FACTORS AFFECTING THE STORAGE, EXCRETION, AND PLACENTAL TRANSFER OF DIELDRIN IN DAIRY CATTLE

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

DARWIN GILBERT BRAUND

1968

This is to certify that the

thesis entitled

FACTORS AFFECTING THE STORAGE, EXCRETION, AND PLACENTAL TRANSFER OF DIELDRIN IN DAIRY CATTLE

presented by

Darwin Gilbert Braund

has been accepted towards fulfillment of the requirements for

PhD degree in Dairy

J. T. Huber
Major professor

Date February 7, 1968

ABSTRACT

FACTORS AFFECTING THE STORAGE, EXCRETION, AND PLACENTAL TRANSFER OF DIELDRIN IN DAIRY CATTLE

by Darwin G. Braund

This study was undertaken to investigate the retention and excretion of dieldrin when fed in equal dosages to lactating dairy cows, and to pregnant dairy heifers during various stages of gestation.

The investigation included analyzing appropriate samples for dieldrin content with electron-capture gas chromatography to: (a) establish a normal dieldrin excretion curve and ascertain whether this excretion pattern could be altered by dietary changes, (b) determine body storage of dieldrin when fed prepartum, and the subsequent appearance and duration in milk following parturition, and (c) ascertain the occurrence and degree of placental transfer, sites of storage in the fetus, and the time required for dissipation of residues from young contaminated in utero.

Varying the dietary energy level did not significantly enhance the rate of dieldrin excretion, although thyroprotein-feeding did decrease the time required to reach a mean residue level of 1.0 part per million in

the milk fat. Residues in whole milk did not change when the fat content of milk was sharply reduced by feeding pelleted hay.

Significantly greater dieldrin storage occurred in the body fat of pregnant dairy heifers contaminated early rather than late in gestation, even though total pesticide intake was essentially equal.

Once non-lactating pregnant dairy heifers were contaminated with dieldrin, very little was eliminated from the body until lactation began. The pattern of residue decline in milk fat was similar for both cows and heifers. Concentrations dropped to about one-half their initial levels after three to four weeks, and to one-third by five to six weeks. At the end of 24 weeks milk still contained detectable levels of dieldrin. Because of plateauing, an exponential decline did not accurately describe the dieldrin disappearance pattern in milk fat. As the dieldrin was eliminated from the body via excretion in the milk, residue levels in shoulder fat of both cows and heifers declined exponentially.

Approximately 2.5% of the daily intake was excreted as unchanged dieldrin in the feces during contamination. Only trace amounts of the original compound were found in the urine.

Placental transfer of dieldrin occurred in each dam, resulting in contamination of all newborn calves.

The carcasses of male calves sacrificed at birth contained about 0.9% of the dieldrin ingested by the dams. Dieldrin levels in omental fat and blood of calves contaminated *in utero* declined with age and at 52 weeks negligible residues in omental fat were detectable in only one of five female calves.

FACTORS AFFECTING THE STORAGE, EXCRETION, AND PLACENTAL TRANSFER OF DIELDRIN IN DAIRY CATTLE

By

Darwin Gilbert Braund

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy

1968

DEDICATED TO

My wife, Carol, and my parents, whose many sacrifices have so meaningfully enriched my life.

BIOGRAPHICAL SKETCH

of

Darwin G. Braund

Born at Towanda, Pennsylvania, July 22, 1934, Darwin G. Braund was raised on a dairy farm, and was graduated from Sayre High School where he was named Outstanding Senior. In September, 1952, he enrolled at The Pennsylvania State University and was awarded a Sears-Roebuck Foundation Scholarship and the Allegheny County Boys' Working Reserve Scholarship.

Following graduation with a Bachelor of Science degree in Dairy Science, he joined The Pennsylvania State University Dairy Extension Staff July 1, 1956, and served four years as Dairy Specialist. In September, 1960, he enrolled in the Dairy Department of the University of Wisconsin and received the Master of Science degree in Dairy Science August, 1961, under the guidance of Dr. L. H. Schultz.

He joined Beacon Feeds, Cayuga, New York in September, 1961, as Dairy and Livestock Specialist, and was named Assistant Director of Dairy and Livestock Research January 1, 1964.

On September 1, 1964, he enrolled in the Graduate School of Michigan State University and completed the requirements for the Doctor of Philosophy degree in December, 1967. On December 15, 1967, he assumed the responsibilities of Project Leader for the Dairy Extension Program in the Department of Animal Sciences at the University of Kentucky.

ACKNOWLEDGMENTS

Because of the duration and scope of this research project, the list is long of those whose minds and hands have contributed to its fruition.

The author wishes to express his gratitude to his former major professor, Dr. L. D. Brown, for encouragement and counsel during the early phases of his graduate study. He is grateful to Dr. J. T. Huber, current major professor, for his guidance and constructive criticism in the preparation of this thesis.

Sincere appreciation is extended to Drs. R. M. Cook, J. L. Gill, and H. A. Tucker (Dairy), N. C. Leeling and M. J. Zabik (Entomology), E. J. Benne (Biochemistry), and D. A. Reinke (Physiology) for their advice concerning experimental techniques and preparation of this manuscript. The contributions of Drs. D. A. Ellis, S. M. Getty, and G. R. Ruth (Veterinary Surgery and Medicine) in performing surgery on the experimental animals are appreciated. Technical assistance was rendered by George Blank, Mrs. Nancy Millard, and Kim Wilson. The feeding and management of the cattle by the Dairy Barn staff and use of the facilities of the Meats Laboratory is acknowledged.

Thanks are extended to Dr. C. A. Lassiter, Chairman of the Department of Dairy, for making available the facilities and animals, and for financial support through a Graduate Research Assistantship and Research Instructorship.

And last, but not least, deepest appreciation goes to my wife, Carol, for her ever ready assistance, moral support, and sacrifice throughout my Ph.D. program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

I. INTRODUCTION

Pesticide residues in foods are nothing new, but interest in the problem by scientific investigators and the public in general has developed only during the past 20 years (116). The main contributing factor for this increased attention is the great number of new organic compounds that have come into general use since 1940. Accompanying this use, a demand has arisen for adequate controls to protect the consumer.

Residues in milk and dairy animals are particularly troublesome. Presently (December 1967) the tolerance for any pesticide, other than DDT and its metabolites, in milk is technically zero. A zero residue level simply means that a particular product contains less residue than the analytical chemist is able to detect with available techniques. However, the more capable the chemist and the better his technique, the lower the residue level that can be detected.

In October 1966 the Advisory Committee to the Food and Drug Administration (FDA) recommended that a tolerance level be established with a limit of 0.05 parts per million (ppm) of DDT, DDD, or DDE, individually or

in combination, for whole milk (40). FDA accepted this recommendation and established the tolerance indicated above.

A difficult situation for the dairy industry exists because of the very widespread use of pesticides on farms, drift problems, the pooling of milk from many dairy farms in a milkshed, and the rapidity with which milk has to be processed and distributed (115). In addition, analytical techniques which can detect parts per billion (ppb) and even less of a particular residue are becoming increasingly available.

The persistence of chlorinated hydrocarbon pesticides in tissues of dairy animals has been well established. Once an animal is contaminated with a chlorinated hydrocarbon pesticide, residues are found in the milk long after intake of the chemical has ceased (8, 12, 15, 16, 20-22, 30, 37, 38, 51, 52, 57, 71, 83, 84, 113, 135). However, there is a paucity of information relative to management practices which might effect a more rapid decontamination of milking cows. In addition, relatively few data are available concerning the body storage and elimination of chlorinated hydrocarbon pesticides fed prepartum; or of their subsequent appearance and duration in milk following parturition. An area of additional interest, which has profound implications for human health and medicine, is placental transfer of these

compounds to the fetus and the time required for dissipation of residues from young contaminated *in utero*.

The purpose of the investigations described herein was to establish a normal dieldrin excretion curve and ascertain whether this excretion pattern could be altered by dietary changes. Furthermore, dieldrin storage, excretion, and placental transfer were studied in prepartum heifers contaminated during three stages of gestation.

II. LITERATURE REVIEW

The latest chromatographic methods for residue analysis with their increased sensitivity give results which are not always directly comparable with those obtained by older colorimetric methods (45). During recent years electron-capture gas-liquid chromatography (GLC) has emerged as the analytical method of choice for chlorinated hydrocarbon insecticide residues (68). For this reason pesticide residue literature obtained since the development of electron-capture GLC is emphasized in this thesis.

A. Pesticide Absorption

Chlorinated hydrocarbon pesticides can enter the biological system through the skin, by inhalation, or ingestion (69, 143).

1. Dermal Application

All forms, including the dry powder of dieldrin and aldrin are absorbed through skin. Absorption of dieldrin through the skin was as rapid for the crystalline as for the solubilized material (62, 63, 143). Presumably the same is true for aldrin. Although the

mechanism of the percutaneous absorption of these materials has not been thoroughly investigated it is probably related to their lipid solubility (143). O'Brien and Dannelley (106) examined the penetration through rat skin of DDT, famphur, carbaryl, malathion, and dieldrin using radioactive compounds. The penetration rates varied markedly, increasing in the above order. Moreover, the rate was not related to the olive oil-water partition coefficient. However, the vehicles in which the compounds were applied did influence penetration rate, being slowest for corn oil, then benzene, and fastest for acetone.

2. Inhalation

Limited data concerning the inhalation of aldrin or dieldrin are available. Following inhalation of an aldrin aerosol, some irritation of mucous membranes was observed in guinea pigs (56). Baker and associates vaporized 0.50 g of aldrin per 1000 cubic feet of air each day and did not find any significant effect on mice, hamsters, guinea pigs, or parakeets (5). Whitacre and Ware (134) exposed chickens, rats, mice, frogs, fish, and seven species of plants to vaporized lindane for periods varying from 7 to 46 days. Nearly all animal and plant tissues showed significant storage of lindane, including egg yolks of chickens. Usually the residue in an animal or plant and the duration of exposure were correlated.

Witt et al. (138) found that respiratory exposure of dairy cows to DDT produced a lower level of DDT and its metabolic products in milk than did alimentary exposures.

3. Intravenous Administration

Intravenous administration of DDT to dairy cows was also studied by Witt et al. (138) who theorized that maximal response would be observed only after the oral dose was absorbed into the blood. They found that both one- and six-dose intravenous administrations produced much larger amounts of DDT and its metabolites in the milk fat than any other method tested. Levels were 162 to 525%, respectively, of those obtained with comparable doses of DDT given by intratracheal infusion or alimentary administration in a corn oil solution.

Hayes (60) stated that intravenous administration of an insecticide may not produce a true maximal response (death); because, if administered as an emulsion of an oil solution, the oil droplets may be physically blocked from passage in small capillaries or taken up by phagocytic cells. Thus, the fraction of the dose immediately available for distribution to critical tissues is unknown but probably small. Witt et al. (138) were unable to determine the effect of the vehicle used on absorption of an intravenous dose of DDT but did show that 33% was excreted in the milk compared to only 3 to 5% appearing in milk from intratracheal infusion or alimentary administration.

4. Oral Ingestion

Heath and Vandekar (67) found that very little dieldrin remained for as long as 24 hours in the gut of rats, but that relatively high proportions of unabsorbed dieldrin were excreted in the feces. Heath and Vandekar (67) suggested that absorption of dieldrin is controlled by the amounts of certain naturally occurring materials in the gut which enable its passage, and not by the quantity of dieldrin present. They found that dieldrin absorption from the gastrointestinal tract varied with the vehicle used and the absorbed compound appeared in the portal vein rather than in the thoracic lymph duct.

Witt et al. (138) concluded that absorption of DDT by dairy cows from aged-residues on alfalfa was about twice as efficient as absorption of DDT from an oil solution. This phenomenon has also been observed by Ely et al. (46). The increased absorption of DDT from an aged-residue on alfalfa may be due to a longer rumen exposure time for the alfalfa compared to the oil. It is not known whether pipetting the acetone solution of the insecticide on to the grain ration as used in this study would be more like administration in an oil solution or as an aged-residue.

B. Pesticide Transport in the Mammalian Body

1. Solubility and Transport in Blood

Moss and Hathway (102) showed that blood serum constituents exerted a powerful solubilizing effect on dieldrin and Telodrin. Solubilities of these two chlorinated hydrocarbon pesticides were 4000 times as great in rabbit serum as in water. These investigators presented evidence for the binding of dieldrin and Telodrin with the soluble proteins of the circulating blood. Ultracentrifugation demonstrated that radioactivity was associated with albumin, but not with chylomicra or lipomicra. In other experiments, DEAE-cellulose-column chromatography separated from rabbit serum three bands of dieldrin-^{14}C or Telodrin-^{14}C which were associated with albumin, the α-globulins, and another protein having the same electrophoretic mobility as albumin, respectively (65, 102).

The percent of total dieldrin and Telodrin bound to constituent proteins was not decreased with increased concentrations of the insecticide. Furthermore, increased concentrations of pesticide were not accompanied by spillover from one protein to another. Dieldrin and Telodrin were located mainly in the plasma and erythrocytes of rabbit and rat blood and not in the leucocytes, platelets or stroma. After treatment of animals with either

dieldrin-^{14}C or Telodrin-^{14}C, the distribution of ^{14}C between the soluble proteins of blood in vivo was identical with that in vitro. Thus, the radioactivity was due to unchanged starting materials and not to metabolites. The absence of metabolites for Telodrin was confirmed by gas chromatographic studies, in which all of the label was accounted for as Telodrin.

2. Transfer from the Blood

Pesticide levels in blood are difficult to interpret because it is not known whether the blood levels are a reflection of an immediately prior overt intake or of an equilibrium with pesticides stored in adipose tissue. The initial rate of removal of dieldrin and Telodrin from blood was very rapid, followed by a slow, roughly logarithmic rate of removal (102). Witt et al. (137) showed that DDT in bovine blood declined rapidly and concluded that equilibrium was nearly re-established 24 hours after cessation of pesticide absorption into the blood, although it is felt that their data did not cover a sufficient period of time (24 hr) to firmly establish the equilibrium point. Cook et al. (28) injected dieldrin into the jugular vein of sheep and goats at a level of 1.2 mg/kg body weight. They found that within 1 hour after administration dieldrin appeared in rumen fluid (2-20 ppb), parotid saliva (2-10 ppb), total saliva (20-80 ppb), and pancreatic juice (30-100 ppb). Dieldrin

in saliva and pancreatic juice remained rather constant for 48 hours. This recycling of dieldrin from the blood to the gut would extend the absorption period and increase the time required for blood to reach equilibrium. Crosby et al. (30) gave two cows single doses of DDT (13.5 and 15.0 mg/kg body weight, respectively) and found whole blood DDT increased sharply within the first two hours after feeding. The levels diminished rapidly after the peak measured at 17 hours, but did not reach a plateau until approximately two weeks post-administration.

Moss and Hathway (102) proposed that unloading of dieldrin and Telodrin in the kidney and liver was caused by breakdown of the carrier proteins, by changes in pH after penetration into the tissues, and by phagocytosis. They argued that since all of the plasma proteins enter phagocytic cells at the same rate (96), a mechanism for the rapid unloading of bound dieldrin and Telodrin from blood involving pinocytosis seemed likely, especially since many water-soluble and water-insoluble substances are rapidly removed from circulating blood. The withdrawal of these substances into the sinusoid spaces of the liver would provide an alternate explanation of their rapid removal from blood (102).

C. Factors Affecting Pesticide Storage in the Body

Most of the chlorinated hydrocarbon insecticides are retained to a greater or lesser extent and for varying periods of time in the body fat. The fact that chlorinated hydrocarbon materials are stored in adipose tissue is not surprising in view of the known solubility of these compounds in fats and fat solvents and their relative insolubility in aqueous solvents (21).

1. Dosage Level

In several species, less DDT was stored at higher than at lower dosage levels (59, 87). Lehman (87) showed that the same was generally true for aldrin, dieldrin, endrin, and isodrin; in contrast, lindane was stored with the same efficiency at all dosages studied. Methoxychlor showed a threshold and was stored only at the higher dosage levels.

Ivey et al. (72) found that 0.25 ppm of aldrin in the diet of steers for 12 weeks resulted in 0.99 ppm of dieldrin in the body fat, and 10 ppm aldrin in the diet increased the dieldrin content of fat to 39.2 ppm. Thus, in this study the storage of dieldrin was almost exactly proportional to the amount of aldrin fed. Not all species show the same storage when maintained on similar dosage levels (53).

2. Duration of Dosage

Soon after a single dose of any chlorinated hydrocarbon insecticide, distribution is relatively equal in most tissues. However, there is a gradual redistribution leading to higher concentrations in fat than in other tissues (34, 67).

Barnes and Heath (7) showed that two equal doses of dieldrin given to rats within three weeks of each other were more toxic than the sum of the two given as a single dose. Rats that survived one dose of dieldrin remained much more sensitive than normal rats to a second dose given up to three weeks later. Heath and Vandekar (67) theorized that after an original high dose of dieldrin, a concentration barrier develops between the blood and fat, so that the dieldrin from the new dose is diverted away from the fatty tissues, and much more dieldrin reaches the central nervous system.

At a constant daily dose, the storage of DDT in fat increased steadily for a period, but eventually reached a maximum and plateaued in the rat (82), monkey (44), cattle (108), dog (111), and man (61, 64).

It had been claimed (22) that cattle and sheep achieve storage equilibrium for a wide range of insecticides in four to eight weeks, but more recent work shows that a steady state is not achieved in cattle fed DDT until 18 to 21 weeks of administration (84). There

is great variation in the different estimates of the time necessary to reach a steady state of storage (or excretion) under any given conditions. Therefore, few if any of these estimates seem completely reliable (60).

The time necessary for the rat to reach a steady state in the storage of DDT was estimated to be as little as seven weeks (140) and as much as 19 to 23 weeks (82). The most likely value was considered to be 17 weeks (44). In the monkey, the time is unknown but it was less than 26 weeks (44). In man, the time was somewhat greater than 52 weeks (61).

Some authors have assumed a steady state when their earliest measurements were made. Others have accepted the highest observed storage level as the steady state. Hayes (60) emphasized that the latter assumption would be erroneous if storage were really incomplete or if a steady state had already been reached and the high value observed represented part of the random variation of a lower mean, which would be ascertained eventually by a longer-term study.

3. Age

Nothing is known about the relation of age to susceptibility of contamination by chlorinated hydrocarbon pesticides. What might have been interpreted as an age effect in previous studies can be explained by the

intensity and duration of dosage. No age is completely immune to contamination. To illustrate this, placental transfer of chlorinated hydrocarbon pesticides has been reported in dogs (48), rabbits (65, 66), rats (13, 65, 66), dairy calves (12, 14, 16, 24, 75, 83), and man (39).

4. Sex

At any substantial level of intake in excess of about 0.05 mg/kg per day, female rats store more DDT and derived compounds than do males (43). Similar results have occurred with benzene hexachloride (36), chlordane (1, 2), aldrin, endrin and isodrin (80), and heptachlor epoxide (110). Female rats did not store significantly more methoxychlor than males (81).

