UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL SCIENCE RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

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Mr. W. P. Papageorge
Monsanto Industrial Chemical Company
800 North Lindberg Boulevard
St. Louis, Missouri 63166

Dear Mr. Papageorge:

I am enclosing a copy of the manuscript on feeding Aroclor 1254 to cows that we discussed during our recent phone conversation. This paper was presented at the Boston ACS meetings and has been submitted to the Journal of Agriculture and Food Chemistry.

At the time I think I told you there was nothing new to report. This is true with regard to the silo problem. However, as you may remember from your visit, we were planning to feed a number of Aroclors to chickens. We now have some preliminary results.

Aroclors 1232, 1242, and 1248 have severe effects on hatchability at 20 ppm in the diet--1242 is probably the most severe. Hatchability dropped from near 90% to less than 10% within three weeks after the start of feeding. We did not have adverse effects when 1242 and 1248 were fed at 2 ppm in the diet. Aroclor 1221, 1254, and 1268 did not have an effect on hatchability at 20 ppm in the diet.

My guess is that there are probably one or two common components in 1232, 1242, and 1248 that are causing the hatchability problems. These compounds are probably missing in the low and high chlorination Aroclors.

I'll try to keep you up to date on any new observations on either one of the problems.

Sincerely,

G. F. Fries
Research Animal Scientist
Dairy Cattle Research Branch

Enclosure

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Excretion of a Polychlorinated Biphenyl (Aroclor 1254) in Milk of Cows

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Abstract

Nine cows were fed 200 mg/day Aroclor 1254 (PCB) for 60 days. Milk and body fat samples were obtained during and for 60 days following feeding. Concentrations of PCB in milk fat approached equilibrium after 40 days. The average concentration in milk from 40 to 60 days was 60.9 ± 4.5 µg/g fat. Concentration in body fat was 41.7 ± 11.5 µg/g at 60 days. When feeding stopped, concentration in milk fat declined 50% within 15 days. After 15 days the rate of the first-order decline in concentration was much less. The average rate constant was 0.010 day^{-1} and varied among cows from 0.005 day^{-1} to 0.016 day^{-1}. The variation could not be related to such parameters as milk fat production or body weight change. Decline in concentration of PCB in body fat paralleled decline in concentration of PCB in milk fat.

Aroclor [2/] 1254 is a polychlorinated biphenyl (PCB) [3/] containing 54% chlorine. It was used in a sealant applied to the interiors of a limited number of concrete stave silos. This source has caused PCB residues in milk. Some producers have been removed from the market with levels exceeding Food and Drug Administration (FDA) guidelines.

1/ Animal Science Research Division, ARS, Beltsville, Maryland 20705.

2/ Aroclor is a trademark of Monsanto Chemical Co.

3/ Unless specifically noted, the abbreviation PCB refers to Aroclor 1254.

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Residues of PCB in silage occur at high levels adjacent to the treated walls (Skrentny et al., 1971). There is some migration of the residue in the silage but residues seldom occur beyond 3 feet from the wall. Gas chromatographic examination of the residues from silage suggest little, if any, microbial or chemical change in the composition of Aroclor 1254 (Skrentny et al., 1971; Fries, 1972).

We have compared the behavior of PCB and DDE residues in milk after environmental exposure (Fries et al., 1972). Only the period after removal of the sources of both contaminants from the diet was studied. The rates of decline in milk concentrations of the two residues were identical. Platonow et al. (1971) have studied the distribution of residues in milk of two cows that received a single dose of PCB. We are not aware of long-term controlled studies on the relationship of dietary intake of PCB to residue accumulation and excretion in the cow.

This study was carried out to determine milk and body fat residues while cows consumed a fixed level of Aroclor 1254 and to determine the rate of decline of milk and body fat residue levels after removing PCB from the diet.

MATERIALS AND METHODS

Cows. Nine first lactation Holstein cows were used and their major characteristics are presented in Table I. The cows were selected to provide observations at three stages of lactation and at various levels of production within a stage of lactation. However, because of the differing rates of production decline the differences in production became less as the experiment progressed.

