indebted to the Animal Science Department for assistance and cooperation in the animal work.

LITERATURE CITED

Chem. Eng. News "Hexachlorophene: Cyclamates revisited?" April 5, 1971, p 14.
Chung, H. L., Ts'ao, W. C., Hsu, H. C., Kuo, C. H., K'o, H. Y., Mo, P. S., Chang, H. Y., Chuo, H. T., Chou, W. H., *Chinese Med. J.* **82,** 691 (1963).
Gump, W. S., *J. Soc. Cosmet. Chem.* **20,** 173 (1969).
Gutenmann, W. H., Lisk, D. J., J. AGR. FOOD CHEM. **17,** 1008 (1969).
Gutenmann, W. H., Lisk, D. J., *J. Ass. Offic. Anal. Chem.* **53,** 522 (1970).
Schlenk, H., Gellerman, J. L., *Anal. Chem.* **32,** 1412 (1960).
Wit, J. G., Van Genderen, H., *Acta Physiol. Pharmacol. Neerlandica* **11,** 123 (1962).

Received for review July 7, 1971. Accepted September 13, 1971.

Metabolism of Xenobiotics in Ruminants. IV.

Storage and Excretion of HEOD in Holstein Cows

Kim A. Wilson* and Robert M. Cook

The recovery of HEOD in milk, urine, blood, feces, and body fat was determined when the pesticide was orally administered in gelatin capsules to Holstein cows at the level of 0.1 mg/kg of body weight for 3 and 6 weeks. Forty to 50% of the pesticide could be accounted for as HEOD at the end of 6 weeks. Most of this was in the feces. HEOD was not detected in urine. By analyzing wet instead of dry feces, HEOD levels were four to seven times higher than when dried feces were analyzed. The main route of HEOD elimination from the body was through the feces and not through the milk. Fifty to 60% of the HEOD unaccounted for was believed to be in the form of hydroxylated metabolites. Thus, 90% of the pesticide can be accounted for in the feces and as metabolites. Phenobarbital lowered HEOD levels in milk and body fat.

As discussed in a previous publication in this series (Cook, 1970), the contamination of livestock with chlorinated pesticides is a serious problem in animal agriculture. In addition to the presence of the parent pesticide compound, metabolites of some pesticides are present in meat and milk. It has been clearly established that DDT is changed in the environment to two major metabolites, DDD and DDE, and these metabolites are present in the blood and tissues of animals and man. Metabolites of DDT are easy to measure. However, metabolites of other pesticides that are hydroxylated are not readily detected. Several years ago HEOD was shown to be metabolized to several different compounds in rats and rabbits (Korte and Arent, 1965; Klein *et al.*, 1968). Recently it was shown that HEOD is readily metabolized to several metabolites in sheep (Hedde *et al.*, 1970). The fact that HEOD is metabolized in the sheep suggests that metabolites of this insecticide may be present in animal food products.

We conducted a HEOD balance trial using lactating Holstein cows with two objectives: to determine the recovery of an oral dose of HEOD in milk, blood, urine, feces, and body fat and the effect of phenobarbital on these measurements; and to determine the recovery of HEOD metabolites in milk, blood, urine, feces, and body fat and the effect of phenobarbital on these measurements. The first phase of the study has been completed and the results are reported in this communication.

Dairy Science Department, Michigan State University, East Lansing, Michigan 48823.

EXPERIMENTAL PROCEDURE

Two groups of four lactating Holstein cows each were contaminated orally for 3 weeks with HEOD at the level of 0.1 mg per kg of body weight per day. During the second 3-week period, two cows in each group continued to receive the pesticide, while the remaining two cows were not given HEOD (Table I). In addition, phenobarbital was superimposed on one group of four cows for the entire 6-week period at the level of 10.0 mg per kg of body weight per day. Daily samples of milk, feces, and urine were collected during the experimental period. During weeks 2 and 6 total urine was collected for 5 and 6 days, respectively. Body fat samples were obtained at 0, 2, 4, and 6 weeks of the experiment; blood samples were obtained at 0, 2, 4, and 5 weeks. All samples were stored at −20°C until prepared for analysis. The animals were fed a ration of hay, corn silage, and grain that was balanced to meet the requirements of maintenance and lactation. Phenobarbital and HEOD were administered orally in a gelatin capsule which also contained 15 g of chromic oxide for determining total fecal dry matter.