The greater storage of most chlorinated hydrocarbon insecticides in the female, as compared with the male rat, is probably characteristic of most tissues and not of fat only. This is documented by findings with DDT (33), aldrin, dieldrin, endrin and isodrin (80).

The difference in the storage of DDT and DDE between male and female rats which had been fed DDT was reduced greatly but not reversed by hormones of the opposite sex, gonadectomy, or a combination of these treatments (43). The difference in DDT storage between the sexes is small or absent in the dog (141), the hog (58), and the monkey (44).

5. Species

Gannon et al. (53) reported species differences in the storage of dieldrin. They found that steers stored more dieldrin than cows or hogs while sheep stored the least for any given level in the diet. In a related experiment (54) dairy cows showed slightly less dieldrin in tissues than did steers. Losses through lactation most likely accounted for this difference. Dieldrin was fed at levels of 0.1 to 2.25 ppm in the diet of steers, hogs, lambs, and dairy cows for 12 weeks and residues were found in renal fat, body fat, heart fat, udder fat, steak, and roast. Six weeks after feeding of the insecticide had ceased levels in most of these tissues had decreased by 50% or more (52, 53).

Bovard et al. (10) fed apple pomace contaminated with 103 ppm DDT to non-lactating beef heifers for 104 days. Average residues of 75.7 ppm DDT were detected in fat samples taken 79 days after pomace feeding began. Presumably some additional residue was deposited during the 25 days of pomace feeding which followed these fat analyses, but no other fat samples were obtained until 80 days after pomace feeding ceased. At this time DDT residues averaged 49.7 ppm. Based on limited data they concluded that DDT residues dissipated most rapidly immediately after pomace feeding was stopped, but the animals still contained 8.6 ppm DDT 566 days after last receiving contaminated feed.

It has been well established that the decline in residue concentration of chlorinated hydrocarbon pesticides in lactating dairy cows following cessation of intake is due primarily to elimination via the milk (12, 14, 16, 21, 30, 52, 54). There is no obvious reason for the faster rate of decline in residue concentration for the non-lactating animals (steers, hogs, lambs) used by Gannon et al. (53) and by Bovard et al. (10) (beef heifers), than occurred in the present studies (12, 16) with non-lactating dairy heifers.

6. Tissue

As previously mentioned the chlorinated hydrocarbon pesticides tend to be stored more extensively in adipose than in other tissues. There is some indication that the occurrence of DDT (85) and dieldrin (53) in tissues other than adipose is determined by their lipid content. The high DDT content of yellow bone marrow (108) is almost certainly explained by the high fat content of the tissue. The rather high levels of DDT reported for the adrenal (89) and ovary (126) may reflect the high lipid concentration in these organs.

Radeleff and Polen (109) fed heptachlor to beef cattle for 16 weeks at levels varying from 1 to 60 ppm. They found that complete conversion of heptachlor to heptachlor epoxide occurred in the animal body and that higher concentrations of the epoxide were noted in the

renal than in subcutaneous fat. Long et al. (88) found slightly more endrin in internal than in external fat of lambs.

Gannon et al. (52, 53) investigated dieldrin accumulation in tissue of steers and cows after feeding the insecticide (0.1 to 2.25 ppm) for 12 weeks. At 12 weeks, brain and kidney were free from residues regardless of dosage level. Highest concentrations attained were 0.6 ppm in the heart, 0.7 ppm in the liver, 6.2 ppm in renal fat, 4.8 ppm in body fat, 7.0 ppm in heart fat, 5.6 ppm in udder fat, 1.3 ppm in steak and 1.2 ppm in roast. Claborn et al. (23) found highest levels of dieldrin in the body and renal fat of steers while less was detected in muscle tissue. Fries and Kane (50) conducted a total balance study of DDT and DDE fed to male calves. They found similar residue levels in subcutaneous and perirenal fat; whereas, lower concentrations were noted in the lipids of liver and brain.

Heptachlor epoxide in the tissues of a male calf and a lactating cow were reported by King et al. (75). Concentrations in 11 different tissues of the newborn calf averaged about 1 ppm (range 0.44 to 2.00) while the residue was 3 to 4 times higher in the hair and hide. Residues in extracted fat of 18 tissue samples removed from a lactating cow were very uniform. The overall average was 0.53 ppm with lowest concentrations in the

brain and highest in liver, hide and hair. Matsumura and Hayashi (93) found that in the German cockroach (Blattella germanica L.) dieldrin formed a complex with nerve components which was inextractable with organic solvents. They suggested that such a binding phenomenon involved a complex formation with nerve components other than simple lipids.

Chlorinated hydrocarbon pesticides accumulate at much higher concentrations in adipose than in other tissues. This raises the question regarding the exact nature of the storage process. The concept has existed for some time that adipose tissue functions primarily in fat accumulation (131). However, studies in recent decades indicate adipose tissue may be part of the reticulo-endothelial system (RES). Under certain conditions adipose tissue has the capacity to develop a hematopoetic function and synthesize antibodies (128). The possibility that adipose tissue is part of the RES suggests that it could play a role in immune processes (130). These considerations caused Wasserman and Wasserman (128) to propose that adipose storage of chlorinated insecticides is an active process in the sense of phagocytosis.

7. Interaction of Drugs and Other Compounds

Durham (42) has recently published the first review on the subject of pesticide interactions with a variety

of factors. In this comprehensive report he discussed the relationship of pesticide contamination to factors such as physiologic state (species, sex, age, nutritional status, and hereditary defects in enzyme systems) disease, environment (temperature and light), route of exposure, formulation of the pesticide and interaction with other chemical agents.

Street (122, 123) demonstrated a reduction in dieldrin storage in fatty tissues of the rat when DDT was administered simultaneously. In further work Street et al. (125) tested selected drugs for their effectiveness in reducing dieldrin retention by rats. They found that oral administration of heptabarbital, aminopyrine, tolbutamide, and phenylbutazone reduced tissue concentration of dieldrin. Heptabarbital was the most effective drug used and resulted in an 80% decrease in tissue dieldrin when fed at a rate of 225 mg/kg rat/day. In comparison, DDT at 4 mg/kg rat/day effected a 72% reduction. Braund (13) obtained similar results with rats fed heptabarbital and dieldrin concurrently. At the end of two weeks contamination, dieldrin storage in rats receiving the drug was reduced 75%. The drug was also effective in eliminating the pesticide already stored in the body. Six weeks after cessation of dieldrin feeding, residues had been reduced 96% in animals receiving the drug during decontamination, while much higher concentrations were detected in groups receiving no drug.

In additional studies Street and co-workers (124) found that interactions among certain organochlorine insecticides influenced their storage in rat adipose tissue. DDT administration markedly depressed storage of dieldrin and heptachlor when the three pesticides were fed simultaneously. DDT also promoted a rapid depletion of pre-existing dieldrin stores. The DDT effect on cyclodiene storage was postulated to result from stimulation of detoxifying enzymes. Supporting evidence for the postulate included the depressing effect on dieldrin storage by several enzyme-inducing drugs, and the stimulation of ascorbic acid biosynthesis by DDT. However, this theory was challenged by the failure of inhibitors of protein synthesis to stop the effect of DDT on dieldrin storage. DDT interaction with dieldrin metabolism was also demonstrated in swine and sheep, but not in chickens (124).

Nutritional interactions in dieldrin toxicity have recently been investigated by Tinsley (127). At a dietary level of 20 ppm he found dieldrin accentuated an essential fatty acid stress in rats due to the action of dieldrin in the metabolism of polyunsaturated fatty acids. A riboflavin deficiency resulted in a depressed growth rate in female rats subjected to dieldrin stress. The vitamin inadequacy accentuated dieldrin toxicity and increased the level of dieldrin in liver. The hypothesis was

proposed that a riboflavin deficiency could limit the detoxification process.

Phillips (107) reported that, in the rat, DDT decreased the utilization of vitamin A and carotene and that rations low in protein accentuated dieldrin toxicity. The combination of the low protein and dieldrin stresses reduced the levels of vitamin A in liver (86).

D. Biotransformation

1. Chemical Nature

Processes concerned with the metabolism and decomposition of pesticides are important for several reasons. First, the duration of toxic action can be related to the rate and manner in which the compound is metabolized. Second, the rate of elimination from the body is dependent upon the physical and chemical properties of the metabolic products. Third, the ability of a compound to reach a site of action may be limited by the rate at which it is metabolized and the character of the metabolic products. Fourth, the toxicity of a compound can be decreased or intensified upon conversion to a metabolite. These factors emphasize the need for knowing the pathways of metabolism as well as the degree of accumulation of metabolic products in tissues.

Menzie (99) has classified metabolic pathways into seven groups that cover the majority of biotransformations which pesticides undergo.

These are:

1. Oxidation (in the sense that oxygen, as hydroxyl, takes part or is postulated to take part in one or more of the steps).
   a. Hydroxylation of aromatic rings
   b. Oxidation of side chains to alcohols, ketones, or carboxyl groups
   c. Dealkylation from oxygen or sulfur (ether cleavage)
   d. Sulfoxide formation
   e. N-oxide formation
2. Dehydrogenation and dehydrohalogenation
3. Reduction
4. Conjugation
   a. Amide formation
   b. Metal complex
   c. Glucoside or Glucuronic acid
   d. Sulfate
5. Hydrolytic reactions
   a. Cleavage of esters
   b. Cleavage of amides
6. Exchange reactions
7. Isomerization

Illustrative examples of substances known to undergo each of the biotransformations were also indicated.

2. Identity of Metabolites

Metabolites of chlorinated hydrocarbon insecticides which have been identified were summarized by Hayes (60). He made no attempt to separate the metabolites according to their certainty of identification. Many metabolites of chlorinated hydrocarbon insecticides have not been identified although they have been clearly recognized by solubility or chromatographic properties. Even when a new metabolite is indicated, further study is usually required to determine whether it is one of the previously unidentified metabolites of the compound.

3. Relation to Species or Strain

Bann et al. (6) found that aldrin was readily converted to dieldrin in the body of beef and dairy cattle, pigs, sheep, rats, and poultry. This change was independent of the mode of entry, since it occurred following oral ingestion or subcutaneous injection.

When aldrin-^{14}C was fed to male rats (25, 91) or administered orally to male and female rats, the active material excreted in feces and urine consisted of aldrin, dieldrin, and considerable amounts (up to 75% of the label appearing in feces and up to 95% in urine) of a mixture of unidentified hydrophilic metabolites. At a feeding level of 4.3 µg per day, a saturation level was reached after about eight weeks and daily excretion of

the active material approximated the entire activity administered daily. When injected intravenously, 16.2% of aldrin-^{14}C and 13.0% of dieldrin-^{14}C was excreted within four hours, via the bile, into the intestinal canal, mainly as hydrophilic compounds (78, 101). Recent studies with rat livers showed that this epoxidation was performed by the microsomes (139). Jensen (73) showed that biliary excretion was responsible for almost all of the DDT metabolites in feces of rats.

Paper chromatography of extracts of feces and urine showed a high percentage of aldrin-^{14}C initially. The percentage of unchanged aldrin decreased while that of hydrophilic metabolites increased for about 12 days. Thereafter, the distribution of aldrin and metabolites remained constant as long as aldrin was administered. After aldrin administration was discontinued, the relative percentage of aldrin in feces decreased and dieldrin increased (91). Paper and thin-layer chromatography showed that two additional compounds appeared in the feces and in the urine, and that one of the compounds in both feces and urine behaved similarly. Hydrolysis of the main compound from urine with alcoholic KOH resulted in a new acidic compound (91). When rabbits were given aldrin intravenously, hydrolysis of the metabolites gave the aldrin diol (78).

Dieldrin was administered internally to rats as a single dose at 20 mg/kg body weight (112). During the first eight days, only unchanged dieldrin was excreted in urine. Within the following four days, a metabolite of dieldrin was excreted. Of the dieldrin administered, 15.7% was excreted within 14 days in the feces. Datta et al. (35) showed that dieldrin was metabolized in rats to two compounds more polar than dieldrin and unstable to alcoholic KOH.

When labeled dieldrin was administered to a rabbit via stomach tube, six metabolites were isolated and purified via thin-layer chromatography. The acute oral toxicity of the main metabolite, which accounted for about 86% of the total activity in the urine, was only one-twelfth to one-sixteenth that of dieldrin (77).

Urine of men with occupational exposure to dieldrin contained at least two neutral, polar, chlorinated metabolites, which are thus far unidentified (31).

E. Excretion and Storage Loss

True excretion, i.e. elimination of previously absorbed material, of chlorinated hydrocarbon insecticides may occur by way of expired air, urine, feces, milk, dermal secretions (136), eggs, and fetus (12, 14, 16, 48, 65, 66, 75, 83).

1. Respiratory Loss

Respiratory excretion may be important for certain fumigants. McCollister (95) found that although monkeys eliminated 20% of absorbed radioactive carbon tetrachloride by exhalation during the first 18 hours following exposure, ^{14}C continued to be measureable in samples of respired air for a period of four weeks. Material derived from the carbon tetrachloride was also found in the urine and feces.

2. Fecal, Biliary, and Urinary Excretion

For a wide range of compounds, urinary excretion has been studied more thoroughly than fecal excretion, yet fecal excretion is more important for certain compounds. Studies by Hayes (60) showed that in the rat fecal excretion of DDT exceeded urinary excretion irrespective of the route of administration. The same was true of Perthane (9), methoxychlor (129), aldrin (94), and dieldrin (67). In fact, 90% of the excretion of dieldrin-derived material was via the feces (67). It has been suggested that most residual chlorinated hydrocarbon insecticides are excreted chiefly in the feces (60).

Biliary excretion has been shown for DDT (73, 112), Perthane (9), methoxychlor (129), aldrin (101), and dieldrin (67). The rate of biliary excretion may be

considerable. Weikel (129) recovered as much as 40% of an intravenous dose of methoxychlor from the bile of rats within six hours after administration.

The existence of an enterohepatic circulation of metabolites of chlorinated hydrocarbon insecticides appears certain. Bleiberg and Larson (9) collected bile from two rats given radioactive Perthane intravenously and in an eight hour period recovered 25 and 55% of the ^{14}C. When the bile samples were combined and given to a third rat, about 70% of the ^{14}C appeared in the feces and 13% in the urine over a three day period. This experiment also showed the relatively rapid excretion of the metabolites compared with the parent compound. Enterohepatic circulation of dieldrin was demonstrated by Heath and Vandekar (67) who found that the proportion of unchanged dieldrin excreted could be increased from 3% to 10% of the total excretion by cannulation of the bile duct.

The bile appears to be the principle route through which DDT metabolites enter the feces. The bile duct was cannulated in a rat before intravenous injection of radioactive DDT and 65% of the labelled dose was recovered in the bile, 2% in the urine, and only 0.3% in the feces (73). Furthermore, the possibility of some contamination of feces by urine could not be excluded.

Biliary excretion of metabolites suggests, but does not prove, that the liver is an important site for their production. For aldrin and dieldrin, the high concentrations of both the parent compound and its hydrophilic metabolite in the liver support this view (101).

However, Mendel and Walton (98) have presented evidence that the normal flora of the gastrointestinal tract may be more important than the liver in conversion of p,p'-DDT to p,p'-DDD. They showed that coliform bacteria isolated from feces of control animals could effect reductive dechlorination of p,p'-DDT to p,p'-DDD.

The urine is a more important route for excretion of several chlorinated hydrocarbon insecticides than for DDT. Stohlman, Thorpe, and Smith (121) recovered about 18% of the chlordane that had been fed from the urine of rabbits. Approximately 80% of the radioactivity derived from both α and γ benzene hexachloride was found in the urine and only about 20% in the feces (76).

3. Excretion in Milk

Gannon et al. (51, 52) found that the first dieldrin appearing in milk of cows which grazed treated pastures was directly proportional to dieldrin intake. Later concentrations were determined by the combined effect of daily intake and amount released from the body fat. Finally, when dieldrin had been removed from

the diet the slowly declining residues that persisted in the milk apparently were associated with dieldrin stored in the body fat.

As would be expected, while pesticides are being ingested, excretion rates in the milk are proportional to the dosage for DDT (22, 144), dieldrin (22, 33), aldrin, heptachlor, methoxychlor (22, 26), heptachlor epoxide (4, 71), and endrin (79). Moreover, the slopes of the lines relating concentration in milk to that in feed were identical for most of the compounds mentioned (22).

Once an animal has been contaminated with chlorinated hydrocarbon pesticides, residue excretion in milk may continue for many months. Bruce et al. (21) fed various heptachlor levels (0.5 to 50 ppm) to cows for 12 weeks. The maximum concentration of heptachlor epoxide appearing in milk fat was 460 ppm. This level occurred after 12 weeks of feeding the pesticide. Detectable residues were still present in both body and milk fat 23 months later. Other studies have shown that after hepachlor epoxide (22, 37, 38, 51, 57, 71, 113, 135), dieldrin (12, 14, 16, 22, 51, 52, 135), Telodrin (8, 57), and DDT (20, 22, 30, 51, 83, 84, 135) are stored in body fat, they continue to contaminate the milk long after chemical intake has been discontinued. Laben et al. (83, 84) calculated that DDT in milk declined at 9 to

11% per week after maximum concentrations of DDT in milk fat were 19, 231 and 812 ppm for three treatment groups. Rate of decline was 6.6% per week for their control group which had a maximum milk fat DDT concentration of about 2 ppm. However, Brown et al. (20) studied DDT excretion in eight cows during the first 59 days of lactation and found no marked change in the level of DDT or its metabolic products in milk. The level of DDT and its degradation products in this study ranged from 0.58 to 0.79 ppm in milk fat.

It has been shown (117, 119) that 10% or more of the DDT derived from residues on forage was excreted subsequently in the milk. When daily DDT intake was approximately 126 mg/cow (7 to 8 ppm on the hay) 15 to 23% was secreted. On some days certain cows secreted up to 32.5% of their DDT intake in their milk (119). Shepherd et al. (117) found the output of DDT in milk varied from 5 to 30% when daily intake varied between 100 and 700 mg per cow. However, these results must be viewed with caution, because they depend not only on analysis of DDT in milk, but also on analysis of DDT residues in hay. Any systematic failure to detect all of the residue in the hay would lead to an over-estimation of the proportion of the dose recovered in the milk. Ely et al. (46) were able to recover in the milk of cows only 1.8 to 7.0% of the DDT administered as an oil solution.

Huber and Bishop (71) reported that cows fed heptachlor and heptachlor epoxide residues on hay secreted 20 to 29% of the insecticide in their milk in the form of heptachlor epoxide. Again, the accuracy of the analysis of the residues in hay has bearing on the credibility of these data. Recent evidence (103, 133) has indicated that routine extraction techniques are inefficient when applied to crops containing solely an internal accumulation of dieldrin. Wheeler et al. (133) emphasized that the extraction of insecticides from plant materials, whether surface or internal residues, is often the weakest link in the entire analytical procedure.

Demott et al. (38) found that the percentage of heptachlor secreted into the milk as the epoxide ranged from 4 to 22, with an overall average of 12. Those cows on alfalfa hay that contained 0.29 ppm heptachlor or heptachlor epoxide secreted an average of 9% of the intake in their milk, whereas those on alfalfa with 0.08 ppm secreted 17% in their milk. This difference in secretion rate as influenced by concentration in the feed was also observed by others (52).

