PHARMACODYNAMICS OF DIELDRIN IN DAIRY CATTLE AS AFFECTED BY PHENOBARBITAL FEEDING

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ABSTRACT

PHARMACODYNAMICS OF DIELDRIN IN DAIRY CATTLE
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By

Kim Alyn Wilson

This study was undertaken to investigate the metabolic fate of HEOD, the major compound in the pesticide dieldrin, when administered orally alone and in combination with phenobarbital to lactating dairy cows.

The experiment included analyzing milk, feces, blood, urine and body fat for HEOD content. The analyses were made in order to determine the sites of normal storage and excretion of HEOD. In addition, the investigation was designed to ascertain the effect of phenobarbital on the metabolism of HEOD.

HEOD residues in body fat were lower in animals receiving phenobarbital during contamination. In addition, the concentration of HEOD in the body fat of those animals receiving phenobarbital was lower during decontamination, and declined at a faster rate.

The treatment of dairy cows with HEOD and phenobarbital at the levels used did not affect feed intake or milk production.

Upon cessation of HEOD administration, the level of the pesticide in the milk fat declined rapidly. During decontamination, cows receiving the drug produced milk containing lower concentrations of dieldrin in the milk fat than animals not treated with phenobarbital. There was no effect of phenobarbital treatment on the concentration of HEOD in the milk fat during contamination.

The major portion of HEOD was excreted via the feces. The maximum excretion of the pesticide occurred during the first week in all groups, which ranged from 22-51 mg HEOD/day. The pattern of residue decline was similar for all groups. Five days after cessation of the HEOD treatment the concentration of pesticide in the feces became undetectable.

The concentration of HEOD in the blood was low and no effects of treatment were noted. There was no HEOD detected in the urine.

From 18.6 to 53.1% of the total HEOD given was accounted for in feces. Body fat contained from 7.3 to 11.9% of the HEOD dose, whereas 3.1 to 3.8% of the total pesticide administered was measured in the milk. Less than 0.1% of the dose was in the blood and body fluids. In all tissues analyzed, a range of 29 to 66% of the dose could be accounted for as HEOD.

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Kim Alyn Wilson

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DEDICATED TO

My wife, Dale, and my parents, whose encouragement has been so meaningful.

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CHAPTER I

INTRODUCTION

Pesticide contamination of livestock is a serious problem in animal agriculture. Concentrations of chlorinated hydrocarbon insecticides in meat and milk exceeding established "safe" levels result in severe economic loss to the producer, for these compounds persist not only in the environment, but in livestock for months and even years.

In the 1940's, organic chemicals were synthesized which were not degraded rapidly, enabling lower levels to be used than was possible with their botanically-derived predecessors. But, the residual character of the new compounds, though beneficial against the target organism, also became their nemesis when non-target organisms became contaminated.

Xenobiotics, compounds foreign to a biological system, have become of greater concern recently both because of their preponderance and also the ability to detect ever smaller amounts. Millions of tons are added to the biosphere annually. Chlorinated hydrocarbon insecticides are easily detected at levels of 0.01 ppb. This concentration is analogous to one second in 32 centuries.

Concentrations this low have been detected in almost all tissues of animals studied. But, although pesticide residues may be present everywhere, the low levels observed may not be metabolically significant. For example, the acceptable level for dieldrin in milk is 0.3 ppm in fat which is 30,000 times higher than detectable limits.

Demand for greater productivity in agriculture has necessitated the increased use of pesticide and herbicide chemicals. Nutrient levels and their availability per land unit have been improved by controlling insect and weed infestations, but continued pesticide use has caused leaching into ground water and recycling at great distances from the original application sites.

It is necessary to study the metabolism of many representative chemicals in the environment in order that new chemical classes be understood for their effect not only on specific targets but on the biosphere as a whole. Pressure has been applied to reduce the use of organochlorines and change to "bio-degradable" products. None of these are completely degradable to carbon dioxide and water--their degradation products may be as harmful as organochlorines. Also, metabolic products of chlorinated hydrocarbons may be beneficial as insecticides, perhaps more than current compounds utilized.

The objectives of this work are to account for the accumulation and excretion of the total dose of HEOD and to study the effects of phenobarbital on HEOD clearance and metabolite formation in cows. This is the first part of a study having the overall objective of determining the metabolic fate of dieldrin in ruminants.

CHAPTER II

LITERATURE REVIEW

A. Pharmacodynamics of Chlorinated Hydrocarbon Insecticides

Pharmacodynamics has been defined, ". . . absorption, accumulation, and elimination and changes in physiological function consequent to those processes . . ." (82). In particular, the pharmacodynamics of dieldrin have been investigated in many biological systems, as has the interaction of dieldrin with other factors (53, 82, 85, 140). Recent reviews on the status of pesticide contamination and metabolism have appeared (20, 29, 56, 83, 84, 110, 123, 127, 131, 132).

1. Chemical properties of HEOD

Dieldrin is a light brown crystalline material containing not less than 85% of the active ingredient (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene), a white crystalline compound commonly abbreviated HEOD. For the purposes of this thesis, both because the contaminant employed was HEOD (99+% pure) and because the analytical method primarily detects HEOD, the abbreviation

will be used to distinguish from the technical grade material, dieldrin.

Dieldrin has a melting point near 145°C. HEOD melts at 175-176°C (7). The molecular weight of HEOD is 380.93. The compound is readily soluble in benzene and acetone and less soluble in hexane and methanol. HEOD solubility in water is less than 0.185 ppm (126). It is stable in strong base but labile in strong acid.

Photodecomposition occurs in sunlight to form dechlorinated products with additional cyclic rearrangements (77, 133, 135). Dieldrin is the epoxide form of aldrin. This epoxidation of aldrin is catalysed readily by sunlight, microbes or in mammals (68).

2. Sources of contamination

The primary route of livestock contamination by pesticides is via oral ingestion of feeds containing pesticide residues (78, 137, 157). Direct contamination of animals or their environs with pesticides for insect control has been of secondary importance. Aerial movement (drift) of insecticides applied to areas adjacent to pasture or croplands is a significant source of feedstuff contamination in many areas (78, 152).

Feeds can become contaminated by the direct application to crops of either excessive amounts of pesticide or by the use of incorrect pesticides (104, 148, 162). The insecticides can also be translocated from

soil to the crop which serves to concentrate these materials prior to consumption (1, 105, 137, 154, 155).

Accidental contamination of livestock appears to be more prevalent than previously thought (19, 28, 119, 121, 150) and represents a severe economic loss to the producer. Often the loss is three-fold: (1) death losses, (2) levels in meat and/or milk prevent disposition of the animals and/or products, and (3) the period of decontamination is long during which no income from the sale of meat and/or milk is received.

3. Absorption

Absorption of chlorinated hydrocarbon insecticides occurs primarily in three ways: (1) dermal or percutaneous, (2) inhalation (respiratory), and (3) oral ingestion (alimentary). Dermal application causes a rapid rise in blood levels (72, 73, 125, 140). The rate of absorption is rapid and depends on the solvent used. The rate is greatest in acetone, less in benzene, slower in corn oil. The structure of the compound also affects absorptive rate, the rate being highest for dieldrin followed by malathion, carbaryl and DDT (77). Inhalation of DDT by dairy cows produces lower levels of DDT and DDE in milk than does alimentary contamination (162).

Oral ingestion of chlorinated pesticides is the most common environmentally encountered absorptive route

and results in the rapid elevation of blood levels. Rapid absorption of a single dose of DDT from the gut produced high blood levels initially which tended to reach equilibrium within 1-3 days (42, 90). In one trial the half-life of a single dose of DDT intravenously in cows initially was 60-80 minutes. The half-life of DDT increased until equilibrium was reestablished in 24 hours (161). Retention of dieldrin in the gut or the regulation of absorption appears to be affected by gut contents (74).

Keane et al. (93) demonstrated that the degree of obesity is directly related to the time required to produce poisoning in animals receiving chronic doses of HEOD. They suggested that this relationship should be valid regardless of whether absorbed orally, by inhalation or through the skin. If the "adipose sink" works in this manner, then the work by Morgan and Roan (116) would suggest that the toxicity of DDT should be affected in a similar manner, and to an even greater degree, by obesity. Chronic doses or low level contamination results in initially high blood levels which continue to rise with contamination, dependent upon dosage level (104).

4. Transport

Pesticides are transported bound to blood lipoprotein (69, 116), are solubilized by blood components, e.g., fatty acids, and have been shown to be, in the case of dieldrin, 4,000 times more soluble in serum than water (68, 118). Dieldrin in blood is located primarily in plasma and erythrocytes, at a ratio of 2:1, respectively. A rapid initial rate of dieldrin removal from blood has been shown to be followed by a logarithmic rate of removal (118). Absorbed pesticides are transported to the liver via the portal vein and their toxicities are controlled by their low solubilities in water and high solubilities in lipoidal material. Slow infusions are necessary in order to permit dieldrin to be absorbed by adipose tissue, because rapid infusion overloads the central nervous system. Shortly after absorption, considerable dieldrin appears not in the fat, but in brain and other organs, and the faster the rate of absorption, the greater the proportion found in the brain (74). In addition, dieldrin is more extensively distributed to non-lipid cellular and blood components than are DDT and DDE (116). This relation conflicts with the relative LD_{50} levels of dieldrin and DDT. The lower LD_{50} of dieldrin implies a rapid association with the neural system, especially the lipoidal fraction.

A recent report on the effects of dietary methionine on DDT metabolism in rats clearly demonstrated that accumulation of DDT and its metabolites in liver tissues was dependent upon dietary methionine level (149). With the knowledge that limiting the availability of methionine reduces the formation of chylomicra thus impairing lipid transport (86), the authors (149) suggest that the opposite may also be true, that stimulation of lipid transport may in fact be responsible for increased tissue storage of DDT.

5. Storage

Studies have attempted to correlate body fat levels of pesticides with blood levels and storage as a measure of environmental exposure (44, 80, 87, 91, 92, 120, 130, 134). Robinson et al. have measured tissue concentration of HEOD in rats at 12 weeks and found that exact levels depend on the type of tissue studied, with adipose > liver > brain > blood (134). The half-life of HEOD was 10.3 days in adipose tissue of rats. Correlations of 0.81-0.96 were noted between levels of HEOD in blood and its concentration in the heart, liver, kidney, lung and fat (130). A high correlation (r=>0.8) was found for dieldrin concentration in different tissues from the same person of workers occupationally exposed to the pesticide (71). Concentrations of dieldrin in body fat and liver are correlated with levels

in the blood (50). Keane and Zavon suggested that "under conditions of equal and constant oral intake, the concentration of a chlorinated insecticide per gram of fat is inversely related to the total body burden of the insecticide and also inversely related to the degree of obesity of the individual sample" (92). Their data also supported the contention of Hunter (82) that the blood concentration of a chlorinated insecticide can be used as an index of the concentration of that insecticide in the fat. Long-term surveys of human residue levels suggest that dieldrin is more extensively distributed to non-lipid and blood components than are DDT and DDE (116).

When aldrin is fed to livestock it is rapidly converted to dieldrin which is stored in adipose tissue up to four times the level ingested (0.25 to 10.0 ppm in diet) (87). In an extensive review (78), Henderson concluded that the concentration of pesticides in the body fat increases rapidly upon ingestion of a pesticide, then levels off at a plateau characteristic for the amount of the insecticide in the feed. The lower the concentration in the feed, the sooner the plateau is reached. The dietary level is concentrated 10-20 fold in milk fat and 5-10 fold in body fat when heptachlor epoxide is fed for 12 weeks, whereas dieldrin is only concentrated 2-fold in each tissue at 12 weeks (27).

Dietary levels of Telodrin of between 0.02-0.15 ppm caused a 4-fold concentration in tail head fat at 88 days in dairy cattle. After 40 days on treatment, milk levels of Telodrin were 30% of those in the ration (8). A series of studies by Gannon et al. (58, 59, 60) showed that dieldrin fed from 0.1-2.25 ppm in the diet was concentrated 2-3 fold in the various body fats of dairy cows after 12 weeks. In the same period, milk levels rose to 1/10-1/5 that in the diet. Accumulated intakes for the 0.1 and 2.25 ppm level were 0.293 and 6.556 mg/kg body weight. The same levels of pesticide were given to steers, hogs, lambs and poultry. Dieldrin concentration in tissues of these species was highest in hens and steers and lower for hogs and lambs. Although hens had the greatest level in body fat, their eggs contained very little dieldrin. After 12 weeks hens concentrated the dietary level 10-fold, steers 2-fold, hogs and cows < 1-fold and lambs to about 1/3 the level in the ration. At 42 days of feeding 10 and 50 ppm dieldrin to dairy cows, the ratio of dietary level to concentration in the whole milk was 6:1 and appeared to be dose dependent. The ratio reached a maximum of 5:1 at 16 weeks. At 50 ppm in the diet the body fat level was 11 times higher than the milk level at 16 weeks (58, 59, 60). Dieldrin at 1 and 10 ppm concentrates in adipose tissue of rats to 15 and 67.5 ppm, respectively, in 10 weeks (142, 145).

DeMott et al.(51) noted a 0.97 correlation between the amount of heptachlor and heptachlor epoxide consumed and the concentration of heptachlor epoxide in milk fat. The ratio between milk fat and body fat levels decreased as time before parturition increased in dairy cattle (15). Contamination of pregnant dairy heifers early rather than late in gestation produced significantly higher body fat levels (15).

6. Biotransformation

The behavior of the parent compound, HEOD, has been reviewed in the preceding sections. HEOD is rapidly absorbed, transported and assimilated by lipoidal material. Almost immediately upon absorption of a toxin the body begins a detoxification process. Detoxification can include any or all of these alternatives: direct elimination of the toxin in the urine as a conjugate; enzymatic alteration and conjugation; absorption into the adipose tissue either as the parent compound or as a metabolite; or recycling the toxin to the gut from the blood via the saliva, bile, gastric or pancreatic juices.

The logical action by the liver is a structural alteration of the compound in order to reduce its toxicity to the host. Often the liver is stimulated by the drug (or other chemical) to increase its capacity for detoxification. However, it is obvious that if

liver action results in a metabolite more biologically active than the parent compound, the net effect has been an enhancement rather than a reduction of toxicity (35). Examples of this toxification have been demonstrated in mammals (21, 22, 23, 26, 47, 48, 160). The classic toxification reactions are the conversion of aldrin and heptachlor to their respective epoxides, dieldrin and heptachlor epoxide. Bann et al. (6) found that aldrin is converted to dieldrin in pigs, poultry, sheep, rats, beef and dairy cattle regardless of the route of entry. The change occurred whether administered orally or subcutaneously. Christensen (32) showed a 30% change of aldrin to dieldrin in rats and dogs by 24 hours. In addition, a photoconversion product of HEOD has been shown to exhibit increased toxicity to rats, mice, guinea pigs and pigeons, but is less toxic than HEOD to domestic fowl or a species of fish, and no discernible difference in toxicity was noted for beagle hounds (26). Likewise, another photo-product of HEOD is more toxic to houseflies and mice (76). Other photo-products have been reported (40, 133, 135). These are readily formed by the action of sunlight on HEOD applied to plants, soil and glass, and by artificial light (mercury lamp) on glass and in solution. The metabolism of one photo-product has been studied (5). Microorganisms in soil degrade dieldrin rapidly (111, 112).

That dieldrin may be degraded to other forms was first suspected when rats fed dieldrin excreted a compound similar to HEOD in the urine (102). From 1962 to 1965 reports appeared on the presence of degradation products which were apparently derived from HEOD but these were not identified (32, 43, 47, 74, 108, 109, 117).

British researchers found that when chlorine-labelled HEOD was fed to rats, very little of the label was excreted as HEOD. The amount of the dose which appeared in the feces probably was excreted as a glucuronide in bile and then reconstituted in the gastrointestinal tract to be incorporated into the feces (74). HEOD is considered to be stable in KOH, yet in alkaline conditions metabolites are readily changed (47). Preliminary trials suggested that less metabolites were excreted in urine from an intravenous dose than from an oral dose (47). The trans-diol was found to be unstable at greater than 200°C (108). Almost all of a dose of aldrin was accounted for in the tissues of rats and dogs at 24 hours. However, only 40% of a dieldrin dose could be accounted for primarily in the fatty tissues. None was found in urine or feces. Since much of the dieldrin was not recovered, it was assumed that it was partially converted and rapidly excreted. It was also suggested that the metabolites of dieldrin are polar in

nature and, as such, are not likely to be located in fatty tissues (32).

The first identification of an HEOD metabolite occurred in 1965 (101). These workers isolated a dihydroxylated form of HEOD (108), which differed by the addition of one oxygen and two hydrogen atoms across the epoxy group. Rabbits receiving dieldrin-^{14}C per os for 21 weeks excreted about 1/3 of the dose in the urine. Of the total radioactivity excreted in the urine, 86% occurred as one of six metabolites, which was shown to be the compound resulting from the hydrolytic cleavage of HEOD. The toxicity of this compound is lower than dieldren for mice. When administered intravenously to rats, it was excreted unchanged in the feces. As was the case in subsequent work, the initial steps of isolation proved as difficult as those of identification. It appeared that the metabolite had been present in earlier experiments, but methods for isolation of the compound had not been established. Further studies in the pharmacodynamics of dieldrin have centered around the development of techniques designed to isolate metabolic degradation products of organochlorines in tissues and excreta of animals.