Milk and blood were prepared for analysis according to the method described by Crosby and Archer (1966), except that hexane was used instead of pentane. The milk fat percentage was determined by the Babcock method. Shoulder fat was prepared by homogenizing 1 part fat to 10 parts hexane in a glass tissue grinder. The homogenate was dried, using anhydrous sodium sulfate. Urine was extracted with three successive portions of hexane (2 parts urine:1 part hexane). The extracts were concentrated to 1 ml on a rotary evaporator under vacuum at 40°C.

Daily samples of feces were thoroughly mixed and aliquots

Table I. HEOD and Dry Matter Intake[a]

	C-C	C-D	PB-C	PB-D
Total HEOD, mg	2722.6	1324.1	2850.6	1252.9
ppm HEOD in DM	4.10	3.47	4.03	4.02
ppm HEOD "as fed"	2.68	2.36	2.71	2.64
Total DM intake during contamination, kg	664.72	381.64	703.73	311.78
Feed consumed "as fed" during contamination, kg	1016.07	560.21	1051.27	474.74
% DM of total ration consumed	65.7	68.1	66.9	65.7

[a] Each figure is the average of two cows. C-C, control, contaminated with HEOD 6 weeks. C-D, control, decontaminated last 3 weeks. PB-C, phenobarbital, contaminated with HEOD 6 weeks. PB-D, phenobarbital, decontaminated last 3 weeks.

Table II. HEOD Excretion *via* Feces

Week	C-C	C-D	PB-C	PB-D
	mg HEOD/cow/day			
Control	0	0	0	0
1	39.7	29.3	21.7	50.9
2	16.6	11.8	11.6	22.9
3	19.0	6.3	6.2	13.3
4	17.6	0.8	19.0	0.7
5	11.2	...	5.4	...
6	11.2	...	7.6	...

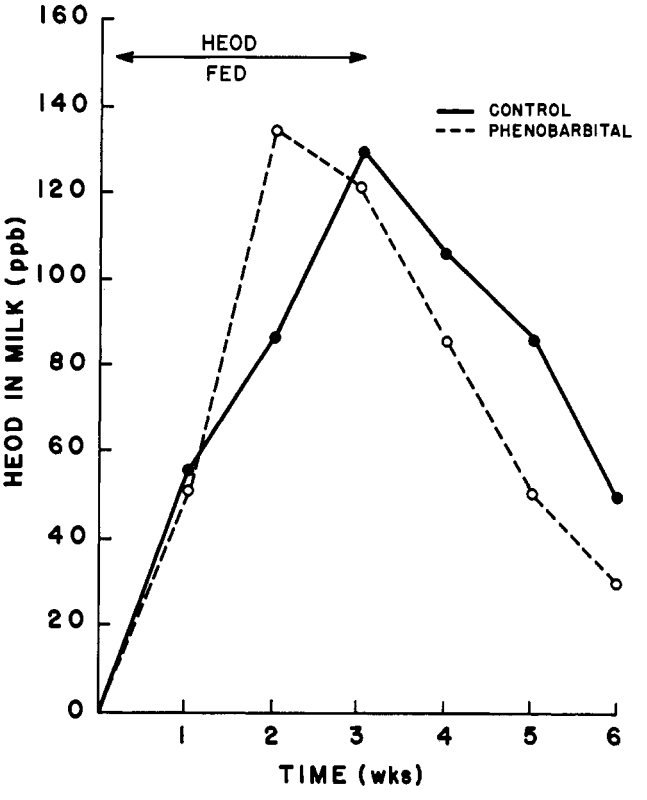

Figure 1. Concentration of HEOD in whole milk from cows fed the pesticide 3 weeks

removed for separate HEOD, percentage dry matter, and chromic oxide determinations. Upon thawing, 1 g of feces was extracted first with 2 ml of isopropyl alcohol:water (1:1). The solution was then extracted with hexane and centrifuged at 1700 × *g* for 15 min. Dieldrin was determined in the hexane extract. Dry matter was determined by drying in a forced-air oven at 100°C for 24 hr. The chromic oxide content of the feces was established according to the method of Kimura and Miller (1957).