Zweig et al. (144) reported finding about 0.3 to 2.2% of the daily dose of DDT in the milk while Laben et al. (83) calculated that 3% of the dose was excreted in the milk. By estimating urinary output of DDA they calculated that urine and milk would account for 17 to

19% of the DDT fed. Witt et al. (138) investigated contamination of milk from different routes of animal exposure to DDT. The amount of administered insecticide which they accounted for in the milk varied from 0.78% to 32.2%. The percent of the dose recovered in the milk did not seem to be correlated with milk production, lactation number, stage of lactation, or weight of the cow.

4. Relation of Excretion to Storage and Storage Loss

High storage is generally correlated with slow excretion; thus, when excretion is relatively inefficient, storage tends to be high and storage loss slow.

Some of the earliest studies on residues of chlorinated insecticides indicated that the rate of storage loss was not constant after dosage was discontinued. In 1949, Kunze et al. (82) reported that following small doses of DDT about one-half remained in storage a month after dosage was discontinued and one-fourth after three months. Male rats, which had stored chlordane in their fat at a concentration of about 40 ppm, reduced stores by about 25% in five days and about 50% in 20 days (2). When these values for storage of DDT and chlordane are plotted on semilog paper the resulting curves are not strictly logarithmic. Similar curves were published by Gannon and Decker (51) in connection with the concentration of

dieldrin in milk following reduction or termination of dosage. Hayes (60) found similar curves for the storage of DDT and the excretion of DDA after dosage was stopped in man.

Greater efficiency in excretion at higher dosage levels accounted for, at least in part, the lesser amounts of DDT stored at high compared with low dosages (59, 87). Similarly, greater excretion efficiency may partially explain the reduced storage of DDT and DDE in males than female rats (60).

Hayes (60) pointed out that although the term "half-life" is frequently used to refer to storage loss, the term "percent per day" is preferable when accounting for both storage loss and excretion. The latter term is far more appropriate for describing changes in rates produced by starvation or some other temporary factor. Moreover, the rate of loss of some residual chlorinated insecticides decreases as storage decreases. The term half-life is completely valid only when stores decrease according to a logarithmic pattern. Hayes (60) cited the data of Heath and Vandekar (67) as an example of the rapid change in loss that might occur. In this study about 5% of a single intravenous infusion of tagged dieldrin was excreted daily in the bile, but the amount excreted daily increased to more than 10% in rats that had been starved for a few days.

F. Placental Transfer

Relatively few data are available on placental transfer of chlorinated hydrocarbon pesticides to the fetus or on the dissipation of residues from young contaminated in utero.

Finnegan et al. (48) showed that both DDT and DDD were transferred across the placenta of the dog. Placental transfer of dieldrin and Telodrin have been demonstrated in the rat and rabbit (65, 66). Clark (24) reported dieldrin in calves born to cows fed contaminated corn silage and cull potatoes. Laben et al. (83) found DDT in three stillborn calves from dams fed DDT prepartum. Heptachlor epoxide was distributed throughout the tissues, but was three to four times higher in the hide and hair than in other tissues, of a calf whose dam had consumed heptachlor-treated alfalfa for 6 to 12 weeks prepartum (75).

Hathway (65) investigated transplacental passage of dieldrin and Telodrin in rabbits. When a single injection of Telodrin-^{14}C was made into an ear vein of 24-day pregnant rabbits, the absence of ^{14}C in the amnionic fluid eliminated the possibility of Telodrin-^{14}C transport occurring from the uterine cavity at this stage of gestation through the vascularized yolk-sac or splanchnopleure. Guinea pig and rabbit antibodies are selectively transferred to the fetus by way of the yolk-sac mechanism (11).

During a 40-minute period, Telodrin concentration in fetal blood did not decline, whereas the concentration of Telodrin in maternal blood decreased. Hathway (65) concluded that Telodrin-^{14}C administered to the mother concentrated on the fetal side of the placenta. The passage of insecticide from mother to the fetus continued while the external to internal concentration gradient was favorable. Conversely, continued uptake of ^{14}C by the fetal blood against an adverse concentration gradient was not indicated. Moreover, maternal blood was constantly unloading insecticide, which was partially taken up by maternal fat depots.

Two-way placental transfer of dieldrin in rabbits was demonstrated by Hathway (65). Seven minutes after a single intravenous injection of dieldrin-^{14}C into 24-day pregnant rabbits, the concentration of ^{14}C in fetal blood was 93% as high as in maternal blood. This relationship between fetal and maternal blood concentrations remained roughly constant throughout the experimental period (40 minutes). As evidence for two-way transplacental passage, Hathway et al. (66) intravenously injected dieldrin-^{14}C into the umbilical vein of a single fetus of a 24-day pregnant rabbit. Radioactivity was found seven minutes later in the blood of the injected fetus, and also in maternal blood and in the blood of a non-injected fetus. It was found that after a single

intravenous injection of dieldrin-^{14}C in pregnant rabbits a very small uptake of insecticide by the free blastocysts rapidly occurred. Following their implantation, the rate of dieldrin-^{14}C uptake by blastocysts from maternal blood was significantly slower. Dieldrin was secreted from the endometria of treated animals irrespective of pregnancy. During the second half of pregnancy allantoic and amnionic fluids were free from ^{14}C.

The transfer of compounds across the placenta is regulated by the same physico-chemical mechanisms which control the transport across other cell walls (66). In this regard, the method of passage of dieldrin across the blood-brain barrier (67), the erythrocyte stroma (102), the wall of the gut (65), and the blastocyst membrane (66) seems related. Non-ionized compounds with high lipid solubility, like dieldrin, can rapidly cross the placenta in either direction, provided that the concentration gradient is favorable. Other compounds of low lipid solubility are only slowly transferred across the placenta, and many larger molecules also soluble in lipids are incapable of crossing the placental barrier.

G. Experimental Decontamination

It has been documented in this review that chlorinated hydrocarbons are stored largely in the body fat and exhibit a relatively long retention time in the

animal. There is a paucity of information relative to management practices which might result in a more rapid decontamination. The possibility of increasing the rate of decontamination via an increased rate of body fat depletion has been suggested. Brown et al. (20) investigated this approach but their results were inconclusive because fat mobilization was not actually measured and the cows studied lost an average of only 17 kg body weight during the first month postpartum.

Fries et al. (49) studied the rate of depletion of DDT and its metabolites when body fat loss was estimated by complete energy balance trials. They concluded that DDT and DDE were depleted from the animal at rates independent of fat mobilization. In contrast, DDD depletion appeared to be closely associated with the fat losses. However, the practical importance of clearing DDD through fat loss is minimal because it is stored to a much lesser degree than DDE and DDT.

Starvation diets in poultry have decreased the level of DDT found in eggs (132). Miller (100) investigated the effect of thyroprotein and a low-energy ration on removal of DDT from lactating dairy cows. He found no appreciable difference in rate of decline of DDT between cows that received normal rations and those fed thyroprotein. However, cows on a low-energy ration showed an appreciable increase in rate of decline.

Miller concluded that cows and heifers contaminated prepartum with DDT were more difficult to decontaminate than cows ingesting the same amount of pesticide postpartum. Prepartum contamination resulted in milk fat containing 7 to 12 ppm DDT compared with five to seven ppm DDT in the milk fat for animals contaminated postpartum.

III. EXPERIMENTAL PROCEDURES

A. Experiment 1--Excretion and Storage of Dieldrin in Dairy Cows Fed Thyroprotein and Different Levels of Energy

1. Experimental Animals--Dosage and Duration of Contamination

Sixteen lactating Holstein cows, averaging 39 months of age and 115 days postpartum were selected from the Michigan State University dairy research herd. Cows were assigned to one of four outcome groups based on daily milk production and stage of lactation.

For 56 days each animal was contaminated with technical dieldrin in grain at the daily rate of 0.11 mg/kg body weight. The technical dieldrin contained 88.4% 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 (HEOD)]. Because the analytical procedure measured HEOD, not dieldrin, all intake and analytical data reported hereafter in this thesis will be expressed as HEOD equivalent. Intake of HEOD was equivalent to 1.94 ppm in the total diet or 3.25 ppm in the dry matter (DM) consumed.

Intake of dieldrin was based on body weights of cows obtained two consecutive days just prior to contamination. Once each week during treatment the total weekly intake of dieldrin for each cow was thoroughly mixed in 6.35 kg of grain by delivering the necessary volume of an acetone solution containing 5 mg/ml dieldrin onto the grain from a buret. Thorough mixing was effected by tumbling the grain in a six-gallon covered polyethylene container. Grain was then divided into seven-0.907 kg (2 lb) units and placed in individual paper bags identified with the appropriate cow number. A bag of dieldrin-containing grain was fed to the appropriate cow in a clean manger at 8:00 A.M. daily, immediately following weighback of unconsumed feed. Only after the contaminated grain had been completely consumed was the remaining portion of the morning grain allotment offered.

During contamination, TDN was furnished to provide 100% of the NRC (105) requirement for maintenance and milk production. The requirement for growth instead of maintenance was used to calculate TDN needed by first lactation heifers.

All cows received a ration of 4.5 kg of alfalfa-grass hay, 13.6 kg of corn silage and sufficient concentrate to balance the TDN requirement. Adjustments in concentrate level were made every two weeks based on

the average milk production for the preceding seven days. Cows remained in a stanchion barn at all times except for two hours each morning when they exercised in a paved lot. Body weights were obtained on two consecutive days at two-week intervals throughout the contamination and decontamination period. Milk yields were recorded each milking and amounts of feed offered and unconsumed were weighed daily.

2. Dietary Treatments and Adjustments

At the end of the eight-week contamination period the four groups of four cows each were randomly assigned to one of the following dietary treatments:

a. 75% of the NRC requirement for TDN
b. 150% of the NRC requirement for TDN
c. 150% of the NRC requirement for TDN plus 22 mg of iodinated casein (Protamone)[1] daily per kg of body weight
d. 100% of the NRC requirement for TDN (Control)

Grain intake was adjusted every two weeks as previously described. Hay and corn silage levels remained constant throughout the experiment except for the low-energy ration in which each was reduced proportionately to achieve 75% of requirement.

---

[1]Supplied by Agri-Tech, Inc., Kansas City, Missouri.

Thyroprotein was fed by placing the required daily dose in gelatin capsules which were injected directly into the rumen with a balling gun. During the first five days thyroprotein was divided daily into two equal portions which were administered at the A.M. and P.M. feedings. Thereafter, the total daily dose was given at the 8:00 A.M. feeding only. At biweekly intervals thyroprotein dosage was adjusted for changes in body weight.

3. Sample Collection

a. Milk.--Aliquot milk samples were collected and composited daily from each cow just prior to contamination, on the third and sixth day following the start of contamination, and at weekly intervals thereafter until the eighteenth week of decontamination. The milking machine buckets were rinsed with water after each cow was milked to reduce cross contamination. Milk fat was determined according to the Babcock method on each composite sample following the P.M. milking. Milk samples were refrigerated overnight and extracted the following day for HEOD analysis.

b. Body Fat and Tissues.--Biopsies of external body fat weighing 5 to 10 g were removed from the shoulder area of two cows in each treatment group at the end of contamination and during the eighth and sixteenth week of decontamination. A 2% Procaine solution was used as local anesthetic. Fat samples were placed in plastic bags and stored frozen until extracted for HEOD analysis.

Following termination of the decontamination period six cows were sacrificed for various reasons not related to the experiment. Samples of external shoulder fat, omental fat, kidney fat, liver, kidney, and udder tissues were removed, frozen, and stored at -15°C until extracted for HEOD analysis.

c. Feces and Urine.--During the third week of decontamination total feces and urine were collected for five days from two cows in each treatment group. Feces were collected in metal pans placed at the rear of each cow and urethral catheters were used for shunting urine to ten-gallon cans.

After thorough mixing, two percent of the feces excreted daily was placed in a plastic bag and frozen. The five daily samples from each cow were thawed, composited, and dried at 80°C for 72 hours. After weighing, the dried samples were ground in a Wiley mill through a 1 mm screen.

Daily urine collections from each cow were stirred and an aliquot sample placed in a plastic jar and frozen. Each subsequent day's sample was added to the original container such that at the end of a collection period approximately two liters of urine had been saved from each cow.

d. Feed.--Prior to and at monthly intervals during the entire experiment, hay (1 kg), corn silage (2 kg), and grain (1 kg) were sampled for dieldrin analysis.

For hay, a Penn State Forage Sampler was used to obtain cores from about 25 bales which were representative of each sampling period. All samples were placed in plastic bags and refrigerated until extracted for HEOD analysis.

4. Sample Extraction

Extraction and clean-up procedures were developed in the Pesticide Analytical Laboratory of Michigan State University (142). All solvents were of reagent grade and were checked for interferring impurities by gas chromatography before use. Extraction proceeded as follows:

a. Milk.--

(i) After warming to room temperature milk was thoroughly mixed by shaking and 30 ml of whole milk were placed in a 500 ml flask, to which was added one gram of sodium or potassium oxalate, 25 ml ethanol, and 100 ml diethyl ether.

(ii) The flask was then shaken for 10 minutes on a Burrell Wrist-Action Shaker, Model CC[2] set at 6 1/2.

(iii) The diethyl ether was decanted and saved.

(iv) A 100 ml equivolume mixture of diethyl ether and n-hexane was added to the original sample two separate times. After shaking for 10 minutes each time at a setting of 2, the diethyl ether was decanted and saved.

---

[2]Burrell Corporation, Pittsburg, Pennsylvania.

The three extracts were combined and dried by the addition of about 5 g anhydrous Na_2SO_4. After standing for 30 minutes, the combined extract was filtered into a graduated cylinder. The amount of extract was then recorded and the Na_2SO_4 was rinsed with about 20 ml of diethyl ether.

(v) Next, the extract was concentrated to approximately 10 ml in a Kuderna-Danish Concentrator in preparation for column chromatographic clean-up.

b. Body Fat.--

(i) The frozen sample (5 to 10 g) was weighed and then blended with Dry Ice in a Waring Blendor[3] until very finely powdered. The powdered sample was then poured through a powder funnel pre-cooled with Dry Ice into a 500 ml flask.

(ii) The Blendor and funnel were slowly rinsed with 250 ml of n-hexane which was allowed to drain into the flask. The flask was then shaken at a setting of 5 on the wrist-action shaker for approximately 30 minutes. Prior to shaking flasks had been covered with Saran-Wrap squares secured by a rubber band. A few pin holes were made in the center of the squares to allow for escape of residual CO_2.

---

[3]The Blendor had been pre-cooled with Dry Ice before the fat sample was added; thus preventing the powder sample from adhering to the sides of the container.

(iii) The extract was then filtered through anhydrous Na_2SO_4 into a graduated cylinder; the flask was rinsed with an additional 50 ml of n-hexane, and the volume of extract recorded. All n-hexane was evaporated from a 25 ml aliquot of extract for determination of percent lipid.

(iv) A volume of extract containing the equivalent of 2 g of fat was then introduced into a 250 ml separatory funnel containing 50 ml of acetonitrile saturated with n-hexane. The mixture was shaken vigorously for two minutes and acetonitrile layer was drained into a 500 ml separatory funnel. The last step was repeated with two successive washings of acetonitrile which were accumulated in the same 500 ml separatory funnel.

(v) One hundred ml of n-hexane and 200 ml of a 10% aqueous NaCl solution were added to the acetonitrile in the 500 ml separatory funnel and the mixture was shaken for two minutes. The lower layer was discarded. Another 200 ml portion of NaCl solution was added, the shaking repeated, and the lower layer again discarded.

(vi) The n-hexane layer was transferred to a 250 ml flask, dried with anhydrous Na_2SO_4, and then introduced into a Florisil®:Celite®* (5:1) column for clean-up.

---

*Standardized Copyright symbol.

c. Body Tissue.--

(i) Approximately 10 g of frozen tissue were weighed and steps (i), (ii), and (iii) for body fat were performed except that all n-hexane was evaporated from a 50 ml aliquot to determine the percent lipid in the extract.

(ii) The extract was concentrated to about 10 ml in a Kuderna-Danish Concentrator and was then introduced into a Florisil®:Celite®* (5:1) column for clean-up.

d. Urine.--

(i) One liter of urine was filtered through a 5 μ millipore filter to remove particulate matter without loss of pesticide. The filtrate was divided into three 1-liter separatory funnels and each portion was extracted in order (left to right) once with the same 100 ml of n-hexane. This "progressive-extraction" was repeated, extracting each portion in reverse order (right to left) with another 100 ml of n-hexane. This procedure was repeated for the third time, adding another 100 ml of n-hexane to the center separatory funnel and then extracting the other two fractions.

(ii) An additional 100 ml of n-hexane was used for quantitative rinsing of the separatory funnels. The four 100 ml n-hexane extracts were combined

*Standardized Copyright symbol.

and concentrated to a 5 ml volume. Two µl were then injected into the gas chromatograph for HEOD quantification.

(iii) The millipore filters were dried in a vacuum oven for 10 minutes at 60°C to 70°C. Next, the filters were placed in a 16 x 150 mm culture tube with a Teflon-lined cap and 5 ml of n-hexane added. The tube was shaken for 10 minutes and 2 µl of the hexane extract were injected into the gas chromatograph.

e. Feed and Feces.--

(i) A 100 g sample of feed or feces was blended for two minutes in a Waring Blendor with 400 ml of a 3:1 mixture of n-hexane and isopropyl alcohol (IPA). The extract was then filtered through glass wool into a 1-liter separatory funnel.

(ii) Next, the IPA was removed with five or six washes of water, or until no odor of IPA could be detected. The extract was then dried for at least 30 minutes over anhydrous Na_2SO_4.

(iii) The extract was then filtered into a graduated cylinder, the volume recorded and the Na_2SO_4 rinsed with an additional 20 ml n-hexane. Next, the extract was concentrated in a Kuderna-Danish Concentrator to 10 ml for introduction into a Florisil®:Celite® * (5:1) clean-up column.

---

*Standardized Copyright symbol.

5. Column Clean-up Procedure for Chlorinated Hydrocarbon Pesticide Residue Extracts

Chromatographic clean-up columns were 20 mm OD by 600 mm Pyrex®* glass tubes equipped with a coarse fritted glass disc and Teflon stopcock. A clean-up column consisted of one-half inch of anhydrous Na_2SO_4, 10 g of Florisil®:Celite®* (5:1), and one-half inch of anhydrous Na_2SO_4 on top. Sufficient n-hexane was used to wet the column, then an additional 10 ml were added. The n-hexane was allowed to flow through the column until the top layer of anhydrous Na_2SO_4 was just covered with n-hexane. The concentrated sample and the rinse of the sample container were then poured into the column. The sample was allowed to drain into a 500 ml volumetric flask until it just covered the top layer of anhydrous Na_2SO_4 at which time n-hexane was added until 500 ml had been eluted. At higher levels of dieldrin an aliquot of the 500 ml sample was saved for injection into the gas chromatograph, but at lower levels this amount was concentrated in a Kuderna-Danish Concentrator to 100 ml or 10 ml.