Since the identification of the dihydroxy metabolic conversion product of dieldrin, several other metabolites have been isolated and the structures elucidated (5, 22, 24, 100, 111, 112, 113, 114, 128,

129, 134). Richardson and coworkers identified a rat urinary metabolite as a ketone which retains the epoxide ring but is monodechlorinated and the carbon skeleton rearranged (128, 129). The fecal metabolite of HEOD from the rat appears to be altered only by hydroxylation at the 4a or 5 carbon. Recent work has confirmed a fecal metabolite hydroxylated at carbon 4a, and the urinary product as a ketone at carbon 3 and an additional carbon-carbon bond between positions 2 and 9 (100, 113). Proposed reaction mechanisms for the formation of each metabolite of dieldrin have been reported by Robinson et al. (134). Damico et al. (46) confirmed the identity of the ketone metabolite from rat urine. They have also established the structure of a ketone metabolite of aldrin in rat urine which differs from dieldrin only by the endo- or exo-orientation of the epoxide ring.

The most recent metabolite of dieldrin to be identified is 9-hydroxy dieldrin (54). Extensive studies on the extraction and isolation of this compound (75) suggested that the 4a or 5-hydroxy dieldrin may have appeared during feeding trials with sheep. But subsequent analysis demonstrated that the 9-carbon of the 5,8-methylene bridge is hydroxylated. This compound, as well as trans-dihydroxy-dihydro-aldrin, was extractable from sheep urine with hexane. Eight metabolites were isolated from sheep urine, four of which were

hexane-extractable. The remaining four were ether and ethyl acetate-soluble, but their structures were not established. Isolation was complicated by the narrow pH range which permitted extraction. A change from pH 3 to pH 5 caused a decline in yield from 100% to 15%.

7. Excretion

a. Normal patterns.--The excretion of unchanged pesticide occurs after residues circulating in the blood, and those mobilized from adipose tissue, are taken up by the liver, kidney and mammary gland to be excreted in the bile, urine and milk. When biotransformation occurs, the compound is usually made more polar, thus it is more readily conjugated and excreted in the bile and urine (74, 75, 114, 134). The primary excretory routes are in feces and urine and in milk during lactation. The extent to which each route contributes to total excretion depends on the obesity, sex, stage of lactation or gestation, nutritional status, species, age, type of contaminants, and duration and intensity of exposure to the contaminant.

Many studies have attempted to provide information on the normal excretion pattern of herbicides and insecticides in livestock (9, 15, 42, 51, 52, 58, 60, 65, 66, 75, 81, 96, 120). The excretion equilibrium among feces, urine and milk is highly variable. The chlorinated

hydrocarbon insecticides have been shown to be excreted primarily in the milk and feces (as the parent compound) and in the urine as metabolites. Any physiological change which affects lipoidal material, such as adipose tissue, should also affect the pesticides incorporated there. This is not always the case. For example, a substantial decrease in milk fat production of dairy cattle produced a corresponding increase in concentration of dieldrin in the milk fat, such that the total excretion of the pesticide was nearly the same (15). A representative sampling of experiments which examined excretion patterns show that thyroprotein feeding caused no effect on the rate of decline of DDT or dieldrin from lactating cows treated for 30 days and 56 days, respectively (15, 115). Heptachlor fed at 0.28 - 0.95 ppm on hay for 30 days caused a rapid rise in milk levels of heptachlor epoxide, reaching a plateau at 10 days, which lasted 20 days. Upon withdrawal of the contaminated feed there was a rapid decline in heptachlor epoxide levels. Twenty to twenty-nine percent of the total intake was excreted in the milk (81). The carbamate Temik® was concentrated in milk to only 1/100 that in the feed, with an excretion distribution of 1, 92, and 3% in milk, urine and feces respectively (52). The herbicide Bromacil® is excreted only in the milk, with highly variable daily concentrations, but 10 times more is excreted in the evening milking than the morning (66). The excretion of

HEOD is reported to occur primarily in the milk (15). Dairy cows fed 10 ppm HEOD for 112 days contained 1.78 ppm HEOD in the whole milk. At one day past withdrawal the level had declined to 1.26 ppm followed by 0.47 ppm at 21 days (60). A single dose of DDT caused a rapid initial rise to 340 ppm in milk fat, then declined to a 6.0 ppm plateau to reach 2.5 ppm in 15 weeks (42). Some of the "metabolically unreactive" compounds such as DDT, DDE and dieldrin have been shown to be excreted in milk at about 1% per day (103). These data suggest that the excretion of foreign compounds is a complex phenomenon. Furthermore, the body is able to adapt to an infinite variety of conditions such that detoxification and eventual elimination of those compounds not essential to basic metabolic process is accomplished. Braund (15) found that contrary to previous reports when animals are contaminated with dieldrin prior to lactation they excrete little of the dose. At parturition the levels in milk are extremely high, reflecting the storage of the high levels which are mobilized upon calving (15). Thus it was reported that milk is the primary excretory route of stored dieldrin in the lactating dairy cow.

There are examples of other pesticide excretion experiments which are of a more basic nature. The amount of dieldrin appearing in milk depends upon the daily intake and the existing concentration in the fat of the

animal (58). The ratio of HEOD concentration in body fat to that in milk is 12 - 18:1 during feeding, but upon withdrawal, milk depends on stored HEOD in body fat for the HEOD and the ratio increases to 55:1. Body fat concentration of HEOD declined 30%, HEOD in milk by 80% in six weeks of decontamination. Correlations have been established, respectively, (51, 96) for body fat and milk fat concentrations (r^2 = .96) and pesticide consumed and milk fat concentration (r^2 = .97).

The above experiments did not investigate the mechanisms of metabolism. However, there are two good examples of a systematic approach to the mechanism of metabolism of xenobiotics. First, Casida (30) presented a comprehensive account of the use of radioisotopes to study the metabolism, degradation and mode of action of radiotracer chemicals. The use of labelled chemicals is essential to the examination of the complete metabolic fate of a xenobiotic. Second, Robinson et al. proposed a two-compartment pharmacokinetic model to simulate the elimination of HEOD from the rat (134). Partitioning of HEOD between blood and tissues is dependent upon the lipophilicity of HEOD. The model proposes the circulating blood and liver constitutes one compartment. The rate of transfer from the compartment is dependent upon the concentration of HEOD in the compartment. The peripheral compartment is conceived to be passive storage areas, contrasted with the central compartment where

biotransformation occurs. The authors conclude ". . . the rate-controlling factor for the elimination of HEOD from the whole body, after the initial period of rapid change in the central compartment, is the transfer of HEOD from the peripheral compartment (of which the adipose tissue is part) to the central compartment." This simplified model of a highly complex series of events may be confounded by the inductive effect that the xenobiotic may have on the liver, thereby altering storage and excretion rate constants.

Also, Hayes (70) and Heath and Vandekar (74) have suggested that most of the excretion of dieldrin, dieldrin-derived material, and most residual chlorinated hydrocarbon insecticides is via the feces. In addition, regardless of route of administration, DDT excretion in feces exceeds that in the urine (70). All metabolites of HEOD in rats (113) and sheep (75) were present in several-fold greater amounts in feces than were the same forms in the urine. Biliary excretion of the compounds most likely accounts for the substantial amount appearing in feces. Thirteen percent of an intravenous HEOD dose appeared in the small intestine in the form of hydrophilic compounds (117). Enterohepatic circulation of dieldrin was demonstrated by total cannulation of the bile duct, which increased the amount of HEOD excreted from 3 up to 10% of the total HEOD-derived material excreted (74).

More recent work has shown that dieldrin metabolites are conjugated in the liver and the conjugates are excreted in the bile (74, 75, 113, 114). Trans-dihydroxyaldrin was conjugated by liver microsomes (40-50% of the total label). The metabolites formed in vitro were shown to be identical to those produced in vivo (113). Others have shown that over 90% of labelled dieldrin injected i.v. appeared in the feces of rats and in the bile of bile-fistulated animals, suggesting that most of the intravenously administered dieldrin (0.25 mg/kg body weight) in the feces was hepatically derived (33). In intact animals, 50% of the dose was excreted within three days (33). In support of the above, another recent study has indicated that HEOD degradation does occur resulting in up to 18% of the dose being excreted per day in feces compared to 8% in the urine. In all animals studied, fecal excretion of the dose was greater than urinary (75).

b. Induced biotransformation and excretion.--Many chemicals are known which induce hepatic microsomal drug metabolizing enzyme systems and also cause a proliferation of smooth endoplasmic reticulum in the cell (16, 31, 34, 35, 36, 39, 62, 63, 124, 142, 145, 163). Of primary concern to this author is the effect of drugs and/or pesticides on the induction of hepatic mixed-function oxidases that metabolize xenobiotics. An excellent review of the mechanism and consequences of enzyme induction has

been presented by Conney (35). Some of the drugs which have caused induction are the barbiturates, diphenylhydantoin, tolbutamide, phenylbutazone, aminopyrine, methylcholanthrene and benzpyrene. This group includes hypnotics, analgesics, tranquilizers, anticonvulsants, alkaloids and antihistiminics. In addition, some steroid hormones induce liver enzymes.

In addition to the above compounds which affect liver enzymes, the chlorinated hydrocarbon insecticides have also been shown to induce microsomal mixed-function oxidases (36, 55, 61, 62, 63, 64, 67, 94, 143, 145). For example, DDT is known to induce hepatic drug metabolizing enzyme systems which will degrade DDT, the inducer. The similar effect by drugs is borne out by the decreased hexobarbital sleeping times noted in animals chronically administered a drug (16, 34, 35, 57, 146). This effect on the duration of drug action was used in early studies as the measure of microsomal enzyme induction. The "no effect" level of DDT induction of microsomal epoxidation enzymes has been established at 2.0 ppm in the diet of 6-week old male rats fed 14 days (61). Street et al. established that oxidase and O-demethylase levels were induced by between 1 and 5 ppm DDT or dieldrin in the diet (146). This study was conducted to better understand the potential hormonal imbalance which may be affected by low levels of chlorinated pesticides in the

environment. Street has also established that the interaction of one pesticide with one or more others can greatly alter normal metabolism (142, 145). The interaction of DDT with dieldrin to minimize storage and/or accelerate metabolism of HEOD (31, 142, 145) demonstrates a toxification effect by DDT which results in a subsequent detoxification of HEOD. Enhanced levels of mixed-function oxidases appear to be responsible for the hydroxylation of several compounds and their subsequent increase in polarity, which enables conjugation and excretion to occur. The effect that each pesticide has on the stimulation of its own degradation is difficult to measure, but Richardson et al. (110) suggested that 100 ppm HEOD in the diet used in their experiments may have caused proliferation of smooth endoplasmic reticulum, hence oxidizing activity. It can be seen from previous work (146) that 1-5 ppm dieldrin induces several enzyme systems, hence their hypothesis would appear to be correct.

Phenobarbital administered at the level of 75 mg/kg body weight i.p. has been shown to be the most effective stimulus for increasing bile flow. The drug caused a 50% increase in bile flow in rats which was higher than all drugs tested (97). The acceleration of biliary flow causes a concomitant increase in biliary excretion and plasma disappearance of several classes of drugs (97, 98, 99). This effect occurs after one day of treatment,

is maintained for at least three weeks, and remains elevated for five days after withdrawal of phenobarbital. A dose as low as 30 mg/kg body weight daily in rats produced in two days a biliary flow which was significantly greater than controls. In addition to the increase in bile flow, there was an increase in the wet weight of the liver (97). Klaassen and Plaa (99) suggest, that in the case of sulfobromophthalein (SBP) the effect of phenobarbital on SBP plasma disappearance is not mediated through its biotransformation by the hepatic microsomal enzyme system. Instead, the effect was accomplished by the combination of the acceleration of biliary flow and the stimulation of glucuronide conjugation. Also, enhanced maximum rates of transport of iodocyanin green and bilirubin via increased conjugation have been demonstrated.

Thus, it is evident that drugs and pesticides play important roles in affecting homeostasis of a variety of organisms. In particular, the enhancement of normal biotransformation by stimulating degradative enzymes in several tissues, especially in the liver, offers a promising means for effectively dealing with pesticide contamination.

8. Toxicity

Acute toxicity of various chlorinated hydrocarbon pesticides is presented as the minimum dose required to

cause death in 50% of the test population, usually mice or rats (LD_{50}). Excellent summaries have been reported by O'Brien (61) and Soto and Deichmann (141) for LD_{50} values of cyclodiene insecticides. The LD_{50} for HEOD given orally to rats ranges from 38-87 mg/kg body weight, and 74-120 mg/kg for the mouse, the lower values applying to females, the higher levels to males. The same relationship was observed by Heath and Vandekar (74) but with levels of 50.3 mg/kg for females and 64.5 mg/kg for males. A comprehensive study of the toxicity not only of HEOD, but of a photoisomer of HEOD in several species revealed that although the photo-isomer was more acutely toxic than dieldrin, the extent of its presence in the environment "does not represent an overall increase in the toxicological significance of residues from the use of dieldrin (26)."

Threshold levels of pesticides in blood for toxicity have been difficult to establish, primarily because of the paucity of agreement of the measure to be used to define toxicity (91). Levels of HEOD in blood which produce a convulsion in man and animals were in the range of 0.15 - 0.20 ppm in the blood (25). Blood concentration of HEOD which caused a 40% reduction in food consumption by dogs ranged from 0.37 - 0.39 ppm; a 10% decrease in body weight, 0.38 - 0.50 ppm; and, at the time of first observed convulsion, 0.74 - 0.84 ppm (91). Differences between the levels in these studies (25, 91) are

attributed in part to differences in time intervals between dosing, intoxication and blood sampling. It was concluded that "a threshold level of dieldrin in the blood which, when exceeded, results in an intoxication, appears to be reasonable . . . regardless of the type or duration of exposure to dieldrin" (91).

The interaction of nutritional status and susceptibility to insecticide poisoning has been investigated extensively (10, 11, 12, 13, 14, 149). In essence, the less satisfactory the nutritional status, e.g., protein-deficiency, the more susceptible the subject is to toxic effects of insecticide chemicals. In addition, increased levels of pesticides mobilized from fat during reduced food intake can cause toxicity, but rarely do permanent lesions result (74).

9. Decontamination

A review of normal rates of decontamination or depletion of body stores in several species has been presented above. Several attempts have been made to accelerate excretion and clearance of stored and circulating pesticides from mammals (9, 15, 16, 17, 18, 19, 31, 49, 57, 79, 103, 147, 149, 158, 159). The reader is referred to three excellent reviews on the subject (106, 144, 151).

Two general approaches have been taken. The first was through the action of drugs on the liver which induces detoxification enzyme systems which should enhance

excretion. Studies have been conducted using drugs such as heptabarbital (16, 17, 31), hexobarbital (57), phenobarbital (19, 159), aminopyrine (31), dephenylhydantoin (49), methionine (149), vitamin A (79), thyroprotein (15, 18, 103, 147), and inositol (150). The second approach to decontamination was the use of an absorbent such as activated carbon to trap pesticides in the gastro-intestinal tract, prevent reabsorption, and thereby increase fecal excretion.

The discovery in this laboratory that dieldrin, subsequent to intravenous administration, was recycled from the blood to the gastro-intestinal tract via saliva, bile and pancreatic juice in several species, illustrated that the clearance of a pesticide from the body would not entirely follow normal routes of excretion (37, 38). Reabsorption of pesticides from the gut may then prolong their half-life in the body, but there are also advantages to recycling. First, recycling means that portion is not being stored, but rather, is mobilized from tissue storage. The compound should then be able to be adsorbed within the gut for excretion in the feces. This would shift the equilibrium even further away from storage and thereby accelerate the decline in total body burden.

Activated carbon has been used successfully to adsorb recycled and exogenous dieldrin in the gut of the bovine, sheep and goat (158, 159). The combination of

phenobarbital and activated carbon has been shown to accelerate the decline of dieldrin in milk from cows accidentally contaminated (19).

The purpose of this study was to obtain information on the effect of phenobarbital on the elimination of HEOD from the lactating dairy cow and to account for the total dose of the contaminant. Braund (15) could account for 50% of a chronic dose of dieldrin as HEOD, and speculated that the majority of the remainder was converted to metabolites. The second part of the study to be reported later will examine the extent to which HEOD is metabolized and identify the major forms produced.

CHAPTER III

EXPERIMENTAL PROCEDURES

A. Experimental Design

Eight lactating cows were selected from the Michigan State University dairy research herd. Averaging 256 days of lactation and 47 months of age, the cows were assigned to treatment groups according to average daily milk yield. Uniformity of physiological state was thereby achieved, which would minimize bias of total pesticide excretion data.

