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Effects of Thyroprotein on Excretion of Polychlorinated Biphenyls by Lactating Cows[1]

L. B. WILLETT and T-T. Y. LIU
Department of Dairy Science
Ohio Agricultural Research and Development Center
Wooster 44691

ABSTRACT

Lactating dairy cows were fed 50 mg daily of commercially available polychlorinated biphenyl Aroclor 1254 for 20 days. Upon withdrawal of this from the ration, groups were given 0, 2 g/100 kg, or 4 g/100 kg body weight of thyroprotein (Protamone) for 57 days. Animals that continued to lactate were studied for an additional 70 days. The average concentration of polychlorinated biphenyls in milk fat on day 20, the last day of dosing, was 6.01 ± .6 ppm. Declines of concentrations in cows not fed thyroprotein were described by a two-component first order equation with half-lives of 5.7 and 68 days. Thyroprotein feeding provided a nearly dose responsive increase in heart and respiration rate and also weight losses among treated animals. Milk and fat production was increased in most animals; however, stage of lactation appeared to influence magnitude of response. Thyroprotein increased clearance of polychlorinated biphenyls during the thyroprotein feeding phase of the study. By day 52 daily excretion by control animals averaged 1.09 mg/day compared to 1.39 and 1.87 for animals receiving 2 or 4 g/100 kg body weight of thyroprotein. Withdrawal of thyroprotein resulted in a sharp decline in excretion of polychlorinated biphenyls from thyroprotein treated animals. On experimental day 98, control animals had an average daily excretion of .87 mg compared to .67 and .59 for the 2 and 4 g/100 kg body weight of thyroprotein groups. These declines ultimately resulted in similar total excretion of polychlorinated biphenyls among all three experimental groups. Thyroprotein was not a practical means to increase rate of excretion of accumulated polychlorinated biphenyls.

INTRODUCTION

The environmental contaminants polychlorinated biphenyls (PCB) are commercially used mixtures that have been the subject of unprecedented amounts of public interest, regulatory action, and research during the past 10 yr. Since these lipophilic compounds are persistent, accumulative, toxic, and identified as potential carcinogens, efforts have been considerable to eliminate these compounds from our ecosystem. As part of this effort to prevent human exposure to PCB, extensive monitoring programs have been implemented and strict standards imposed (17).

Cattle and poultry have been exposed to polychlorinated biphenyls by a number of routes. A PCB-containing herbicide that was sprayed along roadways contaminated dairy cattle in West Virginia (12). Feedstuffs have been contaminated from leaking electrical equipment in several locations (12), and PCB-tainted oil was used for insecticide applicators for beef cattle in Kansas (8). The most prevalent source of PCB contamination for both dairy and beef cattle has been contaminated silos throughout the eastern half of the United States. A silo coating which contained PCB was used on many thousands of silos (22). The PCB from this coating was dissolved in silage juices and migrated into the feed (22). The PCB then was accumulated in adipose tissue of the animals and subsequently deposited in the milk of lactating cattle.

When cattle are identified as having residues of PCB, rapid methods must be taken to eliminate this residue from the food chain and to return to within government tolerances

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for unavoidable contamination (17). Attempts to purge livestock of residual pesticide and industrial chemicals by treatment with an additional exogenous compound have been made. Some of the treatment regimens utilized include feeding activated charcoal (1, 2, 3); feeding and injecting barbiturates (1, 2); feeding thyroactive compounds (13); including mineral oil in the diet (2, 6); and providing high energy rations (9). Some of these treatments have been successful for specific pesticides or chemicals whereby a specific metabolic or excretory route can be enhanced. Unfortunately, most have been unsuccessful or impractical. The polychlorinated biphenyls and the closely related polybrominated biphenyls have been particularly resistant to efforts to increase clearance rates (1, 3).