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Cows were fed corn silage, alfalfa hay, dehydrated alfalfa pellets, and concentrate. A description of the feeding regime has been presented by Miller et al. (1971). There were some differences in ration composition between cows and within cows during the experiment. These differences did not affect results and only total dry matter intake is presented (Table I).

Dosing and Sampling. All cows were fed 200 mg of Aroclor 1254 per day for 60 days. Aroclor 1254 in acetone solution was pipetted on a small portion of the concentrate and the acetone was allowed to evaporate. The spiked concentrate was hand mixed with the remainder of the concentrate at the time of feeding. Feed refusals were not a problem. Based on the measured dry matter intake and the body weight of the animals, this dose rate was equivalent to 12.4 ± 0.9 mg/kg dry matter or 0.37 ± 0.2 mg/kg body weight.

Milk samples were collected at 5-day intervals during the feeding period and the following 60 days. More frequent milk samples were obtained both when feeding was initiated and when feeding was stopped. Body fat biopsy samples from the tailhead area were obtained at 30, 60, 90, and 120 days after the start of the study.

Analysis. Fat from the milk and biopsy samples was isolated and cleaned up using FDA (1968) multipesticide residue methodology. The concentration in most samples was relatively high and there were no significant interferences. Thus, it was not necessary to use more elaborate clean-up methods such as silicic acid chromotography (Armour and Burke, 1970) or dehydrochlorination (Fries et al., 1972).

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Concentrations of PCB were determined by gas liquid chromatography using electron capture detection. A Hewlett-Packard Model 7600A instrument equipped with ^{63}Ni electron capture detector and an electronic integrater was used. The 5 foot by 1/4 inch OD glass column was packed with 10% DC-200 on 80 to 100 mesh Gas-Chrome Q. A 95% argon-5% methane mixture was used as both carrier and purge gas. The column temperature was 200°C.

Typical chromatograms of an Aroclor 1254 standard and a milk fat residue sample obtained under these conditions are presented in Figure 1. There were no significant peaks with retention times less than 12.4 minutes in residue samples. The peaks with greater retention times occurred in residue samples at approximately the same proportions that were present in Aroclor 1254. The peaks with the short retention time are presumably the less chlorinated compounds (Zitko et al., 1971).

The concentration of PCB in samples was calculated by comparing the areas of the peaks with retention times of 12.4 minutes or greater with the areas of the corresponding peaks of Aroclor 1254 standards. Thus, the values that we report tend to overestimate the true quantity of PCB in the samples.

RESULTS AND DISCUSSION

Residues While Feeding. The average levels of PCB in milk fat and body fat during the 60-day feeding period are presented in Figure 2. The shape of the milk fat concentration curve is typical of the curves for other chlorinated hydrocarbon compounds (Fries et al., 1969). After 40 days the level of milk fat approached equilibrium but was still rising slowly.

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Concentration of PCB in body fat rose more slowly and was always lower than concentration in milk fat. The ratio of concentration in body fat to concentration in milk fat became closer to unity at 60 days. At equilibrium one would expect a ratio of one if the findings of Stull et al. (1971) with DDT can be applied to PCB.

The evidence indicates that the cows were not at equilibrium at 60 days. However, the response from the 40th to the 60th day is adequate to determine if any characteristics of the cows significantly affect levels excreted in milk or accumulated in body fat.

Average concentrations of PCB in milk fat from the 40th to 60th day were similar for all cows (Table II). The average for all cows was 60.9 ± 4.5 µg/g and the minor variations were not related to production or any of the parameters listed in Table 1. Because of the small variation in concentration, the amount excreted per day was directly related to fat production. The average excretion was 42.3 mg/day accounting for 21% of intake.

Concentrations in body fat at 60 days, averaging 41.7 ± 11.5 µg/g, were more variable than concentrations in milk fat (Table 2). With all cows concentration in body fat was lower than concentration in milk fat. As in the case of levels in milk fat, variation among cows cannot be explained by the parameters measured in this experiment.

The total amount of body fat, one of the major parameters that could affect body fat concentration, cannot be measured with east. We have shown that the total retention of chlorinated hydrocarbons by the rat is quite constant under a given set of conditions (Fries et al., 1971). Concentrations in body fat were inversely related to the total amount of body fat. If a similar phenomonen held true for these cows, one would expect that cows with smaller body fat pools would have higher body fat concentrations of PCB.