The duration of the experiment was six weeks. This was divided into two three-week periods (see Figure 1). Animals were assigned to the following groups:

Period I - 3 weeks	Designation in text
a. Control - dieldrin only - 4 cows	Control
b. Treatment - dieldrin + phenobarbital - 4 cows	PB
Period II - 3 weeks	
a. Control - dieldrin only - 2 cows	Control - C
Control - no dieldrin - 2 cows	Control - D
b. Treatment - dieldrin + phenobarbital - 2 cows	PB - C
Treatment - no dieldrin + phenobarbital - 2 cows	PB - D

During contamination, each animal received the principle active ingredient in dieldrin, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo,5,8-dimethanonaphthalene, abbreviated HEOD. The HEOD was greater than 99% pure and was a gift of Shell Chemical Co.[1]

Each day animals were given a gelatin capsule orally with a balling gun which contained 15.0 gm chromic oxide. In addition, the following was administered daily by capsule to the designated cows:

HEOD: 0.10 mg/kg body weight

Phenobarbital: 10.0 mg/kg body weight

Intake of HEOD and phenobarbital was based on body weights of cows taken for two consecutive days at weekly intervals. The weights were averaged and then used to determine the dosages for the following seven days. The gelatin capsules for each animal were made up for the next week containing the appropriate doses.

B. Dietary Treatments

All cows received a ration of 4.54 kg alfalfa hay, 9.08 kg urea-treated corn silage and sufficient concentrate to meet requirements for maintenance and lactation. Concentrate levels were adjusted weekly based on the previous week's daily milk yield. Daily weights of each feed offered and unconsumed were recorded for each cow.

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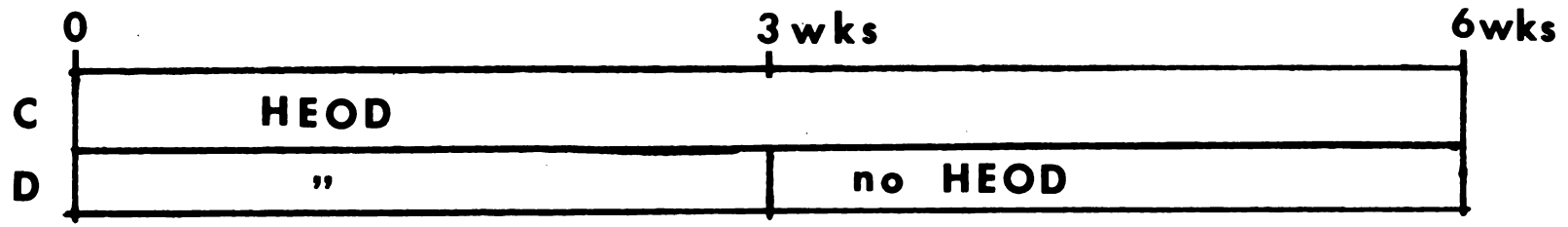
[1]Shell Chemical Co., New York.

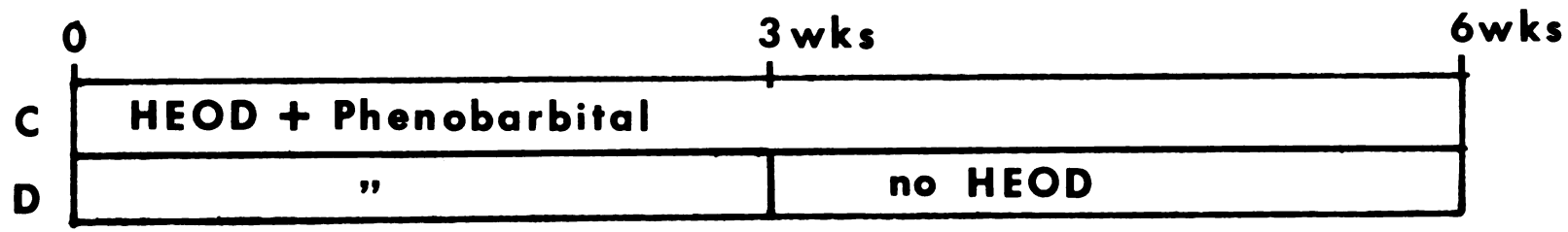
Figure 1.--Experimental design

EXPERIMENTAL DESIGN

GROUP C



GROUP PB



All animals were housed in the same stanchion barn throughout the experiment. With the exception of weeks 2 and 6, when total urine production was measured, the cows were turned out to exercise 1-2 hours each day.

C. Sample Collection

1. Feed

Three times per week, 1-2 kg of each feed were obtained at the time of feeding. For hay, small grab samples were made from several bales at the time of feeding and were composited weekly. Each feed sample was placed in a polyethylene bag, sealed, and frozen at -20°C until analysed for HEOD and dry matter.

2. Milk

In the evening of each day and the morning of the next, the milk from each cow was weighed, thoroughly mixed, and an aliquot (1%) removed for a daily composite for % milk fat and HEOD analyses. In addition, approximately 2 liters of milk were saved from each cow at each milking as a reserve for subsequent HEOD metabolite analyses. The milking machine buckets and claws were thoroughly rinsed between cows in order to minimize cross-contamination. Milk fat percentage was determined by the Babcock method on each daily composite. Remaining samples were then treated with 3-4 drops of formaldehyde and stored frozen at -20°C until extracted for HEOD.

3. Blood

Five samples from each animal were obtained at approximately weekly intervals except for samples obtained at the time of fat biopsy. Samples were drawn from the tail or mammary veins into 15 ml vacuum tubes containing sodium heparin as an anticoagulant. They were then frozen at -20°C until extraction for HEOD analysis.

4. Body fat

External body fat samples were obtained at 0, 2, 4 and 6 weeks of the experiment. Biopsies performed in the scapular region, were alternated at each two week period between left and right sides in order to minimize trauma and infection. Weights of 10-30 grams were obtained and attempts were made during surgery to avoid removing fat from a previous biopsy site. The first biopsy on each side went high on the scapular fat pad; the second went low. A local nerve block was achieved using 2% procaine + epinephrine.

Internal body fat samples were also obtained at the time of the last biopsy. Samples of omental fat and abdominal fat from cows in each group were surgically removed during the laparotomy performed to facilitate a liver biopsy. Samples were stored in small plastic bags and frozen at -20°C until extraction for HEOD analysis.

5. Feces

Daily grab samples of feces were taken from each cow, placed in plastic bags and stored frozen at -20°C until extraction for HEOD, dry matter and chromic oxide determinations.

6. Urine

During weeks 2 and 6 of the experiment total urine collections were made for periods of 5 and 7 days respectively. Two-liter samples were saved from each day's collection from each cow for later compositing. Urine collecting bags made of polyethylene were surgically attached to the periphery of the vulva and sealed with branding cement. At the base of each bag a small funnel was sealed and connected to a rubber hose which drained into a polyethylene-lined stainless steel container. Daily urine production was weighed each morning of the total collection period.

On the days when urine was not totally collected, attempts were made to take one urine sample from each cow. Vulvular stimulation usually induced urination. After total collection periods the vulva was too tender for urinary stimulation. Over the experimental period, urine was obtained on the average every other day from each cow.

Each sample was treated with formaldehyde to contain 0.1% in the final sample as a preservative. All samples were then thoroughly mixed and stored frozen until later HEOD analysis.

7. Liver

Laparotomies were performed at the time of the last biopsy for shoulder fat. This was the method of choice to facilitate removal of 20 to 30 grams of liver from each cow with a minimum of trauma. Each sample was immediately homogenized in 1.15% KCl buffer containing 0.02% nicotinamide (4 ml buffer per gram wet tissue). The homogenate was then centrifuged at 15,000xg for 15 minutes. The supernatant was decanted to cellulose nitrate centrifuge tubes for centrifugation at 105,000xg for 1.5 hours. The supernatant from centrifugation was decanted from the microsomal pellet. The microsomal pellets were suspended in 0.1 M sodium phosphate buffer in 50% glycerol, pH 7.5, at the rate of 1 ml buffer per 1 gm tissue homogenized. The suspension was made using a teflon-glass tissue grinder, then decanted into a screw-capped test tube, gassed with pure nitrogen, sealed and stored frozen at -20°C until analyzed.

D. Sample Extraction

All sample extractions were performed in this laboratory employing methods previously established and found applicable to the particular type of samples

studied. References to the procedure are duly noted and the method as well as the modifications employed are described. All reagents used were reagent grade; all solvents were reagent grade or Nanograde® [2] and were routinely checked for interference with HEOD analysis.

1. Feed

a. HEOD.--Ten to twenty grams of each feed was placed in a 250 ml ground-glass stoppered erlenmeyer flask to which was added respectively, 10, 2 and 10 ml of water, hexane and isopropyl alcohol. This mixture was shaken for 30 minutes on a Burrell Wrist-Action Shaker, Model CC [3] set at 10. The hexane layer was then aspirated off, dried over anhydrous sodium sulfate and 1-2 microliters of the extract were injected into the chromatograph.

b. Percent dry matter.--Fifty to one hundred gram samples of each feed were placed in a forced air oven set at 95°C for 24 hours, then removed and placed in a desiccator to cool and then rapidly weighed.

2. Milk

a. HEOD.--Milk samples were warmed to room temperature then thoroughly agitated and 1.0ml of each was immediately removed by volumetric pipet and placed in 16x125 mm screw-capped tubes to which was added 2.0 ml

[2] Mallinkrodt Chemicals, St. Louis, Missouri.

[3] Burrell Corporation, Pittsburg, Pennsylvania.

ethanolic potassium hydroxide (EtKOH) prepared by dissolving 25 grams of KOH in 15 ml of distilled water and diluted with 85 ml of absolute ethanol. The tubes were then sealed with teflon-lined screw caps and placed in a water bath at 75°C for 15 minutes during which time the tubes were agitated 2-3 times to insure complete mixing for hydrolysis. The tubes were removed and cooled to room temperature. After cooling, 1 or 2 ml of hexane was added, the tubes shaken vigorously by hand for three 1-minute periods permitting the layers to separate between shakings. All samples were then routinely centrifuged at 1700 xg for 15 minutes to remove any emulsions that may have formed and to provide complete separation. One to three microliters of the hexane layer were then injected into the chromatograph for HEOD analysis. This procedure is similar to that reported by Crosby and Archer (41).

b. Percent milk fat.--The standard method of milk fat percentage determination is the Babcock procedure. This technique was used on all daily samples, and is the same method used by DHIA.

3. Blood

The extraction of blood was essentially the same as the extraction of milk, except that 1.0 ml blood were used, and 2 ml distilled water were added after extraction with hexane. The tubes were shaken again and then

centrifuged at 1700 xg for 15 minutes. One to three microliters of the hexane layer were injected into the chromatograph for HEOD analysis.

4. Body fat

Body fat samples were warmed to room temperature and 1-2 grams were weighed and placed in a teflon-glass tissue grinder. The sample was then ground in hexane and transferred to a 16x125 mm screw-capped test tube to a final dilution of 5 ml hexane to 1 gm of fat. Approximately 0.5 gm anhydrous sodium sulfate were added and the tube shaken vigorously for 30 seconds. The tubes were then centrifuged at 1700 xg for 15 minutes to clarify the solution. Appropriate dilutions were made as 1-2 microliters of the hexane layer were injected into the chromatograph without prior cleanup.

5. Feces

a. HEOD.--Upon thawing, each fecal sample was thoroughly mixed to provide a uniform sample, then 1-5 grams were weighed into a 16x125 mm screw-capped tube. A 1:1 mixture of isopropyl alcohol:distilled water was pipetted into the tube at the rate 2 ml per gram of wet sample. The tube was capped and shaken vigorously for 3-5 minutes. One to two milliliters of hexane were pipetted into the tube which was then capped and shaken vigorously for 3-5 minutes with 2-3 interruptions to

permit layers to separate. All tubes were then centrifuged for 20 minutes at 1700 xg. One to three microliters of the hexane layer were injected into the gas chromatograph after appropriate dilutions, in order for the values to fall within the linear range of the instrument.

b. Percent dry matter.--A 20-30 gram aliquot of each daily sample of wet feces was placed in an aluminum drying dish, weighed and dried in a forced air oven at 100°C for 48 hours. After drying the sample was removed and placed in a dessicator to cool, then rapidly reweighed for dry weight. Samples were stored at 60°C until subsequent chromic oxide analysis.

c. Chromic oxide determination.--One to two grams of each sample used for percent dry matter determination were placed in a 100 ml volumetric flask to which was added 5.0 mg sodium molybdate (0.1 ml of 50 mg/sodium molybdate/ml double-distilled water) and 10.0 ml concentrated nitric acid. The flask was heated slowly on a hot plate until the initial foaming stopped and vigorous boiling occurred. Boiling was then continued for 10 minutes, the flask was cooled and 5 ml 70% perchloric acid added. This second digest was continued until 1-2 minutes past the point when the predominantly green color changed to yellow, orange or red (the color at this point is concentration dependent). During each digestion, the flasks were frequently swirled. Total time for the second

digest was usually around 20 minutes. The flask was then cooled and made up to volume with water and mixed well. The precipitate formed was permitted to settle, then each sample was pipetted into spectrophotometer tubes and read at 440 mu wave length on a Coleman Jr. II spectrophotometer.

Standards were prepared by digesting 0, 10, 40, 70, and 100 milligrams of Cr_2O_3 in nitric and perchloric acids, and making final concentrations of 0, 10, 40, 70, and 100 ug/ml. These levels were checked against the same levels of potassium dichromate in double-distilled water. The zero level was used as the reagent blank; all samples were read against this solution. Recovery data indicated that Cr_2O_3 standards gave satisfactory results. Standards prepared this way provided constant readings for as long as 4 months, and were linear with each analysis. The concentration of each sample was divided by its dry weight, the result of which was divided into the 15 grams Cr_2O_3 given each cow to obtain the dry matter excreted per day. Hence, concentration of Cr_2O_3 was inversely proportional to the dry matter excretion. The method employed was that reported by Kimura and Miller (95). A more recent investigation of the absorption maxima for the color reaction reported that a wave length of 350 mu was more sensitive than 440 mu (45). This difference was also noted in the present study. However, 440 mu gave

more repeatable results. The sensitivity of the method using 350 mu was not needed in this experiment due to the high concentration of chromic oxide used.

6. Urine

One-hundred milliliter aliquots of urine were tracted three times with 50 ml portions of hexane. The hexane extracts were concentrated on a rotary evaporator under vacuum at 50°C and brought up in 1-10 ml of hexane. The alkaline hydrolysis procedure for milk and blood was also used.

7. Liver

a. HEOD.--Liver tissue was analyzed for HEOD according to the alkaline hydrolysis procedure used with milk and blood. One to two ml of 4:1 homogenate were added to 2-4 ml ethanolic KOH and the mixture was heated for 15 minutes at 75°C and cooled and extracted with 1-2 ml pentane. The pentane extract was injected into the gas chromatograph with no prior cleanup.

b. Hepatic drug metabolizing enzyme activity.--The enzyme activity was determined by measuring formation of formaldehyde from aminopyrine by nitrogen-demethylase (122). The enzyme incubation mixture contained 0.1M phosphate buffer pH 7.4, aminopyrine (10 mM), $MgCl_2$ (6 mM), isocitric acid (10 mM), NADP (0.5 mM), isocitric acid dehydrogenase (0.05 units per ml) and microsomal protein (0.1 to 1.0 mg/ml). Protein was determined by the method

of Lowry (107). The reaction mixture was incubated 15 minutes and stopped by adding 10% trichloroacetic acid. The specific enzyme activity was expressed as milli-micromoles of formaldehyde formed per milligram of protein per minute.

E. Electron-capture Gas-liquid Chromatography

All HEOD analyses employed a Varian Aerograph 1520B[4] dual column gas-liquid chromatograph equipped with electron-capture detectors containing tritium (H^3) foils. The recorder was a dual-channel Westronics Model LD 11A[5] operating at 1 mv range and chart speed of 20 in. per hour. The most frequently used columns were 5' x 1/8" stainless steel containing 5% QF-1 on 100/120 mesh Varaport 30, or 5% QF-1 on 100/120 mesh DMCS-AW Gas-Chrom Q. Operating temperatures were: injector, 230°C; column, 195°C; and detector, 195-200°C. Carrier gas was high purity nitrogen using a flow rate of 40-50 cc per minute through the detector, with a head pressure of 65 p.s.i. The retention time of HEOD under these conditions ranged from 3-5 minutes. Recoveries of HEOD were determined by fortification of the samples prior to extraction with amounts of HEOD roughly equivalent to the amount in the sample such that the final concentration would be twice that of the unfortified sample. Identification was made by matching

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[4]Varian Associates, Walnut Creek, California.

[5]Westronics, Inc., Fort Worth, Texas.

retention times and peak characteristics with pure standards of HEOD. The minimum measurable amount of HEOD was 10^{-12} gram (1 picogram) at maximum sensitivity of the instrument.

HEOD standards were prepared in glassware thoroughly rinsed with solvents. Concentrations ranged from 0.01 ppm to 2.0 ppm HEOD in hexane. Injections were made with a Hamilton 701 microsyringe, using 1-5 microliters per injection. Low-bleed septums were changed at least once daily. Concentration of HEOD was determined by a standard curve utilizing a 10-fold range of standards.