Iodinated casein or thyroprotein increased metabolic rate of lactating cattle and thus increased the production of milk and milk fat (11, 15, 16). Milk fat has been one of the more important excretory routes for the polychlorinated biphenyls and related polybrominated biphenyls (3, 21). Therefore, studies were to determine if the feeding of thyroprotein to lactating dairy cows could increase significantly the rate of clearance of polychlorinated biphenyls.

MATERIALS AND METHODS

Experimental Design

This experiment was designed to simulate the farm situation where a herd of cows was contaminated with PCB. With the discovery that this xenobiotic is contaminating food products, the PCB source would be removed and treatment (thyroprotein) instituted to increase the rate of clearance. Subsequently, thyroprotein treatment would be withdrawn.

The experiment was in three phases. In the first phase, days 1 through 20, experimental cows were given 50-mg daily doses of commercial PCB Aroclor 1254.[2] The Aroclor was dissolved in acetone, and contents of premeasured dose vials were added on top of each cow's silage. Control animals received sham doses which contained only acetone.

During the second phase, days 21 through 77, designated cows received daily doses of thyroprotein[3] (iodinated casein) at 2 or 4 g/100 kg of body weight (BW). Dosages were calculated individually and adjusted for changes in body weight as the experiment progressed. The thyroprotein was contained in a concentrate mix which was substituted for an equal portion of the regular grain mixture.[4] Thyroprotein was withdrawn gradually during the last 7 days of this experimental phase.

The last phase, days 78 through 147, was used to evaluate the excretion of PCB by cows following thyroprotein treatment. Seven animals completed their lactations during this phase of study.

Cows

Fourteen mature Holstein cows were assigned to one of four treatment groups (Table 1). Group 1 animals that received no PCB served as controls. Two of these animals received no thyroprotein while the others received 2 or 4 g/100 kg of BW of thyroprotein.

Cows in Groups 2, 3, and 4 all received 50 mg of Aroclor 1254 daily for 20 days. Cows in treatment 3 did not receive thyroprotein whereas those in treatments 2 and 4 received 2 or 4 g/100 kg of BW of thyroprotein.

Size, production, and stage of lactation have been important factors influencing responsiveness of cattle to thyroprotein. Thus, these factors were considered when cows were allotted to treatment groups and are shown in Table 1.

Cattle were maintained in individual comfort stalls where they could be fed individually and excrement from dosed animals could be contained. They were fed corn silage, alfalfa hay, and a grain mix (1:1:1 as fed basis). Amounts fed were adjusted so that there was no refusal during the PCB dosing phases of the experiment, but they were increased to allow some refusal during thyroprotein feeding. Daily feed intake and milk production were recorded. Measure-

[2]Monsanto, St. Louis, MO. Lot No. KK602.
[3]Protamone, Agri-Tech Inc., Kansas City, MO.
[4]Grain mixture consisted of rolled corn 50%, whole ground oats 23.7%, dicalcium phosphate 1%, iodized salt .58%, Vitamin A–30 .05%, Vitamin D .02%, soybean meal 18%, urea .72%, alfalfa meal 2.93%, and molasses 3%. Thyroprotein ration was the same except soybean meal 15.36%, and Protamone 2.64%.

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TABLE 1. Allocation of animals to treatments for dosing with PCB and thyroprotein. Stage of lactation, body weight, and milk production at the beginning of experiments.

Group	Cow no.	Experimental dosing		Day of lactation	Body weight	Daily milk yield
		PCB (days 1–20)	Thyroprotein (days 21–77)	(day 1)	(day −2)	(day −2)
		(mg/day)	(g/100 kg BW)		(kg)	(kg)
	1662		0	265	653	17.3
1	1722	0	0	146	700	20.9
	2154		2.0	173	607	20.0
	2160		4.0	81	492	26.1
	1993			98	657	17.1
2	2110	50	2.0	119	502	18.5
	2163			70	663	26.8
	2039			239	632	12.6
3	2114	50	0	182	519	18.6
	2158			82	495	20.9
	2164			123	472	16.1
	2147			174	608	18.7
4	2148	50	4.0	198	582	15.3
	2156			128	518	16.4

ment of body weights, respiration, heart rates, and body temperatures were at scheduled intervals.