All chemicals used were either reagent grade or Nanograde (Mallinckrodt). Analyses for HEOD were as described by Cook (1970). The pesticide dieldrin contains not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene (abbreviated HEOD). Pure HEOD was used in this study and was a gift of Shell Chemical Co., New York.

RESULTS

The primary route of HEOD excretion was *via* the feces. Although there was considerable variation among groups, the total daily excretion of HEOD in the feces was highest in the first week and steadily declined during contamination (Table II). The percentage of the weekly dose that was excreted that week declined during HEOD contamination. HEOD ceased to be excreted within 4 days after withdrawal (Table III). Because fecal dry matter excretion was constant, the changes observed for fecal HEOD excretion are primarily a function of the concentration of the pesticide.

The large excretion of HEOD in the feces is in contrast to previous reports (Braund *et al.*, 1968). A difference in the fecal extraction method for HEOD analysis can account for most of the increased fecal output of HEOD. Previous workers have dried the feces in order to measure percentage dry matter and to facilitate thorough sample mixing prior to residue analysis. Archer and Crosby (1968) have shown that

Table III. Percentage of Weekly HEOD Dose Excreted in Feces That Week

Weeks	C-C	C-D	PB-C	PB-D
	%			
1	66.5	47.1	35.1	86.0
2	27.7	18.8	18.1	38.4
3	31.1	11.4	9.5	25.7
4	28.1	...	28.8	...
5	17.8	...	8.2	...
6	17.7	...	11.8	...

Table IV. Extraction of HEOD from Wet and Dried Feces

	Dried	Normal
Range of HEOD in dry matter, ppm	0.670–0.807	2.613–6.019
Relative extraction		
As a percentage of "dried"	100	390–740
As a percentage of "normal"	26–13	100

DDT and dieldrin codistilled with water. In addition, it is possible that some of the pesticide may become tightly bound to the particulate matter during drying. The combination of these effects would greatly decrease the amount of pesticide recovered. A simple change to the extraction of wet feces with isopropyl alcohol and subsequent extraction with hexane gave good recoveries of HEOD (Table IV). The recoveries of HEOD from dried samples were highly variable and low, whereas all recoveries from wet feces were greater than 85%.

There were no significant differences among treatments for dry matter intake, body weight change, decline of milk and

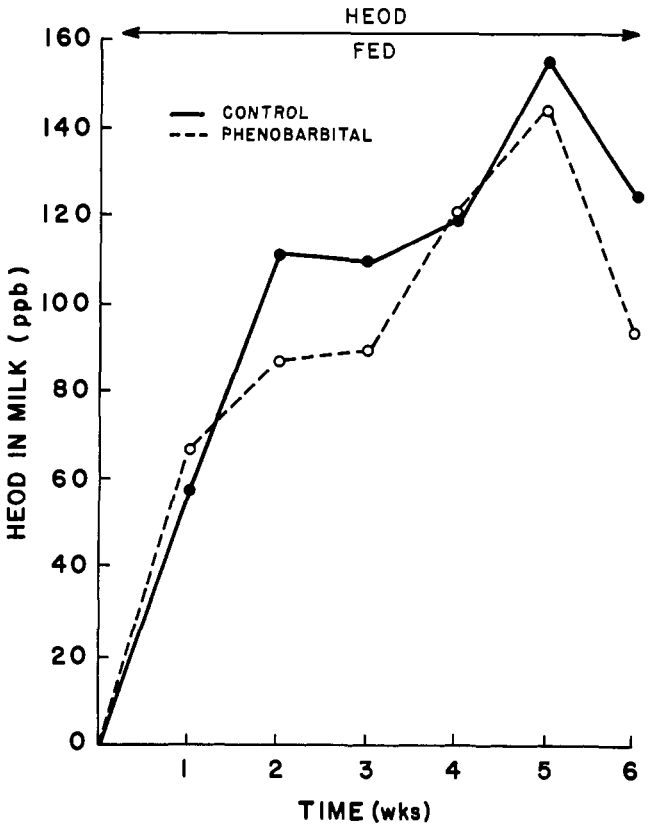

Figure 2. Concentration of HEOD in whole milk from cows fed the pesticide 6 weeks