CHAPTER IV

RESULTS

During the entire six-week feeding period, there appeared to be no toxic effect by either HEOD or phenobarbital, nor did there appear to be a synergistic effect manifested between the two chemicals on normal maintenance and production parameters. Period I indicates weeks 1-3 of treatment; period II, weeks 4-6.

The average age and day of lactation on the first day of the experiment are presented in Table 1. Differences were small, with little effect on performance.

A. Feed Intake

Feed intakes expressed on a dry matter basis are presented in Table 2. Dry matter consumption tended to decline regardless of treatment. The highest intakes occurred in the decontaminated control group (C-D). The decontaminated phenobarbital group (PB-D) had the lowest intakes, expecially for hay. Groups C-D and PB-C consumed more grain per day, whereas the remaining two groups preferred hay (Table 3). This may account for the differences in consumption per unit of body weight (Table 4) observed for groups C-D and PB-C. The control group consumed

TABLE 1.--Average age and day of lactation on first day of experiment.

	C-C	C-D	PB-C	PB-D
Average age (months)	47	45	51	44
Average day of lactation	263	248	237	244

TABLE 2.--Dry matter consumption.

Week	C-C	C-D	PB-C	PB-D
	kg DM/cow/day			
0	16.93	16.97	18.31	15.81
1	15.71	18.02	18.78	16.14
2	14.93	18.06	15.36	14.67
3	15.43	18.44	16.88	13.73
4	14.89	18.15	13.81	13.81
5	14.77	17.65	15.75	13.38
6	14.98	17.41	15.49	13.42
mean (0-6)	15.38±0.29[a]	17.81±0.19	16.34±0.66	14.42±0.43

[a] Standard error of the mean.

TABLE 3.--Feed DM intake as a percentage of total DM intake.

Week	C-C	C-D	PB-C	PB-D
			Grain	
0	58.9	61.8	63.3	59.1
1	55.8	63.6	65.6	61.2
2	53.4	63.6	61.5	55.8
3	55.5	62.6	66.8	58.4
4	53.5	62.5	56.7	56.6
5	53.5	61.8	61.1	55.2
6	56.1	63.2	60.0	55.3
(0-6) mean	55.2±0.8[a]	62.7±0.3	62.1±1.3	57.4±0.9
			Silage	
0	18.1	18.1	16.8	19.4
1	19.5	17.0	16.3	18.7
2	20.6	17.0	18.8	20.6
3	19.8	16.6	16.1	20.7
4	20.6	16.8	21.0	22.2
5	20.8	17.4	19.2	22.8
6	20.2	17.6	19.3	22.6
(0-6) mean	19.9±0.4	17.2±0.2	18.2±0.7	21.0±0.6
			Hay	
0	23.0	20.1	19.9	21.5
1	24.7	19.4	18.1	20.1
2	26.0	19.4	19.7	23.6
3	24.7	20.8	17.1	20.9
4	25.9	20.7	22.3	21.2
5	25.7	20.8	19.7	22.0
6	23.7	19.2	20.7	22.1
(0-6) mean	24.8±0.4	20.1±0.3	19.6±0.6	21.6±0.4

[a] Standard error of the mean.

TABLE 4.--Dry matter intake as a per cent of body weight.

Week	C-C	C-D	PB-C	PB-D
	----------% BW----------			
0	2.87	2.76	3.00	2.72
1	2.63	2.89	3.02	2.71
2	2.49	2.87	2.39	2.47
3	2.50	2.84	2.60	2.25
4	2.37	2.80	2.08	2.23
5	2.37	2.66	2.38	2.18
6	2.36	2.65	2.38	2.15
(0-6 mean)	2.51±0.07[a]	2.78±0.04	2.55±0.13	2.39±0.09

[a] Standard error of the mean

about 1 kg more dry matter per day than the phenobarbital group. Also when expressed as a per cent of body weight, the intakes vary considerably (Table 4). Groups C-D and PB-D consumed the most and least amount of dry matter per unit body weight, respectively.

The average per cent dry matter of each feed during the control period and weeks 1-3, 4-6, (periods I and II, respectively) is presented in Table 5. A decline in dry matter consumption occurred over the entire experiment which was primarily attributable to the reduction of grain offered in response to the decline in milk production.

It was anticipated that feed intakes might increase with phenobarbital feeding. As noted in Table 2 there appeared to be no effect of phenobarbital on feed intake.

TABLE 5.--Per cent dry matter of feedstuffs consumed.

Period	Hay	Silage	Grain
	----------% DM----------		
Control	85.61	34.06	86.35
I	85.60	33.24	85.97
II	85.13	32.85	85.71
(Control II mean)	85.45±0.16[a]	33.38±0.36	86.01±0.19

[a] Standard error of the mean.

Over the entire experiment each group consumed a ration which consisted of the following overall per cent dry matter: C-C, 65.7; C-D, 68.1; PB-C, 66.9; and PB-D, 65.7.

B. Body Weight Changes

Changes in body weight from the first period to the second are shown in Table 6. All animals, regardless of treatment, gained weight over the entire experiment. The animals in the control group averaged a 5.0% increase compared to the phenobarbital-treated group which gained 4.3%.

The expected increase in feed intake by phenobarbital treatment was also expected to produce a concomitant increase in body weight. This effect did not materialize. Rather, the opposite effect was noted.

The ration met all nutrient requirements of maintenance and lactation while roughage intake was kept at

a minimum in order to superimpose a $MgCl_2$ infusion experiment designed to correct slight depressions in milk fat percentage.

TABLE 6.--Body weights of experimental animals.

Period	C-C	C-D	PB-C	PB-D
	----------kg----------			
Control	590	615	611	582
I	605	634	640	600
II	628	656	659	619

C. Dosage

Contamination of the cows at 0.10 mg HEOD/kg body weight/day provided each group with between 59 and 65 mg/cow/day (Table 7). Because of differences in body weight, PB-C received the highest total HEOD intake per day, PB-D the lowest. Gain in body weight caused an increase in total daily and period HEOD intake. These values represent the amount administered to each animal. However, on the fourth day of the experiment, one cow in group PB-D regurgitated the bolus for it was found in the orts the next morning. The data do not include this lost dose. It is assumed that all other doses were retained by all animals for there is no evidence to the contrary.

The contamination level in the feed is shown in Table 8. The total dose administered divided by the

TABLE 7.--HEOD dosage.

Period	C-C	C-D	PB-C	PB-D
	mg HEOD/cow			
I	1334.3	1324.1	1400.3	1252.9
II	1388.3	0	1450.3	0
Total	2722.6	1324.1	2850.6	1252.9
Per day of contamination	61.88	63.05	64.79	59.66

TABLE 8.--HEOD intake.

	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

amount of dry matter consumed during the contamination period gives the concentration on a dry matter basis. When corrected for per cent moisture or placed on an "as fed" basis, group C-D continued to be contaminated at the lowest level per unit of feed intake. This appears to be

primarily a function of not only consumption of more hay and grain relative to silage but also of a lower daily dose of HEOD.

The cumulative mean total dose given to each group is enumerated in Table 9. It can be seen that the dose given up to each succeeding week is essentially the same for each group. The quantity of HEOD administered represents the actual amount weighed out to the nearest 0.1 mg, and not the amount calculated for each animal to receive.

TABLE 9.--Cumulative HEOD dose administered.

Week	C-C	C-D	PB-C	PB-D
	----------mg/cow----------			
1	477.6	498.0	495.6	473.4
2	906.9	938.8	944.3	890.8
3	1334.3	1324.1	1400.3	1252.9
4	1774.3	--	1864.8	--
5	2277.4	--	2395.2	--
6	2722.6	--	2850.6	--

D. Milk Production, and Concentration and Excretion of HEOD in Milk

Milk production was quite persistent for each period, except for a marked decline noted during the second period for PB-D (Table 10). The daily milk yields declined throughout the experiment for all treatments.

This decline was expected since all cows were in the sixth to tenth month of lactation when contamination began.

TABLE 10.--Average daily milk production.

Week	C-C	C-D	PB-C	PB-D
	kg/cow/day			
Control	14.80	19.10	19.40	16.80
1	13.95	18.00	19.15	16.30
2	13.40	18.20	15.20	13.80
3	13.60	16.25	16.20	13.50
4	12.95	16.65	13.70	11.45
5	12.50	15.25	14.95	11.65
6	12.15	14.25	15.25	12.05
(1-6) mean	13.09±0.28[a]	16.43±0.63	15.74±0.76	13.13±0.75

[a] Standard error of the mean.

In Table 10 it can be seen that there was a substantial drop in milk production in the phenobarbital-treated groups from week one to week two. This may reflect an adjustment to phenobarbital treatment and the transient decline in dry matter intake. Average daily production throughout the experiment was highest for C-D, lowest in C-C and PB-D. Again, these differences probably are a reflection of energy intake. Milk production may have been been affected by treatment with phenobarbital (Table 11). The average persistency of milk yield on phenobarbital treatment was 75% compared to 83% for the controls. A similar change in milk production

was noted in the first period from the production observed prior to the initiation of treatment. Throughout the experiment the animals receiving the drug appeared to decline at a faster rate than did the control group. This may be a reflection of a decrease in dry matter intake noted for the drug-treated group. The great decline in daily yield for group PB-D is primarily a reflection of one cow's production dropping to one-third the control level while the other cow maintained normal production. No abnormality was noted for the first animal.

TABLE 11.--Percentage decline in milk production.

Period	C-C	C-D	PB-C	PB-D
	----------% of control----------			
Control	100.0	100.0	100.0	100.0
I	92.8	91.6	86.9	86.5
II	85.3	81.1	79.6	69.8

In order to obtain a different view of milk production standardized to eliminate body size and level of production effects, the daily amount of milk produced per kg of dry matter consumed is presented in Table 12. These values represent a measure of efficiency of conversion. All animals tended to decline in efficiency as the experiment progressed. However, no notable differences appear between the control and phenobarbital groups. The

averages suggest that possibly PB-C and D were more efficient utilizers of feed, which is supported by the relative differences in preference for grain, i.e., those that preferred grain produced more milk per unit of intake.

TABLE 12.--Ratio-milk produced per day vs dry matter consumed.

Week	C-C	C-D	PB-C	PB-D
	kg milk/kg DM intake			
Control	0.87	1.13	1.06	1.06
1	0.89	1.00	1.02	1.01
2	0.90	1.01	0.99	0.94
3	0.88	0.88	0.96	0.98
4	0.87	0.92	0.99	0.83
5	0.85	0.86	0.95	0.87
6	0.81	0.82	0.93	0.90
(1-6) mean	0.87±0.01[a]	0.92±0.04	0.98±0.01	0.92±0.03

[a] Standard error of the mean.

The concentration of HEOD in whole milk is presented in Table 13. The levels rose rapidly in each group during the first week, continued to increase in the six-week contamination groups, but declined rapidly when HEOD was withdrawn at the end of the third week in groups C-D and PB-D. HEOD concentration declined at a considerably greater rate in PB-D than in C-D. The variable response in HEOD concentration among groups during the first three weeks is almost as great as within-group differences from one week to the next. The lower HEOD levels in milk

observed when contamination ceased appears to be due more to withdrawal of HEOD rather than to drug treatment. The decline in the sixth week for group PB-C may be due to one cow which dried off rapidly.

TABLE 13.--Concentration of HEOD in whole milk.

Week	C-C	C-D	PB-C	PB-D
	ppb			
1	57.6± 6.9[a]	56.6±7.7	64.2± 7.3	52.3± 3.4
2	110.5±13.9	87.7±7.7	89.4± 7.6	135.7±13.3
3	108.7± 9.9	129.8±8.2	90.2± 8.3	124.6± 9.1
4	118.9± 8.3	106.9±6.9	122.6±12.9	89.7± 7.0
5	154.1±11.5	86.4±4.0	147.3±15.3	52.1± 2.9
6	121.6±14.2	50.0±5.7	92.0± 8.9	38.6± 3.1

[a] Standard error of the mean.

In Table 14, daily excretion of HEOD per cow rose rapidly and remained elevated as contamination continued. The most dramatic elevations, in both concentration and excretion, occurred during the first two weeks. Clearance of HEOD via milk appears to be more a function of milk production than HEOD concentration. Whereas phenobarbital appeared to have a slight effect on milk concentration of HEOD, the most pronounced differences due to phenobarbital were noted for weekly excretion data. Levels of HEOD in the milk were lower in group PB-D especially when contamination ceased. This would suggest that phenobarbital both minimizes the amount of

HEOD excreted via the milk and accelerates excretion upon withdrawal of HEOD.

TABLE 14.--Daily excretion of HEOD via milk.

Week	C-C	C-D	PB-C	PB-D
	mg/cow/day			
1	0.80	1.02	1.23	0.85
2	1.48	1.60	1.36	1.87
3	1.48	2.11	1.46	1.68
4	1.54	1.78	1.68	1.03
5	1.93	1.32	2.20	0.61
6	1.48	0.71	1.40	0.47

When the average daily excretion values are combined to obtain period averages the same trends appear (Table 15). When HEOD was withdrawn (groups C-D and PB-D) the excretion of HEOD in milk was 40% less when phenobarbital was administered. The total HEOD excreted by period again suggests the above conclusions (Table 16).

The amount of the total HEOD received during the entire experiment that was excreted in the milk amounted to one day's dose or about 2.5%.

TABLE 15.--HEOD excreted in milk.

Period	C-C	C-D	PB-C	PB-D
	mg/cow/day			
I	0.989	1.577	1.350	1.467
II	1.650	1.270	1.760	0.703

TABLE 16.--HEOD excreted in milk.

Period	C-C	C-D	PB-C	PB-D
	mg/cow/period			
I	20.8	33.1	28.4	30.8
II	38.0	29.2	40.5	16.2
I and II (total)	58.8	62.3	68.9	47.0

E. Concentration of HEOD in Milk Fat and Milk Fat Production

The average per cent milk fat of all groups varied considerably (Table 17). The values at the end of the contamination period for both decontamination groups were high, whereas at the end of the experiment the percentage milk fat was lowest. Across all treatments the percentage milk fat declined, which is possibly a reflection of the modified restricted-roughage ration imposed on all animals. The higher value for PB-D in period I was primarily due to one individual which initially tested very high throughout lactation, but whose percentage milk fat declined rapidly after initiation of the experiment. This depression may have been due to phenobarbital but data from other cows in this experiment do not suggest that the drug affected milk fat content. All other animals, though declining in percentage milk fat, were not as severely depressed as is usually observed with restricted-roughage rations. Certainly, there appeared

to be no synergistic depression by the use of phenobarbital with a restricted roughage diet. The reverse situation does not seem to exist either. That is, the data do not support the possibility that phenobarbital acted counter to the restricted-roughage ration and minimized the depressing effect on milk fat caused by the diet. As was illustrated by Braund (15) changes in milk fat percentage did not significantly alter total excretion of HEOD. His observations seem to be supported by the present experiment. During the course of the experiment, two official tests by the DHIA supervisor were conducted. The official values obtained for milk production agreed very closely with the weights recorded before and after the test. Likewise, the results of our own Babcock test varied no more than ±0.1 percentage point from that obtained by the DHIA tester. Comparison of experimental values with official results not only served as a check on the conduct of the trial, but also indicated that sampling techniques were adequate.

TABLE 17.--Average percentage milk fat.

Period	C-C	C-D	PB-C	PB-D
Control	3.09	2.99	3.73	3.86
I	3.75	3.76	3.36	3.99
II	3.30	2.96	3.38	3.19
I and II (mean)	3.53	3.36	3.37	3.59

Maintenance of milk fat production was achieved in each C group but was markedly reduced in each D group (Table 18). These differences may be due to the ration, but probably are due more to the combination of per cent milk fat decline and decline in milk production seen to a greater degree in the two D treatments.

TABLE 18.--Milk fat production.

Period	C-C	C-D	PB-C	PB-D
	kg/cow/period			
I	10.92	13.02	11.97	12.92
II	10.70	10.24	12.19	8.05
I and II (total)	21.62	23.26	24.16	20.97

The concentration of HEOD in milk fat shows trends similar to those for whole milk concentrations (Table 19). Again, phenobarbital-treated animals had a lower HEOD concentration than the control groups and the rate of decline appeared to be faster due to phenobarbital treatment. These differences did not appear during the first week of the trial. Since differences in milk fat percentage were small, the expected correction of milk concentration for fat content was also negligible.

F. Concentration of HEOD in the Blood

Samples of blood analyzed for HEOD contained very low amounts of the pesticide (Table 20). The values are

TABLE 19.--HEOD concentration in milk fat.

Week	C-C	C-D	PB-C	PB-D
	ppm			
1	1.565	1.545	1.815	1.324
2	2.757	2.440	2.807	3.491
3	3.260	4.080	3.081	3.497
4	3.569	3.032	3.334	3.026
5	4.512	2.994	4.078	1.866
6	4.691	2.319	3.245	1.298

TABLE 20.--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	2.83	1.50
5	5.50	1.37	5.37	1.59

are highly variable and appear to be related more to the duration of HEOD dosage than to any other treatment variable. Analytical problems evolved which caused the values presented to be estimates rather than precise figures. The fact that they are all much lower than usually encountered with the dosage intensity used in this experiment, may be more a function of the large body weight increases, thus shifting equilibrium toward deposition in adipose tissue.

