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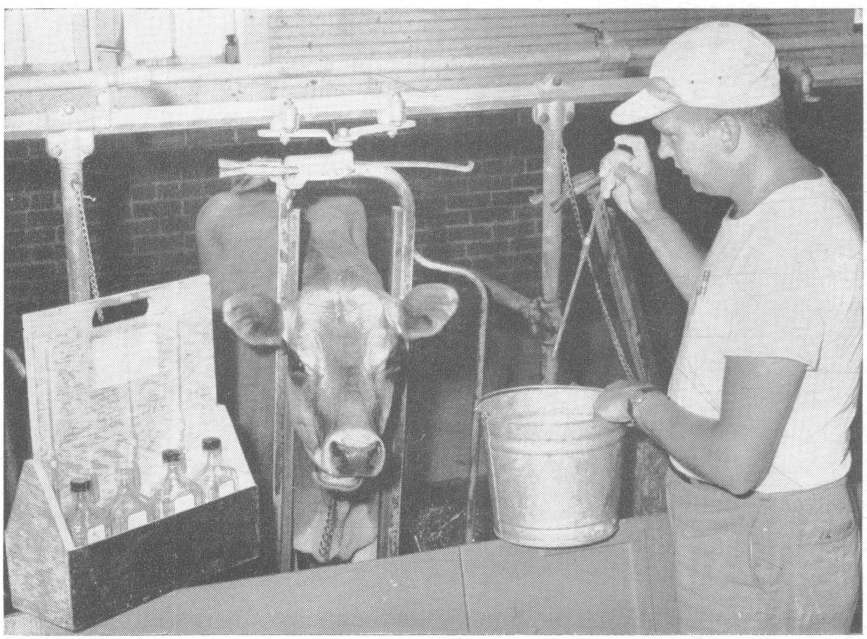

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EXCRETION OF BHC IN MILK FROM DAIRY COWS FED KNOWN AMOUNTS OF BHC[1]

GEORGE W. WARE and L. O. GILMORE

In conjunction with the studies of BHC residues on alfalfa (Ware, 1958) a joint project was conducted in which a group of lactating dairy cows were fed known amounts of benzene hexachloride (BHC) in their daily rations. Milk samples were taken at regular intervals during the BHC feeding period and following termination, to determine the amount of BHC excreted in milk during feeding and its persistence when removed from the animals' ration. BHC residue determinations were assayed both chemically and biologically. The experiment was initiated on May 4, 1957.

Methods:

Ten dairy cows in various stages of the declining phase of lactation were used to test the effect of feeding different levels of BHC[2] on the amount of this compound passing through the mammary epithelium with time. Five pairs of cows were fed respectively 0, 1, 5, 25 and 125 ppm of BHC daily with the morning grain. For each Holstein there was paired either a Jersey or Guernsey. The BHC acetone solution was prepared so that the desired concentration of BHC was delivered onto the grain in 5 ml. of solution using a standard pipette. The test solution was placed on the grain to insure its being consumed. Mixed hay was fed slightly in excess of requirements twice daily, and that refused was weighed back daily. From these data the feed consumed was calculated from which the ppm of BHC consumed was determined. The cows were withheld from pasture during that part of the experiment in which BHC was fed. At the end of 50 days the BHC feeding was discontinued and the cows were turned out to pasture.

The body weights used in the calculation were the respective mean values of three consecutive daily weighings at the beginning and end of the 50 day phase of the experiment during which BHC was fed.

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[1]Contribution to Regional Project NC–33.

[2]The 40 percent gamma isomer BHC used was supplied by United Cooperatives, Inc., Alliance, Ohio.