Samples for PCB Analysis

Samples of milk for PCB analyses were collected from each cow as follows: prior to and on even-numbered days during PCB feeding; twice daily on days 21 through 24; daily on days 25 through 34; every other day on days 36 through 42; on days 45, 48, 52, 56, 63, 70; and once a week thereafter. At each sampling, complete collections of milk were made, thoroughly mixed, and a sample taken. Samples were stored frozen until analyzed.

Quantification of PCB Residues

All solvents used to extract samples were analytical reagent grade. These were redistilled in glass before use until a volume equivalent to the quantity used for extraction would yield no interfering peaks after being reduced to 1 ml when 5 μl was injected into the gas-liquid-chromatograph (GLC).

Samples of whole milk (25 ml) were extended with ethanol and extracted with petroleum and diethyl ethers as described in (23). The fat content of each sample was measured gravimetrically after evaporation of the solvent. Interfering pesticides, pigments, and fat were removed by column chromatography procedures utilizing Florisil and silica gel (19). Selected samples were subjected to saponification and acid extraction procedures described by Monsanto (7) as modified by Willett (19) to confirm the presence of PCB.

An F & M Model 402 GLC with a ^{63}Ni detector was used for all analyses. A 2.44 m glass column of 4% DC–200 and 6% QF–1 (1:1) on Gas-Chrom Q (80/100 mesh) was used. All samples were diluted so that they were detected within one linear attenuation range of the detector.

Aroclor 1254 was the reference standard. Changes in the chromatographic profile of standard Aroclor 1254 and Aroclor 1254 in cows milk have been reported (22). Quantifications were based on the total area of chromatograms for total retention time of all major PCB peaks. If the more recently accepted methods of individual peak quantification described by Webb and McCall (18) had been utilized, the PCB quantities would be approximately 50% of those reported in this paper.

Corrections for method loss were based on

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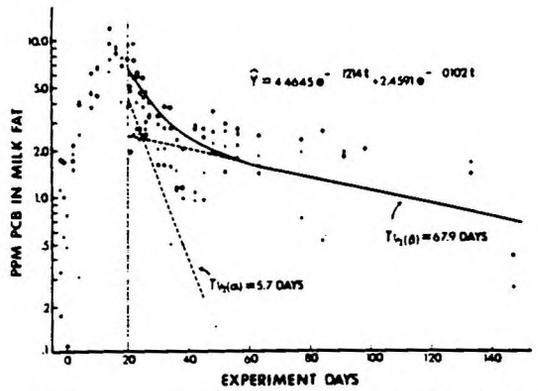

Figure 1. Concentrations of PCB in milk fat of Group 3 control cows. A first order equation was used to describe declines in concentration beginning at the end of PCB dosing. Individual cows are represented: ● = 2164, ◆ = 2158, ■ = 2114, ▲ = 2039.

the extraction of milk samples to which known quantities of Aroclor 1254 were added. Average recovery was 72.0 ± 6.5% (SE).

RESULTS AND DISCUSSION

Allotment of cows to experimental groups was designed to create a balance in size, production, and stage of a lactation. Initial allotment was different from that in Table 1. During the PCB dosing period when cows in Groups 2, 3, and 4 were all receiving 50 mg of PCB per day, problems developed with two animals in Group 2. Cow 1993 became nervous and responded adversely to experimental manipulations, and milk production from this cow declined rapidly. Data for PCB excretion was of little value to the study. Cow 2114 contracted an apparent cystitis during dosing which also adversely affected her production. This dysfunction responded to therapy; however, it was questionable whether the cow's performance would be reliable throughout the experiment. Since the loss of two animals virtually would destroy Group 2 and contamination of additional animals with this persistent xenobiotic was prohibited by cost, animals 2110 and 2114 were switched between Groups 2 and 3, respectively, prior to the onset of thyroprotein treatment. Although this change ensured at least two animals with normal productivity in Group 2, it resulted in a disproportionate grouping of early lactation cows within this group. However, this was not critical to the study.