The Florisil®:Celite®* (5:1) mixture for column clean-up was prepared as follows: 2000 g of Florisil[4] and 250 ml of distilled water were thoroughly mixed in a large plastic bag. This deactivated Florisil and 400 g of acid-washed Hyflo Super Cel Celite[5] were poured

---

[4] Floridin Company, Hancock, West Virginia.

[5] Johns Manville Co., Manville, New Jersey.

*Standardized Copyright symbol.

into a 4-liter Erlenmeyer flask and shaken for approximately one hour or until all lumps had disintegrated. After standing overnight the mixture was shaken for an additional hour.

For calibration, three columns were prepared with new Florisil®:Celite®* mixture as previously described. Instead of adding the concentrated sample, 25 ml of 100 ppm HEOD were added. The HEOD was then eluted with 500 ml of n-hexane and the eluate collected in 50 ml aliquots. These aliquots were analyzed by gas chromatography to ascertain that no HEOD appeared before the third or after the ninth 50 ml aliquot. If either of these conditions was not met, more water or Florisil®:Celite®* was added to the mixture and re-calibration performed.

6. Electron-capture Gas Chromatographic (ECGC) Analysis

Analytical columns (one-eighth inch i.d. by 6 ft Pyrex®* glass) were packed with 5% Silicone DC-11[6] on 60/80-mesh Gas-Chrom Q.[6] The instrument used for analysis was a Beckman GC-4 gas chromatograph[7] equipped with an electron-capture detector and a Bristol 1 mV recorder.[8]

---

[6] Applied Science Laboratories, State College, Pennsylvania.

[7] Beckman Instruments, Inc., Fullerton, California.

[8] Bristol Instruments, Waterbury, Connecticut.

* Standardized Copyright symbol.

Operating temperature parameters were: injection port - 250°C, column - 200°C, and detector - 350°C. Ultra-pure helium[9] was used as a carrier gas at a flow rate of 40 ml per minute. HEOD residue recoveries were determined by comparison of retention time (about 2 1/2 min) and peak height with known standard samples injected into the gas chromatograph. Sensitivity was such that two picograms (2×10^{-12} g) of HEOD gave a measurable peak.

Recovery of HEOD for various types of materials was calculated by fortifying samples with known amounts of HEOD and then extracting. Percent HEOD recovered for different materials were: milk, 93%; body fat, 96%; body tissue, 90%; urine, 98%; feed and feces, 104%.

B. Experiment 2--Storage, Excretion, and Placental Transfer of Dieldrin by Dairy Heifers Contaminated During Three Stages of Gestation

1. Contamination of Heifers

Thirty-one pregnant heifers utilized in this experiment were fed technical dieldrin in grain at the daily dose of 0.11 mg/kg body weight (5 mg/cwt) for 60 days. The dieldrin was mixed in the grain and contamination

---

[9]Matheson Co., Joliet, Illinois.

accomplished as described for Experiment 1. Based on total feed consumption during the contamination period, HEOD intake was equivalent to about 5.4 ppm in the total diet. The contamination scheme is shown in Figure 1. During contamination and until parturition, all heifers were fed hay at a daily rate of 1.5 kg/100 kg of body weight. Grain was fed in sufficient amounts to bring TDN intake up to 115% of the NRC requirement (122).

a. Contamination During 60 to 0 Days Prepartum.--Eighteen heifers were contaminated for 60 days starting 60 days prepartum. Problems were encountered with three of the animals which necessitated that they be replaced in the experiment. The specific reasons for removal were: one animal calved to an earlier breeding date and had only 34 days of contamination; a second heifer had a non-functional udder after calving; and the third heifer suffered from calving paralysis a week after parturition.

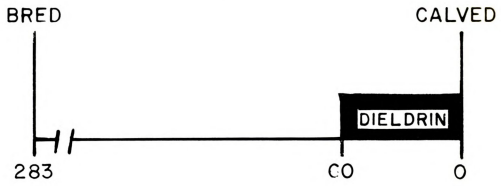
The calving span for the 18 heifers used on the experiment extended from November 3, 1965, to April 15, 1966. Based on actual calving order, heifers were assigned at parturition to one of three dietary groups.

Some variation occurred in the duration of dieldrin feeding. Feeding commenced on the 60th day preceding the projected calving date. If calving

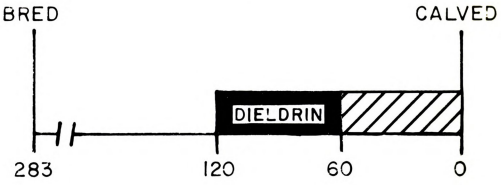
Figure 1.--Experimental design of prepartum contamination.

Plan for Pesticide Contamination

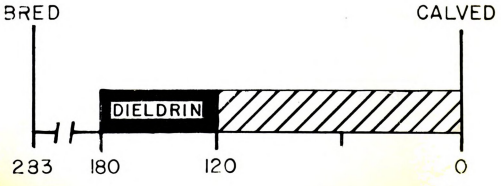
GROUP I



GROUP II



GROUP III



occurred before 60 days of contamination had elapsed, the feeding of dieldrin was discontinued. If, however, calving did not occur by the expected date, dieldrin was fed until the animal calved. If parturition had not occurred by 8:00 A.M. the dieldrin for that day was fed. Thus, the contamination period for individual animals ranged from 52 to 74 days with an average of 58.7 days.

b. Contamination During 120 to 60 Days Prepartum.-- Seven heifers were contaminated for 60 days at the previously described level (0.11 mg/kg) commencing 120 days prior to the expected calving date. The extra animal included in this group was originally scheduled for the 180-day contamination group but calved two months early. The calving span ran from March 31, 1966, to May 10, 1966. The mean number of days at which contamination commenced was 112.1 days prepartum, and the time between end of contamination and parturition averaged 52.1 days (Table 1).

c. Contamination During 180 to 120 Days Prepartum.-- An additional group of six heifers was contaminated at the same rate as the other two groups for 60 days beginning 180 days before expected parturition. For this group, contamination began an average of 177.5 days prepartum resulting in a mean time of 117.5 days between end of contamination and calving. The calving span for these animals extended from June 9, 1966 through October

12, 1966. Data on each individual animal are provided in Tables II, III, and IV of the Appendix. Mean group values for the three stages of contamination are shown in Table 1.

2. Dietary Treatments Imposed During Lactation

The following dietary treatments were employed to evaluate specific feeding practices which might affect the rate of residue persistence and excretion during lactation:

a. Contamination During 60 to 0 Days Prepartum.--

(i) Group A (6 animals--medium energy)--Alfalfa hay was fed at 2.0 kg/100 kg body weight and grain at 1.0 kg/3.5 kg milk produced.

(ii) Group B (6 animals--high energy)--Alfalfa hay was fed at 1.5 kg/100 kg body weight and grain at 1.0 kg/2.0 kg milk produced.

(iii) Group C (6 animals--depressed milk fat)--These heifers were fed the same as Group B except that dehydrated alfalfa pellets replaced long hay for the purpose of depressing percent milk fat.

b. Contamination During 120 to 60 and 180 to 120 Days Prepartum.--The 120-day (7 animals) and 180-day (6 animals) groups were fed similarly to Group A previously described. All heifers were fed 9.1 kg of grain daily for two weeks postpartum. After calving, grain

TABLE 1.--Contamination data for groups of heifers fed dieldrin during three stages of gestation.

HEOD Fed Prepartum	No. of Heifers	Contamination Period				No. Days Between End Contamination and Calving	Actual No. Days Contamination Started Prepartum
				HEOD Fed			
		Avg. B.W.	No. Days	Total	Body Wt.		
days		kg		mg	µg/g		
60-0	18	565	58.7	3111	0.094	0	58.7
120-60	7	547	60.0	3145	0.096	52.1	112.1
180-120	6	503	60.0	2939	0.097	117.5	177.5

was fed in accordance with specific treatment regimes. Biweekly adjustments were made based on milk production during the preceding week. All heifers were weighed twice within 48 hours following parturition, and on two consecutive days at biweekly intervals during lactation.

3. Sample Collection

a. Milk.--For most animals aliquot samples of colostrum milk were collected from each of the first eight consecutive milkings following parturition. This procedure was initiated after five heifers had calved, thus the data on colostrum are not complete. Colostrum samples were frozen and stored in pint cardboard containers of cylindrical shape. Once each week samples were analyzed for milk fat and extracted for HEOD analysis. After the colostrum period, daily milk composites were collected from each cow at weekly intervals for the first six weeks, and thereafter at 8, 10, 12, 16, 20, 24, and 30 weeks of lactation. The milking machine buckets were rinsed with water after each animal was milked to reduce cross contamination. Percent milk fat was determined on each composite sample following the evening milking as previously described.

b. Body Fat.--Approximately 5 to 10 g of external body fat were taken from each heifer prior to contamination, at the end of contamination, at partuirition, and at 8, 16, and 24 weeks postpartum. These samples

were removed, handled, and extracted as previously described.

c. Blood.--About 10 ml of blood was collected from the tail vein of each animal at the same time that the biopsy operation was performed. Ethylenediaminetetraacetic acid (EDTA) was used to prevent clotting and the samples were frozen until extracted for HEOD analysis.

d. Feces and Urine.--Total collections of urine and feces were made during two 5-day periods, one at the midpoint of contamination for the 180-day group and the second at 30 days postpartum for two animals in each of the 60-day dietary groups. Collections were conducted in digestion stalls equipped to automatically separate urine and feces. Sampling procedures were identical to those described earlier.

e. Feed.--Throughout the duration of the experiment, samples of grain, hay, and alfalfa pellets were taken at monthly intervals for HEOD analysis. Procedures used were the same as discussed previously.

f. Body Tissues from Newborn Calves.--Seventeen newborn male calves were prevented from nursing, trucked to the Michigan State University Meats Laboratory, and slaughtered soon after birth. Each calf was stunned with a captive-bolt gun and immediately exsanguinated. The carcasses were skinned, eviscerated, and placed in large plastic bags. The bags were then sealed and frozen.

The whole frozen carcasses were sliced into one-eighth inch slices on a band saw and then ground through a heavy-duty meat grinder with a one-half inch die. After mixing the ground tissue in a large plastic tub, the tissue was reground through the one-half inch die five consecutive times with thorough mixing after each grinding. Then the tissue was twice ground through a one-fourth inch die with mixing as before. It was then ground through a five-sixty-fourth inch die, and after thoroughly kneading this finely ground material about 10% was placed in a plastic bag as a subsample. From this amount, 100 g were analyzed for HEOD content as previously described. The liver, kidney, renal fat, and brain were also weighed, ground, and frozen for HEOD analysis.

Omental fat samples were removed by surgical biopsy from all female calves at birth prior to ingestion of colostrum. Both omental and shoulder fat biopsies were taken at 8, 16, 24, and 52 weeks of age. Blood samples were taken concurrently with fat biopsies similar to the manner previously discussed for the heifers. Methods for fat, tissue, and blood extractions and analyses were the same as described for the heifers.

4. Sample Extraction, Clean-up, and Analysis

Procedures for extraction, column clean-up, and analysis of samples taken during Experiment 2 were

identical with those described earlier under Experiment 1 for milk, body fat, body tissue, feces, urine, and feed.

Blood samples were extracted according to Crosby and Archer (124). All solvents were of Nanograde® quality. The gas chromatograph used was an Aerograph Model 1520[10] equipped with an electron-capture detector and a Westronics Model LD11A recorder.[11] The most satisfactory chromatographic column used was a 5' x 1/8" o.d. stainless steel tube packed with 8% Silicone DC-11 and 4% QF-1 on 60/80 mesh Gas-Chrom Q. Carrier gas was ultra-pure nitrogen[12] (40 p.s.i.) with a flow of 30-40 ml/minute. Operating temperatures were: injection port, 190°C; column, 175°C; and detector, 190°C. HEOD residue recoveries were determined by comparisons of retention time and peak height with known standard samples injected into the gas chromatograph. Sensitivity was such that 2 picograms (2 x 10^{-12}g) of HEOD gave a measurable peak.

5. Feeding Contaminated vs "Clean" Milk to Calves Contaminated in Utero

To ascertain the effect of feeding milk from contaminated animals on residue levels in growing calves,

---

[10]Varian Aerograph, Walnut Creek, California.

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

[12]Liquid Carbonic, Division of General Dynamics, Detroit, Michigan.

*Standardized Copyright symbol.

female calves born to dams contaminated during the last 60 days of gestation were alternately assigned to either a contaminated or non-contaminated milk diet. The calves receiving contaminated milk were allowed to nurse their dams for 24 hours and were then removed and fed contaminated colostrum from their dams for an additional 24 hours. Starting 48 hours after birth these calves were fed the dieldrin-containing milk from their own dams until weaned at 12 weeks of age.

Calves fed "clean" milk were removed from their dams immediately after birth without being allowed to nurse. Dieldrin-free colostrum, frozen from non-contaminated cows, was fed for the first 48 hours. Thereafter, dieldrin-free milk was fed until the calves were weaned at 12 weeks of age.

A sample of the milk fed to each calf was taken five days per week and composited for HEOD analysis. Hay and grain fed to these calves were sampled at monthly intervals for HEOD analysis. All calves were weighed on two consecutive days at four-week intervals. Milk, hay, and grain feeding were done according to the regular Michigan State University calf rearing program.

IV. RESULTS

A. Experiment 1--Excretion and Storage of Dieldrin in Dairy Cows Fed Thyroprotein and Different Levels of Energy[13]

1. Energy Intake

Energy intakes for each treatment during contamination and decontamination are calculated as percent of the 1966 NRC requirement for TDN and shown in Table 2.

TABLE 2.--TDN intake of cows as percent of the NRC requirement.[a]

Treatment	Contamination	Decontamination				
	8 wks	First 4 wks	Second 4 wks	Third 4 wks	Fourth 4 wks	Last 2 wks
	----------	----------	----------	----------	----------	----------
			%			
150% NRC	97.7	110.5	115.4	115.2	125.2	130.3
75% NRC	99.6	80.2	79.1	76.4	73.9	73.9
100% NRC	101.0	104.9	100.9	100.8	98.1	96.8
150% NRC + thyroprotein	101.4	98.7	106.4	127.1	138.4	138.0

[a]National Academy of Sciences - National Research Council (NRC). 1966. Nutrient Requirements of Dairy Cattle, Publication 1349.

[13]Presented in part at the Sixty-First Annual Meeting of the American Dairy Science Association, Oregon State University, Corvallis, June, 1966 (15).

All cows were offered 100% of the NRC requirement during the eight-week contamination period. During decontamination it was intended to feed two groups 150%; but, because of appetite limitations during the first eight weeks, intake in proportion to requirement increased only slightly. This was especially true for the cows fed thyroprotein because an increased response in milk production resulted in a correspondingly higher requirement. During the latter weeks of treatment, when lower production resulted in decreased energy requirements, appetite appeared less limiting and TDN intake was considerably above requirements for both the thyroprotein and high energy groups, but it never reached the desired 150%.

2. Body Weight Changes

Thyroprotein administration caused a rapid loss of body weight of cows averaging 57 kg four weeks after initiation of feeding. Thereafter, body weight plateaued until the twelfth week of decontamination and small gains were noted between 12 and 18 weeks. Body weight remained constant for the control cows until late in the treatment period after which slight increases were noted, while the 150% group exhibited an early and sustained increase. Weights of the 75% group decreased slowly during the entire decontamination period. Larger weight losses for this treatment were anticipated but they did not materialize. Body weight data are shown in Figure 2.

3. Milk Production

The average daily milk production is presented in Figure 3. A sharp initial increase was obtained for the thyroprotein treatment with a smaller increase for the 150% group. The definite drop in production during the fourth week of decontamination and subsequent recovery was due to several days of extremely hot weather which adversely affected most animals in the herd. As a result of decreased energy intake, the 75% group responded with a larger production decline than the other groups. These decreases preceded body weight loss. The precipitous decline in production for the 150% group during weeks 9, 10, and 11 occurred because the highest producing cow in the group suffered an udder injury and acute mastitis.

4. Excretion of HEOD in Milk Fat

The percent of the HEOD intake excreted during the contamination period is presented in Table 3. By the end of contamination approximately 19% of the total HEOD fed had been excreted in the milk fat.

HEOD residues in the milk fat of weekly samples fluctuated considerably and as indicated in Figure 4, dropped rapidly for all cows, regardless of treatment, to about one-third the initial levels after five weeks of decontamination. Thereafter, the rate of decline was slower with apparent plateaus in concentrations

Figure 2.--Body weight changes during decontamination of cows fed thyroprotein and different levels of energy.

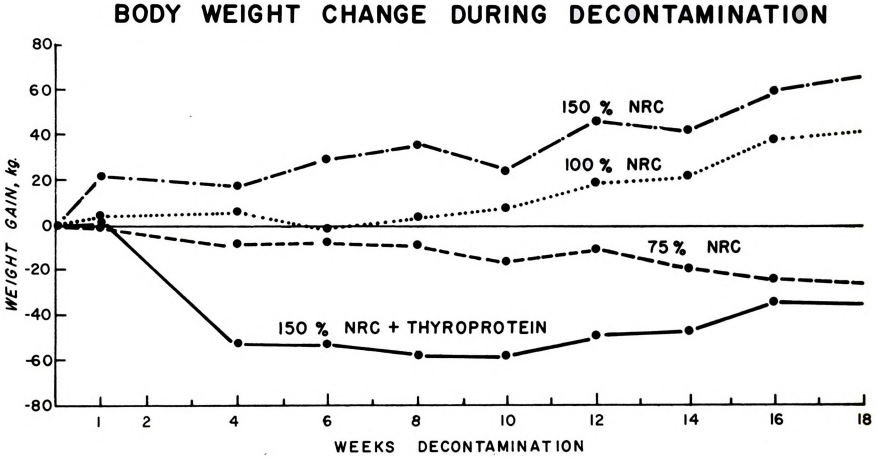

BODY WEIGHT CHANGE DURING DECONTAMINATION
WEIGHT GAIN, kg.
80
60
40
20
0
-20
-40
-60
-80
150 % NRC
100 % NRC
75 % NRC
150 % NRC + THYROPROTEIN
1
2
4
6
8
10
12
14
16
18
WEEKS DECONTAMINATION

Figure 3.--Average daily milk production during contamination and decontamination of cows fed thyroprotein and different levels of energy.

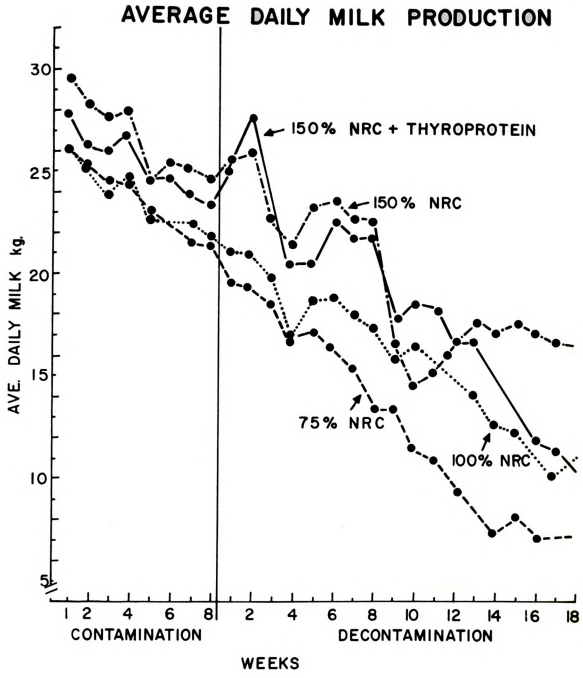

AVERAGE DAILY MILK PRODUCTION
AVE. DAILY MILK kg.
30
25
20
15
10
5
150% NRC + THYROPROTEIN
150% NRC
75% NRC
100% NRC
1 2 4 6 8 2 4 6 8 10 12 14 16 18
CONTAMINATION
DECONTAMINATION
WEEKS

Figure 4.--Levels of HEOD in milk fat of cows contaminated during lactation and fed thyroprotein and different levels of energy.