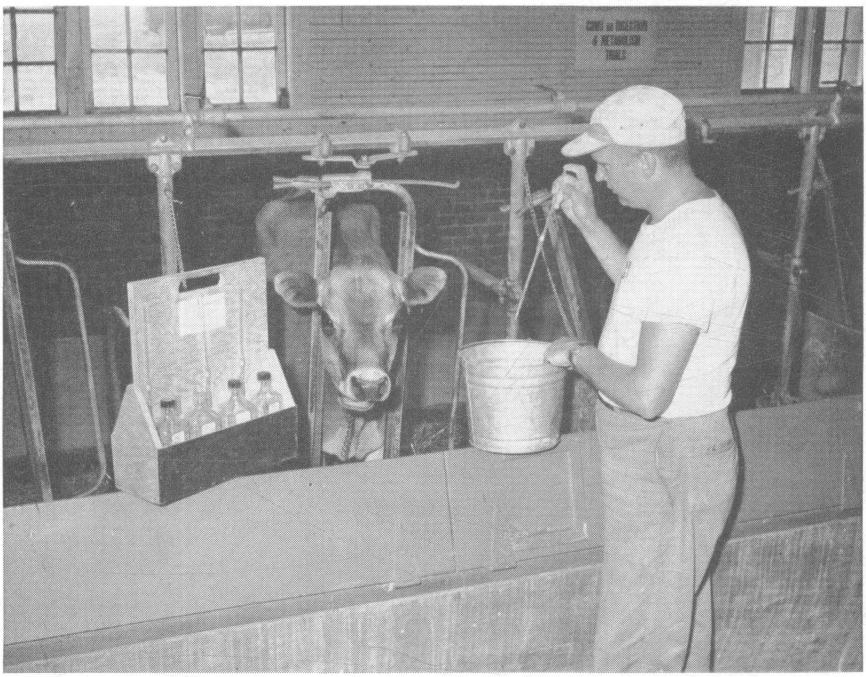

Figure 1.—The morning grain ration was fortified with BHC by placing it directly on the grain using a standard pipette.

One-quart milk samples were collected from each animal at 1, 3, 5, 7, and 10 days following the initiation of BHC feeding and at weekly intervals (through the feeding period) and thereafter until lactation ceased or BHC disappeared from the milk. For the first five weeks of sampling, one-quart, plastic-coated, cardboard cartons were used as sample containers. However, these were easily damaged and difficult to handle. The remainder of the samples were collected in pint prescription bottles with aluminum foil-lined caps. Two bottles were used for each sample, one for bioassay and one for chemical determination. The samples were preserved by the addition of one ml. of 40 percent formaldehyde to each pint and held at 40° F.

Chemical Analysis:

The colorimetric BHC analysis method and corresponding glassware follow closely that described by Wylegala **et al.** (1956), which is a modification of that originally described by Schechter and Hornstein

TABLE 1.—Average Amounts of Technical Grade BHC Fed Daily to Test Animals

PPM Planned	PPM Actual	
	Jersey–Guernsey	Holstein
1	0.86	0.89
5	4.17	3.84
25	21.84	21.71
125	103.98	100.28

(1952). Since this method deals exclusively with milk fat rather than whole milk, the authors reported the sensitivity to be 0.01 ppm BHC in 4 percent fat corrected milk.

Three changes worthy of mention were introduced into the method. They were the composition of the nitrating acid, purification of malonic acid, and use of glacial acetic acid substituted for phosphoric acid as the glass fittings lubricant. The above method calls for a nitrating acid made 1:1 (vol/vol) of concentrated sulfuric and fuming nitric acids. Consistent results could not be obtained in this study until a nitrating acid of 20:1, sulphuric to fuming nitric, was used as suggested by Hancock and Laws (1955).

It was observed that Eastman Organic Chemicals' practical grade malonic acid produced a blank equal to that of malonic acid recrystallized from ethyl acetate, as suggested by Gunther and Blinn (1955).

Glacial acetic acid served as the ground glass-joint lubricant as suggested by Gehrke and Bevirt (1956) and Gehrke **et al.** (1957). They consistently obtained lower lindane readings when the joints were lubricated with 85 percent phosphoric than with acetic acid. However, Wylegala **et al.** (1956) and Fahey and Rusk (1958) experienced no difficulty when phosphoric acid was used.

Standard Curve:

A standard curve was established using purified milk fat fortified with technical BHC (40% gamma isomer)[3] dissolved in glass distilled methylene chloride. The methods used in purifying and fortifying milk fat followed those of Wylegala **et al.** (1956). This standard curve was somewhat lower than that obtained by using lindane, as observed by Gunther and Blinn (1955).