Consumed PCB are absorbed readily from the GI tract (23), are detectable in blood, and ultimately are deposited in depot fats. Simultaneously, portions of the residue are excreted in milk. Studies in our laboratories (unpublished) showed that distribution and clearance of PCB from Aroclor 1254 are similar over wide ranges of concentrations fed. We demonstrated that secretion of PCB in milk after cows were fed 3.5, 7, and 14 mg daily (20) were similar to those reported by Fries (4) after feeding 200 mg/day. Cows that consume silage from a PCB-contaminated silo generally receive between 1 and 20 mg/day (22). However, daily intakes exceeding 50 mg/day have been estimated in some of our studies (unpublished). The magnitude of contamination from possible future incidents cannot be predicted.

The dose of 50 mg/day of PCB fed over 20 days provided a total consumption of 1000 mg. This provided sufficient PCB residue to determine the kinetic behavior in conjunction with thyroprotein feeding. Figure 1 shows that PCB was absorbed rapidly, as it was quantifiable in the milk at the first sampling on day 2. Concentrations rapidly increased and began approaching equilibrium by day 20. Average concentration of PCB in milk fat on day 20, the last day of dosing, was 6.01 ± .6 ppm. These concentrations are similar to those reported when animals consumed feed from contaminated silos (3, 19, 22). Concentrations of PCB in milk from cows of Group 3, which received no thyroprotein, are in Figure 1. Although the same dose was given to all animals, residue concentrations and clearance in milk were variable. Analysis of halogenated hydrocarbons excreted in milk has been inherently variable even when samples were collected sequentially from the same animal (13). Recently, stage of lactation has been identified as an important factor influencing apparent residue concentration in tissue and apparent clearance in milk of the closely related polybrominated biphenyls (24). These sources of variation must be considered whenever predictive equations are used to estimate kinetics of a xenobiotic. This is particularly true when a heterogeneous group of animals is included in the experiments.

A two-component first order equation was used to describe the decline of PCB concen-

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tration in milk. The first component having a half-life of 5.7 days occurred right after withdrawal of PCB from the diet. The second component had a half-life of 68 days. These changes in the PCB concentration in milk were similar to (20) and to studies by Fries and co-workers (3, 4).

Whereas actual residue concentration may provide an indication of the clearance of a xenobiotic residue, in actuality productivity among other factors can influence concentration without necessarily changing clearance. Therefore, it was important to evaluate effects of a treatment designed to influence productivity of the animal not on a concentration basis but on actual milligrams of xenobiotic excreted. Since residue concentrations were determined at frequent intervals (see Methods and Materials) and milk production was recorded daily, total milligrams excreted were estimated. Residue concentrations for days where residue determinations were not, were interpolated between sequential points. In Table 2, the milligrams of PCB excreted by animals in Groups 2, 3, and 4 during the PCB dosing period can be seen. Cow 1993 was deleted from the group mean because her productivity declined sharply and appeared to be responsible for reduced excretion of PCB.

Although there were no significant differences between groups, animals that were in early lactation excreted more PCB during the dosing period. During this period, average accumulated PCB excreted in milk from these animals of these three groups was approximately 6.6% of the doses. For day 20 the average daily excretion was 3.74 mg.

Beginning on experimental day 21 no additional doses of Aroclor 1254 were given, and thyroprotein feeding was initiated. The PCB that remained in the gastrointestinal tract apparently was the source of the PCB in blood and subsequently milk. Clearance of gastrointestinal tract of most of the residues of polybrominated biphenyls occurred by 72 h after the last dose (23). Thereafter, concentrations of the halogenated biphenyl declined rapidly in feces and plasma (21). A corresponding decline in concentration (Figure 1) and milligrams of residual PCB excreted via

TABLE 2. Total PCB excreted during and after dosing with PCB and during and after dosing with thyroprotein.