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Residue Elimination. There was a rapid initial decline in the level of PCB in milk fat when PCB feeding stopped (Fig. 3). After 15 days the rate of decline was quite slow. The shape of this curve is similar to curves that have been established for other chlorinated hydrocarbon compounds (Fries et al., 1969).

The decline in concentration is adequately described by a 2-component first-order system with the equation:

$$(1) \quad C = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}$$

where C is the concentration at any time, C_1 is the initial concentration of the first compartment, C_2 is the initial concentration of the second compartment, k_1 and k_2 are rate constants, and t is time in days.

After 15 days the contribution of the first term to the total concentration is negligible and can be ignored. The values of the constants for the second term were estimated by fitting the conventional linear regression of the logarithmic form of the second term.

$$(2) \quad \ln C = \ln C_2 - k_2 t$$

After subtracting the values for the second term from the overall concentration, it was possible to estimate the constants of the first term. The equation estimated using the averages of all cows was

$$(3) \quad C = 30.6\, e^{-0.32t} + 32.3\, e^{-0.010t}$$

With individual cows, it is not possible to precisely evaluate the first term of equation (1) because of the limited number of observations. In practice this is not of great importance. The level of PCB in milk fat of all cows decline about 50% in the first 15 days and there was little variation among cows (Table 3). This is in agreement with our field studies (Fries et al., 1972).

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Unlike the initial decline in concentration, the rate of decline for the slower compartment is quite variable among cows (Table 3). We have also observed this variation in previous studies (Fries et al., 1972). These differences are of practical importance. If two cows start at the same level, the time required to return to the FDA guide-line is inversely proportional to the magnitude of the rate constant during the second phase. In this study the range between the extreme cows was a factor of three. Even wider ranges were observed in the field studies.

One purpose of this study was to obtain information on possible causes of the variation among cows. It can be assumed that the only significant source of PCB for this compartment is the body fat stores. This is supported by the decline in concentration in body fat that paralleled the decline in concentration in milk fat (Fig. 3). The close positive relationship between concentrations of chlorinated hydrocarbons in milk fat and body fat is presented elsewhere (Fries and Marrow, 1972).

If the model is appropriate there are several factors that could influence the rate of decline in concentration in milk fat. Cows with higher fat production would tend to excrete more PCB and this could be expected to increase the rate of decline. However, there was no significant relationship between the rates of decline and the amount of fat produced (Table 3).

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A second factor that could affect the rate is change in body weight. All the cows were gaining weight in this study (Table 3). The amount of PCB remaining in the body would be diluted by this added body fat. Thus, one would expect the dilution as indicated by weight gain to enhance the rate of decline in milk fat concentration. However, there was no consistent relationship between these two factors (Table 3).

The third factor, not evaluated in this study, is the total amount of body fat. A given rate of milk fat production would clear a larger fraction of body fat if a cow has a small amount of body fat than if she has a large amount of fat. Some indication of the amount of body fat can be obtained from the concentration of PCB in the body fat at 60 days feeding if it is assumed that all cows absorb PCB with the same efficiency. Concentration would be inversely related to the amount of body fat and the most rapid decline would occur with the cows with the highest initial concentration in body fat. It is of some interest that the two cows (6 and 9) with the most rapid rate of decline were among the highest in initial concentration of PCB in body fat. However, overall, the relationship was not consistent (Table 3).

The rate of decline is probably controlled by a combination of the above and/or other factors rather than a single factor. With the small number of animals involved, we did not consider it worthwhile to attempt a more elaborate multiple factor analysis of the data.

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Similarity to DDE. In earlier work (Fries et al., 1972), we suggested that residue behavior of Aroclor 1254 was similar to DDE, the most persistent degradation product of DDT. The conclusion was based on the similar rates of elimination after the cows had been removed to clean feed. The results of this study confirm and extend the conclusion.