[3]United Cooperatives, Inc., Alliance, Ohio.

Bioassay:

The method of testing followed closely that of Sun and Sun (1953). Pint samples of formalinized mik were heated to 70° C. and shaken by hand before storage at 4° C. Before testing, the milk was heated to 65-70° C. and homogenized by hand[4] or with a Waring blendor. Standard mortality curves were run with each test using 0.5-2.0 ppm of lindane in acetone solutions in the milk from the check animal of the same breed group. All milk samples from BHC-fed cows, except those receiving 125 ppm, were fortified with 0.8 ppm lindane before testing, since milk from cows on the lower feeding rates contained, at the most, sublethal concentrations of the gamma isomer.

Starved adult house flies, **Musca domestica** L., of mixed sex, 18-24 hours old and fed only water, served as the test animal. They were reared by the Chemical Specialties Manufacturers Association standard method. The test and lindane standard milk was fed to the flies on cellucotton in a small portion cup in wide mouth Mason pint jars, covered with cheese cloth. Four jars were used in each test with 50 flies to a jar. After 48 hours in a refrigerator-incubator at 25±1° C. the flies were counted. Concentrations of lindane in the samples were estimated by plotting mortalities of the unknowns on a log probit analysis curve derived from the standard for that day. Final results were multiplied by 2.5 to convert ppm lindane to ppm BHC (Fisk, 1958).

Results:

The data involving the amounts of BHC in milk fat during BHC feeding have been analyzed statistically. The slopes of BHC in milk fat against time in days appear in Figure 2. The slopes of 1, 5, and 25 ppm in both breeds are not significantly different from zero. These data indicate that lactating dairy cows fed from 1 to 25 ppm of technical grade BHC in their diets do not excrete BHC in increasing quantities with time. However, both breeds on 125 ppm showed an increase of BHC excretion with time, significant at the 0.01 level of probability.

The data of BHC disappearance from milk fat after termination of BHC feeding have also been analyzed statistically and are shown in Figure 3. The slopes of ppm BHC are plotted against time, on a log-log basis. All slopes when considered as a group are not alike. However, the pairs of 1, 5, and 25 ppm are the same. Both slopes at 125 ppm are significantly different from each other at the 0.01 level of probability.

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[4]Hand homogenizer, 1-quart, by Club Aluminum.

The residues of BHC in milk standardized to 4 percent fat during and after BHC feeding appear in Table 2. It will be seen that BHC was detectable in milk from cows fed 1 ppm up to 34 days following