Group	Cow no.	During dosing with PCB (days 1–20) PCB excreted	Group mean	During dosing with thyroprotein (days 21–77) PCB excreted	Group mean	Total after dosing with PCB (days 21–147) PCB excreted	Group mean
		(mg)					
	1993	17.3[a]		7.3[ab]		7.3[ab]	
2	2110	80.5	87.1	80.9	90.6	127.8	137.3
	2163	93.7		100.3		146.8	
	2039	42.8		37.3		37.3[ad]	
3	2114	37.1	54.5	56.3[ac]	59.6	56.3[ac]	131.6
	2158	76.0		78.3		139.0	
	2164	62.1		63.2		124.2	
	2147	69.7		123.9		140.1[ae]	
4	2148	59.5	68.5	93.7	107.2	103.8[ae]	138.9
	2156	76.2		104.0		138.9	

[a]Not included in average.

[b]Total PCB excreted during day 21 to day 48.

[c]Total PCB excreted during day 21 to day 70.

[d]Total PCB excreted during day 21 to day 77.

[e]Total PCB excreted during day 21 to day 98.

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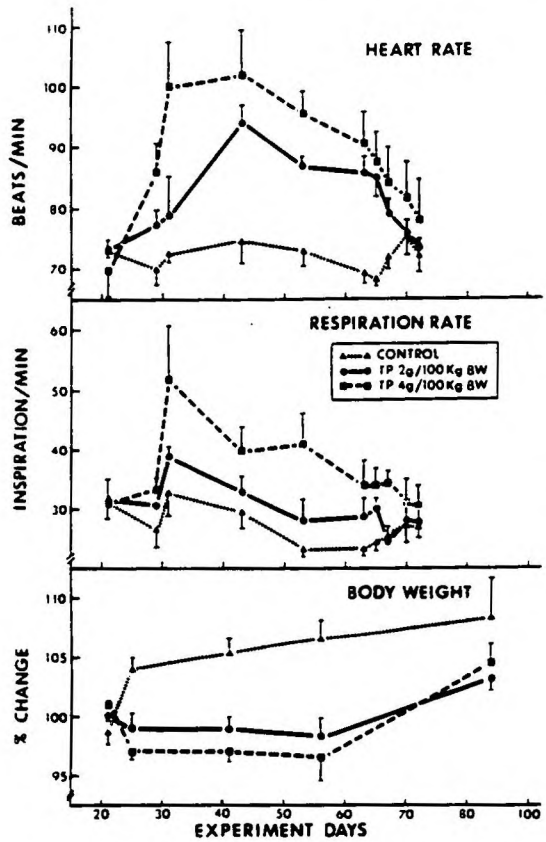

Figure 2. Changes in means ± standard error for heart rate, respiration rate, and percent change of body weight in response to thyroprotein feeding.

milk occurred until new equilibria were achieved between PCB in blood and depot fats.

The rapid decline in residue excretion, resulting from clearance of large quantities of PCB from the gut, occurred simultaneously with the apparent lag in response of treated animals to thyroprotein. Important changes in the heart and respiratory rates did not occur during the first 3 days of thyroprotein feeding (Figure 2). Similar response lags have been reviewed and reported (10). Changes in body weight were apparent in response to thyroprotein feeding and removal of feed limitations imposed during the PCB dosing period. Body temperature changes were not important.