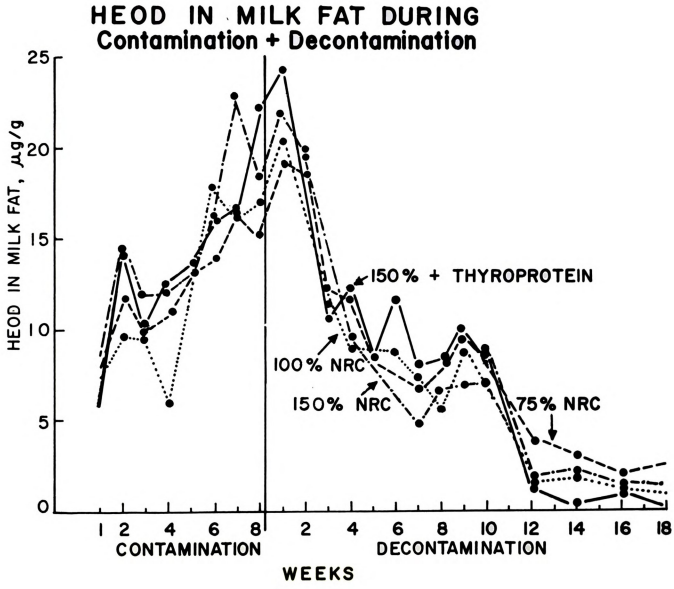

HEOD IN MILK FAT DURING
Contamination + Decontamination
HEOD IN MILK FAT, µg/g
25
20
15
10
5
0
150% + THYROPROTEIN
100% NRC
150% NRC
75% NRC
1 2 4 6 8
2 4 6 8 10 12 14 16 18
CONTAMINATION
DECONTAMINATION
WEEKS

TABLE 3.--Percent of HEOD intake excreted in milk fat during the eight-week contamination period of all cows.

	Weeks of Contamination							
	1	2	3	4	5	6	7	8
% of intake excreted weekly	11.7	19.6	15.2	15.4	16.8	21.5	23.9	24.4
% of intake excreted to date	11.7	15.6	15.5	15.4	15.7	16.7	17.7	18.6

occurring between weeks 5 to 10 and 12 to 18 of decontamination. Eighteen weeks after dieldrin feeding stopped, the milk from twelve cows still contained measurable levels of HEOD (0.01 ppm). Calculation of regression coefficients for the HEOD levels in milk fat, with transformation to log Y + 1, and statistical tests for goodness-of-fit, showed that the HEOD residues in milk fat did not decline at a straight line, exponential rate.

During the 18 weeks of decontamination an average of about 19% of the total HEOD intake was excreted in the milk fat (Table 4). There was no significant treatment difference in the percent of HEOD intake excreted. The mean percent excretion of HEOD was almost equally divided between the eight weeks of contamination and 18 weeks of decontamination. Therefore, 26 weeks after initiation of contamination about 37% of the total HEOD ingested had been accounted for in the milk fat (Table 4).

TABLE 4.--HEOD excretion pattern for cows contaminated during lactation.

Treatment Group	HEOD Intake	Percent of Intake Excreted During: 8 wk Cont.	18 wk Decont.	26 wk Total
	mg	%		
150% NRC	2970	22.5	19.0	41.5
75% NRC	2692	19.4	19.2	38.6
100% NRC	2939	14.7	16.6	31.3
150% NRC + thyroprotein	2939	16.9	21.0	37.9
S. E. of Mean	160	3.5	2.8	5.4
Overall Mean	2885 ± 80	18.6 ± 1.7	19.0 ± 1.4	37.4 ± 2.7

5. HEOD Levels in Body Fat

During decontamination HEOD residues were determined in samples of external shoulder fat removed from two cows in each group at eight-week intervals. These data are plotted in Figure 5. Initial values averaged 10 ppm, but declined exponentially to approximately 5 ppm at eight weeks and 1 ppm at 16 weeks. Regression coefficients of ppm HEOD in body fat on days of decontamination are shown in Table 5. Although the cows fed thyroprotein declined at the fastest rate, numbers were probably insufficient to detect a significant difference.

Figure 5.--Levels of HEOD in external body fat during decontamination of cows fed thyroprotein and different levels of energy.

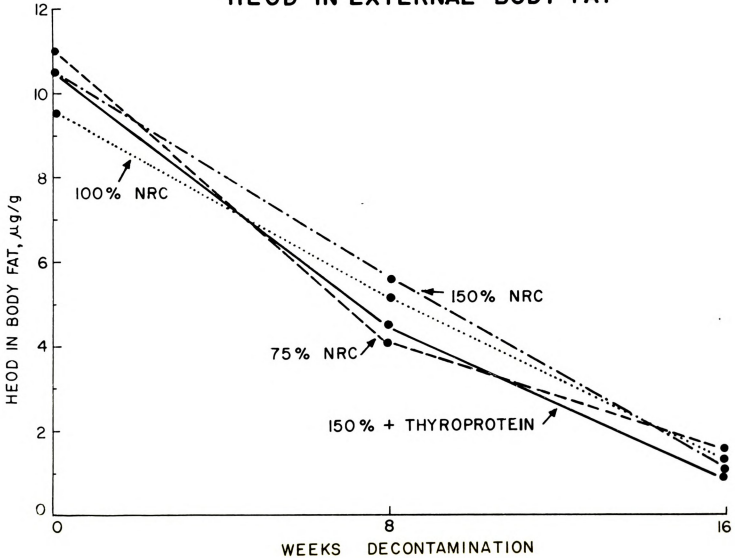

HEOD IN EXTERNAL BODY FAT
HEOD IN BODY FAT, µg/g
12
10
8
6
4
2
0
100% NRC
150% NRC
75% NRC
150% + THYROPROTEIN
0
8
16
WEEKS DECONTAMINATION

TABLE 5.--Regression coefficients of ppm HEOD in body fat on days of decontamination for cows contaminated during lactation.

Treatment % of NRC	Regression Coefficient	Standard Error	Correlation R^2
150	-.00677	.00126	.88
75	-.00648	.00188	.75
100	-.00590	.00161	.77
150 + thyro-protein	-.00705	.00261	.65

6. HEOD Levels in Tissues and Body Fat of Slaughtered Cows

After 18 weeks of decontamination six cows were slaughtered for various reasons. By coincidence three animals were from the thyroprotein-fed group and one from each of the other three treatments. Residue levels in fat at slaughter indicated a definite difference between thyroprotein-fed cows compared with the three others; therefore, the data for cows which did not receive thyroprotein were pooled and mean values are presented in Table 6.

Residues in kidney, liver, and udder tissues showed little difference between the two groups. However, residues in shoulder, kidney, and omental fat were three to five times higher for the thyroprotein-fed

cows. The differences for concentrations in omental fat were highly significant ($P < .01$) and levels in the kidney fat approached significance ($P < .10$).

TABLE 6.--HEOD residues in tissues and body fat of cows fed thyroprotein or no thyroprotein after 18 weeks of decontamination.

	PPM in Tissues			PPM in Fat		
	Kidney	Liver	Udder	Shoulder Fat	Kidney Fat	Omental Fat
Mean for 3 non-thyro-protein cows	.03	.13	.07	1.24	.57[a]	.72[c]
Mean for 3 thyro-protein cows	.03	.15	.10	3.69	2.80[b]	2.42[d]
S. E. of Mean	.01	.03	.02	1.2	.8	.2
Ratio: Thyro-protein / non-thyro-protein	1.0	1.2	1.4	3.0	4.9	3.4

[a,b] Difference approaches significance ($P < .10$).

[c,d] Means are significantly different ($P < .01$).

B. Experiment 2--Storage, Excretion, and Placental Transfer of Dieldrin by Dairy Heifers Contaminated During Three Stages of Gestation[14]

1. Experimental Treatments and Body Weight Changes During Lactation

Lactation rations, mean HEOD intake, and stage of gestation during which contamination occurred are summarized in Table 7. Body weight data obtained for each heifer on two consecutive days at biweekly intervals

TABLE 7.--Contamination and ration schedules for heifers.

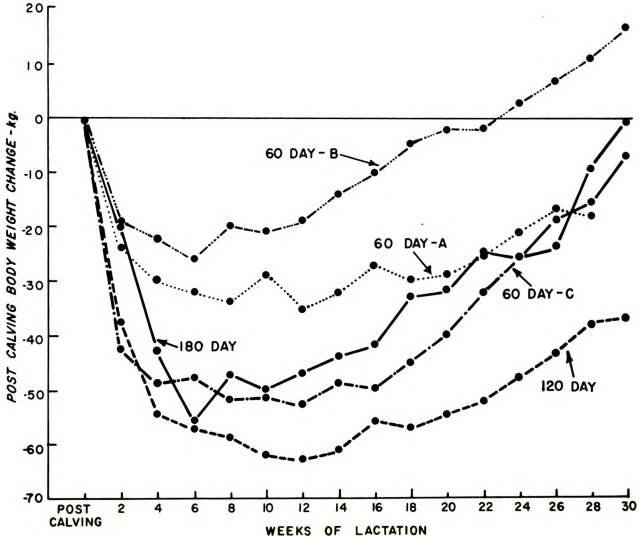
HEOD Fed Prepartum	Total Intake	Conc. in Feed	Gest. Stage at Contam.	Lactation Ration
days	mg	mg/kg	months	
60-0 A	3164	5.6	8-9	Normal
60-0 B	2982	5.4	8-9	High Grain
60-0 C	3189	5.4	8-9	Pelleted Hay
120-60	3145	5.3	6-7	Normal
180-120	2939	5.4	4-5	Normal

during lactation are plotted in Figure 6. The 60-day B group, receiving the highest level of energy intake reached minimum body weight at six weeks postpartum,

[14]Presented in part at the Sixty-Second Annual Meeting of the American Dairy Science Association, Cornell University, Ithaca, New York, June, 1967 (16).

Figure 6.--Body weight changes of heifers contaminated at various stages of gestation and fed different rations during lactation.

BODY WEIGHT CHANGE DURING LACTATION



but regained post-calving weight between 22 and 24 weeks. Losses in weight for other groups were of a greater magnitude and more persistent.

2. Milk and Milk Fat Production

Table 8 shows the total yield of milk, milk fat, and percent milk fat during the first 24 weeks of lactation. Although milk production was highest in those groups fed the diet of high grain and pelleted hay, mean production was not significantly different among the five treatment groups.

TABLE 8.--Total milk and milk fat production of heifers during the first 24 weeks of lactation.

HEOD Fed Prepartum	Milk	Fat	Milk Fat
days	kg	%	kg
60-0 A	3718	3.27[a]	120[ab]
60-0 B	3880	2.78[b]	106[bc]
60-0 C	4149	2.21[c]	92[c]
120-60	3908	3.65[a]	142[a]
180-120	3494	3.52[a]	123[ab]
S. E. of Mean	285	0.16	8

[a,b,c]Means sharing a common superscript are not significantly different ($P < .05$).

Milk fat percentages for the three groups on the normal rations (60-A, 120-, and 180-day) were significantly higher than for 60-B or 60-C heifers ($P < .05$). Milk of the 60-C group contained less fat than that of the 60-B animals ($P < .05$). Milk fat yields for the groups on the normal ration did not differ, but were significantly higher ($P < .05$) than for the group receiving pellets; moreover, fat yield for the 120-day group was significantly higher ($P < .05$) than for the high-grain group.

3. Body Storage of HEOD

a.--Pre- and Postpartum HEOD Concentrations in External Body Fat.--HEOD levels in external body fat samples removed from the shoulder area of each experimental animal at different pre- and postpartum stages are presented in Table 9 and Figure 7. Although total intake was very similar, the stage of gestation during which the pesticide was ingested exerted a highly significant effect upon storage levels in body fat ($P < .005$). Mean HEOD levels at the end of contamination were 10, 18, and 34 ppm, respectively, for the three stages of gestation. During the two- and four-month period between end of contamination and parturition, concentrations for the 120- and 180-day animals changed only slightly.

TABLE 9.--HEOD concentrations in body fat of heifers contaminated for 60 days at various stages of gestation.[a]

HEOD Fed Prepartum	HEOD Intake	Concentration in Body Fat				
		End Contam.	At Calving	During Lactation 8 wk.	16 wk.	24 wk.
days	mg	µg/g				
60-0 A	3164 ± 116[b] (6)	9.0 ± 1.5 (6)	9.0 ± 1.3 (6)	5.5 ± .9 (6)	2.3 ± .5 (6)	.4 ± .1 (6)
60-0 B	2982 ± 116 (6)	10.6 ± 1.7 (5)	10.6 ± 1.5 (5)	5.5 ± .9 (6)	2.8 ± .5 (6)	1.4 ± .1 (6)
60-0 C	3189 ± 116 (6)	10.2 ± 1.7 (5)	10.2 ± 1.5 (5)	8.6 ± .9 (6)	3.9 ± .5 (6)	.9 ± .1 (6)
120-60	3145 ± 107 (7)	18.4 ± 1.4 (7)	16.8 ± 1.2 (7)	7.9 ± .8 (7)	3.6 ± .5 (7)	1.8 ± .1 (7)
180-120	2939 ± 116 (6)	33.8 ± 1.5 (6)	31.7 ± 1.3 (6)	14.5 ± .9 (6)	4.2 ± .5 (6)	1.4 ± .1 (6)

[a]Number of animals each value represents is given in parentheses.

[b]Standard error of the mean.

Following parturition, body fat residues declined exponentially as the pesticide was excreted in the milk. At eight weeks postpartum HEOD concentrations were approximately one-half their level at parturition with the relative differences highly significant as indicated in Table 10. By 16 weeks postpartum no significant differences existed in body fat residues among treatments.

TABLE 10.--Orthogonal contrast comparisons of HEOD concentrations in body fat of heifers.

Treatment Contrast	End Contamination	At Calving	During Lactation		
			8 wk.	16 wk.	24 wk.
60 A, B, C vs 120, 180	***[a]	***	***	N.S.	N.S.
60 A vs 60 B, C	N.S.[b]	N.S.	N.S.	N.S.	N.S.
60 B vs 60 C	N.S.	N.S.	***	N.S.	N.S.
120 vs 180	***	***	***	N.S.	N.S.

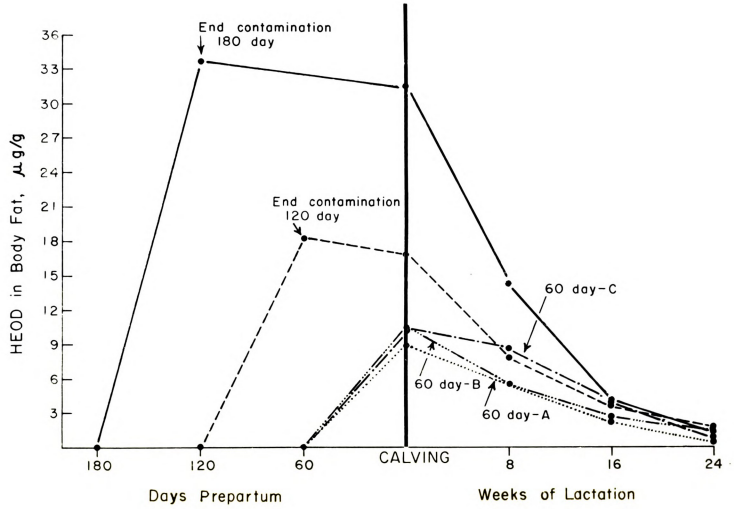
[a] Treatment comparisons significantly different ($P < .005$).

[b] Treatment comparisons not significantly different ($P < .05$).

After 24 weeks of lactation the average HEOD concentration in body fat of all heifers was approximately 1 ppm with no significant differences among groups. Three of 31 animals had less than .001 ppm in body fat at 24 weeks postpartum.

Figure 7.--Pre- and postpartum levels of HEOD in external body fat of heifers contaminated at various stages of gestation.

HEOD Concentration in External Body Fat of Heifers Contaminated for 60 Days at Various Stages of Gestation



Regression coefficients of ppm HEOD in body fat on days of lactation are shown in Table 11. Residues in heifers contaminated during 180- to 120-days prepartum declined at a significantly faster rate ($P < .01$) than those in any other group.

TABLE 11.--Regression coefficients of ppm HEOD in body fat on days of lactation for heifers contaminated during various stages of gestation.

HEOD Fed Prepartum	Regression Coefficient	Standard Error	Correlation R^2
days			
60-0 A	-.00498[a]	.00046	.85
60-0 B	-.00414[a]	.00034	.88
60-0 C	-.00481[a]	.00053	.80
120 - 60	-.00514[a]	.00041	.88
180 - 120	-.00723[b]	.00051	.90

[a,b]Regression coefficients sharing a common superscript are not significantly different ($P < .01$).

b.--Pre- and Postpartum HEOD Concentrations in Blood.--Blood samples obtained simultaneously with fat biopsy samples were analyzed for HEOD and the results are presented in Table 12 and in Figure 8.

In contrast to the highly significant differences in body fat residue levels found at the end of contamination, blood HEOD concentrations were not significantly different. Mean blood levels were approximately 15, 18,

TABLE 12.--Effect of stage of gestation on blood HEOD concentrations of heifers.[a]

HEOD Fed Prepartum	HEOD Intake	End Contamination	At Calving	During Lactation		
				8 wk.	16 wk.	24 wk.
days	mg	----------	----------mμg/ml----------			
60-0	3111	15.2 ± 1.6[b] (14)	15.2[c] ± 1.2 (14)	7.8 ± .7 (15)	3.3 ± .5 (16)	1.2 ± .2 (18)
120-60	3145	18.4 ± 2.2 (7)	6.9[d] ± 1.7 (7)	5.8 ± 1.0 (7)	4.9 ± .8 (6)	0.9 ± .4 (7)
180-120	2939	16.2 ± 2.4 (6)	7.4[d] ± 1.8 (6)	5.1 ± 1.2 (5)	2.1 ± .8 (6)	1.1 ± .4 (6)

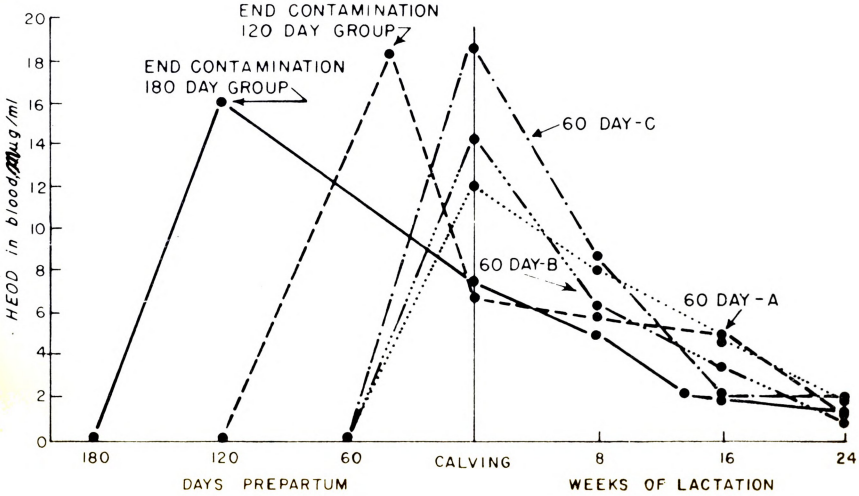
[a]Number of animals each value represents is given in parentheses.