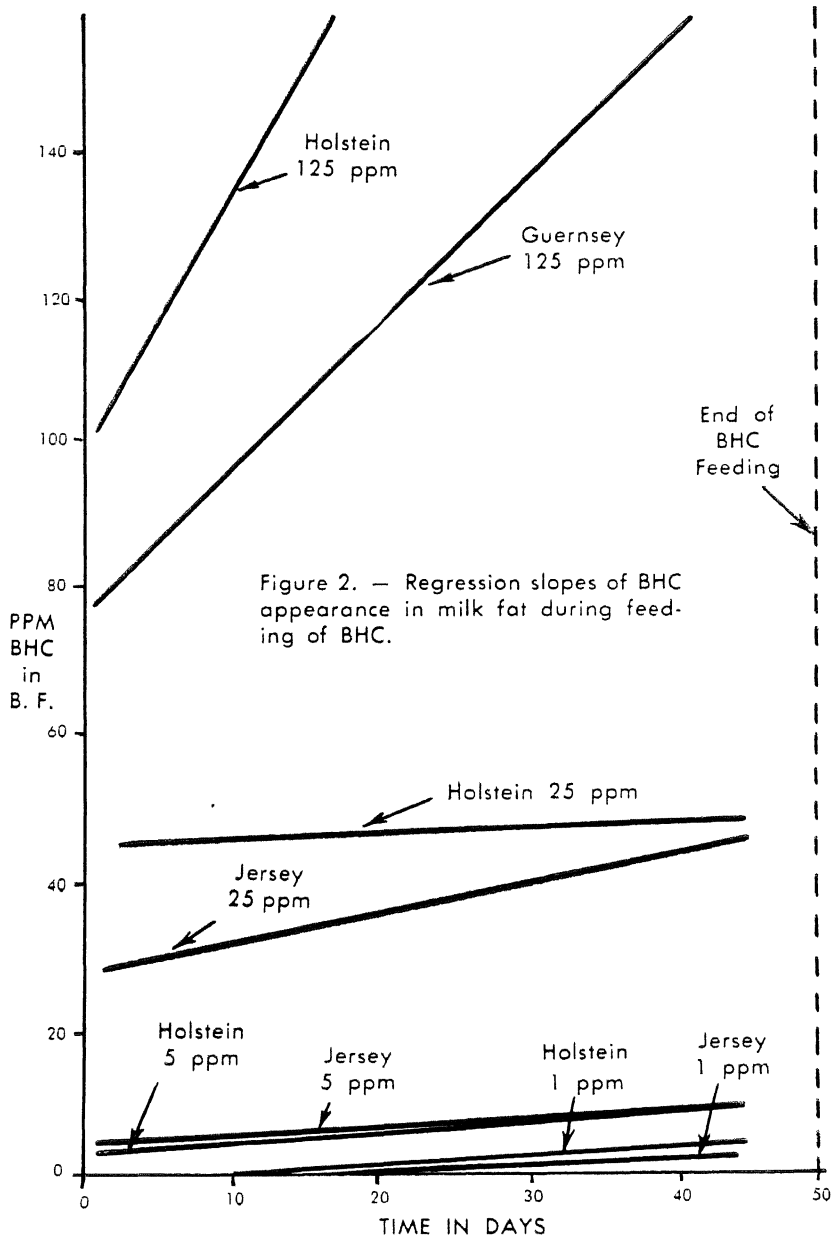


Figure 2. — Regression slopes of BHC appearance in milk fat during feeding of BHC.

BHC termination, up to 52 days in cows fed 5 ppm, beyond 102 days with cows fed 25 ppm and beyond 171 days in one cow fed 125 ppm. Milking was discontinued 87 days after BHC was removed from the test ration of the other animal fed 125 ppm.