Feeding 2 or 4 g/100 kg BW of thyroprotein resulted in a dose responsive change in metabolic rate as indicated by respiration and heart rate. Following the initial lag in response, there was a rapid increase in both heart and respiration rate during the 1st mo of thyroprotein feeding. However, they declined and approached the same rates as the Group 3 control cows by the end of the 77-day thyroprotein feeding period (Figure 2). Declines in body weight in Figure 2 were not as great as in (5, 11, 16). As weight losses were not greater during the thyroprotein feeding period, the amount of PCB cleared from these animals may have been influenced. Body weights rapidly increased upon withdrawal of thyroprotein, a response that has been reported often (14, 16).

Changes in milk production and fat production are in Tables 3 and 4, respectively. The actual milk production of each cow and the number of days into lactation at the onset of the experiment are in Table 1. This information was used for computerized projections[5] of milk production through the various experimental periods. From these projections (Table 3) responses of individual cows to thyroprotein feeding were variable. This was predictable and consistent with reports that show that animals in various stages of lactation respond differently to thyroactive agents (15). As expected, cows in later stages of lactation were more responsive to thyroprotein treatment. During the thyroprotein feeding period, three control animals in Group 3 exceeded projected production. This response may be from removal of restricted feeding at the end of the PCB dosing period.

Whereas two of the animals fed thyroprotein did not exceed projected milk yields (Table 3) during the thyroprotein period, only one animal failed to exceed projected milk fat production (Table 4). For the most part, substantial increases in actual fat production resulted from feeding thyroprotein, which was important to excretion of a lipophilic compound. Simple correlation coefficients between total excretion of PCB versus milk and milk fat production for all animals were .76 and .90 during thyroprotein treatment.

During the first few days after removal of PCB from the diet, the amount of PCB excreted declined rapidly among all three groups. Then the quantity excreted daily increased for animals receiving either 2 or 4 g/100 kg BW of

[5]Projections were made utilizing Program PROJECT, Statistical Laboratory, O.A.R.D.C.

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TABLE 3. Projected and actual changes in milk production after dosing with PCB, then during and following thyroprotein feeding.

Group	Cow no.	Milk production during thyroprotein feeding (days 21–77)			Milk production after thyroprotein feeding (days 78–98)		
		Projected	Actual	Difference	Projected	Actual	Difference
		(kg)					
1	2154	1118.4	1187.9	69.5	379.5	367.2	−12.3
	2160	1255.4	1410.9	155.5	484.6	414.4	−70.1
	1993	....	....	....	....	....	....
2	2110	1056.1	1304.9	248.8	322.7	251.0	−71.7
	2163	1500.2	1419.9	−80.3	547.3	483.2	−64.1
	2039	465.3	545.2	79.9	....	....	....
3	2114	789.6	707.1	−82.5	....	....	....
	2158	847.2	967.9	120.7	295.3	359.4	63.9
	2164	914.1	1026.3	112.2	314.1	378.4	53.3
	2147	798.5	1294.0	495.5	312.5	276.2	−36.3
4	2148	786.5	1156.4	369.9	164.2	264.8	100.6
	2156	951.9	947.3	−4.6	332.6	225.2	−107.4

thyroprotein. By day 52, which is near the midway point in the thyroprotein feeding phase of the study, daily excretion by control animals averaged 1.09 ± .20 mg/day compared to 1.39 ± .23 and 1.87 ± .08 for Groups 2 and 4. Total PCB excretion for Groups 2 and 4 animals was greater than for controls during thyroprotein treatment (Table 2). However, differences in PCB excretion by Group 2 cannot be attributable entirely to thyroprotein treatment. As compared to controls, these animals were earlier in lactation and were excreting more PCB before and during the thyroprotein feeding period.

After withdrawal of thyroprotein, milk production of stimulated animals declined

TABLE 4. Projected and actual changes in milk fat production after dosing with PCB, then during and following thyroprotein feeding.