We have conducted experiments with DDE under conditions similar to this experiment (Fries et al., 1969). The parameters of the DDE experiments are in good agreement with this PCB experiment when all values are normalized to an equal intake (Table 4). Further studies are required to determine the extent to which these conclusions can be extended to PCB formulations other than Aroclor 1254.

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Table 1. Days in lactation, weight, intake and production of the cows 1/

Cow	Days in lactation	Weight	Dry matter intake	Milk	Fat	Fat
		(kg)	(kg/day)	(kg/day)	(%)	(kg/day)
1	211	559	15.9	17.7	4.1	0.72
2	207	533	16.3	15.3	4.1	0.62
3	206	557	15.0	11.9	4.3	0.51
4	143	537	16.5	18.3	3.9	0.72
5	161	577	15.8	14.5	4.2	0.61
6	159	528	17.9	20.8	3.8	0.78
7	39	587	17.8	22.7	3.9	0.88
8	46	495	16.3	22.9	3.5	0.79
9	48	507	17.1	21.9	3.4	0.75

1/ Days in lactation are at the start of the study. All other values are averages for the 120 days of the study.

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Table 2. Fat production, PCB excretion in milk, and PCB accumulation in body fat of cows consuming 200 mg PCB per day 1/

Cow	Milk fat production	PCB in milk fat		Body fat
		Concentration	Excretion	
	(kg/day)	(µg/g)	(mg/day)	(µg/g)
1	0.72	59.2	42.6	34.5
2	0.61	58.3	35.6	39.0
3	0.53	57.9	30.7	39.5
4	0.72	60.1	43.3	25.3
5	0.58	64.2	37.2	54.0
6	0.77	63.8	49.1	53.2
7	0.89	56.6	50.4	37.1
8	0.70	57.6	40.3	32.3
9	0.73	70.6	51.5	60.2

1/ Milk fat production and concentrations in milk fat are averages for the 40th to 60th day of feeding. Concentrations in body fat are at the 60th day of feeding.

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Table 3. Decline in concentration of PC3 in milk fat after feeding stopped

Cow	Initial concentration		Milk fat production 1/	Weight change 1/	Initial decline 2/	Rate 3/
	Milk fat	Body fat				
	(μg/g)	(μg/g)	(kg/day)	(kg/day)	(%)	(day^{-1})
1	59.2	34.5	0.61	0.22	57	0.011
2	58.3	39.0	0.60	0.42	55	0.005
3	57.9	39.5	0.44	0.73	55	0.007
4	60.1	25.3	0.67	0.29	64	0.008
5	64.2	54.0	0.56	0.58	54	0.008
6	63.8	53.2	0.69	0.31	52	0.013
7	56.6	37.1	0.81	0.07	58	0.010
8	57.6	32.3	0.72	0.07	58	0.008
9	70.6	60.2	0.70	0.31	49	0.015

1/ 15 to 60 days post feeding.

2/ 100 [(concentration at 0 days - concentration at 15 days)/concentration at 0 days]

3/ k_2 in equation (1) calculated for 15 to 60 days post feeding.

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Table 4. Comparison of DDE and PCB accumulation and excretion by cows 1/

Parameter	DDE	PCB
Milk fat level 20 days (μg/g)	0.21 ± 0.04	0.20 ± 0.02
Milk fat level 60 days (μg/g)	0.29 ± 0.05	0.32 ± 0.03
Body fat level 60 days (μg/g)	0.23 ± 0.02	0.21 ± 0.06
C_1 (μg/g)	0.12	0.15
k_1 (day^{-1})	0.31	0.32
C_2 (μg/g)	0.17	0.16
k_2 (day^{-1})	0.013	0.010

1/ DDE values are from Fries et al. (1969). Intakes are normalized to 1 mg/day. C_1, k_1, C_2 and k_2 are the constants of equation (1).