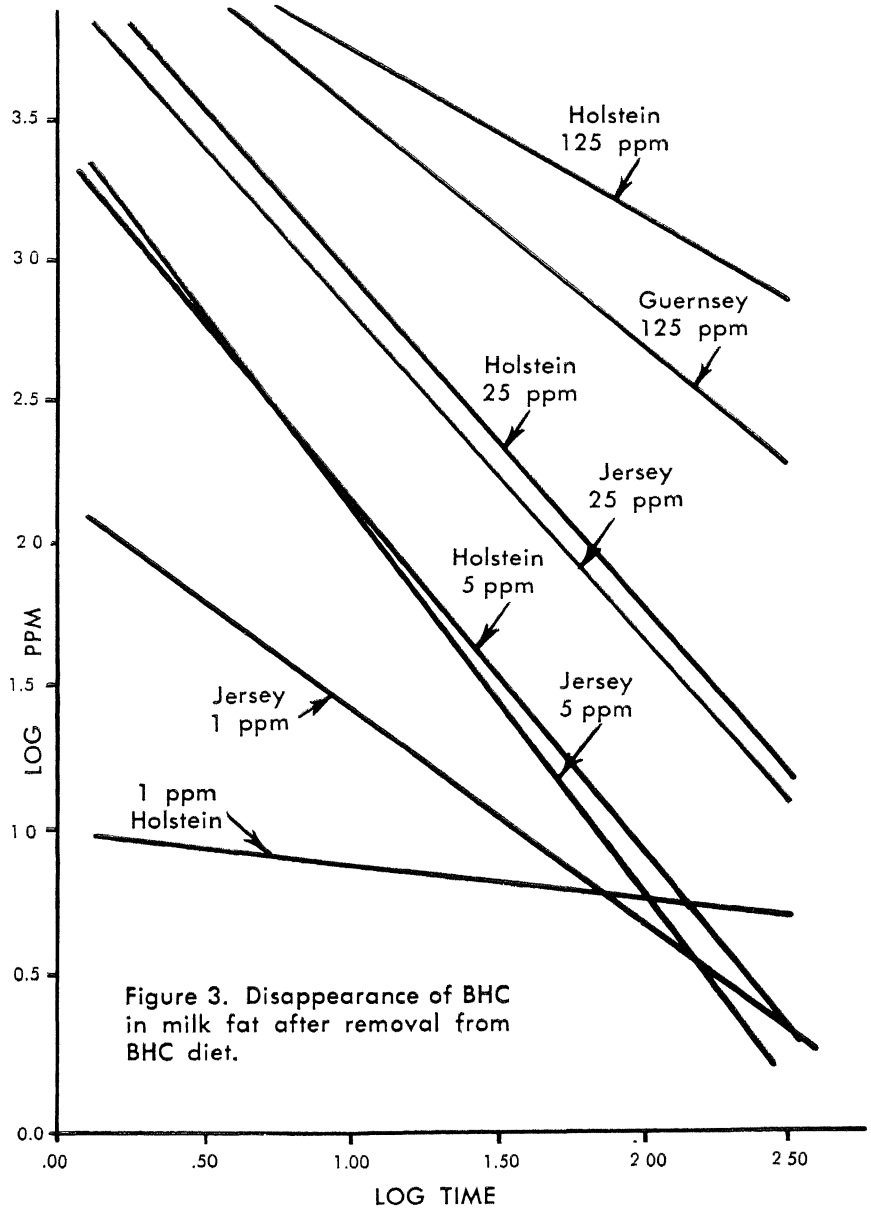


Figure 3. Disappearance of BHC in milk fat after removal from BHC diet.

TABLE 2.—PPM of BHC Based on Milk Standardized to 4 Percent B. F. During and After Daily Feeding for 50 Days

BHC IN DIET		1 PPM		5 PPM		25 PPM		125 PPM	
Cow	No.	H-1036	J-1107	H-1195	J-1132	H-1196	J-1184	H-1197	G-1342
Date	Days Fed								
5- 5	1	____	0.11	0.00	0.24	1.42	0.68	4.57	1.74
5- 7	3	0.02	0.01	0.29	0.15	4.01	____	3.36	2.64
5- 9	5	____	0.03	0.19	0.16	1.20	1.24	5.62	3.00
5-11	7	0.02	0.02	0.22	0.27	1.11	1.59	4.08	4.56
5-14	10	0.04	0.22	0.14	0.23	1.00	1.46	5.37	4.11
5-21	17	0.04	0.03	0.23	0.25	1.85	1.43	____	5.43
5-28	24	0.06	0.03	0.36	0.34	2.04	1.38	7.42	3.79
6- 4	31	0.10	0.02	0.37	0.17	1.84	1.14	7.00	6.57
6-14	41	0.07	0.04	0.22	0.28	1.70	1.84	9.00	5.18
6-18	45	0.10	0.01	0.28	0.31	1.99	1.70	9.88	5.48
6-22	50			BHC REMOVED FROM DIET					
6-25	3	0.03	0.01	0.13	0.12	0.94	0.88	3.62	2.63
7- 2	10	0.03	0.02	0.08	0.11	0.37	0.36	2.23	1.64
7- 9	17	0.00	0.01	0.03	0.03	0.40	0.22	2.15	1.01
7-12	20	0.00	0.01	0.02	0.02	0.27	0.16	1.40	0.79
7-19	27	0.01	0.01	0.04	0.14	0.15	0.11	1.60	0.71
7-26	34	0.01	0.01	0.05	0.02	0.18	0.09	1.47	0.86
8- 6	45	0.00	0.00	0.02	0.02	0.06	0.04	0.95	0.42
8-13	52	0.00	0.00	0.01	0.01	0.02	0.02	0.79	0.38
8-20	59	0.00	0.00	0.00	0.00	0.03	0.01	0.70	0.37
8-26	67					0.03	0.01	0.84	0.41
9- 3	73					____	____	0.69	0.27
9-10	80					0.08	0.04	0.58	0.43
9-17	87					0.04	0.11	DRY	0.22
9-24	94					0 01	0.05		0.24
10- 2	102					0.21	0.02		0.22
10- 9	109					DRY	DRY		0.14
10-15	115								0.13
10-22	122								0.11
11- 5	136								Positive
11-14	145								Positive
11-19	150								Positive
11-26	157								Positive
12- 3	164								Positive
12-10	171								Positive
									DRY