Group	Cow no.	Milk fat production during thyroprotein feeding (days 21–77)			Milk fat production after thyroprotein feeding (days 78–98)		
		Projected	Actual	Difference	Projected	Actual	Difference
		(kg)					
1	2154	39.4	44.8	5.4	13.6	14.6	1.0
	2160	52.0	63.4	11.4	17.8	15.7	−2.1
	1993	....	....	....	....	....	....
2	2110	37.5	53.1	15.6	12.4	10.9	−1.5
	2163	62.4	56.3	−6.1	21.4	18.2	−3.2
	2039	27.7	21.0	−6.7	....	....	....
3	2114	27.3	26.5	−.8	....	....	....
	2158	29.2	35.6	6.4	10.0	13.8	3.8
	2164	27.7	33.2	5.5	9.5	12.4	2.9
	2147	32.5	64.1	31.6	11.2	11.3	.1
4	2148	30.8	53.8	23.0	8.7	11.4	2.7
	2156	39.7	42.0	2.3	13.7	10.1	−3.6

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sharply (Table 3). This was consistent with (5, 14, 15, 16). There was also a corresponding decline in excretion of PCB in milk of animals that had received thyroprotein. On experimental day 98 (21 days after complete withdrawal of thyroprotein) the daily PCB excretion of the remaining Groups 2, 3, and 4 animals averaged .67, .87, and .59 mg. This reversal in excretion of PCB by thyroprotein treated animals as compared to controls effectively eliminated gains in excretion during treatment. For animals that lactated at least to day 98 or through day 147, total excretion, after the end of PCB dosing, was similar, irrespective of thyroprotein treatment (Table 2).

GENERAL DISCUSSION

We hypothesize that thyroprotein treatment may be successful in increasing the clearance rate of PCB for two reasons. First, this agent increases production of milk and simultaneously milk fat in treated animals. Because milk fat is the most important excretory route for PCB in lactating animals, it is the most feasible route to alter to achieve increased excretion. Simply increasing milk production, i.e., via feeding of high grain rations, was not an approach deemed plausible because milk fat production generally is suppressed. Second, feeding of thyroprotein generally results in the utilization and loss of body fat by the animal. By metabolizing the adipose tissues, the concentration of PCB in adipose tissue that remains should increase. This phenomenon has been demonstrated in cattle fed the closely related polybrominated biphenyls (21). With concentration of PCB in the remaining adipose tissue we hypothesize that a change in equilibrium between PCB concentrations in body fat and blood would occur. Concentrations of PCB would be elevated in blood plasma and would be available for excretion in milk fat. Through this process tissues would be depleted more rapidly of residual PCB. Once the thyroprotein was withdrawn at the end of treatment, animals would be predicted to gain weight rapidly, replacing and diluting PCB residues with additional body fat. This would shift the equilibrium again and reduce PCB available to milk fat.

Despite the limited number of animals in this experiment, a farm situation was represented effectively by involving animals of all stages of lactation. Responses, although variable, were sufficient to provide a conclusion on the practicability of this treatment.

Addition of thyroprotein to the ration is not practical to remove PCB residues from cattle. Treatment with 4 g/100 kg body weight did increase PCB excretion during the time it was fed. This occurred simultaneously with an increase in milk production and milk fat production. The treated animals did lose body weight; however, weight losses were less than the 7 to 10% expected from accounts (5, 11, 16). This study was begun in early January which may have contributed to reducing weight loss. However, even if weight losses had been as expected, it is doubtful that results would differ. Evidently there was not a major shift in the equilibrium of PCB during these studies. Resources were not available to determine changes in blood or adipose concentrations of PCB during and following thyroprotein treatment.

None of the previously tried treatments, activated charcoal (3), barbiturates (1, 3), vitamins (1), and thyroprotein, has been effective as an agent to increase the excretion of halogenated biphenyls from dairy cattle. Until an effective agent is found, the most desirable action for a contamination incident is to determine residue concentrations in the animals. By clearance equations presented here and by others (3, 4), clearance time can be estimated. Based on these estimates, decisions can be made whether to milk the cows until sufficient residue is cleared or to destroy the animals.

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