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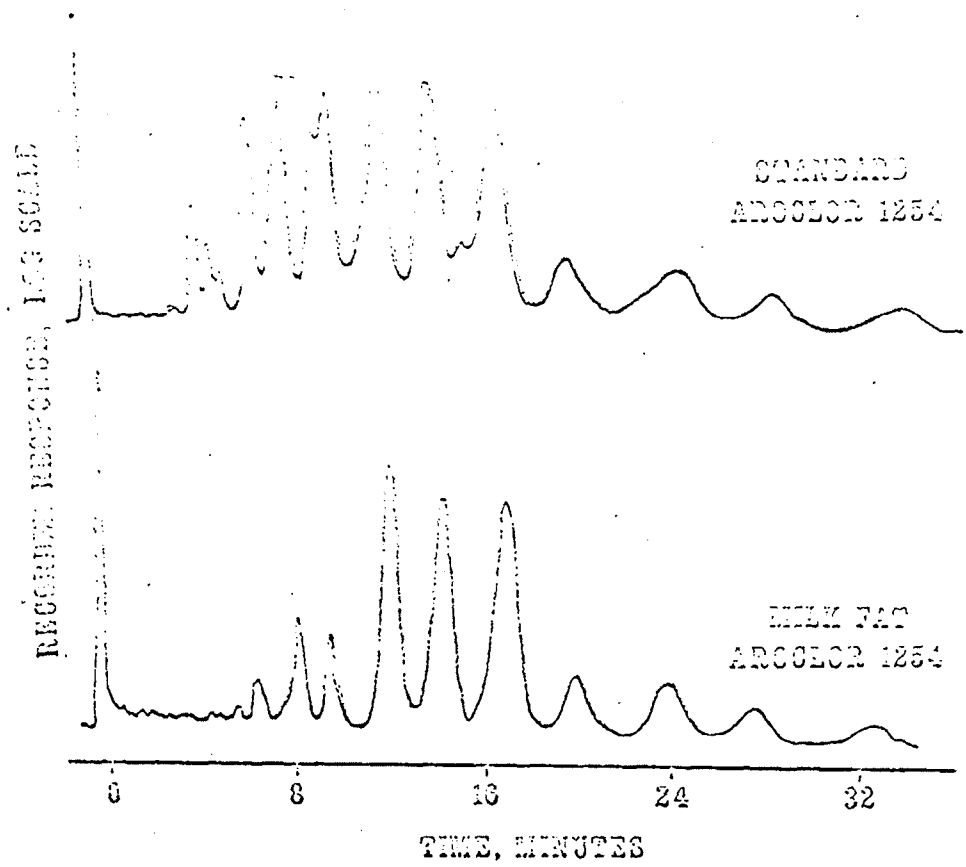

Figure 1. Typical gas chromatograms of an Aroclor 1254 standard and a milk fat residue sample.

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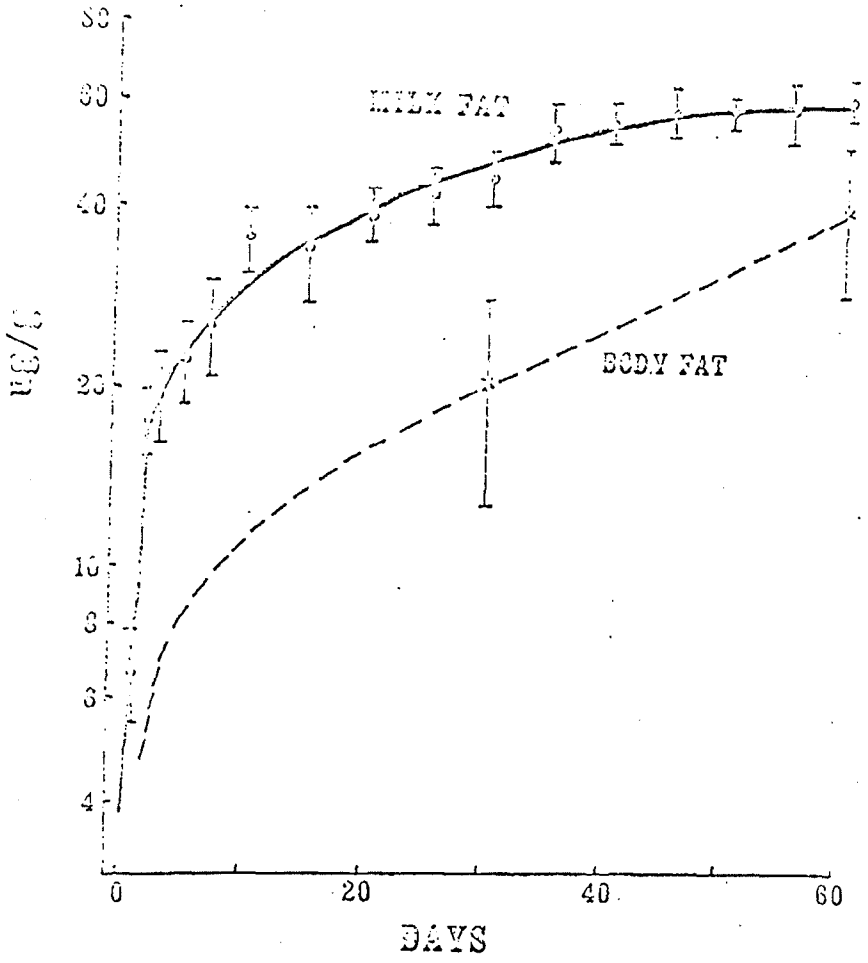