The results of the house fly bioassay appear graphically in Figure 4. It will be observed by comparing this with Figure 5 that the bioassay data for the animals fed 125 and 25 ppm BHC in the diets are generally lower than the chemical analyses, but that the data from cows fed 1 (not illustrated) and 5 ppm are higher than those obtained from the chemical analyses. The bioassay data as they appear in Figure 4 were obtained by using a factor of 2.5 times the gamma isomer detected, since the gamma isomer was 40 percent by weight of the technical BHC. This is only an approximation because the isomers of BHC have different rates of fat deposition, and consequently different rates of fat release (Metcalf, 1955).

Discussion:

Knipling (1950) reported that there was little or no lindane in the milk from cows fed lindane in amounts of 7.5 ppm in their total diet. However, the limit of accuracy of the methods used was considered to be 0.2 ppm. Furman and Hoskins (1948) fed one cow a single dose of 45 grams of BHC (12 percent gamma) and by means of house fly bioassay detected a maximum of 5.5 ppm gamma isomer in the cream on the following day. The animal excreted gamma BHC from 5 to 11 days following ingestion.

Ely **et al.** (1952) fed crystalline lindane in soybean oil continuously to several lactating cows. They compared their work with that from other workers who had fed DDT to cows and found that approximately the same amounts were excreted in milk when comparable dosages were administered. In this same work when 94 and 95 ppm lindane were fed in the diet, an average of 3.02 and 2.69 ppm respectively were observed in 4 percent fat corrected milk. When 23 and 18 ppm were fed, an average of 0.39 and 0.38 ppm was observed, and when 5 ppm was fed, 0.11 ppm was observed in the milk. They reported that the lindane in milk dropped rapidly when the dosage was discontinued, but that lindane was detectable in the milk for approximately 30 days from the cow which received 2 grams of lindane daily.

In the above reference lindane intake was plotted as mg/kg body weight versus ppm excretion of lindane in 4 percent fat corrected milk. It was observed that there was a linear relationship of intake to excretion.

The data of the present study were plotted in a similar fashion on a log-log basis. It is seen in Figure 6 that these data also form this same straight-line relationship. The regression slope of Ely **et al.** (1952) is given by the equation $Y = 1.44X - 0.49$, whereas the regression slope

Figure 4. — Housefly bioassay of BHC in milk from cows fed 5, 25, and 125 PPM technical grade BHC (40% gamma) in their rations.
1 PPM not illustrated.