[b]Standard error of the mean.

[c,d]Means sharing common superscript are not significantly different (P < .01).

Figure 8.--Pre- and postpartum levels of HEOD in blood of heifers contaminated at various stages of gestation.

HEOD CONCENTRATION IN BLOOD OF HEIFERS CONTAMINATED FOR 60 DAYS AT VARIOUS STAGES OF GESTATION



and 16 ppb (parts per billion) at the end of contamination for the groups contaminated at the respective stages of gestation (60-, 120-, and 180-days prepartum). During the two- and four-month period between cessation of HEOD intake and parturition, blood levels dropped from 18.4 to 6.9 ppb for the 120-day group and from 16.2 to 7.4 ppb for the 180-day heifers.

At parturition, blood HEOD concentrations were significantly higher ($P < .01$) for the 60-day heifers than for either the 120- or 180-day groups as indicated in Table 12. Stages of contamination had no significant effect on blood HEOD levels at 8, 16, or 24 weeks postpartum and by 24 weeks postpartum concentrations for all groups averaged about 1 ppb.

c. Content of HEOD in Body Fat and Blood at Parturition.--Presented in Table 13 are the calculated amounts of HEOD contained in the body fat and blood at parturition based on concentrations determined for each tissue. To determine the total body burden of pesticide, body fat content of heifers was estimated at 10%, whereas 57.4 ml/kg body weight was assumed for blood volume.

Based on these calculations, approximately 17, 30, and 59% of the pesticide consumed was accounted for at parturition in the blood and body fat of the 60-, 120-, and 180-day groups, respectively. These differences are highly significant ($P < .01$).

TABLE 13.--Effect of stage of gestation on HEOD content in body fat and blood of heifers at parturition.[a]

HEOD Fed Prepartum	Concentration in		Amount in		% of Intake Found in Body Fat and Blood at Calving
	Body Fat	Blood	Body Fat[b]	Blood[c]	
days	μg/g	mμg/ml	----------mg----------		%
60-0	9.9[e] ± .9[d] (16)	15.2[e] ± 1.2 (14)	523[e] ± 56 (13)	.47[h] ± .05 (13)	16.8[e] ± 1.7 (13)
120-60	16.8[f] ± 1.3 (7)	6.9[f] ± 1.7 (7)	934[f] ± 76 (7)	.22[i] ± .07 (7)	29.6[f] ± 2.3 (7)
180-120	31.7[g] ± 1.0 (6)	7.4[f] ± 1.8 (6)	1748[g] ± 82 (6)	.24[i] ± .07 (6)	59.4[g] ± 2.5 (6)

[a]Number of animals each value represents is given in parentheses.

[b]Body fat composition estimated at 10%.

[c]Whole blood volume calculated at 57.4 ml/kg body weight. Source: Biology Data Book, 1964. Federation of American Societies for Experimental Biology.

[d]Standard error of the mean.

[e,f,g]Means sharing a common superscript are not significantly different ($P < .01$).

[h,i]Means sharing a common superscript are not significantly different ($P < .05$).

d. Distribution at Parturition of HEOD Fed During Gestation.--Calculated in Table 14 is the distribution at parturition of the HEOD fed during gestation. Based on these values about 34% of the total intake was accounted for at parturition. The major amount was found in body fat and blood, with approximately 2.5% excreted in the feces during contamination and about 1% recovered in the calf carcasses.

TABLE 14.--Distribution at parturition of HEOD fed to heifers during gestation.

HEOD Fed Prepartum	Excreted in Feces During Intake[a]	In Blood and Body Fat At Calving[b]	Recovered in Calf Carcasses	Total % of Intake Accounted for at Calving
days	----------	---------- % ----------	----------	----------
60-0 A	2.5	14.8[c]	1.0	18.3[c]
60-0 B	2.5	19.3[c]	1.0	22.8[c]
60-0 C	2.5	16.7[c]	1.0	20.2[c]
120-60	2.5	29.6[d]	1.0	33.1[d]
180-120	2.5	59.4[e]	1.0	62.9[e]
Mean	2.5	30.1	1.0	33.6

[a]Calculated from data obtained in total collection trial at midpoint of contamination period for 180-day group (see Table 19).

[b]Body fat composition estimated at 10%; whole blood volume calculated at 57.4 ml/kg body weight. Biology Data Book, 1964.

[c,d,e]Means sharing a common superscript are not significantly different ($P < .01$).

4. Excretion of HEOD

a. HEOD in Colostrum Milk.--It is apparent that HEOD was present in the first milk available after parturition for all animals as summarized in Table 15. Mean concentrations in milk fat of the first eight milkings were 17.6, 23.7, and 30.9 ppm for the respective stages of contamination. These values reflect the body fat levels of HEOD at parturition (9.9, 16.8, and 31.7 ppm, respectively).

b. Concentrations of HEOD in Milk Fat During Lactation.--Residue levels in milk fat during lactation are presented in Figure 9. Concentrations dropped rapidly for all heifers with similar, but less definite patterns than noted for Experiment 2. As with Experiment 2, the regression of HEOD level in milk fat on days of lactation did not decline at a straight line, exponential rate. Concentrations in milk fat for heifers in groups 60-A and 60-B were not significantly different; whereas, group 60-C animals, which averaged 2.2% fat for the first 24 weeks (compared with 3.2% for the control group), exhibited higher HEOD concentrations ($P < .01$).

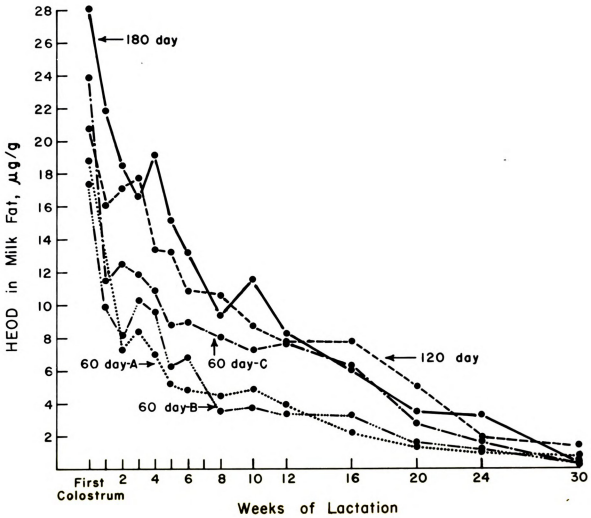
Residue concentrations for the 120- and 180-day heifers were significantly greater than for any of the 60-day treatments ($P < .01$). Moreover, HEOD levels for the 180-day animals were higher than for the 120-day heifers, again reflecting a higher ($P < .05$) prepartum

TABLE 15.--HEOD levels in colostrum milk of heifers contaminated during three stages of gestation.

Milking	60-Day Contamination				120-Day Contamination				180-Day Contamination			
	No. Samples	% Fat	PPM in Milk	PPM in Milk Fat	No. Samples	% Fat	PPM in Milk	PPM in Milk Fat	No. Samples	% Fat	PPM in Milk	PPM in Milk Fat
First	13	4.82	1.08	22.83	7	7.13	1.66	23.98	5	5.66	2.20	51.01
Second	13	5.32	0.83	16.60	7	6.64	1.47	25.15	6	8.13	1.70	21.98
Third	13	4.74	0.83	18.68	7	5.40	1.42	27.86	6	5.77	1.64	32.40
Fourth	10	4.25	0.82	19.42	7	4.93	1.37	27.80	6	5.18	1.66	35.85
Fifth	13	4.04	0.69	17.61	7	5.03	1.19	24.50	6	6.37	1.53	27.43
Sixth	13	3.95	0.68	17.45	7	5.13	1.06	21.29	4	5.68	1.37	25.58
Seventh	12	4.06	0.62	15.50	7	5.20	1.00	19.76	6	4.97	1.22	29.22
Eighth	13	4.21	0.53	13.30	7	5.27	0.98	19.01	6	4.70	1.14	25.57
Mean		4.43	0.76	17.64		5.59	1.27	23.67		5.82	1.55	30.93

Figure 9.--Levels of HEOD in milk fat of heifers contaminated at various stages of gestation.

HEOD Concentration in Milk Fat During Lactation



storage of the pesticide in the body. At 30 weeks post-partum, milk fat residues had declined to less than 1 ppm for nearly all groups.

c. Excretion of HEOD During Lactation.--The mean daily excretion of HEOD is shown in Figure 10. Of the three 60-day groups, the amount of pesticide excreted daily was slightly higher for the group fed pellets because of the increased concentrations in milk (Figure 9). Although the total amount of pesticide excreted during 24 weeks of lactation was about 43% greater for the pellet-fed than the other 60-day groups, differences were not significant. Both the 120- and 180-day groups excreted significantly larger amounts of pesticide during lactation than the 60-day groups ($P < .01$).

Stage of gestation during contamination had a significant effect on the cumulative percent of total pesticide ingested which was excreted during lactation (Table 16). After 24 weeks of lactation, the 120- and 180-day heifers had excreted over 40% of their total intake compared with about 18% for the 60-day groups. The means for the 60-day heifers were significantly less than for the 120- and 180-day groups at each six week interval ($P < .01$).

In Table 17 are shown ration effects on pesticide excretion. Animals fed pelleted hay had excreted a higher percentage ($P < .05$) of their pesticide intake in milk fat by 18 and 24 weeks than those on normal or high grain diets.

Figure 10.--HEOD excreted daily in milk fat of heifers contaminated at various stages of gestation.

Daily HEOD Excretion in Milk Fat During Lactation

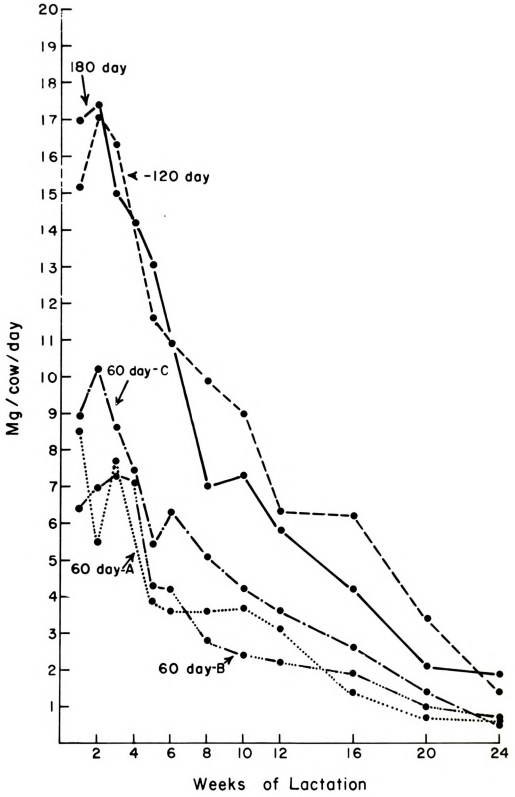


TABLE 16.--Cumulative excretion of HEOD in milk fat as affected by the stage of gestation during which heifers were contaminated.

HEOD Fed Prepartum	HEOD Fed	6 wks	12 wks	18 wks	24 wks
days	mg	----------% of intake----------			
60-0	3111	8.7[b] ± 1.7[a]	13.5[b] ± 1.7	16.4[b] ± 1.7	17.6[b] ± 1.7
120-60	3145	19.2[c] ± 2.9	30.2[c] ± 2.9	37.9[c] ± 2.9	41.1[c] ± 2.9
180-120	2939	20.7[c] ± 2.9	31.0[c] ± 2.9	37.3[c] ± 2.9	40.3[c] ± 2.9

[a]Standard error of the mean.

[b,c]Means sharing a common superscript are not significantly different ($P < .01$).

TABLE 17.--Cumulative excretion of HEOD in milk fat as affected by the ration.

Ration	HEOD Fed	6 wks	12 wks	18 wks	24 wks
	mg	----------% of intake----------			
Normal	3164	7.6	12.2	14.5[a]	15.4[a]
High grain	2982	8.2	11.9	14.5[a]	15.8[a]
Pelleted hay	3189	10.2	16.4	20.0[b]	21.5[b]
S.E. of Mean		2.9	2.9	2.9	2.9

[a,b]Means sharing a common superscript are not significantly different ($P < .05$).

d. Fate of HEOD Found in Body Fat and Blood at Parturition.--Presented in Table 18 is the disposition of the pesticide assumed present in the body fat and blood at parturition. The assumptions made for body fat composition and blood volume required for calculations of these data were discussed earlier. Of the amount present in the body at parturition, 116% was excreted in the milk, 7% eliminated via feces, and 8% remained in body fat and blood after 24 weeks of lactation.

e. Excretion of HEOD in Feces and Urine.--To investigate possible excretory pathways of HEOD, total collections of urine and feces were made during two 5-day periods; one at the midpoint of the contamination period for the 180-day group and the second at 30 days postpartum for two animals in each of the 60-day groups.

TABLE 18.--Fate of HEOD found in body fat and blood of heifers at calving.

HEOD Fed Prepartum	Amount in Blood and Body Fat at Calving[a]	Excreted During Lactation		Amount in Blood and Body Fat After 24 wk. Lactation[a]	Total % of Amount in Body at Calving
		In Milk	In Feces[b]		
days	mg	----------	----------	%	----------
60-0 A	447	132.6	7.8	7.5	147.9
60-0 B	577	84.0	8.6	8.9	101.5
60-0 C	565	132.8	6.5	8.0	147.3
120-60	934	153.5	6.6	10.4	170.5
180-120	1748	68.2	3.8	4.3	76.3
Mean		115.9	7.1	7.9	130.9

[a]Body fat composition estimated at 10%; whole blood volume calculated at 57.4 ml/kg body weight. Biology Data Book, 1964.

[b]Calculated from data obtained in total collection trial conducted 30 days postpartum for 60-0 A, B, and C groups; mean used to calculate excretion for 120- and 180-day groups (see Table 20).

Shown in Table 19 are data on excretion of HEOD by non-lactating dairy heifers during contamination. A mean concentration of 0.52 ppm HEOD found in the feces accounted for an average of 2.5% of the daily intake. Only trace amounts (<.001 ppm) of HEOD were found in the urine. Mean HEOD excretion for two heifers in each of the 60-day treatment groups at 30 days postpartum are presented in Table 20. Milk was definitely the major excretory pathway of HEOD from lactating animals. A much smaller amount was eliminated in feces while only trace amounts were found in urine.

5. Placental Transfer of HEOD

a. Tissue Residue Levels in Newborn Male Calves.--Placental transfer of HEOD occurred in each of 33 dams, resulting in contamination of all newborn calves. Seventeen male calves were prevented from nursing and slaughtered immediately after birth. Mean levels in body tissues from these calves are presented in Table 21. Neither differences in HEOD in blood or body fat of the dams at parturition significantly affected the mean residue level in whole carcass, carcass fat, brain, or renal fat of the male calves. HEOD concentrations in the kidneys of the calves born to heifers contaminated at 180 days were significantly greater than those of the 60-day group ($P < .01$). However, residues in livers of calves born

TABLE 19.--Daily excretion of HEOD in feces and urine of non-lactating heifers at midpoint of 60 day contamination period.[a]

Heifer	HEOD Concentration		HEOD Excreted in Feces	
	Feces	Urine	Total	% of Amount Fed
	---------mg/kg--------		mg	%
H-786	.47	<.001	1.18	2.3
H-788	.59	<.001	1.27	2.6
H-783	.65	<.001	1.68	3.2
H-814	.35	<.001	1.04	2.0
Mean	.52	<.001	1.29	2.5

[a]Data are from four heifers contaminated 180 to 120 days prepartum.

TABLE 20.--Daily excretion of HEOD in feces, urine, and milk 30 days postpartum of heifers contaminated during the last 60 days of gestation.

Treatment Group[a]	Feces			Urine		Milk			Total HEOD Excr.
	D.M. Prod.	HEOD Concn.	HEOD Excr.	Prod.	HEOD Concn.	Prod.	HEOD Concn.	HEOD Excr.	
	kg	mg/kg	mg	kg	mg/kg	kg	mg/kg	mg	mg
60 day A	4.7	.08	.38	7.1	<.001	26.7	.13	3.47	3.86
60 day B	4.0	.13	.52	7.2	<.001	27.1	.12	3.28	3.80
60 day C	2.7	.17	.46	5.1	<.001	30.3	.06	1.72	2.18

[a]Two heifers per group.

TABLE 21.--Mean HEOD concentrations in body tissues of male calves born to contaminated dams.[a]

HEOD Fed Prepartum	HEOD in Dam at Calving		HEOD Recovered in Calf as % Fed to Dam	Calf Tissue						
	Body Fat	Blood		Whole Carcass	Liver	Kidney	Brain	Renal Fat	Carcass Fat	Whole Blood
days	μg/g	mμg/ml	%	μg/g						mμg/ml
60-0	9.2[c] ± 1.0[b] (10)	12.8[j] ± 1.4 (7)	.9 ± .1 (10)	.84 ± .11 (11)	.89[f] ± .12 (10)	.22[h] ± .06 (11)	.15 ± .03 (8)	25.7 ± 5.5 (11)	25.2 ± 3.4 (11)	9.1 ± 1.4 (5)
120-60	19.4[d] ± 1.8 (3)	6.7 ± 2.1 (3)	.9 ± .2 (3)	.93 ± .22 (3)	.37[g] ± .21 (3)	.34 ± .11 (3)	.05 ± .05 (3)	34.2 ± 10.5 (3)	26.8 ± 6.5 (3)	3.2 ± 1.9 (3)
180-120	31.7[e] ± 1.6 (4)	7.3 ± 1.8 (4)	1.0 ± .2 (3)	1.02 ± .19 (4)	.44 ± .18 (4)	.57[i] ± .09 (4)	.09 ± .04 (4)	14.9 ± 10.5 (3)	33.7 ± 5.7 (4)	3.7 ± 2.3 (2)
Average			.9 ± .1	.89 ± .09	.69 ± .09	.32 ± .04	.11 ± .02	25.3 ± 4.4	27.4 ± 2.7	6.2 ± 1.0

[a]Number of animals each value represents is given in parentheses.

[b]Standard error of the mean.

[c,d,e]Means significantly different from each other (P < .01).

[f,g]Difference approaches significance (P < .05).

