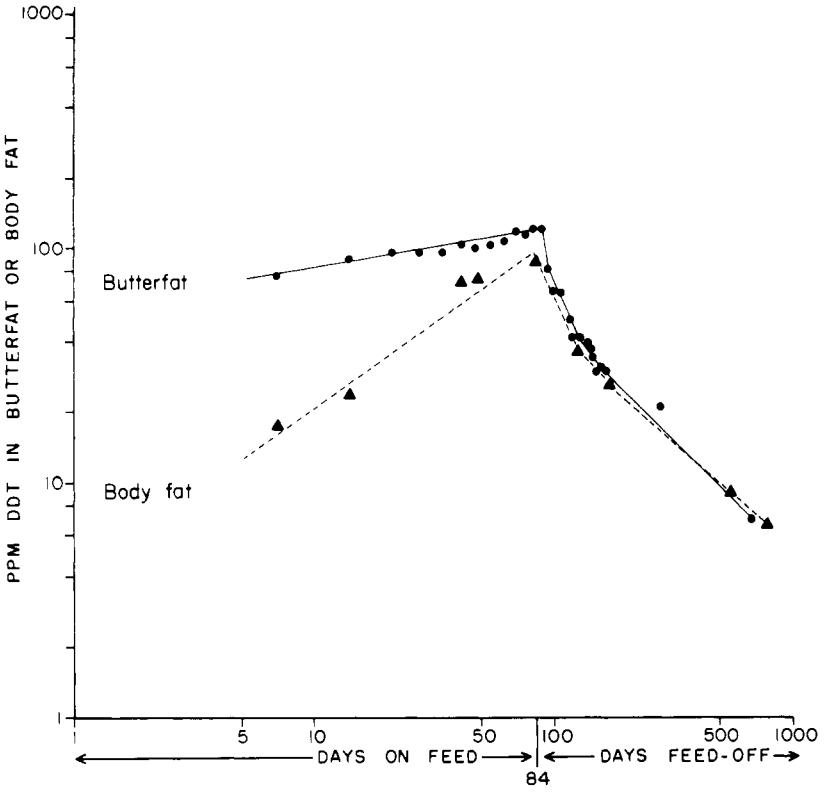

Figure 4. DDT in butterfat and body fat from cows on continuous intake of 100 p.p.m. of DDT in daily ration for 84 days

Log-log relationship

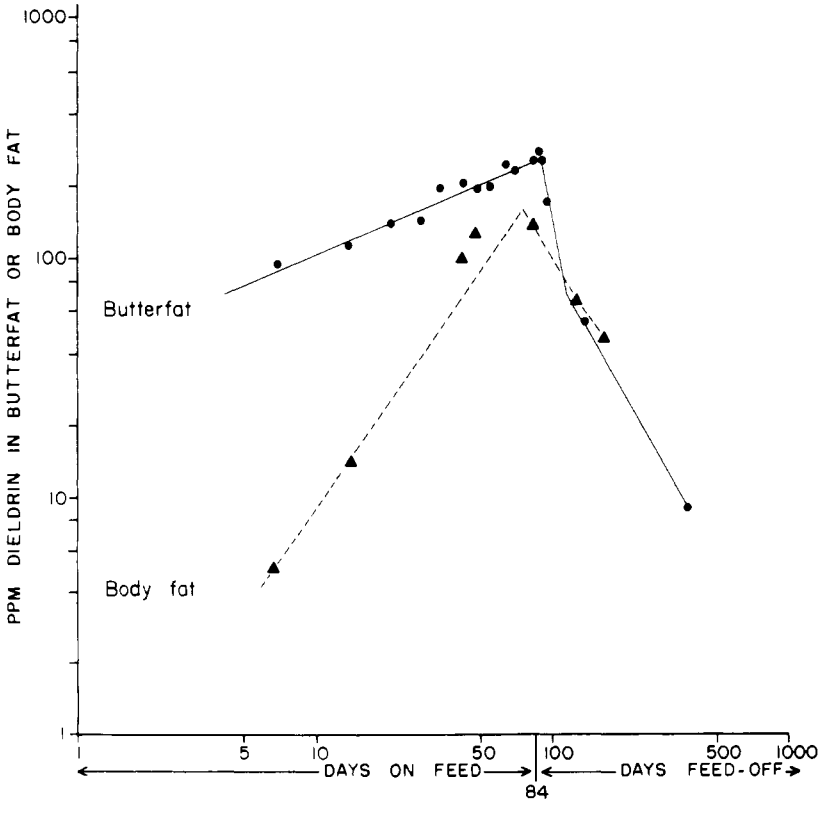

Figure 5. Dieldrin in butterfat and body fat from cows on continuous intake of 50 p.p.m. of dieldrin in daily ration for 84 days