The low value for PB-D in the second week would appear to agree with the high excretion in the feces

during this time, but the levels in the other three groups do not agree with the relationships noted in feces. Certainly, the effects due to withdrawal of the pesticide were rapidly seen. As early as seven days after cessation of the dose blood levels had declined. In the fourth week it appeared that phenobarbital may have accelerated the decline of HEOD in blood, but values had increased again in the fifth week.

G. Concentration of HEOD in Body Fat

The levels of HEOD in body fat from the scapular fat pad are presented in Table 21. Particular care was taken to minimize trauma and to ensure that newly deposited adipose was not used in the samplings from each surgical site. The first samples were removed from the right side of each animal, the second from the left side, each sample being taken from the upper region of the incision. The third biopsy was performed on the right side, the fourth from the left side, both just below the previous biopsy, on the respective side.

No HEOD was detected in control biopsies. As early as two weeks there was considerable variation in the HEOD concentrations. Although the absolute values were different initially, a trend developed by the fourth week, a week after cessation of HEOD feeding in group C-D and PB-D. Upon withdrawal of the pesticide, HEOD was depleted

more rapidly from group PB-D than group C-D. The rise in HEOD concentration was greater throughout the experimental period for group C-C than for group PB-C. In addition, levels in PB groups were consistently lower than corresponding controls. All values are somewhat lower than those observed by Braund (15) using the same level of contamination as employed in this experiment.

TABLE 21.--Mean HEOD concentration in shoulder fat.

Week	C-C	C-D	PB-C	PB-D
	(ppm)			
Control	0	0	0	0
2	1.21	1.78	1.07	1.53
4	1.56	1.65	1.27	1.10
6	2.96	1.35	2.53	1.18

H. Concentration of HEOD in Urine

No HEOD was detectable in urine (<0.1 ppb). Solvent extraction (ether, petroleum ether, hexane, ethyl acetate) extracted no HEOD. However, peaks appeared in the chromatograms which may have been metabolic products of the parent compound. Alkaline hydrolysis was also employed on large quantities of urine, in order to destroy conjugates of HEOD and improve extractability of the compound. Acidification to pH 2 did not appear to enhance HEOD extraction because HEOD is labile in acid conditions.

Daily urine production did not appear to be affected by treatment or stage of lactation (Table 22). Individual variation among animals was considerable, with average daily outputs ranging from 9-29 kg/cow, but variation within each animal was low in each period. One animal in group PB-C had a high urine output throughout the experiment. There was no trend due to time on treatment, with some animals increasing urine output, others decreasing during the progress of the experiment.

TABLE 22.--Daily urine production.

Week	C-C	C-D	PB-C	PB-D
	kg/cow/day			
2	11.3±0.7[a]	14.3±1.8	20.0±3.3	12.1±1.1
6	11.7±0.6	12.4±0.9	19.4±1.6	11.3±0.8

[a] Standard error of the mean.

I. Feces

1. Dry Matter Excretion

Dry matter excretion of feces expressed as kg/cow/day is shown in Table 23. The method used to determine chromic oxide gave recoveries of 93-98%. The concentration of chromic oxide ranged from 1-5 mg/kg dry matter, and was more variable than per cent dry matter. During the six weeks of the experiment the mean dry matter excretion for groups C-C, C-D, PB-C, and PB-D was 8.26, 8.83, 6.14 and 7.78 kg DM/day, respectively. The reason

for the higher dry matter excretion in the control than in the phenobarbital-treated groups is not known. A general decline was seen for both phenobarbital groups whereas the control groups were variable.

TABLE 23.-- Excretion of dry matter in the feces.

Week	C-C	C-D	PB-C	PB-D
	kg/cow/day			
Control	--	--	--	--
1	8.05±0.59[a]	8.74±0.41	7.73±0.47	8.58±0.71
2	7.33±0.58	9.79±0.59	5.34±0.29	8.52±0.58
3	8.70±1.52	7.07±0.81	6.86±1.10	8.80±0.63
4	8.95±0.62	7.75±0.41	6.08±0.70	7.84±0.39
5	8.13±0.48	10.86±1.08	5.12±0.37	7.15±0.46
6	8.41±0.77	8.75±0.85	5.72±0.35	5.77±0.32

[a] Standard error of the mean

2. Percentage Dry Matter

The overall average of percentage dry matter was around 19 with a range of 14-24. Some variation among animals was noted, but very little within-animal variation occurred. There appeared to be no trend or differences due to treatment. Excellent repeatability was obtained within samples under the conditions employed and drying longer than 24 hours gave no significant change in the dry matter values.

J. Fecal Concentration and Excretion of HEOD

Extraction efficiency of HEOD from feces was investigated and a summary of results is presented in Table 24. Previous analyses (15) had been performed on feces that had been oven-dried, ground, subsampled and extracted with a 3:1 mixture of hexane:isopropyl alcohol. Alkaline hydrolysis of dried feces caused interfering peaks to occur during gas chromatography, and at times appeared to produce alteration of HEOD. Regardless of the extraction procedure used, recoveries of HEOD from dried feces were highly variable. A simple change to the extraction of wet feces with the same solvent systems and no addition of water gave improved recoveries. At no time was the improvement in extraction efficiency between wet and dry material less than 390%. Water was added to the tubes during extraction to prevent emulsions from forming and to permit the isopropyl alcohol to solubilize the feces.

TABLE 24.--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

HEOD concentration in feces on a dry matter basis was highest during the first week of treatment for all

TABLE 25.--Concentration of HEOD in fecal dry matter.

Week	C-C	C-D	PB-C	PB-D
	ppm			
Control	0	0	0	0
1	4.611±1.669[a]	3.463±1.075	3.263±1.106	6.400±1.972
2	2.486±0.884	1.442±0.287	2.078±0.328	2.618±0.485
3	2.720±0.640	0.995±0.125	1.296±0.282	1.667±0.289
4	2.048±0.618	0.111±0.026	3.557±0.672	0.092±0.028
5	1.306±0.401	---	1.200±0.185	---
6	1.490±0.169	---	1.433±0.169	---

[a] Standard error of the mean.

cows, except for those in group PB-D which peaked during the fourth week (Table 25). The low level in the third week and the high level in the fourth week for the group PB-C are difficult to reconcile.

Examination of fecal concentration of HEOD (Table 25) and fecal excretion of HEOD (Table 26) indicates that fecal dry matter output was much less variable than concentration or total elimination of HEOD. The same relative differences exist regardless of which measure is used. Phenobarbital-treated animals in the first week moved in feces the most and least amounts of HEOD. Simple averages of each treatment indicate that phenobarbital caused a slightly greater elimination of HEOD in the first two weeks, but lesser amounts than shown for the controls were observed during the following four weeks. Individual variation was sufficient to mask any subtle effects of phenobarbital treatment. Analysis of variance failed to indicate differences between groups in any one week. Of primary interest are the extremely high values observed for each group initially. Usually very little HEOD is seen in the feces, but concentrations achieved in this experiment equal and even surpass those seen when activated carbon is administered.

Tables 27 and 28 present fecal excretion data on the basis of percentage of the dose excreted per week, and cumulative, respectively. It can be seen that these

TABLE 26.--HEOD excretion via feces.

Week	C-C	C-D	PB-C	PB-D
	mg HEOD/cow/day			
Control	0	0	0	0
1	39.73±15.59[a]	29.34±8.14	21.72±7.45	50.90±13.89
2	16.68± 5.75	11.86±1.86	11.61±2.23	22.92± 3.92
3	19.01± 4.77	6.30±0.74	6.20±0.78	13.31± 1.55
4	17.68± 5.41	0.82±0.19	19.09±2.71	0.71± 0.23
5	11.21± 4.01	---	5.49±0.74	---
6	11.23± 0.99	---	7.67±0.76	---

[a] Standard error of the mean.

TABLE 27.--Percentage of weekly HEOD dose excreted in feces that week.

Week	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 28.--Cumulative excretion of HEOD as a percentage of the total amount administered.

Week	C-C	C-D	PB-C	PB-D
	----------%			----------
1	66.5	47.1	35.1	86.0
2	47.9	33.8	27.0	63.7
3	42.5	27.3	21.3	52.7
4	39.0	27.8	23.2	53.1
5	34.3	--	19.9	--
6	31.6	--	18.6	--

values approximate the relations seen in Tables 25 and 26. The elevated excretion of group PB-D during the first week represents 86 per cent of the total dose administered. Almost two-thirds of the dose was excreted by C-C in the first week. A precipitous decline in excretion of HEOD in all groups occurred in the second week when from one-fifth to two-fifths of the dose given that week was excreted in the feces. The amount declined as the experiment progressed with no definitive differences among groups. The decline is reflected in a marked reduction in fecal HEOD when expressed as a percentage of the cumulative dose. At no point in the trial did the amount excreted to date fall below 50% for group PB-D. Total excretion of HEOD and total excreted to date (Tables 29 and 30) also indicate trends similar to the foregoing tables.

TABLE 29.--Total HEOD excretion in feces.

Week	C-C	C-D	PB-C	PB-D
	----------mg/cow/week----------			
1	317.84	234.72	173.76	407.20
2	116.76	83.02	81.27	160.44
3	133.07	44.10	43.40	93.17
4	123.76	5.74	133.63	4.97
5	89.68	--	43.92	--
6	78.61	--	53.69	--

TABLE 30.--Cumulative excretion of HEOD in feces.

Week	C-C	C-D	PB-C	PB-D
	----------mg/cow----------			
1	317.84	234.72	173.76	407.20
2	434.60	317.74	255.03	567.64
3	567.67	361.84	298.43	660.81
4	691.43	367.58	432.06	665.78
5	781.11	--	475.98	--
6	859.72	--	529.67	--

An estimate of the digestibility of the feed consumed is presented in Table 31. The data are calculated from the weekly dry matter consumption divided into the weekly dry matter excretion as determined by the chromic oxide method. Significant differences exist among the mean per cent intake digested. Each group shows a marked depression in digestibility for one week, but these occur at different times for the different groups.

TABLE 31.--Dry matter digestibility.

Week	C-C	C-D	PB-C	PB-D
	----------%			
1	48.8	51.5	58.8	46.8
2	50.9	45.8	65.2	41.9
3	43.6	61.7	59.4	35.9
4	39.9	57.3	56.0	43.2
5	45.0	38.5	67.5	46.6
6	43.9	49.7	63.1	57.0
mean	45.4±1.6[a]	50.8±3.4	61.7±1.8	45.2±2.9

[a] Standard error of the mean.

Point plots of mean daily concentration of HEOD in fecal dry matter are presented in Figures 2, 3, 4 and 5. Wide variations exist from day to day, which are primarily a function of variability of the pesticide movement in the gastro-intestinal tract. Fecal concentration of HEOD appears to be cyclic for each treatment. A change in concentration is manifested every 3-6 days. Even if the days of extremely high concentrations are ignored, there appears to be no definitive differences among groups which could be attributed to treatment rather than individual variation.

K. Body Burden and Distribution of HEOD

The distribution of the HEOD administered to the cows is listed in Table 32. A significant amount of the

Figure 2. Concentration of HEOD in feces of cows contaminated for six weeks.

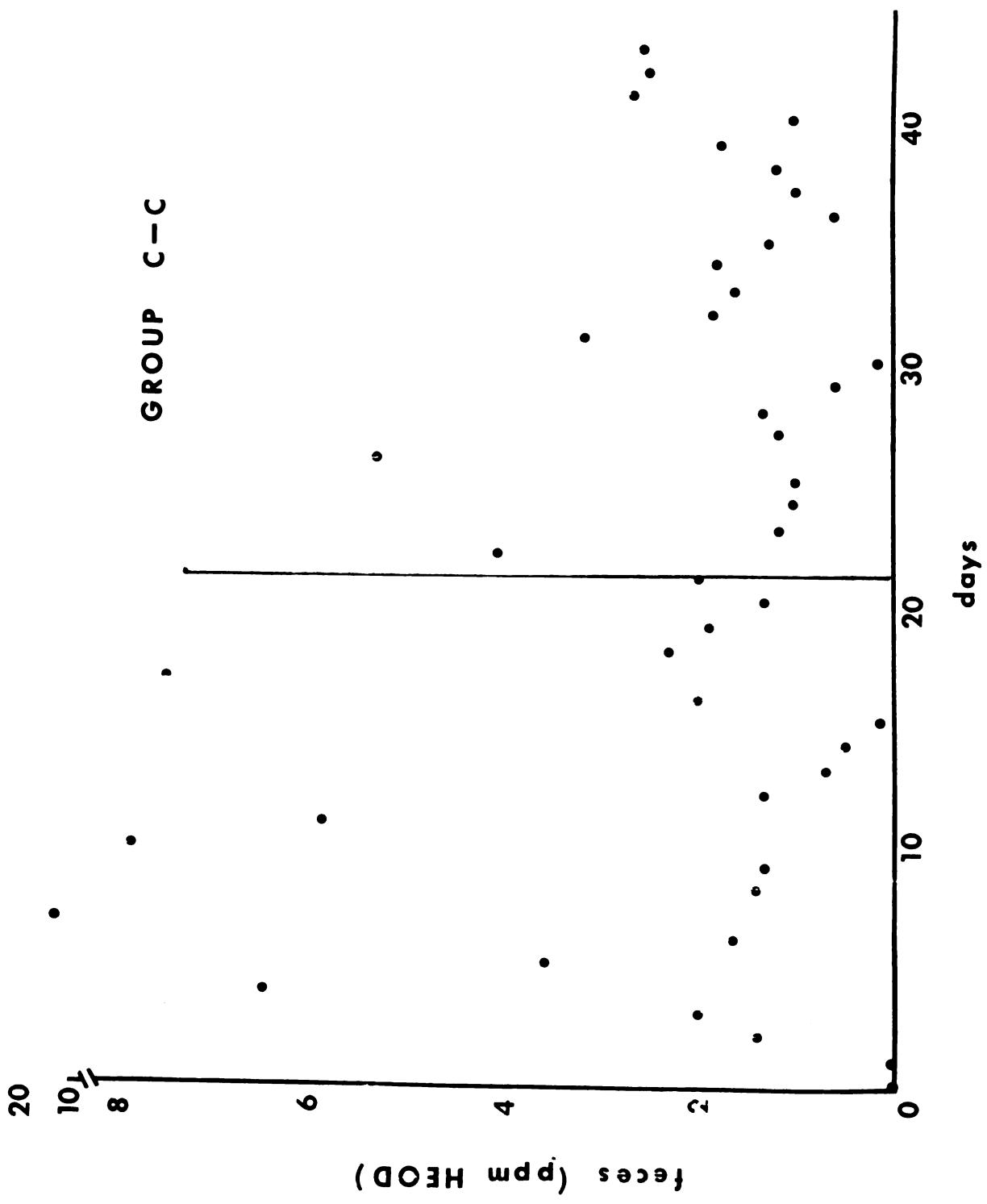
GROUP C—C
feces (ppm HEOD)
20
10
8
6
4
2
0
10
20
30
40
days

Figure 3. Concentration of HEOD in feces of cows contaminated for three weeks.