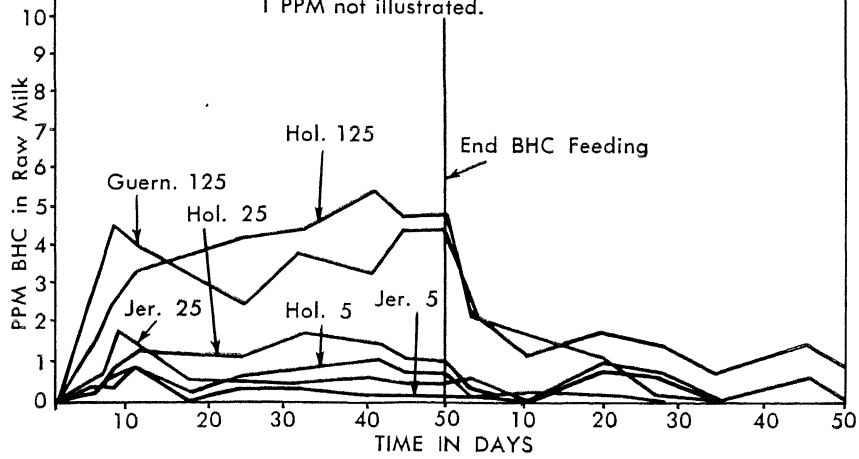
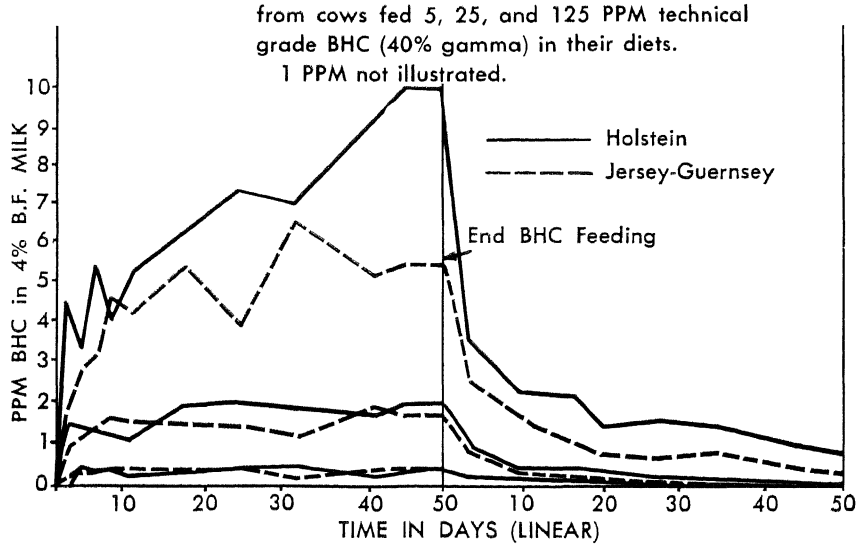


Figure 5.—Chemical assay of BHC in 4% fat corrected milk from cows fed 5, 25, and 125 PPM technical grade BHC (40% gamma) in their diets.
1 PPM not illustrated.



of this study is Y = 1.65X, both plotted linearly. The difference in angles of slope is thought to be due to isomer composition of insecticide. Ely **et al.** (1952) used lindane which is retained only for a short time in fatty tissues, while technical BHC was used in this work which contained only 40 percent of the gamma isomer. Other isomers which are

held longer in body fat than lindane would appear more slowly in the milk than lindane, thus accounting for the difference in slope.

Radeleff (1951) fed lindane at 1, 10 and 100 ppm in the diet of beef cattle, and observed that the stored lindane was metabolized or otherwise eliminated at a rapid rate, the rate being roughly proportional to the quantity stored in body fat. The sensitivity of these tests was only ± 2 ppm in the fat. Lactating animals fed single dosages of 100 mg lindane were observed to excrete 0.6 ppm in the milk at the end of the first day and none 3 days following oral treatment (USBEPQ E-800, 1950). Here the sensitivity was 0.21 ppm.