Log-log relationship

Table I. Heptachlor Epoxide Residues Found in Milk Samples Taken Morning and Evening

Comparison	Heptachlor Epoxide, P.P.M. in Butterfat			
	A.M.	P.M.	+ A.M.	+ P.M.
1	3.98	2.83	1.15	
2	4.33	1.48	2.85	
3	4.03	3.52	0.51	
4	5.08	3.23	1.75	
5	6.94	6.86	0.08	
6	8.41	5.03	3.38	
7	6.26	6.41		0.15
8	6.25	5.90	0.35	
9	22.75	20.97	1.78	
10	24.38	20.13	4.25	
11	22.22	21.68	0.54	
12	24.23	20.70	3.53	
13	280.23	274.76	5.47	
14	269.17	276.35		7.18
No.			12	2

Table II. Analytical Methods Used in Butterfat Analysis

	Davidow Specific Colorimetric				Electron Capture				Difference Av.		Difference Plus	
	Sample		Difference		Sample		Difference		D.		D.	
Test	A.M.	P.M.	+A.M.	+P.M.	A.M.	P.M.	+A.M.	+P.M.	spec.	E. C.	spec.	E. C.
A 1	4.15	3.95	0.20		4.00	3.65	0.35		4.05	3.83	0.22	
2	4.11	4.49		0.38	4.07	3.80	0.27		4.30	3.93	0.37	
3	4.18	3.27	0.91		3.32	3.42		0.10	3.73	3.37	0.36	
4	4.30	4.21	0.09		4.38	4.33	0.05		4.26	4.36		0.10
5	4.15	3.59	0.56		4.34	4.36		0.02	3.87	4.36		0.49
6	5.60	3.79	1.81		4.49	4.49	...	...	4.70	4.49	0.21	
7	4.61	4.41	0.20		4.38	4.29	0.09		4.51	4.33	0.18	
8	5.96	3.58	2.38		4.64	4.79		0.15	4.77	4.72	0.05	
9	2.50	2.04	0.46		2.20	1.85	0.35		2.27	2.03	0.24	
10	2.83	2.43	0.40		2.35	1.70	0.65		2.63	2.03	0.60	
No.			9	1			6	3			8	2

	Davidow Specific Colorimetric				Mills Paper Chromatographic				Difference Av.		Difference Plus	
	Sample		Difference		Sample		Difference		D.		D.	
Test	A.M.	P.M.	+A.M.	+P.M.	A.M.	P.M.	+A.M.	+P.M.	spec.	P. C.	spec.	P. C.
C 1	2.43	2.23	0.20		2.13	2.33		0.20	2.33	2.23	0.10	
2	2.50	1.76	0.74		2.00	1.93	0.07		2.13	1.97	0.16	
3	1.35	1.35	...	...	1.13	1.00	0.13		1.35	1.07	0.28	
4	1.55	0.81	0.74		1.13	1.07	0.06		1.17	1.10	0.07	
5	0.34	0.08	0.26		0.18	0.19		0.01	0.21	0.19	0.02	
6	0.59	0.15	0.45		0.18	0.22		0.04	0.37	0.20	0.17	
7	0.43	0.42	0.01		0.24	0.18	0.06		0.42	0.21	0.21	
8	0.46	0.40	0.06		0.20	0.22		0.02	0.43	0.21	0.22	
9	0.67	0.20	0.47		0.23	0.27		0.04	0.44	0.25	0.19	
10	0.18	0.26		0.08	0.21	0.22		0.01	0.22	0.22	...	...
No.			8	1			4	6			9	

or inaccurate results, we considered it desirable to analyze some samples by two methods. Data presented in Table II show the results obtained in such comparisons. While the standard and generally accepted Davidow colorimetric method may be less sensitive and may give slightly higher readings than either the Mills paper chromatographic or the electron capture methods, the differences are not great and do not unduly bias the data.

By paper chromatography as little as 0.1 μg. of heptachlor epoxide could be accurately measured on the densitometer. Gas chromatography and detection by electron capture can easily measure 0.000025 μg. The electron capture apparatus we used has a retention time of 48 to 50 seconds for heptachlor epoxide and produces a sharp lineal spike of 15 seconds' duration on the recorder. Injection and instrumental error is about 3%. Consequently, most of the error we encountered with the electron capture apparatus was associated with sampling and cleanup.

Reaction of Animals to Treatment. All of the cows maintained normal health throughout the period of study. One cow in the group receiving 50 p.p.m. of heptachlor epoxide was carrying a calf, which she delivered normally, but it died a few days after birth. It is not known whether death of the calf occurred because of heptachlor epoxide storage prior to birth, as a result of nursing and thus consuming milk containing about 20 p.p.m. of heptachlor epoxide, or from other causes. Both cows receiving 100 p.p.m. of DDT were pregnant and both produced normal calves that lived.

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