Table V. Concentration of HEOD in Blood

Week	C-C	C-D	PB-C	PB-D
	ppb			
Control	0	0	0	0
2	3.82	3.48	3.26	3.07
4	7.24	3.90	2.88	1.50
5	5.50	1.37	5.37	1.59

fat production, fecal dry matter, and urine excretion during the 6 weeks of the experiment.

The concentration of HEOD in milk was reduced by phenobarbital (Figures 1, 2). Animals receiving phenobarbital had lower levels of HEOD in their milk during the fourth through sixth weeks, regardless of HEOD treatment. The induction of liver microsomal drug metabolizing enzymes by daily phenobarbital treatment reaches a significant level by the fifth day and continues to increase through 14 days (Cook and Wilson, 1970). This may account for the absence of differences during the first 3-week period in this trial.

The levels of HEOD in the blood were quite low (Table V), especially during the decontamination period. Although several solvent extraction methods were employed, HEOD could not be detected in urine.

HEOD levels in the body fat from the scapular fat pad are presented in Figures 3 and 4. HEOD was not detected in control biopsies. Phenobarbital prevented the normal accumulation of HEOD in body fat.

The distribution of the total HEOD dose at 3 and 6 weeks is presented in Table VI. The percentage of the dose ad-

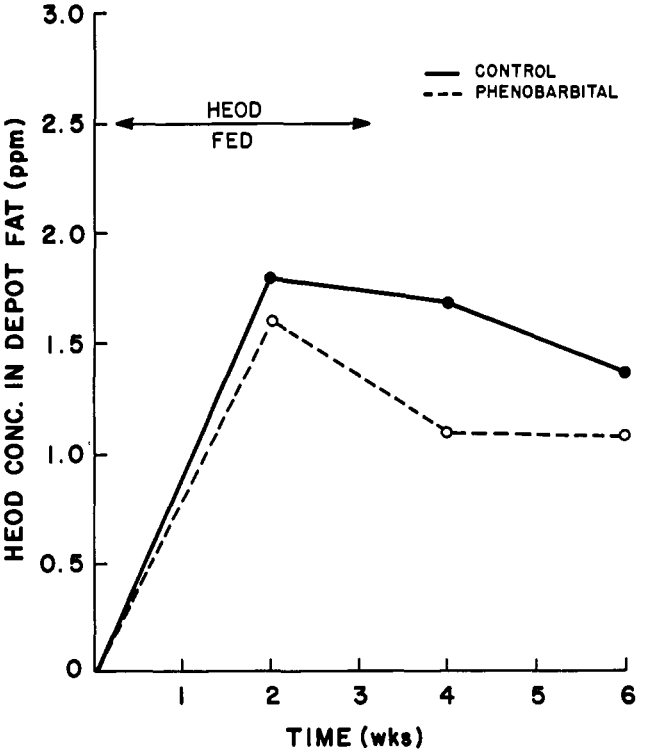

Figure 3. Concentration of HEOD in depot fat from cows fed the pesticide 3 weeks

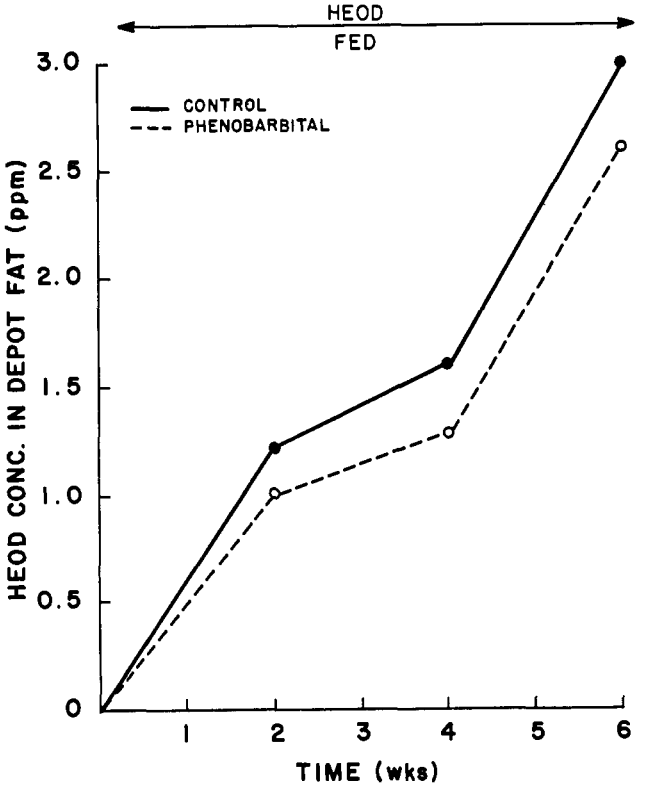

Figure 4. Concentration of HEOD in depot fat from cows fed the pesticide 6 weeks

ministered that was excreted by 6 weeks in the feces ranged from 18.6 to 53.1. Body fat was assumed to be 10% of the body weight. Thus, the percentage of the HEOD dose stored in body fat was determined by multiplying the highest biopsy concentration of HEOD detected in fat for each group by 10% of the body weight and dividing by the total dose ad-

Table VI. Distribution of the Total HEOD Dose

Location	C-C	C-D	PB-C	PB-D
	% of HEOD dose			
Feces	31.6	27.8	18.6	53.1
Body fat				
2 weeks	8.0[a]	11.9[a,b]	7.3[a]	10.2[a,b]
6 weeks	6.9[b]	...[c]	5.8[b]	...[c]
Milk	3.1	3.8	3.3	3.1