[h,i]Means significantly different from each other (P < .01).

[j]The general analysis of variance test indicates a significant (P < .05) difference among treatment means but Duncan's New Multiple Range test does not indicate so at the same level of probability. If, in fact, these differences are real it is apparent which means are different.

in the 60-day group were higher for those born to 120- and 180-day animals.

Calculated recoveries of HEOD in the male calves averaged 0.9% of the total ingested by the dams. Total placental transfer was probably higher than the recovery figures indicate because the calves were skinned prior to grinding of the carcasses for HEOD analysis. Furthermore, HEOD in the placental tissues and fluids was not determined.

Blood residue levels taken at birth and averaged for all calves are given in Table 22. It is apparent that calves born to heifers contaminated during the last 60 days of gestation had higher blood HEOD levels than those born to heifers contaminated for a similar time, but starting at 120 and 180 days prepartum. A direct relationship between blood residue levels in the dams and their calves was shown.

Seven female calves were sacrificed or died during the growing period. Concentrations of HEOD in liver, kidney, brain, and renal fat from these calves averaged 0.75, 0.29, 0.10, and 26.20 µg/g, respectively. These values agreed closely with the comparable means observed for newborn males as shown in Table 21.

b. Age Effect on HEOD Concentrations in Blood and Omental Fat of Female Calves.--The effect of age on blood residues in female calves is presented in Table 23.

TABLE 22.--Comparison of HEOD concentrations in body fat and blood of dams at parturition with blood levels in all newborn calves.[a]

HEOD Fed Prepartum	HEOD in Dams at Calving		Blood HEOD in Calves at Birth
	Body Fat	Blood	
days	µg/g	mµg/ml	mµg/ml
60-0	9.9[c] ± .8[b] (16)	15.2[c] ± 1.2 (14)	6.2[f] ± .9 (12)
120-60	16.8[d] ± 1.2 (7)	6.9[d] ± 1.7 (7)	1.8 ± 1.2 (7)
180-120	31.7[e] ± 1.3 (6)	7.4[d] ± 1.8 (6)	2.8 ± 1.8 (3)

[a]Number of animals each value represents is given in parentheses.

[b]Standard error of the mean.

[c,d,e]Means sharing common superscript are not significantly different ($P > .01$).

[f]The general analysis of variance test indicates a significant difference ($P < .05$) among treatment means but Duncan's New Multiple Range Test does not indicate so at the same level of probability. If, in fact, these differences are real it is apparent which means are different.

TABLE 23.--Effect of age on HEOD concentrations in blood of female calves born to contaminated dams.[a]

HEOD Fed Prepartum	HEOD in Blood of Dam at Calving	Age of Calves			
		Birth	8 wks	16 wks	24 wks
days	----------mμg/ml----------				
60-0	17.5[c] ± 1.9[b] (7)	4.1[c] ± .6 (7)	1.2 ± .7 (5)	.07 (3)	<.001 (3)
120-60	7.1[d] ± 2.6 (4)	0.7[d] ± .8 (4)	0.4 ± .7 (5)	<.001 (5)	<.001 (1)
180-120	7.7[d] ± 3.6 (2)	1.0[d] ± 1.7 (1)	----	----	----
Average		2.7 ± .5	0.8 ± .5	0.003	<.001

[a]Number of animals each value represents is given in parentheses.

[b]Standard error of the mean.

[c,d]Means sharing a common superscript are not significantly different ($P < .05$).

Calves born to dams in the 60-day group contained significantly higher levels of HEOD in their blood than those of dams from the other two groups ($P < .05$). HEOD in the blood disappeared more quickly than that found in the omental fat (Table 24). At 16 weeks of age HEOD in blood was detectable in only one of seven calves, but none was found at 24 weeks in the four calves sampled.

HEOD in omental fat removed from female calves at various ages was not significantly affected by the stage of gestation during which their dams were contaminated nor by the concentration of HEOD in the body fat of the dams (Table 24). The average rate of decline in HEOD concentration in body fat was similar for calves born to both the 60-day and 120-day groups. Prediction equations for rate of decline of HEOD in omental fat for calves from the 60- and 120-day groups were

$$\hat{Y} = 7.13 + (-.221)X$$

and

$$\hat{Y} = 9.69 + (-.210)X \quad ,$$

respectively. Thus, under the conditions of this study, approximately 40 weeks were required before residues in omental fat of calves contaminated in utero declined below detectable limits. At 52 weeks of age, HEOD was detectable in only one of five calves. Neither did

TABLE 24.--Effect of age on HEOD concentrations in omental fat of female calves born to contaminated dams.[a]

HEOD Fed Pre-partum	HEOD in Dam at Calving		Age of Calves				
	Body Fat	Blood	Birth	8 wks	16 wks	24 wks	52 wks[b]
days	μg/g	mμg/ml	μg/g				
60-0	9.5[d] ± 1.4[c] (7)	17.5[g] ± 1.9 (7)	7.39 ± 1.8 (9)	5.00 ± 2.4 (5)	2.90 ± 2.7 (4)	2.44 ± 2.7 (4)	.09 (4)
120-60	14.9[e] ± 1.8 (4)	7.1[h] ± 2.6 (4)	10.95 ± 2.4 (5)	6.21 ± 2.4 (5)	6.06 ± 2.7 (4)	5.89 ± 3.1 (3)	<.001 (1)
180-120[b]	31.7[f] ± 2.5 (2)	7.7[h] ± 3.6 (2)	9.37 (2)	4.31 (1)	----	----	----
Average			8.74 ± 1.3	5.49 ± 1.6	4.48 ± 1.9	3.92 ± 2.0	.07

[a]Number of animals each value represents is given in parentheses.

[b]These data were not included in the two-way analysis of variance because the number of values for the 180 day stage was limited and only 1 of 5 calves had detectable residue at 52 weeks of ages.

[c]Standard error of the mean.

[d,e]Difference approaches significance ($P < .05$).

[f]Mean significantly different from d,e ($P < .01$).

[g,h]Means significantly different ($P < .05$).

stage of gestation during which dams were contaminated affect residue levels in samples of shoulder fat taken simultaneously with the omental fat from female calves. These data are consistent with the similar concentration of HEOD found in renal and carcass fat of male calves, regardless of when the dams were contaminated.

6. Effect of Feeding Dams' Contaminated Milk or Non-Contaminated Milk to Calves Contaminated In Utero

Residue levels in omental fat of female calves fed either contaminated milk from their dams or non-contaminated milk for 12 weeks are shown in Table 25. Mean

TABLE 25.--Comparison of HEOD levels in omental fat of calves contaminated in utero when fed dams' HEOD-contaminated milk or non-contaminated milk for 12 weeks.[a]

Milk Fed	HEOD Intake	Age of Calves				
		Birth	8 wks	16 wks	24 wks	52 wks
	mg	ppm				
Non-Contaminated	0	6.38 (5)	5.06 (2)	3.23 (2)	2.66 (1)	0.17 (2)
Dams' Contaminated	79	8.60 (4)	4.97 (3)	2.58 (2)	2.37 (3)	<.001 (2)

[a]Number of animals each value represents is given in parentheses.

HEOD intake from dams' milk was 79 mg with 342 kg milk consumed per calf during the 12-week feeding period. Mean HEOD concentration in the whole milk averaged 0.49 ppm during the first week and declined to 0.06 ppm during the twelfth week of feeding. No detectable residues were found in the non-contaminated milk fed to calves. No significant difference in residue concentrations of omenal fat resulted from the two milk feeding regimes.

V. DISCUSSION

An important finding of these studies was that the earlier in gestation dairy heifers were contaminated with dieldrin, the greater ($P < .005$) the storage of HEOD in their body fat. For example, storage resulting from the 120- and 180-day prepartum contamination was approximately two and three times, respectively, that found for heifers contaminated 60 days prepartum, even though total pesticide intake was essentially equal (Table 26).

Explanations for the stage of gestation effect upon HEOD storage are not readily apparent, but various theories are proposed. First, the body weight data in Table 27 indicate that the 60-day prepartum heifers gained more weight during the contamination period (56 vs 43 and 28 kg, respectively) than the 120- and 180-day groups. Thus, the HEOD available for storage might have been diluted by a larger body fat mass in the 60- than the 120- and 180-day groups. However, much of this weight gain, especially for the 60-day heifers, was undoubtedly due to the rapidly developing fetus, with minimal fat deposition, since more than half of the fetal-weight increase occurs during the last two months of gestation (114). Therefore, the

TABLE 26.--Contamination data for Experiments 1 and 2 with resulting HEOD levels in body and milk fat.

Exp. No.	No. Animals	Contamination Period					Maximum HEOD Conc.		Ratio
		Start: Pre- or Post-partum	Length	HEOD Fed			Body Fat[a]	Milk Fat	Milk fat / Body fat
				Body Weight	In Feed	Total			
		--------days--------		------µg/g------		mg	------µg/g---		
1	16	115-post	56.0	.097	1.9	2885	10.4	22.0	2.1
2	18	60-pre	58.7	.094	5.5	3111	9.9	20.1	2.0
2	7	120-pre	60.0	.096	5.3	3145	18.4	20.9	1.1
2	6	180-pre	60.0	.097	5.4	2939	33.8	28.1	0.8

[a]Values at end of contamination.

TABLE 27.--Body weight data for Experiments 1 and 2.

Exp. No.	During Contamination				Between End Contamination and Calving		Body Weight at Calving
	Start: Pre- or Post-partum	Initial Weight	Total Gain	Daily Gain	Total Gain	Daily Gain	
	days	kg					
1	115-post	532	19	0.34	----	----	----
2	60-pre	541	56	0.95	----	----	597
2	120-pre	528	43	0.71	45	0.87	616
2	180-pre	488	28	0.47	91	0.78	607

dilution effect of increased body fat deposition on HEOD storage would be negated.

Secondly, the possibility exists that the HEOD was stored in dissimilar body fat depots during the three different stages of contamination. Hence, the external body fat biopsies might have not truly represented the total body burden of the pesticide.

Alternatively, the endocrine changes caused by advancing pregnancy might have stimulated a greater metabolism of the pesticide resulting in less unchanged HEOD. Data in Table 28 indicate a dramatic increase (6 to 16 fold) in estrogen production during the last five weeks of pregnancy (47, 70) which corresponds to the contamination period of the 60-day groups. Nalbandov (104) pointed out that estrogens cause profound changes in liver metabolism such as increased lipemia. Estrogens also have a marked effect on certain enzyme systems in the rat as shown by a quantitative increase in seven different enzyme systems activating amino acids of rat uteri three hours following a single injection of the hormone (104). Enzyme activities continued to increase for the ensuing 24 hours. The estrogen was thought to act by stimulating enzyme synthesis de novo, by activating already existing enzymes, or by uncoupling the enzymes which, after being "released," could participate in cellular metabolism (97). It is conceivable that the

TABLE 28.--Relative amounts of estrogen found in the urine of cows at various stages of pregnancy compared with prepartum contamination periods.

Stage of Pregnancy	Ratio Relative to Amount Found at 101-123 Days		Contamination Period
			Days Prepartum
days	a	b	
101-123	1.0	1.0	——————180
165-175	1.9	3.1	}
200-212	1.9	0.7	}——————120
226-237	2.4	---	
250-254	6.7	6.1	}—————— 60
271-285	11.3	16.1	

[a] R. E. Erb et al. (47)

[b] F. L. Hisaw, and R. K. Meyer (70).

high levels of estrogen in late pregnancy might stimulate enzyme systems capable of metabolizing dieldrin; thus, less intact dieldrin would remain for storage as was the case with the 60-day heifers compared with those contaminated earlier in gestation.

Coinciding with the rapid rise in estrogen at about 250 days of pregnancy is a decrease in progesterone. Short (118) found that progesterone levels in cows remained remarkably constant from the 32nd to about the 256th day of pregnancy, ranging from 0.74 to 0.98 µg/100

ml plasma. Subsequently, there was a decrease in the level and on the day before calving only 0.4 to 0.1 µg/100 ml was found. The effect of decreased progesterone and a shift in the estrogen:progesterone ratio on dieldrin-metabolizing enzymes needs investigation.

Conney et al. (27) studied the effects of pesticides on drug and steriod metabolism. They emphasized that the drug-metabolizing enzymes in liver microsomes may also play a role in the metabolism of other body substrates and thereby regulate important biochemical reactions in the body. If such is the case, drugs and insecticides may alter normal body metabolism through their action on liver microsomal enzymes.

In general, the biotransformations of foreign chemicals which are catalyzed by the liver microsomal enzymes result in the conversion of foreign substrates to less toxic, more water-soluble (or less lipid-soluble) materials (17, 55). These changes usually involve an increase in urinary excretion of the foreign compound by decreasing tubular reabsorption, a process that depends on solubility in the lipoidal membranes of the kidney tubules. Also, the lipid-insoluble metabolites are less able to penetrate other membranes to reach sites of storage. Thus, the more rapid excretion rate and the lower capacity for penetration of membranes would decrease the amount of both the original compound and its

metabolites found in body storage sites. It is conceivable that in our studies biotransformation of HEOD was stimulated by hormonal changes characteristic of impending parturition. Hence, less unchanged HEOD would have been available for storage in body fat during the latter stages of gestation with the resulting metabolite(s) excreted via the urine and escaping detection by our analytical methods.

Non-specific oxidizing enzymes in the microsomal fraction of liver cells are responsible for oxidative conversion of many lipid-soluble compounds. Brooks (18) recently found a species difference in the action of these enzymes. He showed that pig liver, in addition to producing the normal oxidation products, cleaved the epoxide ring of cyclodiene metabolites to yield the diol products; whereas, rat liver was incapable of the epoxide change. This reaction is not catalyzed by microsomal mixed-function oxidases; thus, the pig has some additional mechanism(s) which elicit(s) this cleavage. If such a mechanism exists in the bovine, cleavage of the epoxide ring of HEOD would yield a more hydrophilic compound that could be excreted in the urine leaving less unchanged HEOD available for body storage. The possibility that similar mechanisms exist in other species, including the bovine, merits investigation.

A most interesting phenomenon in these studies was the lack of change in the level of stored HEOD during the two- or four-month period between end of contamination and parturition in the 120- and 180-day groups (Table 9, Figure 7). These results are contrary to data obtained in other studies which showed a rapid decline in body storage of chlorinated hydrocarbon pesticides upon cessation of intake (10, 52, 54, 87). If estrogen does stimulate metabolism of HEOD, it would appear to do so before the pesticide molecule is stored in the fat depot, but the hormone probably is much less effective once storage has occurred.

In contrast to the highly significant differences in body fat residues found at the end of contamination, blood HEOD levels were not significantly different. Mean blood levels were approximately 15, 18, and 16 ppb (parts per billion) at the end of contamination for the three stages of gestation (Figure 8, Table 11). Thus, it would appear that similar levels of circulating pesticide were presented to body storage sites of all groups.

The finding of about 7 ppb HEOD in the blood two and four months after cessation of intake in the 120- and 180-day groups indicates either continuous turnover and release of stored pesticide, or a very long circulation time following absorption. Why blood HEOD levels did not increase during lactation in the 120- and 180-day

animals, thus reflecting the greater amount of HEOD removed from their body fat compared to the 60-day groups in not clear at this time. It is peculiar that HEOD levels were highest in the blood and lowest in the milk fat at parturition for the 60-day groups. The situation was reversed for the 120- and 180-day heifers.

A change might have occurred in the partition coefficient or rate of transfer by which the HEOD was removed from the blood by the mammary gland. Also it is possible that the mammary gland performed selective withdrawal of certain lipid fractions which might transport a disproportionate amount of HEOD in the blood. All blood-lipid fractions contributing to milk fat might have been saturated with HEOD because of the higher body storage in the 120- and 180-day heifers compared with a lesser saturation of these particular fractions in the 60-day heifers due to lower body stores.

Perhaps the HEOD carrier in the blood involves not only the lipid portion, but also the soluble proteins or lipoproteins as suggested by Moss and Hathway (102) for rat and rabbit blood. Factors such as chain length and saturation of specific fatty acids might also be involved in the transfer mechanism of HEOD between body fat, blood, and milk. These details need elucidation.

Another factor complicating the comparison of body storage of HEOD between the three stages of pregnancy is that the 60-day heifers were fed dieldrin up to and including the day of parturition (if parturition had not occurred by 8:00 A.M., the daily dose was administered). Therefore, part of the ingested dieldrin was still in the digestive tract or circulating in the blood at the time lactation was initiated. Thus, milk secretion would have provided an immediate, major route of elimination and minimized body storage of dieldrin ingested during the concluding days of contamination.

However, even though less pesticide might have reached body storage sites in the 60-day heifers, it still should have appeared in milk following parturition. After 24 weeks of lactation, only 18% of the dieldrin consumed by the 60-day groups could be accounted for in the milk, compared with more than 40% for the 120- and 180-day groups (Table 15); suggesting that conversion to a metabolite(s) was much greater in the heifers contaminated immediately before calving.

The 16 cows in Experiment 1 which were contaminated postpartum had body fat residues comparable to those of the 60-day prepartum heifers (Table 26) even though about 19% of the pesticide ingested by the cows was excreted during contamination (Table 3). For animals in both experiments the ratio between body fat and milk

fat was about 2:1 when body storage was 10 ppm. With body fat HEOD residues of about 18 ppm or above, milk fat levels were approximately equal to body fat concentrations. The narrowing of the ratio at higher levels of HEOD in body fat could have been due to saturation of all blood lipid fractions contributing to milk fat.

Shown in Table 29 and Figures 4 and 9 are the HEOD concentrations in milk fat for the various dietary treatments used in both experiments. The initial pesticide concentrations were similar for all seven treatment groups. Within-cow variation of HEOD residues in milk fat from samples taken at weekly intervals fluctuated considerably as was noted also with DDT (83).

The pattern of residue decline was similar for the seven treatment groups. For all treatments, concentrations had decreased to about one-half their initial levels after three to four weeks and to one-third by five to six weeks. Thereafter, the rate of decline was slower with plateaus occurring between weeks 5 to 10 and 12 to 18. The plateaus were less definite in Experiment 2 than Experiment 1.

Laben et al. (83) stated that DDT residues in milk fat declined exponentially after cessation of intake at rates of 9 to 11% per week when maximum concentrations were 19, 231, and 812 ppm for the three treatment groups. Rate of decline was 6.6% per week for their control

TABLE 29.--HEOD decline in milk fat during decontamination for all dietary treatments.

Week	Experiment 1[a]				Experiment 2[b]		
	% of TDN Requirement				Diet		
	150	75	100	150 + Thyro-protein	Normal	High Grain	Pelleted Hay
	μg/g						
1	24.9	18.2	21.0	24.0	18.8	17.4	23.9
2	19.9	19.1	16.2	19.9	13.4	9.9	11.4
3	13.4	11.8	11.8	10.7	7.3	8.2	12.5
4	9.7	11.7	8.8	12.2	8.4	10.3	11.9
5	8.0	6.9	9.1	8.6	7.0	9.6	10.9
6	6.4	7.5	8.8	12.2	5.2	6.3	8.8
7	4.6	6.4	7.4	8.0	---	---	---
8	6.5	7.7	5.5	8.6	4.5	3.6	8.1
9	7.1	10.0	8.9	9.5	---	---	---
10	7.2	7.7	8.6	8.8	4.9	3.8	7.3
12	1.6	4.2	1.6	1.0	4.0	3.4	7.8
14	2.2	2.6	1.8	0.4	---	---	---
16	1.2	2.8	0.9	1.0	2.2	3.3	6.3
18	1.3	2.6	0.8	0.2	---	---	---
20	---	---	---	---	1.3	1.6	2.8
24	---	---	---	---	1.0	1.2	1.7
30	---	---	---	---	0.8	0.2	0.3

[a] Four animals per treatment.