Figure 2. Concentration of PCB in milk fat and body fat of cows fed 200 mg PCB per day. Each point is an average of nine cows ± standard deviation.

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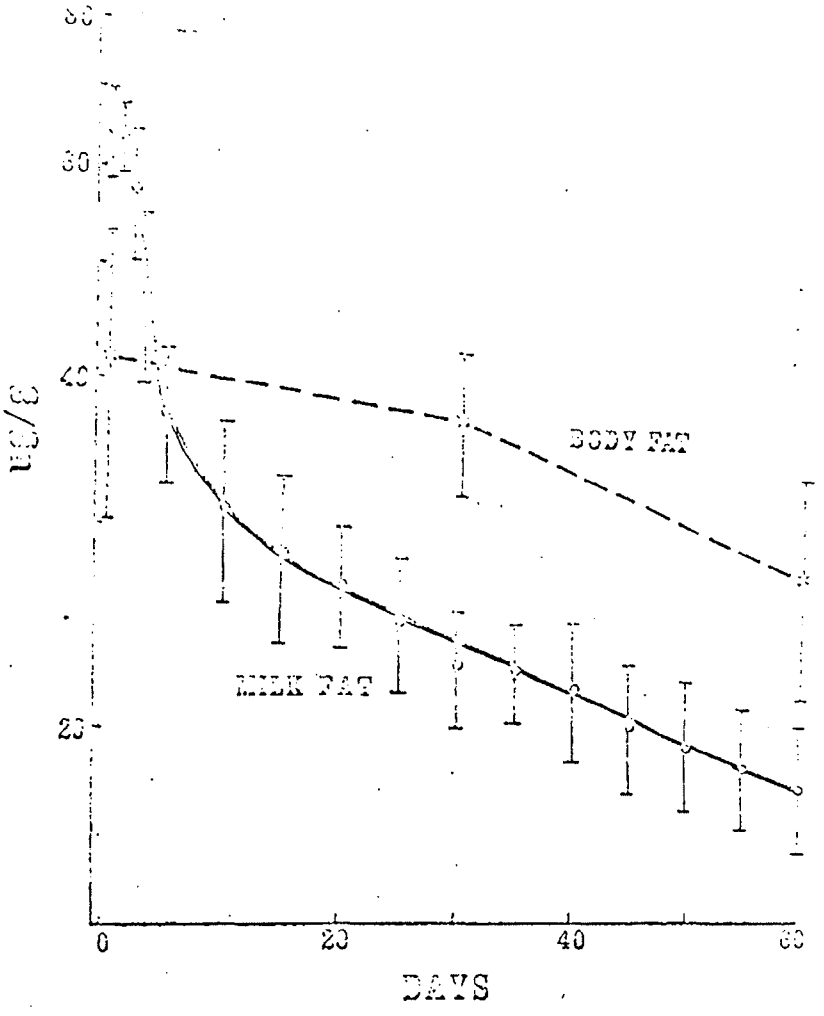

Figure 3. Concentration of PCB in milk fat and body fat of cows after feeding stopped. Each point is an average of nine cows ± standard deviation.

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