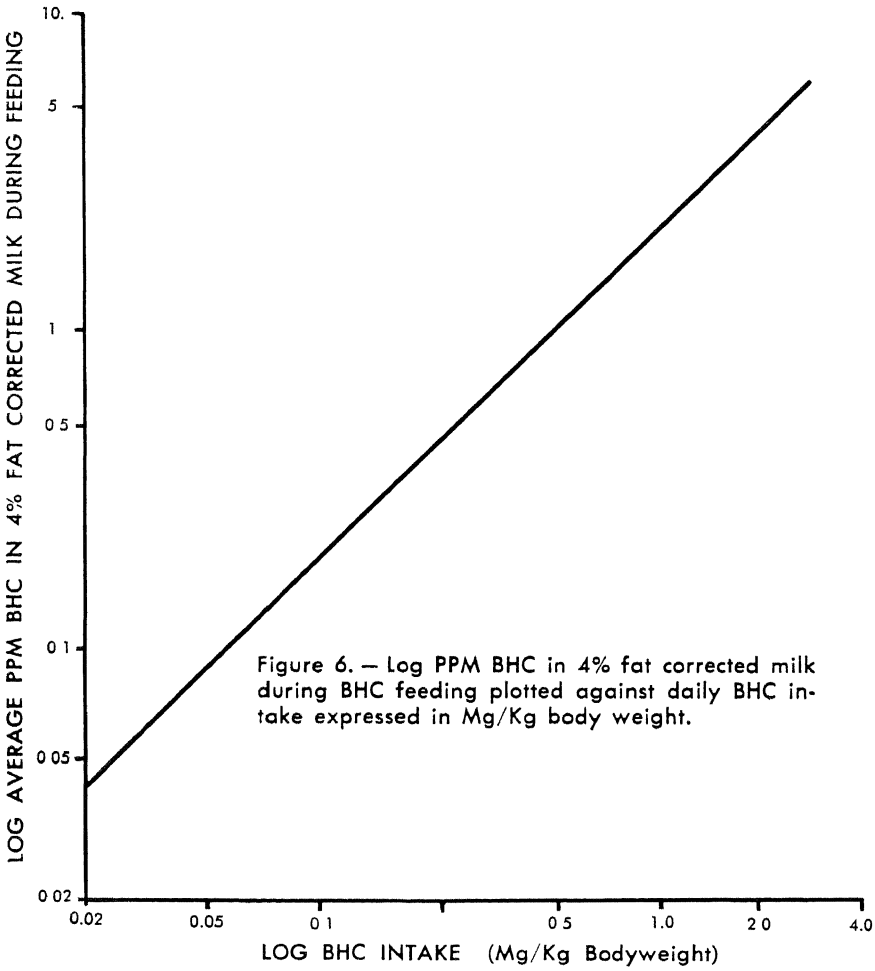


Figure 6. — Log PPM BHC in 4% fat corrected milk during BHC feeding plotted against daily BHC intake expressed in Mg/Kg body weight.

It is thought that the rapid disappearance of lindane and BHC from the milk of animals following termination of continuous feeding is due more to the low sensitivity of detection rather than to true disappearance. The method of analysis used in the present study deals only with butter fat, and therefore is readily sensitive to 0.01 ppm BHC in fat corrected milk.

In the present study, the cows seemed unaffected in any way during and after BHC feeding. The milk and fat production, appetites, disposition and weight gains were all normal in relation to the check animals. This was also reported in all of the references.

Gamma BHC was not detected in any of the samples by bioassay beyond 52 days following termination of BHC feeding. However, chemical analyses indicate that BHC is present up to 171 days after feeding. Apparently the BHC excreted after 52 days is composed of isomers other than gamma, probably beta and some delta and epsilon, since gamma and alpha are stored for the shortest length of time (Metcalf, 1955).

Sternburg and Kearns (1956) found that pentachlorocyclohexene is an intermediate in the metabolism of lindane by house flies. In the Schechter-Hornstein method this compound is reduced to chlorobenzene. The chlorobenzene is nitrated and forms a colored compound which also absorbs at 565 mu. The colored compound from pentachlorocyclohexene absorbs at 420 mu while that from BHC does not. This allows estimation of both lindane and pentachlorocyclohexene.

Considering the possibility that cows may metabolize BHC to the pentachloro-compound, each sample was read at 420 mu as well as at 565 mu. In few instances did the samples read higher than the check. and these did not form a pattern or read highest in the highest feeding levels of BHC. From this work it does not appear that the possible metabolite, pentachlorocyclohexene is excreted in the milk fat of cows fed BHC. In contrast, Bradbury and Standen (1957) contended that the pentachloro-metabolite cannot be detected under the conditions described by Sternburg and Kearns. As a result, it can be stated only that in using the method suggested by Sternburg and Kearns the pentachloro-metabolite was not detected.

CONCLUSIONS

1. Lactating cows fed 1 to 25 ppm BHC in their diets for 50 days did not excrete BHC in their milk in increasing amounts with time.

2. Lactating cows fed 125 ppm BHC in their diets for 50 days excreted BHC in their milk in increasing amounts with time.

3. Following termination of feeding, BHC was detectable in the milk up to 34 days in animals fed 1 ppm, up to 52 days on 5 ppm, beyond 102 days on 25 ppm and beyond 171 days on 125 ppm.

4. A direct relationship exists between the dosage (mg/kg body weight) and the amount which appears in milk during BHC feeding.

5. Gamma BHC was not detected in any of the milk samples by bio-assay beyond 52 days following termination of BHC feeding.

6. Pentachlorocyclohexene, a possible BHC metabolite, was not detected in any of the samples during or following feeding.

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