Body fluids	0.07	0.06	0.04	0.06
Total	42.77	43.56	29.24	66.46

[a] These values are based on the concentration of HEOD in the body fat and the total HEOD administered up to the day of biopsy and used to estimate total burden. [b] These values represent the recovery obtained at the time of highest HEOD concentration in the fat. [c] The values are not presented because contamination had ceased 3 weeks prior to this biopsy.

ministered up to that particular biopsy. Estimations of HEOD in body fat were calculated using the values at 6, 2, 6, and 2 weeks, respectively, for groups C-C, C-D, PB-C, and PB-D. Also estimates were made for all groups using the second biopsy value in order to examine the effect of standard exposure duration for each group. Even though the body fat levels of HEOD were the greatest at 6 weeks for group C-C and PB-C, a larger percent of the dose could be accounted for in body fat at 2 weeks for these groups (Table VI, Figures 3 and 4). At the end of 6 weeks, only 6 to 7% of the HEOD dose could be accounted for in body fat.

In contrast to the high values calculated for percent of the dose in the feces, considerably less HEOD was accounted for in the milk (Table VI). Milk was not a primary excretory route for HEOD in this experiment. For estimation of the body fluid burden of HEOD, it was assumed that the body fluids in equilibrium with the blood represented 30% of the total body weight. The extremely low values show that body fluids contribute little as an HEOD sink.

DISCUSSION

This work shows that on the average about 40% of the HEOD dose was accounted for as HEOD. Almost 80% of this was in the feces. Only 3 to 4% was eliminated in the milk and only 6 to 7% was stored in the body fat. Sixty percent of the HEOD was unaccounted for. If this HEOD were metabolized to other compounds which were excreted in the urine and feces, then almost 90% of the ingested HEOD was eliminated through the urine and feces and only 3 to 4% through the milk. This accounts for 93 to 94% of the ingested HEOD. The remaining 6 to 7% of the dose was stored in body fat.