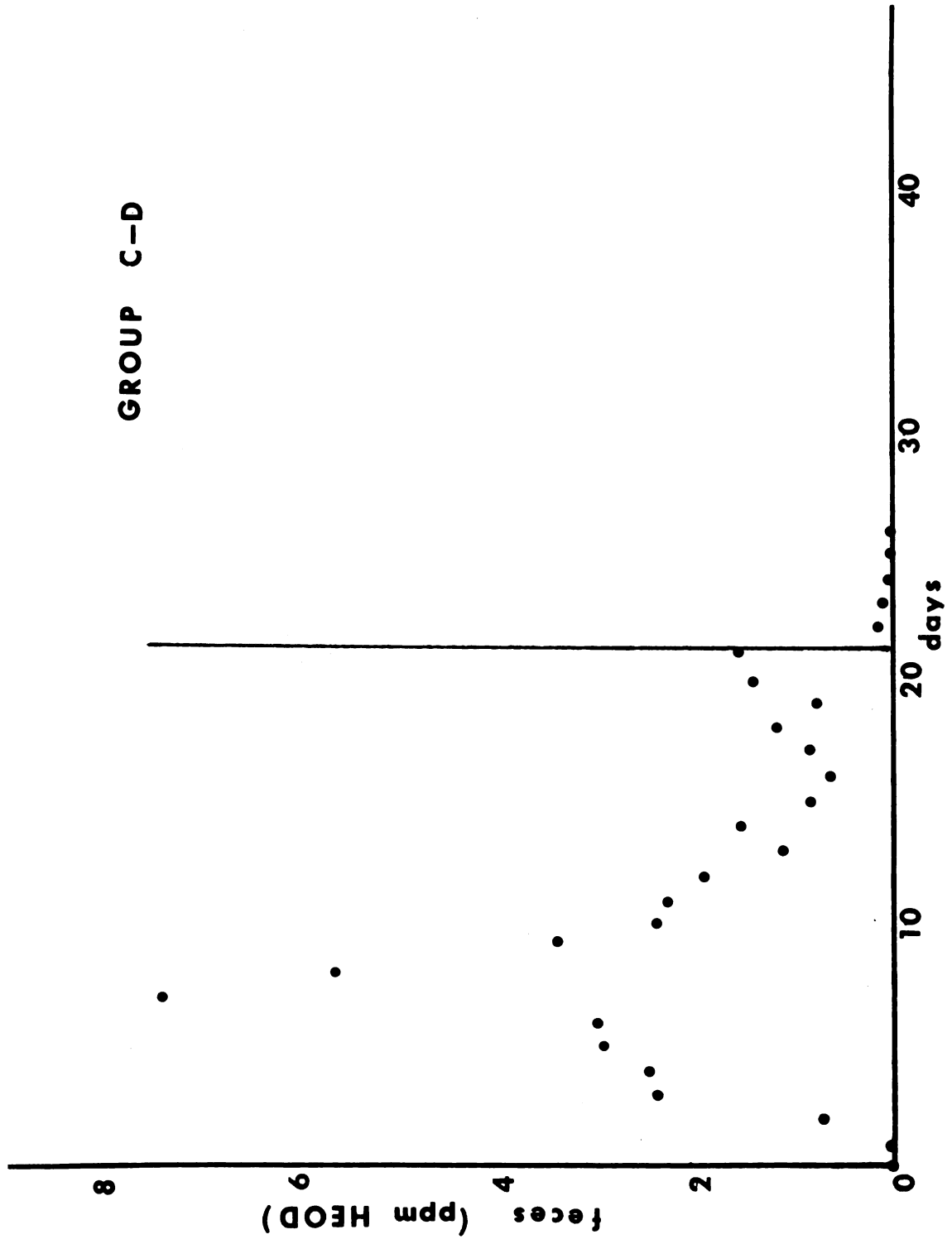
GROUP C-D
faeces (ppm HEOD)
0
2
4
6
8
10
20
30
40
days

Figure 4. Concentration of HEOD in feces of cows contaminated for six weeks and treated with phenobarbital for six weeks.

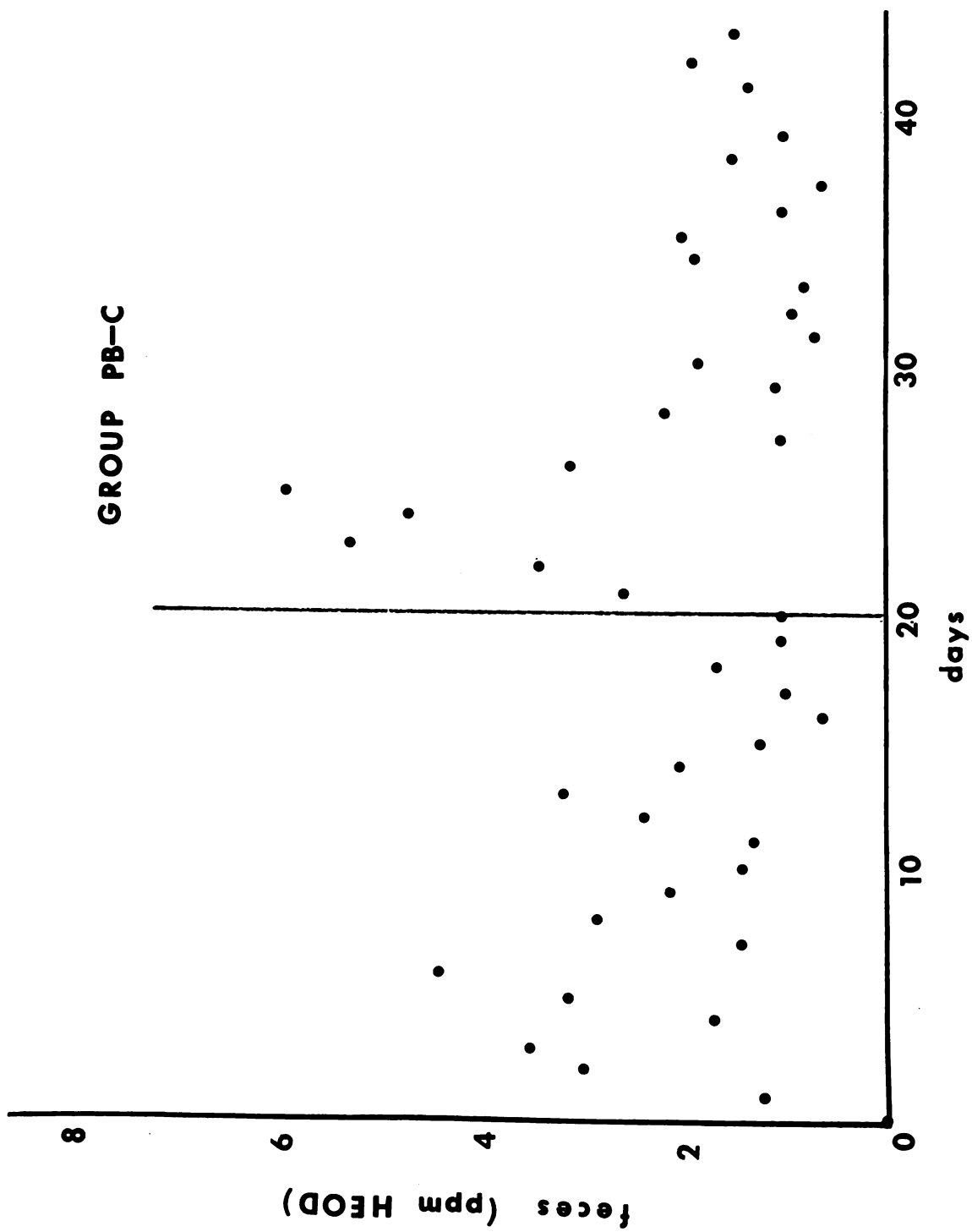
GROUP PB-C
feces (ppm HEOD)
0
2
4
6
8
10
20
30
40
days

Figure 5. Concentration of HEOD in feces of cows contaminated for three weeks and treated with phenobarbital for six weeks.

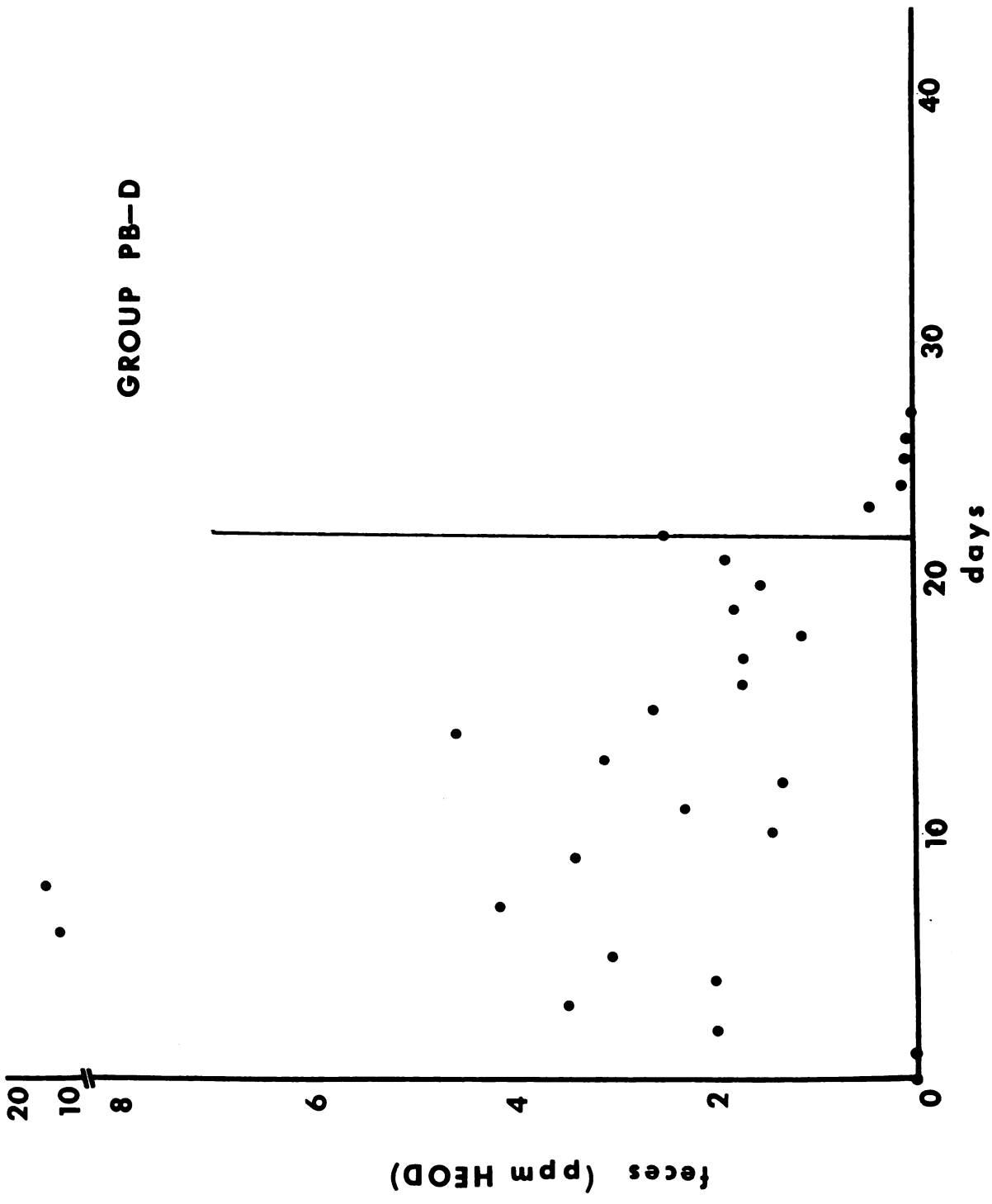
GROUP PB—D
feces (ppm HEOD)
0
2
4
6
8
10
20
0
10
20
30
40
days

TABLE 32.--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 three weeks prior to this biopsy.

parent compound was excreted in the feces. The value was determined by dividing the total amount given to each animal by the total amount of HEOD excreted for the whole experiment. Group PB-D had the highest per cent of the dose in the feces, but group PB-C had the lowest. On the average, the drug had very little effect on the fecal excretion of HEOD. Body fat was assumed to be 10% of the body weight. Thus, the burden of HEOD in body fat was determined by multiplying the highest biopsy concentration of HEOD detected in the fat for each group by 10% of the body weight and dividing by the total dose administered up to that particular biopsy. Estimations

of HEOD in body fat were calculated using the highest values which occurred at six, two, six and two 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 a standard exposure duration for each group. Even though the body fat levels of HEOD were the greatest at six weeks for groups C-C and PB-C, a larger per cent of the dose could be accounted for in body fat at two weeks for these groups.

In contrast to the high values calculated for per cent of the dose in the feces and body fat, considerably less HEOD was accounted for in the milk. The milk is not a primary excretory route for HEOD in this experiment. The values for milk were obtained by dividing the total HEOD excreted in the milk by the total amount of HEOD administered. A value of 30% body fluids which were in equilibrium with the blood levels was assumed for estimation of body fluid burden of HEOD. Extremely low values were obtained showing that body fluids contribute little as an HEOD sink.

In summary, the dispostion of the original HEOD was primarily through feces, body fat and milk. Individual cow variation appeared to exert a greater influence than phenobarbital treatment on HEOD distribution.

L. Induction of Hepatic Microsomal Enzyme by Phenobarbital and Metabolism of HEOD

Since much of the total dose of HEOD in cows was unaccounted for, and metabolism of dieldrin has been shown to occur in other species, it is assumed that a large portion of the remaining dose was converted to metabolites. Even if complete information on metabolites were available, a small portion of the dose would still be unaccounted for due to errors in estimation and inability to analyze the entire animal (e.g. hide and hair, bone marrow, etc.).

In the metabolite fraction, attempts were not made to quantify gas chromatographic responses for two reasons: (1) at present, no metabolite standards were readily available for identification and measurement of the peaks; and, (2) the extraction and analytical techniques were not favorable for obtaining all of the original HEOD and still extract the metabolite(s) quantitatively. Some of the metabolites are base-labile whereas HEOD is relatively nonlabile in strong bases. Some metabolites are best extracted at pH 2, with selected solvent systems. It is known that a large portion of each metabolite exists as a conjugate of glucuronic acid; hence it is unextractable if not treated first with glucuronidase, then extracted with solvents of differing polarity.

During the normal analysis of these samples for HEOD, extra peaks appeared shortly before and after HEOD on the chromatogram. The responses were sharp and fast, and their

behavior was similar to metabolic products of DDT, but were subsequently found different. Even so, they behaved as polychlorinated compounds which could absorb electrons. These extraneous responses were especially noted in feces, which suggests that the primary route of HEOD metabolite excretion is the same as for HEOD itself. This theory would be supported by the work of Cole (33) who noted that 50% of an HEOD dose was excreted via the bile in three days by intact rats. Data published from our own laboratory (37, 38, 158) also suggest that biliary excretion may account for a significant part of the excretion of an HEOD dose.

The use of phenobarbital has shown an increase in drug metabolizing activity of the liver in previous experiments. It was expected that it would do so in this trial. The method employed for assaying the induction of enzyme activity measured the end product formation of nitrogen-demethylase on aminopyrine. This assay is one of several which are routinely employed to measure activity of hepatic microsomal mixed-function oxidases. The results are tabulated in Table 33. There were no significant differences among the values and no effect of phenobarbital was detected. All values were low for untreated animals. Storage of the tissue prior to assay for nine-ten months after sampling may partially account for the low levels of activity but, past experience has shown that the activity will remain for up to six months.

TABLE 33.--Nitrogen demethylase activity of liver microsomes.

	C-C	C-D	PB-C	PB-D
Specific activity (muM HCHO/mg protein/min.)	0.60	0.97	0.76	0.70

CHAPTER V

DISCUSSION

A major effect of phenobarbital on body fat levels of HEOD was observed in this study. Phenobarbital not only prevented HEOD concentration from reaching the level seen in the untreated animals, but it also accelerated the decline in HEOD upon withdrawal of the pesticide. This effect has been noted by other workers using heptabarbital and DDT (16, 142). The weight gains noted for each group could have markedly diluted the pesticide concentration. Since most body weight gain is as adipose tissue a significant increase in the adipose pool could have caused the dilution. The possibility exists that newly deposited adipose tissue would contain less HEOD due to the lack of time exposed for incorporation of HEOD into fat. Although attempts were made to avoid sampling from fat that had been laid down since the previous biopsy some of this new tissue may have been removed. Alternatively, since HEOD is associated with the lipid fraction of tissues, the combined effect of general adipose deposition due to body weight gain and the need to replace fat removed at a particular site may cause newly deposited fat to contain greater residue levels than fat

which becomes contaminated after deposition. Mobilization and storage occur continuously, but the equilibrium is toward storage unless the animal is under a stress such as lactation. In addition to the above factors, lactation must have influenced body fat levels of HEOD in this experiment. The decline in milk production diminishes the excretion via the milk which would shift the equilibrium toward storage or excretion via the other routes. The combination of preferential movement of HEOD with newly deposited adipose as represented by body weight increases and the concurrent fall in milk production could well have exaggerated the storage levels observed. It was assumed old tissue was sampled. However, if new tissue had a lower HEOD level and had been included in the biopsy then the total HEOD in body fat as it was calculated for Table 21 would be underestimated. Even so, relative differences would still be expected to remain. Another factor which can play a role in HEOD storage is the effect of gestation. Placental transfer can account for 0.9% of a 60-day dose of HEOD (15). The transfer to the fetus or fetal fluids of almost 1% of a 60-day dose is 10-20 times greater than the HEOD estimated to be contained in blood and body fluids (Table 32). In addition, the data of Braund (15) suggested that as gestation proceeds, the efficiency of fat uptake of HEOD may have decreased. Part of the weight gain noted in all groups must

have been due to fetal growth, yet the fetus is of negligible size until the 6-7th month of gestation. The major effect of fetal growth would occur during the last two months of gestation when more than half of the birth weight is achieved.

The net deposition of fat may not reflect net storage of a chlorinated insecticide such as dieldrin. A two-compartment model within the fat cell has been proposed which suggests that the primary function of one compartment is the storage and mobilization of lipids while the other compartment serves as a storage area for compounds such as chlorinated hydrocarbons (153). This may explain why starvation of obese subjects has failed to produce toxicity symptoms due to released HEOD circulating in the blood at greatly elevated levels. If the two-compartment situation does exist then the effect phenobarbital has on decreasing the storage of HEOD in fat must be substantial because of the difficulty in moving the pesticide with the mobilized lipids.

The second important finding was that, contrary to previous reports, HEOD was excreted in significant amounts in the feces when chronically administered orally over a period of time. An investigation of extraction techniques in the present study indicated that drying of samples prior to extraction provided the opportunity for the pesticides to co-distill with water. Endrin has been

shown to decompose on dried soils (4). Heptachlor epoxide and dieldrin require exhaustive ultrasonic extraction from dried soil particles due to the strong adsorption of the pesticide by the soil (88, 89). The addition of water to undried soils in order to standardize all extractions at 20-80% moisture, depending on organic matter content, has vastly improved recoveries and extraction efficiency (139). A study of several methods for extracting carbon-14 labelled dieldrin from soil has been reported (136). The addition of water to the soil prior to extraction improved the recovery of the pesticide. Saha et al. reported that fortification of air-dried soil should not be used for measuring the true recovery rates (138). The addition of water to soil samples prior to soxhlet extraction has increased the efficiency of extraction (156). Recent studies have demonstrated that 85% of the DDT residues in alfalfa hay could be removed by washing with water or steam (2,3). Wheeler has stated that extraction is the weakest part of analysis (154). However, the increased extraction efficiency in this investigation did not account for the difference in fecal HEOD excretion from that reported in past experiments.

Results in the present study support the above experiments. The data in Table 16 emphasized the extensive improvement that was obtained upon extraction of feces

which had not been dried. The feces in this experiment had an average moisture content of 81%. The process of drying this material removed 4/5 of the original weight. The fact that HEOD can co-distill with the water phase must have accounted for a significant portion of the increase in extraction efficiency. The recommended amount of water for the extraction of soils "high in organic matter" is 80%. The dry matter in feces is almost entirely organic matter, therefore extraction of the feces in the same condition as collected from the animal seems to be a reasonable method.

In addition to the loss encountered by co-distillation of HEOD with water during drying, it would be expected that HEOD would be tightly bound to organic matter when dried. In soil, the binding is of such magnitude that it requires rigorous ultrasonic treatment in order to obtain adequate recoveries (88, 89). The binding of HEOD to fecal dry matter has been observed in our laboratory. A usual method employed to check analytical efficiency is that of adding known amounts of the compound being analysed to the unknown sample prior to extraction or at various other stages in the analytical process. This procedure is commonly referred to as "fortification" or "spiking" of the sample. An aliquot of the original feces sample was removed after thorough mixing and split into five samples. One sample was untreated while another was

fortified but not dried. The third sample was dried only while the fourth was fortified before drying. The fifth sample was spiked after drying. The results of this study showed significant differences among the methods used (Table 34).

TABLE 34.--Relative differences in extraction efficiency.

Treatment		Relative recovery of HEOD	
Dried	Fortified	Endogenous	Added
-	-	100	-
-	+	100	100
+	-	20	-
+	before drying	20	20
+	after drying	20	90

The differences above are only relative but they demonstrate two points. First, the drying of feces markedly reduces the amount of HEOD detected in the sample. Second, the fortification of a wet sample which is subsequently dried and used to measure extraction efficiency would lead one to falsely assume no loss occurred even though 80% of the original and added HEOD were not recovered. Thus, it can be seen that HEOD was lost and/or bound when dried, but little binding occurs once the sample has been dried. Drying the sample prior to extraction has been used in the past in order to provide adequate mixing and subsampling. This process of obtaining a truly representative

aliquot of the whole sample is desirable in any situation. However, if representative subsamples are obtained by drying which substantially reduces the amount of extractable residues, then drying negates the accuracy initially sought. Thus, oven drying of feed or feces samples prior to extraction of chlorinated hydrocarbons is not recommended.

There are factors other than improved efficiency of extraction that may have accounted for the excessive fecal excretion of HEOD. Chromic oxide will bind HEOD in a hexane solution in an amount equivalent to less than 0.1 percent of each daily dose, but it is possible a larger amount was bound in the gastro-intestinal tract. Binding of HEOD by chromic oxide may have been enhanced in the rumen. This idea appears unlikely since fecal excretion of HEOD declined with time while the chromic oxide dose remained constant. The determination of the chromic oxide content in feces gave recoveries of less than 100%. The nature of the calculation of fecal dry matter output by the chromic oxide technique is such that the lower the recovery the greater the value will be an overestimation of dry matter excretion. Total daily dry matter excretion did not change appreciably during the experiment. Thus, rate of passage was not affected sufficiently to account for the decline in daily HEOD elimination in the feces. Some of the variation in fecal dry matter excretion was

due to differences in preference for hay, grain or silage. The variability of excretion of HEOD in the feces appeared to be more a function of HEOD concentration than it was of changes in percent dry matter, dry matter excretion or rate of passage. All feces samples were analysed in random order; therefore, any effect of time of analysis should be distributed over the entire experiment. The 6-week means in Tables 29 and 31 suggest that HEOD excretion was inversely related to digestibility of the ration. Because many times more HEOD has been accounted for in the feces in this study than has been the case reported previously, the following question arises: if in fact the dynamic movement of fecal HEOD is associated with the particulate matter in the gut, why is there a precipitous decrease in fecal excretion of HEOD beginning the first day after withdrawal of HEOD from the diet? This rapid decline would suggest that there are other effects involved.

The first effect which may account for the movement of HEOD in feces is the action of phenobarbital on biliary flow (97, 99). Phenobarbital increased biliary flow by 50% in rats. An increase in biliary excretion of several compounds can occur but to varying degrees. Phenobarbital can stimulate increases in conjugation of compounds for excretion. Thus, the combined effects of greater bile flow and increased transport maxima due to the elevated

rate of hepatic microsomal conjugate formation are synergistic effects which result in extensive changes in the rate of elimination of a compound. The substantial saliva flow in a cow results in a turnover of large quantities of water. Bile flow may also be great due to the need for elimination of the waste products of energy utilization, emulsification and digestion in the lower gut.

Changes in bile flow noted above can not entirely account for the decline in HEOD excretion in the feces. Animals not receiving phenobarbital also eliminated large quantities of HEOD in the feces. Therefore, if changes in biliary flow due to drug treatment did occur, this phenomenon should be evident in the several tables presented. The cumulative percent of the dose excreted in the feces declined steadily regardless of treatment. This suggests that if bile was a major source of HEOD in the feces, then it may have a saturation point for HEOD (a transport maximum) which was reached early and was unable to deal with the continual dosing. But, if there was a maximum rate of transport, why was there a decline in fecal excretion of HEOD? Perhaps the hepatic system was able to adapt to the increased flow rates due to phenobarbital treatment by decreasing biliary excretion of HEOD. It is more likely that degradation of HEOD occurred which caused increasing portions of HEOD-derived material to be excreted.

In addition to the changes in bile flow which may cause general shifts in excretion patterns, individual variation probably played enough of a role to produce artificial effects due to phenobarbital. That is, all four animals in each group were treated alike during the first three weeks. The large difference between PB-C and PB-D must have reflected individual responses rather than a response due to drug treatment. In spite of a lack of a definitive phenobarbital response for excretion of HEOD, except during the decontamination period, the individual variation was much greater for phenobarbital-treated cows. It can be seen in Figures 2 through 5 that the scattering of points was much greater for the animals receiving the drug. The untreated group had small 3-6 day cycles of excretion of HEOD whereas definitive short-term changes were difficult to see in the drug-treated groups. It is difficult to explain this response. Possibly this was a manifestation of the efforts of the body to eliminate the compound. Increased stress could have caused inconsistent responses to that stress. Adaptations or compensations may have varied greatly due to the drug stimulus. The drug probably affected many metabolic processes.

In addition to the definitive responses in body fat storage of HEOD due to phenobarbital and the substantial fecal excretion regardless of treatment, the concentration

and excretion of HEOD via milk was unexpected. The production or percent milk fat appeared to have no effect on the relative differences observed for HEOD concentration and excretion in the whole milk. The great initial increases in concentration were not matched by later rises in HEOD content. Again, only during decontamination did phenobarbital have an effect on pesticide clearance. The HEOD values for the control groups were much higher during decontamination than were those on drug treatment. All concentrations were above the legal tolerance for HEOD. However, at no time did they reach the levels noted by Braund (15) in a trial with lactating cows. Perhaps phenobarbital inhibits excretion of HEOD in the milk. However, this seems unlikely because there was little difference in HEOD excretion among groups.

The argument used earlier that previous extraction techniques were not always able to obtain all the residue present could be a pitfall for the author in milk analysis. The method used by Braund for extraction of HEOD from milk was different from that employed in this study. He used an oxalate:ethanol:ether system with a cleanup procedure prior to injection into the chromatograph (15). In this study the procedure of Crosby and Archer was used. This method involved an alkaline hydrolysis of the whole milk (41). Recoveries of HEOD were adequate when the samples were fortified prior to extraction. Values were

obtained from milk samples which were from other experiments of herds which were 10-20 times higher than the levels in this experiment. It is possible that this technique was not satisfactory for samples obtained in this experiment. The authors of this method developed it for the dechlorination of DDT to DDE and it is effective for the extraction of several chlorinated pesticides from proteinaceous or fatty tissues. Without the use of radioisotopes it is difficult to be certain that the extraction method is entirely effective. The high excretion in the feces of HEOD and its considerable storage in body fat may explain the low levels seen in the milk. But, it is difficult to believe that milk is not a better route for excretion than is seen in this experiment. HEOD should readily diffuse across the mammary membranes or be easily transported with lipoidal material in milk.

In considering the possible effects of phenobarbital on the various tissues no mention has been made of its effect on the rumen microbial population. Although there is little evidence that this drug can affect microorganisms, it could be possible that the changes resulting from drug administration on fecal excretion of HEOD may be due in part to an alteration of the fermentation occurring in the rumen. If this did occur it apparently was not detrimental to the animals since their milk production and feed intakes were not different from expected normal values.

Although it is doubtful that there could have been induction of microbial enzymes which would degrade HEOD, the evidence exists that the chronic administration of phenobarbital will induce hepatic enzymes capable of pesticide degradation. The data in this experiment suggests a similar action on HEOD in lactating cows. The concentration and excretion patterns in the several tissues indicate that either HEOD was assimilated into some unanalysed tissue or else it was converted to forms which were undetectable under the conditions employed for analysis. It is unlikely that a substantial portion of the HEOD is located in tissues which were not analyzed, according to the work of Braund (15) and King et al. (96). The most logical expectation would be that degradation of HEOD was by induced hepatic-microsomal enzymes to an extent commensurate with the unaccounted-for portion of the dose. The effects of phenobarbital on the microsomal enzymes of the liver are known to be significant within two days, but the activity of these enzymes on HEOD may be slow. These enzymes have also been shown to metabolize HEOD in vitro (113, 114). Therefore it is assumed that the in vivo action of these enzymes would be at least as great as in vitro. The best measure of this activity was to have been the nitrogen-demethylase activity assay on liver microsomes obtained from each of the eight cows. However, due to an apparent loss of activity in storage,

the microsomal preparations showed very little activity regardless of treatment. In fact, the values were lower than those normally observed for fresh cow liver. This fact indicates a general loss of enzyme activity, which is well documented for the mixed-function oxidases. Had the activity remained elevated a correlation could have been established between the decline in HEOD in all tissues except for fat and the increased mixed-function oxidase activity of the liver.

The low level of HEOD administered has not been shown to have an inductive effect as noted with phenobarbital, but it is possible that the HEOD may have also caused induction of other enzymes which could have degraded it to some extent. It is difficult to determine the low level effects which could have occurred due to HEOD and phenobarbital in combination. Phenobarbital may act in a subtle way on the permeability of HEOD in various tissues. This effect can be inferred by the body fat data. Rather than causing the metabolism of HEOD the phenobarbital could minimize the incorporation of the pesticide in adipose tissue. Further, it may act to enhance mobilization from the adipose tissue.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Two groups of four cows each were contaminated for three weeks with HEOD at the level 0.1 mg/kg body weight per day orally. Two of the cows in each group were contaminated for three more weeks, while the remaining two animals received no HEOD. Phenobarbital was superimposed on one group of four cows for six weeks. During contamination and decontamination samples of feces, milk, body fat, blood, urine and liver were taken in order to study the metabolic fate of the pesticide. All cows were fed a ration of hay, grain and corn silage which was balanced to meet the requirements of maintenance and lactation.

A major finding was that from 18-53% of the HEOD was excreted in the feces. Phenobarbital treatment appeared to have its most pronounced effect during decontamination when it was able to accelerate the excretion of HEOD over that of the untreated group. Phenobarbital had no effect on dry matter intakes or excretion compared to the control group. The concentration of the pesticide in the fecal dry matter was highly variable both among cows and from one day to the next. The response to withdrawal of the pesticide was a rapid decline

in fecal HEOD concentration and total HEOD excretion. The decline appeared to be as much a function of withdrawal of HEOD as of phenobarbital treatment. Phenobarbital-treated cows had a much greater variability of HEOD concentration in the feces than did untreated controls. Independent of drug administration was the peak fecal excretion of HEOD in the first week of the experiment. Mean daily excretion ranged from 22-51 mg/cow/day during the first week, which declined to 12-23 mg/cow/day in the second week.

The reason that substantial fecal elimination of HEOD had not been detected previously is attributed to the use of extraction procedures in this experiment that maximized the recovery of the endogenous pesticide. Former methods used as the first step a drying of the feces which has been shown to not only cause losses via co-distillation of the pesticide with the water being removed but also through inextractable binding of the compound to organic matter in the drying process.

The concentration of orally administered HEOD in body fat was lower in phenobarbital-treated animals during contamination than in controls. It was also lower in those animals which were decontaminated and receiving the drug. Maximum levels of HEOD in the body fat were approximately the same as the level in the diet as fed, ranging from 1.53-2.96 ppm at the highest point.

The level of HEOD in the milk was approximately 1/20th that in the diet as fed. A general increase in concentration and daily excretion of HEOD was noted during contamination. Upon decontamination, the decline in these parameters appeared to be a function of withdrawal of HEOD as well as phenobarbital treatment, as was indicated for feces. Milk fat percentage and production declined slightly, presumably due to the slightly restricted-roughage diet, but this alteration did not affect the overall movement of the pesticide in the milk.

Very low and highly variable levels of HEOD were found in the blood. No HEOD was detected in the urine of any of the experimental animals. It is assumed that the degradation products of the pesticide will be found in the urine as conjugates of glucuronic acid.

The formation of metabolites of HEOD was maximized by the use of phenobarbital to induce hepatic microsomal drug metabolizing enzymes. Although liver microsome preparations had low enzyme activity, it is concluded that activity was lost upon extended storage. The appearance of unidentified compounds having a behavior in the gas chromatograph similar to chlorinated hydrocarbon pesticides, suggests that metabolites may have been formed in this experiment. These additional peaks occurred primarily in the feces. They must have been conjugated in the bile and subsequently excreted in the feces.

It is suggested that phenobarbital may be used with discretion to aid in the decontamination of animals containing chlorinated hydrocarbon pesticide residues above the legal tolerance. The drug appears to be particularly effective when ingestion of the pesticide has ceased.

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APPENDIX

TABLE A1--Daily milk production.

Day	C-C		C-D		PB-C		PB-D	
	771	833	820	832	799	991	819	837
1	6.49	23.15	15.35	22.93	23.38	15.44	14.67	18.84
2	4.77	19.98	13.62	18.84	21.34	14.53	15.21	19.52
3	6.81	22.02	14.76	20.66	22.47	15.44	14.53	19.75
4	7.04	22.02	13.85	21.11	22.25	16.80	12.49	22.02
5	6.36	22.25	14.98	21.34	22.93	16.12	13.17	20.20
6	5.90	21.34	14.98	22.47	20.88	17.93	11.58	19.07
7	6.13	22.93	13.85	23.84	21.57	17.25	9.31	21.57
8	5.90	22.25	13.62	23.61	20.20	14.07	9.76	19.75
9	5.90	22.70	13.62	23.61	19.07	14.30	9.53	18.84
10	5.90	22.47	13.85	24.06	18.84	12.26	8.85	19.07
11	5.90	23.15	14.07	22.93	16.57	12.71	9.31	17.71
12	6.13	21.11	13.62	22.70	15.89	12.49	9.76	16.80
13	5.22	20.43	13.17	22.47	17.48	13.39	9.76	19.07
14	6.34	19.30	14.30	22.25	16.12	13.17	8.63	19.30
15	5.22	17.71	12.71	21.34	16.34	13.17	8.85	17.25
16	5.68	20.20	12.26	19.75	15.66	13.17	8.63	17.93
17	5.45	22.02	12.71	21.11	17.25	14.53	6.58	19.30
18	5.90	21.57	10.67	19.98	17.48	14.30	9.99	18.84
19	4.77	22.47	12.49	20.20	19.52	14.53	7.04	18.61
20	5.68	23.15	11.80	21.11	19.52	14.98	9.08	20.20
21	5.22	22.02	11.35	22.47	19.75	14.07	6.81	18.61
22	4.54	21.79	12.49	18.84	18.39	13.17	8.85	18.84
23	4.77	22.25	11.58	22.93	17.48	12.71	6.36	13.93
24	4.99	22.25	11.35	22.70	18.16	11.58	5.68	17.93
25	4.54	22.93	11.58	21.57	18.84	10.22	7.04	14.30
26	3.41	22.25	11.35	23.61	19.30	7.04	6.36	15.89
27	4.31	21.34	10.22	21.11	19.30	4.99	7.04	17.03
28	4.77	21.79	10.22	22.70	21.34	6.58	7.95	18.16
29	4.09	17.71	10.22	22.02	18.39	5.45	6.36	16.57
30	4.54	21.11	9.53	22.25	18.84	7.72	6.13	17.93
31	4.09	19.75	9.99	21.57	19.52	9.53	6.13	17.03
32	4.09	18.61	9.76	22.25	19.07	10.67	5.22	17.71
33	3.86	21.57	8.85	21.34	19.98	10.90	6.13	18.16
34	4.54	20.20	9.31	19.07	18.61	11.35	4.99	17.48
35	3.86	21.57	9.53	20.66	18.61	12.94	5.68	18.61
36	4.09	21.34	8.63	20.66	19.30	11.35	5.90	17.03
37	4.33	20.88	8.85	21.34	19.07	11.58	4.99	17.71
38	3.41	20.43	8.85	22.02	19.30	13.17	5.22	17.71
39	3.41	20.66	8.63	22.02	19.07	13.39	5.90	18.61
40	4.31	20.88	8.17	21.11	18.61	13.17	4.77	18.39
41	3.18	19.98	8.17	19.75	18.16	12.94	4.54	17.93
42	4.09	20.88	8.40	19.98	16.80	13.62	5.45	19.75
43	3.86	20.66	8.17	18.39	15.21	13.62	4.99	19.52
44	3.41	21.11	7.72	19.52	14.30	13.85	4.99	19.52
45	3.18	20.66	7.95	19.98	14.76	14.53	5.45	19.30

TABLE A2--Percentage milk fat.