The first attempt to quantitate the total body burden of HEOD in animals was reported by Keane and Zavon (1969). They fed HEOD to mongrel dogs at the level of 1.0 mg/kg of body wt/day for 0–5 days and then at the level of 0.2 mg/kg of body wt/day for 6 to 59 days. The total burden of HEOD was directly related to the total kilograms of fat in the body and inversely related to the concentration of HEOD in body fat. It is believed that our work is the first attempt at estimating the total body burden in cows and at accounting for the total HEOD fed. Keane and Zavon (1969) determined the lean body mass of the experimental dogs by measuring ^{40}K in order to calculate the total amount of adipose tissue. This method is based on the fact that 0.012% of all naturally occurring potassium (^{39}K) is radioactive potassium (^{40}K). By applying appropriate conversion factors, the lean body mass can be calculated. We estimated total adipose tissue by assuming that adipose tissue represents 10% of the body weight. This figure is a good estimate of adipose tissue for cows in average condition, such as used in these trials.

The measurement and identification of the metabolite (or metabolites) which accounts for about half of the HEOD fed were not resolved in this study. At present, aldrin diol has been identified as a major metabolite of HEOD in urine using the procedure reported by Hedde *et al.* (1970). Although it is not established that HEOD metabolites are eliminated in the feces in these trials as suggested above, the data reported by other investigators support this idea. Quaife *et al.* (1967) have reviewed the studies on the fate of ^{14}C- or ^{36}Cl-labelled aldrin or dieldrin in monogastric animals. These pesticides are deposited in the body fat in the form of HEOD. They are readily metabolized to hydrophilic metabolites which are found in the bile, urine, and feces. The main route of excretion of HEOD is *via* the feces. Although HEOD metabolites were found in several body tissues, this appears to be of a transient nature. From a consideration of the studies with monogastric animals, it is logical to conclude that with Holstein cows most of the HEOD dose (60%) was eliminated in the urine and feces in the form of hydrophilic metabolites.

Therefore it can be concluded that HEOD was readily metabolized and eliminated by cattle. Contrary to classical concepts, very little HEOD, when administered orally in a gelatin capsule, was stored in the body or eliminated in the milk. Even so, the tolerance for HEOD in milk is so low (0.3 ppm in the milk fat) that a concentration of HEOD in body fat of 1.0 ppm is high enough in our experiments to produce milk that was above tolerance.

LITERATURE CITED

Archer, T. E., Crosby, D. G., J. AGR. FOOD CHEM. **16,** 623 (1968).
Braund, D. G., Brown, L. D., Huber, J. T., *J. Dairy Sci.* **51,** 982 (1968).
Cook, R. M., Wilson, K. A., J. AGR. FOOD CHEM. **18,** 441 (1970).
Cook, R. M., J. AGR. FOOD CHEM. **18,** 434 (1970).
Crosby, D. G., Archer, T. E., *Bull. Environ. Contam. Toxicol.* **1,** 16 (1966).
Hedde, R. D., Davison, K. L., Robbins, J. D., J. AGR. FOOD CHEM. **18,** 116 (1970).
Keane, W. T., Zavon, M. R., *Bull. Environ. Contam. Toxicol.* **4,** 1 (1969).
Kimura, F. T., Miller, V. L., J. AGR. FOOD CHEM. **5,** 216 (1957).
Klein, A. K., Link, J. D., Ives, N. F., *J. Ass. Offic. Anal. Chem.* **51,** 895 (1968).
Korte, F., Arent, H., *Life Sci.* **4,** 2017 (1965).
Quaife, M. L., Windbush, J. S., Fitzhugh, O. G., *Food Cosmet. Toxicol.* **5,** 39 (1967).

Received for review April 30, 1971. Accepted September 15, 1971. Supported by the Michigan Agricultural Experiment Station (Publication No. 5433) and by FDA Grant FD-00223. The data are from a thesis submitted by the senior author to the Graduate School in partial fulfillment of the requirements for the M.S. degree.