Day	C-C		C-D		PB-C		PB-D	
	771	833	820	832	799	991	819	837
	percentage milk fat							
1	2.5	3.7	3.3	2.7	4.0	3.4	2.5	5.2
2	2.4	3.7	3.1	2.0	4.0	3.5	2.8	3.9
3	2.6	3.0	5.6	3.6	4.1	3.2	2.7	6.9
4	3.1	4.0	8.7	2.3	4.4	3.2	2.3	5.6
5	7.7	3.2	5.9	3.5	4.5	2.5	3.0	4.3
6	2.9	3.9	3.3	2.6	4.0	3.1	3.0	4.3
7	4.5	4.5	4.8	2.8	4.4	3.0	3.0	8.5
8	4.3	4.2	8.1	3.1	4.3	2.9	4.2	9.2
9	11.0	4.4	8.7	3.4	5.0	3.2	3.6	6.9
10	3.4	4.0	5.4	3.8	3.8	3.4	3.3	4.6
11	4.0	4.1	4.4	2.8	3.3	3.1	3.6	4.6
12	2.9	4.0	3.5	2.1	2.5	3.2	3.8	4.2
13	2.7	3.4	3.2	2.6	2.7	3.0	3.0	3.1
14	2.7	4.2	3.3	2.3	3.2	3.2	3.3	3.8
15	2.3	4.2	3.5	1.5	2.0	3.4	3.5	3.9
16	3.4	4.0	3.4	2.2	2.3	3.8	3.4	3.9
17	2.8	4.0	3.5	2.6	2.3	3.1	3.0	3.9
18	2.4	4.3	3.5	2.7	2.8	3.7	4.4	3.7
19	2.8	3.8	3.5	3.2	2.8	3.2	3.1	4.0
20	3.1	4.1	3.4	2.9	3.3	3.5	3.7	3.9
21	3.0	4.0	3.3	3.7	3.4	3.6	3.3	3.0
22	3.0	4.0	3.3	4.4	3.5	3.2	3.2	2.4
23	2.7	3.8	3.4	4.6	3.2	3.4	3.7	2.8
24	2.5	3.8	3.3	4.1	2.5	3.2	3.6	2.4
25	2.0	2.5	3.1	3.1	3.4	3.1	3.3	2.6
26	2.4	4.0	3.4	2.6	3.5	4.3	3.7	2.6
27	3.0	4.3	3.4	3.3	3.6	4.3	3.7	3.1
28	3.0	4.0	3.2	3.3	3.6	6.7	3.6	2.3
29	2.9	3.9	3.1	3.5	3.7	6.7	3.2	1.9
30	3.0	4.1	3.2	3.2	3.3	4.8	3.7	2.8
31	3.2	4.1	3.7	3.0	3.9	4.1	4.1	2.5
32	3.2	4.1	3.3	2.7	3.4	3.8	3.1	2.2
33	3.0	3.9	3.4	2.6	3.7	3.6	3.5	2.0
34	2.9	4.1	3.2	1.6	3.8	3.8	3.7	1.6
35	2.5	4.0	2.5	2.2	3.5	3.3	3.8	2.0
36	2.3	3.7	3.1	2.6	3.5	3.2	3.3	1.8
37	2.7	3.9	3.0	2.7	3.8	3.4	3.2	2.5
38	3.1	3.7	3.2	2.5	3.8	3.2	3.4	2.5
39	2.5	4.1	3.5	1.6	4.0	3.2	4.7	3.1
40	2.7	3.9	3.1	2.2	4.0	3.2	3.7	3.4
41	3.2	3.8	3.1	2.1	4.3	3.3	4.1	3.3
42	2.8	3.6	3.1	1.7	3.9	3.0	4.2	3.5
43	2.8	3.9	2.9	2.5	4.1	3.1	2.9	3.9
44	3.2	3.6	3.2	2.2	3.7	3.3	3.2	3.7
45	2.9	3.8	2.7	2.3	4.1	3.4	3.8	3.6

TABLE A3--Concentration of HEOD in whole milk.

	C-C		C-D		PB-C		PB-D	
Day	771	833	820	832	799	991	819	837
				ppm				
1	0	0	0	0	0	0	0	0
2	0.0141	0.0630	0.0392	0.0493	0.0571	0.0669	0.0319	0.0408
3	0.0441	0.0871	0.0396	0.1126	0.0742	0.0988	0.0640	0.0552
4	0.0539	0.1144	0.0564	0.0994	0.1045	0.0856	0.0792	0.0480
5	0.0489	0.0418	0.0531	0.0359	0.0499	0.0312	0.0467	--
6	0.0807	0.0504	--	0.0489	0.0541	0.0490	0.0472	0.0595
7	0.0462	0.0435	0.0508	0.0373	0.0667	0.0352	0.0473	0.0567
8	0.0611	0.0576	0.0654	0.0488	0.0678	0.0581	0.0525	0.0507
9	0.0935	0.1184	0.0990	0.0847	0.0958	0.0830	0.1043	0.1219
10	0.0948	0.1213	0.0926	0.0825	0.0892	0.0896	0.1495	0.1671
11	0.0830	0.1000	0.0906	0.0596	0.0915	0.1050	0.1120	0.0733
12	0.0740	0.2300	0.0948	0.1440	0.0840	0.0880	0.1340	0.1780
13	0.0720	0.1360	0.0860	0.0623	0.1000	0.1150	0.1040	0.1420
14	0.0680	0.1410	0.0970	0.0615	0.0492	0.0539	0.1352	0.1660
15	0.0956	0.1139	0.1012	0.0619	0.0707	0.1349	0.1762	0.1357
16	0.0766	0.1667	0.1262	0.0820	0.0845	0.1262	0.1131	0.1381
17	0.0629	0.1104	0.1436	0.1080	0.0525	0.1158	0.0734	0.1267
18	0.0560	0.1143	0.1053	0.1306	0.0756	0.0643	0.1225	0.1361
19	0.0802	0.1446	0.1330	0.1376	0.0802	0.0567	0.1130	0.1520
20	0.0974	0.1688	0.1277	0.1016	0.0834	0.0902	0.1544	0.1112
21	0.1012	0.1640	0.1400	0.2096	0.0680	0.1230	0.1683	0.1234
22	0.0775	0.2015	0.1390	0.1800	0.0928	0.1262	0.1015	0.1111
23	0.0760	0.1180	0.1100	0.1615	0.0830	0.0945	0.1030	0.0920
24	0.0850	0.1130	0.0870	0.1080	0.1290	0.0800	0.1490	0.0990
25	0.0910	0.1370	0.1200	0.0990	0.1940	0.1040	0.0700	0.1260
26	0.0780	0.1383	0.1020	0.0940	0.1100	0.1260	0.0760	0.0760
27	0.0960	0.2000	0.1070	0.0910	0.0860	0.1350	0.0820	0.0720
28	0.0940	--	--	0.0770	0.0940	0.1200	0.0947	0.0644
29	0.1240	0.1320	0.0960	0.0830	0.1228	0.2820	0.0843	0.0628
30	0.1050	0.1450	0.0910	0.0870	0.0970	0.3360	0.0850	0.0644
31	0.1210	0.1590	0.1050	0.0770	0.1230	0.2100	0.0590	0.0552
32	0.1030	0.1630	0.0980	0.0856	0.1150	0.2044	0.0520	0.0506
33	0.1480	0.1750	0.0872	0.0770	0.1270	0.1370	0.0540	0.0550
34	0.1370	0.1860	0.1080	0.0700	0.1080	0.0970	0.0490	0.0390
35	0.1060	0.2960	0.0740	0.0746	0.1800	0.1094	0.0560	0.0460
36	0.1600	0.1748	0.1260	0.0700	0.1216	0.1132	0.0480	0.0414
37	0.1058	0.1968	0.0810	0.0708	0.1308	0.1472	0.0386	0.0396
38	0.0965	0.1705	0.0612	0.0444	0.1525	0.0785	0.0298	0.0372
39	0.0835	0.2010	0.0740	0.0346	0.1535	0.0590	0.0530	0.0432
40	0.0970	0.2155	0.0792	0.0542	0.1425	0.0965	0.0418	0.0452
41	0.1016	0.1418	0.0360	0.0640	0.1221	0.0623	0.0480	0.0280
42	0.1306	0.1374	0.0593	0.0222	0.0684	0.0622	0.0432	0.0396
43	0.1572	0.0796	0.0393	0.0245	0.0662	0.0800	0.0247	0.0340
44	0.0662	0.1105	0.0978	0.0298	0.0712	0.0662	0.0204	0.0548
45	0.1126	0.1327	0.0410	0.0284	0.0921	0.0654	0.0375	0.0385
46	0.0585	0.1310	0.0460	0.0460	0.1300	0.0880	0.0300	0.0360

0 = no HEOD detected

-- = sample was not analysed for HEOD

TABLE A4--Fecal dry matter excretion.

	C-C		C-D		PB-C		PB-D	
Day	771	833	820	832	799	991	819	837
				kg/day				
1	--	--	--	--	--	--	--	--
2	--	--	--	--	9.69	9.36	--	--
3	11.26	6.15	8.11	10.04	6.05	9.56	11.01	5.00
4	9.67	6.95	9.21	8.14	3.65	8.83	6.47	6.61
5	10.16	5.74	7.52	6.41	6.22	6.42	10.41	12.39
6	5.64	8.60	10.33	9.48	8.04	8.53	10.77	7.84
7	6.24	6.66	7.20	7.94	6.88	7.00	7.42	9.81
8	8.92	10.65	9.32	11.17	9.50	8.45	9.94	5.24
9	7.11	9.31	14.51	8.52	5.64	6.11	6.21	7.24
10	6.90	11.25	10.33	5.64	6.46	6.43	7.06	9.51
11	8.96	2.66	11.40	7.58	3.57	4.72	8.08	9.74
12	6.21	8.44	10.88	11.34	3.98	5.74	9.83	5.90
13	6.25	9.84	11.18	8.56	4.76	6.36	7.77	8.93
14	5.53	5.85	9.48	8.06	4.64	6.41	8.95	7.10
15	6.41	7.88	11.27	8.29	3.68	6.28	8.30	14.68
16	27.39	9.57	7.44	3.26	3.56	16.51	5.34	11.95
17	6.58	8.20	11.95	3.11	6.85	9.62	6.28	9.34
18	5.63	8.71	7.45	4.97	6.52	5.33	10.12	6.48
19	9.15	3.98	8.94	12.65	3.43	6.40	10.49	7.70
20	8.29	8.56	5.85	4.35	3.39	14.31	12.89	9.37
21	6.76	8.70	10.40	6.27	5.47	5.73	10.39	5.39
22	3.85	6.42	7.39	4.96	6.64	2.21	9.65	7.85
23	9.62	6.48	7.24	6.33	9.89	8.64	8.15	5.65
24	10.09	9.87	8.62	5.83	10.03	3.71	7.27	--
25	6.52	12.65	10.11	5.84	3.37	8.01	9.94	8.44
26	6.37	8.69	7.44	6.03	5.92	3.70	8.56	6.45
27	7.52	13.20	8.87	7.65	5.25	4.56	7.38	9.93
28	--	8.44	7.42	8.43	7.44	2.45	6.24	8.63
29	6.91	10.04	10.83	7.84	6.07	--	--	7.40
30	5.08	8.78	11.40	6.64	5.29	3.05	5.94	4.74
31	5.47	6.41	11.67	8.67	3.34	--	5.04	8.49
32	10.25	11.08	23.55	14.46	5.33	7.04	8.15	6.52
33	7.77	10.41	14.42	5.15	5.12	4.65	6.90	7.47
34	5.82	7.31	11.75	6.56	6.48	5.68	4.82	11.32
35	7.79	8.98	8.40	12.38	3.81	4.74	7.22	6.23
36	7.48	11.37	8.31	9.20	4.43	3.49	8.86	8.07
37	8.17	7.91	10.82	10.42	6.23	8.10	9.25	5.37
38	10.85	10.62	9.47	12.81	6.65	7.30	4.48	5.72
39	7.35	11.76	7.99	8.28	5.58	5.97	5.71	8.52
40	4.25	6.49	11.75	13.05	5.88	7.83	6.32	6.31
41	7.33	10.42	7.92	4.26	6.21	4.40	5.60	4.72
42	5.83	8.11	6.33	6.19	5.87	6.57	5.19	6.34
43	12.30	5.62	8.20	--	3.38	6.03	5.83	4.50

TABLE A5--Concentration of HEOD in feces.

	C-C		C-D		PB-C		PB-D	
Day	771	833	820	832	799	991	819	837
				ppm in dry matter				
1	0	0	0	0	0	0	0	0
2	--	--	--	--	2.002	0.386	--	--
3	0.893	1.809	0.709	0.604	4.449	1.577	5.724	1.314
4	1.232	4.093	3.844	0.908	6.139	0.805	2.254	1.682
5	11.128	1.880	0.650	5.088	2.244	1.157	5.280	0.737
6	4.133	4.074	2.672	3.285	2.041	2.461	1.364	20.197
7	1.384	1.774	13.875	1.006	3.100	16.639	3.621	4.822
8	20.730	2.205	3.348	5.565	0.964	1.716	10.220	19.585
9	2.314	0.534	0.506	0.705	1.258	4.548	3.471	2.981
10	0.550	1.952	1.804	3.828	1.969	2.246	1.446	1.363
11	4.337	10.182	1.078	3.680	1.967	0.591	4.235	0.284
12	0.666	9.553	1.261	0.448	1.030	0.361	1.384	1.175
13	0.722	1.749	0.554	1.686	1.615	3.106	5.266	0.785
14	0.473	0.762	1.536	1.439	2.066	4.369	6.534	2.630
15	0.712	0.293	0.982	0.686	2.055	1.909	3.474	1.626
16	0.182	--	0.634	0.490	2.019	0.242	3.082	0.506
17	2.433	1.616	0.324	1.351	0.224	0.807	2.524	0.862
18	7.329	7.610	0.985	1.429	1.480	0.592	1.240	1.124
19	1.324	3.274	0.924	0.549	3.050	0.478	2.202	1.443
20	3.141	0.736	1.752	1.138	1.547	0.424	0.551	2.261
21	1.193	1.414	0.387	1.002	1.429	0.599	0.598	4.263
22	2.046	3.057	1.667	1.298	1.542	3.715	1.301	1.375
23	1.643	2.666	0.172	0.213	3.351	2.155	0.095	0.186
24	1.705	0.740	0.150	0.094	1.986	9.250	0.071	--
25	1.706	0.423	0.090	0.033	4.765	2.548	0.161	0.016
26	1.601	9.065	0.026	0	4.920	6.847	0	0.022
27	0.351	2.053	0		1.861	4.320		0
28	--	1.091			0.661	1.342		
29	2.089	0.587			2.245	--		
30	0.715	0.455			0.572	1.602		
31	0.169	0.281			1.770	--		
32	6.482	0.166			1.122	0.157		
33	0.902	0.185			0.672	1.004		
34	1.010	2.308			0.845	0.894		
35	3.163	0.426			2.805	1.089		
36	1.495	1.121			2.098	1.401		
37	1.297	0.713			1.741	0.223		
38	1.022	1.620			2.011	0.634		
39	1.484	0.801			2.279	0.807		
40	1.981	1.703			1.002	1.042		
41	0.863	1.260			--	1.586		
42	2.789	1.338			1.231	1.316		
43	--	1.526			2.621	0.995		

0 = no HEOD detected

-- = sample was not analysed for HEOD