[b] Six animals per treatment.

group which had a maximum concentration in milk fat of 2 ppm. However, when their data on milk fat residues were plotted on a semi-log scale a plateau similar to that observed in our study occurred between weeks 5 to 18 for the high, medium, and control groups. Furthermore, these workers (83) did not indicate statistical treatment of regression lines to test if the rate of residue decline observed in their studies could be described but by a linear function.

Other workers (30, 51, 100, 138) have found that after discontinuation of DDT intake, levels in the milk fat fell rapidly for the first three to five weeks, then leveled off, and thereafter declined at a much slower rate. In our study linear regression coefficients were calculated for milk fat residue decreases by transforming to the log Y + 1 basis. Tests for goodness-of-fit showed that, because of the plateauing, a straight line did not accurately describe the DDD disappearance pattern.

Witt et al. (136) reported that the depletion rate of DDT was uniform for low-level accumulations (< 2 ppm in milk fat), but found two disappearance rates for higher levels of storage (6-8 ppm) as well as for storage resulting from six-day compared to one-day exposures, regardless of the contamination level reached. The presence of two distinctly different dissipation rates of DDT suggests a two-compartment storage with different

rates of exchange. Therefore, it is possible that immediate storage could occur in a site with rapid depletion potential; and then upon longer exposure the pesticide would accumulate in less labile sites.

Wertheimer (131) has pointed out that the fat cell consists of two compartments: a large one serving as a relatively inert storage site in which the exchange or turnover is slow; and a second, smaller compartment, containing lipids in a very rapid state of turnover and in close metabolic association with the serum and liver lipids. The existence of these two compartments in the fat cell might also explain the different rates of elimination of dieldrin suggested in this study, and of DDT shown by Witt et al. (138).

It is known also that adipose tissue occurs in sites where mechanical support is needed and the metabolic activity of this adipose tissue is much lower than that of mesenteric fat. The metabolic activity of subcutaneous fat is intermediate between these two extremes (3). Thus, a differential in fat mobilization from storage sites could contribute to two different elimination rates for residues and explain the plateauing of HEOD levels found in each of the nine treatment groups used in Experiments 1 and 2.

There is a dearth of information concerning methods which might effect a more rapid decontamination of

pesticide residues from the animal. The possibility of increasing the rate of decontamination by an increased depletion of body fat has been suggested. Conversely, it was theorized that energy intake in excess of requirements might minimize body fat turnover, thereby reducing release and decreasing concentrations of residues in milk fat. Increasing the exchange in body stores of residue-free lipids for contaminated fat by increasing the catabolic rate via the use of thyroprotein in conjunction with a high energy intake has also been suggested. Diets designed to depress milk fat, thereby reducing the rate of pesticide elimination in milk, is another possible way of reducing concentrations below detectable levels.

Thyroprotein feeding in Experiment 1 caused a rapid and marked loss of body weight (57 kg/cow within four weeks) (Figure 2), and decreased the time required to reach a mean residue level in milk fat of 1.0 ppm to 12 weeks compared with 16 weeks for the control group. A slower decline was noted for the groups receiving 150% and 75% energy requirement which averaged 1.3 and 2.6 ppm, respectively, after 18 weeks of decontamination (Table 29). The effect noted for thyroprotein is in contrast to the data of Miller (100) who reported that the hormone did not decrease the time required for the milk fat levels of either pre- or postpartum cows

contaminated with DDT to reach 1.25 ppm. A difference in mobilization of dieldrin and DDT may partially explain this apparent conflict.

Residue levels in milk fat for the 75% energy group in Experiment 1 declined at a slower rate than all other groups and did not change significantly during the last six weeks of decontamination. This observation is also in contrast with Miller's data for DDT (100), which indicated that cows on a low-energy ration declined at a more rapid rate than those receiving normal energy. This difference, however, might be explained by the different effect of the low-energy rations on milk yields in the two studies. Miller did not present production figures or exact energy levels; however, it was stated that cows on the low-energy treatment produced about the same amounts of milk and milk fat during the experimental period as in previous lactations. These cows received only 9 kg of alfalfa hay as the sole energy source for the 7th through the 42nd day of lactation and lost an average of 68 kg body weight. Apparently the inherent stimulatory effects of early lactation maintained nearly normal milk yields at the expense of body fat. It is well known that cows tend to mobilize large quantities of body stores to maintain high-level milk production during the first four to six weeks of lactation. However, in the latter stages of lactation,

milk yields decline as the result of low energy intake even though body fat is being deposited.

Our 75% group averaged six months postpartum at initiation of the low-energy diet. These cows responded with a marked decline in production which preceded small losses in body weight (Figures 2 and 3). Because of a decreased excretion of HEOD during the early weeks of decontamination in the low-energy group, a greater body burden of pesticide resulted in the more persistent levels noted at 18 weeks. It would appear that the efficacy of a low-energy diet on enhancing the rate of elimination of chlorinated hydrocarbon pesticides from dairy cows depends on the stage of lactation at which the low-energy ration is imposed.

After 18 weeks of decontamination all dietary treatment groups in Experiment 1 had excreted a similar percentage of the ingested dieldrin but concentrations in milk fat were still highest for the low-energy group. This should emphasize the importance of not imposing a treatment to cause an appreciable decrease in milk production while attempting to eliminate residue from cows contaminated with chlorinated hydrocarbon pesticides.

In Experiment 2, heifers contaminated during the last 60 days of pregnancy were assigned to either the normal (A), high grain (B), or pelleted hay (C) diets. Residue levels in milk fat of heifers in groups A and

B were not significantly different; whereas, group C animals, which averaged 2.2% fat for the first 24 weeks (compared with 3.2% fat for the control group A) exhibited higher HEOD concentrations on a milk fat basis ($P < .01$). Despite this marked depression of milk fat for group C, it is noteworthy that the mean residue level in the whole milk was not different from groups A and B. Heifers fed the pelleted hay produced the most milk (Table 8) which contributed to a greater removal of pesticide. Although the total amount of HEOD excreted during 24 weeks of lactation was about 43% greater for the pellet-fed than the other 60-day groups, differences were not statistically significant ($P < .10$).

Since it is generally accepted that HEOD is associated with the lipid portion of milk, the observation that a depression in percent milk fat did not alter the residue level in whole milk is of special significance. It indicates that HEOD excretion via the bovine mammary gland is related to factors other than the total amount of fat synthesized in the gland.

Laben et al. (83) suggested that various pools or fractions of body fat may vary widely in their concentrations of residues and that a particular lipid fraction may be an important precursor of milk fat and also contain a disproportionately higher concentration of pesticide than would be revealed by analysis of external body fat samples.

Stanley and Morita (120) reported that blood lipids contributed to a larger percentage of the total milk fat in thyroprotein-fed cows than in those on a control ration. Hence, it is possible that HEOD concentration in milk can be altered by relative amounts of the different fat precursors taken up by the mammary gland. This is a possible explanation for the observation on the pelleted ration that, even though fat content of milk was drastically reduced, HEOD levels in whole milk did not change.

As the pesticide was eliminated from the body via secretion in the milk, residue levels in samples of external shoulder fat declined. Only two animals in each group of four were biopsied for fat residue analysis in Experiment 1, thus, estimates based on these data are not complete, but definite trends were noted. Residue concentrations in samples taken at 0, 8, and 16 weeks showed an exponential decline. Equations for the respective energy treatments of 150%, 75%, 100%, and 150% plus thyroprotein were: $Y = 11.6e^{-0.0198t}$, $Y = 10.8e^{-0.0198t}$, $Y = 10.0e^{-0.0178t}$, and $Y = 10.0e^{-0.0224t}$. Thus, the coefficients of these equations indicate that residue levels declined at rates of 1.78% to 2.24% per day. Although body fat levels of HEOD in thyroprotein-fed cows declined at the fastest rate, numbers were insufficient to detect a significant difference.

Experience gained from Experiment 1 dictated that every animal in Experiment 2 should be biopsied for body fat analysis. Body residue concentrations decreased exponentially for all treatments after lactation was initiated. Equations for the 60-day, A, B, and C, and 120- and 180-day groups were: $Y = 10.0e^{-0.0147t}$, $Y = 10.0e^{-0.0116t}$, $Y = 13.2e^{-0.0133t}$, $Y = 16.0e^{-0.0139t}$, and $Y = 34.0e^{-0.0182t}$, respectively. The five groups, therefore, declined at rates of 1.16 to 1.82% per day (8.1 to 12.7% per week). The rate for the 180-day heifers was significantly faster ($P < .01$) than any other group. These elimination patterns compare with 8.1 to 9.8% per week as reported by Laben et al. (83) when initial body fat levels of DDT were 16, 253, and 590 ppm for three treatment groups.

The difference in the disappearance pattern of HEOD from body fat compared with milk fat (exponential decline versus plateau periods) might be explained in two ways. First, the eight-week interval between fat biopsies might have masked any plateau in tissue residue concentrations which could have occurred during the intervening weeks. However, Laben et al. (83) took biopsy samples at two week intervals and did not find a plateauing of DDT residues in body fat. Secondly, a decrease in residue concentrations of shoulder fat might not accurately describe the decline of total pesticides in the body because of differential mobilization and re-location of HEOD within other fat storage sites.

From the heifer data (Experiment 2), one might associate the plateaus observed for milk residues (Figure 9) with the change in body weight during lactation (Figure 6). The rapid decline in milk HEOD during the first four to six weeks corresponds closely with the precipitous loss of body weight during the same period. All groups reached minimum weights at approximately six weeks postpartum and generally stabilized at this level until about 14 weeks after which some weight gains were noted.

Conceivably, the large initial losses of body fat containing low levels of HEOD might account for the steep decline in milk residues early in lactation. After body weight and fat loss had somewhat stabilized, mobilization of storage sites containing higher HEOD concentrations would result in a leveling-off of the fast rate of decrease in milk fat residues.

This suggestion fits the pattern for the thyroprotein-fed cows in Experiment 1, but does not explain the similar pattern for milk residues observed for the 100 and 150% groups. These latter groups increased in body weight from the beginning of dietary treatment. Since decontamination for many cows in Experiment 1 occurred after six months postpartum, body metabolism may have been considerably different than in the heifers, which were decontaminated in early lactation. The differences in

metabolic states of the animal in the two experiments, as influenced by stages of lactation, growth requirements, and hormonal changes related to pregnancy and estrous cycles, advise caution in developing explanations for decontamination patterns applicable to both experiments.

Limited evidence for differential storage and mobilization of HEOD was obtained with tissues removed from six cows at slaughter (Table 6). Residues in shoulder fat were higher than those in kidney or omental fat. Thyroprotein-feeding appeared to affect mobilization of fat without stimulating release of stored HEOD, because residues in shoulder, kidney, and omental fat were three to five times higher for the thyroprotein-fed cows. The markedly higher concentration of HEOD in fat at slaughter of the thyroprotein-fed cows might be explained on the basis that thyroprotein differentially stimulated mobilization in the two-compartment fat cell and/or at various fat depots. Thus, the residual HEOD was more concentrated in the lipid remaining in the fat cells of the thyroprotein-fed cows.

Averaging the values for all heifers in Experiment 2, approximately 34% of the total HEOD ingested was accounted for at parturition as the unchanged compound. This proportion ranged from about 20% for the 60-day group to 63% for the 180-day heifers (Table 13).

Pesticide stored in the body at parturition was apparently underestimated, since an average of 131% of the calculated storage had been accounted for after 24 weeks postpartum. Only in the 180-day group was less than 100% of the estimated amount of stored pesticide accounted for at 24 weeks of lactation. It is possible that assumed body fat content of 10% was too low which would have resulted in more stored pesticide at parturition than indicated. Differential storage in various fat depots could have also been a contributing factor to underestimation of total body residues. However, even a large error in estimating blood volume would have made little difference in the total body pool of HEOD due to the very low blood concentrations (7 to 15 ppb).

Investigation of possible excretory pathways of HEOD from dairy animals revealed that about 2.5% of the daily intake was excreted as unchanged HEOD in the feces during contamination. Heath and Vandekar (67) reported that in rats fed HEOD only 3% of the ^{36}Cl was excreted as HEOD in feces unless the bile was cannulated. Only minute amounts were found in the urine. Once lactation in the cows was initiated, milk was the primary excretory pathway (Table 17).

The appearance of HEOD in feces 30 days after contamination ceased indicates a metabolic origin and recycling of the pesticide. Biliary excretion has been

shown for a number of chlorinated hydrocarbon pesticides (9, 67, 73, 101, 112, 129). Moreover, it has been suggested that the amount excreted in bile may be quite high. Heath and Vandekar (67) found that the proportion of HEOD excreted as the unchanged compound could be increased from 3% to 10% by cannulation of the bile duct. Cook et al. (28) have demonstrated that dieldrin is recycled and appears in the bile, pancreatic juice, and saliva of goats. These were probable pathways through which dieldrin appeared in the feces of heifers during decontamination.

Placental transfer of dieldrin occurred in each of 33 dams, resulting in comtamination of all newborn calves (12, 14, 16). Although total HEOD intake was very similar, the stage of gestation during which the pesticide was ingested exerted a highly significant effect ($P < .01$) upon the concentration of HEOD in the body fat of the dams at parturition. However, the differences in HEOD in body fat of dams did not significantly affect the mean residue levels in whole carcass, carcass fat, brain, or renal fat of the male calves.

These data are in contrast to those of Laben et al. (83), who found that DDT in the body fat of three stillborn calves was proportional to the estimated body fat level of their dams. HEOD levels in blood of the dams at parturition did not parallel those of body fat.

Blood of heifers contaminated during the last 60 days of gestation contained 12.8 ppb as opposed to 6.7 and 7.3 ppb for the 120- and 180-day groups, respectively. The difference between these means approached significance ($P < .05$).

Since blood is the only avenue by which the dieldrin could be transferred from the dam to the fetus, the tissue residue picture in the newborn male calves is interesting in view of the differences in dieldrin concentrations found in blood and fat of dams at parturition. The similar residue levels found in all calves, regardless of stage and degree of contamination of the dams, suggests that fetal residues were a function of both concentration and length of time that blood containing the pesticide was in contact with the developing fetus.

Heifers contaminated during the last 60 days of gestation had higher blood levels and lower body fat concentrations than those contaminated for a similar time, but starting at 120- or 180-days prepartum. The greater HEOD levels found in the blood of calves born to the 60-day group reflect the higher blood levels present in the dams at parturition.

Calculated recoveries of HEOD in the male calves averaged 0.9% of that ingested by the dams. Total placental transfer was probably higher than recovery figures indicate because the calves were skinned prior

to grinding the carcasses for HEOD analyses. King et al. (75) reported that heptachlor epoxide concentration in one newborn calf was three to four times higher in the hide and hair than in several other tissues analyzed. Furthermore, HEOD in the placental tissues and fluids was not determined.

Effect of age on blood residues in female calves was similar to that for the males. Females born to dams in the 60-day group contained significantly higher levels of dieldrin in their blood than the other two groups ($P < .05$). As with the males, this is a reflection of the higher blood levels circulating in the dams contaminated during the last 60 days of gestation. HEOD in the blood of females disappeared more quickly than that found in the omental fat. At 16 weeks of age, HEOD in blood was detectable in only one of seven females, and none was found at 24 weeks in the four calves sampled.

No evidence of dieldrin poisoning was found in any of the calves or dams. Kadis and Jonasson (74) fed single doses of dieldrin (17 mg/kg body weight) and aldrin (15 mg/kg body weight) to two calves and found that blood levels of dieldrin at six weeks in both calves had declined to about one-half of initial values (from 95 and 200 ppb to 46 and 92 ppb, respectively). They did not indicate whether any symptoms of dieldrin poisoning occurred in the calves, although Brown et al. (19)

proposed a "threshold for dieldrin intoxication" of 150 to 200 ppb for men and dogs. Dale et al. (32) reported poisoning symptoms and blood dieldrin levels in humans which corresponded to the threshold suggested by Brown et al. (19).

It is encouraging that HEOD levels in omental fat and blood of calves contaminated in utero declined with age, and that at 52 weeks of age small residues were detectable in only one of five calves. This would indicate that at the dieldrin levels used in these studies, female calves contaminated in utero will not contain sufficient HEOD at parturition for its detection in their milk.

Based on limited data it appears that whole milk having up to 0.50 ppm can be fed to growing calves contaminated in utero without increasing body fat residues. These data agree with the findings of Finnegan et al. (48) who showed that dog pups contaminated with DDT in utero and suckling contaminated dams had lower DDT levels after two weeks of nursing than litter mates at two days of age.

VI. SUMMARY AND CONCLUSIONS

A total of 51 Holstein cows and heifers were contaminated for similar periods of time (56 to 60 days) with equal levels of dieldrin (about .096 mg HEOD/kg body weight) in two experiments. Sixteen lactating animals were fed dieldrin daily for 56 days. Dietary treatments during an 18-week decontamination period as percent of the NRC requirement for TDN were: 75, 100, 150, and 150 plus 22 mg of thyroprotein daily per kg of body weight. Thirty-one pregnant heifers were contaminated at the same level for 60 days beginning either 60, 120, or 180 days prepartum. During contamination and decontamination samples of milk, external body fat, feces, and urine were analyzed for HEOD to determine storage and excretory pathways.

A major, previously unreported, finding was that significantly greater ($P < .005$) HEOD storage occurred in body fat when pregnant dairy heifers were contaminated early compared to late in gestation. Storage resulting from the 120- and 180-day prepartum contamination was approximately two and three times, respectively, (18 and 34 vs 10 ppm) that found for heifers contaminated 60

days prepartum. It is suggested that endocrine changes caused by advancing pregnancy might have stimulated a greater metabolism of the pesticide resulting in less unchanged HEOD.

Once non-lactating pregnant dairy heifers were contaminated with HEOD, very little was eliminated from the body until lactation began. The pattern of residue decline in milk fat was similar for all groups in both experiments. Concentrations dropped to about one-half their initial levels after three to four weeks, and to one-third by five to six weeks. Because of the plateauing, a linear decline did not accurately describe the HEOD disappearance pattern in milk fat. The presence of two distinctly different dissipation rates for HEOD suggests a two-compartment storage with different rates of exchange.

Varying the dietary energy level did not significantly enhance the rate of decontamination, although thyroprotein-feeding did decrease the time required to reach a mean residue level of 1.0 ppm in the milk fat (12 weeks compared to 16 weeks for the control group). A low-energy intake at six months postpartum caused a marked decline in production which preceded small losses in body weight. Because excretion of HEOD decreased with the lowered production, pesticide residues in milk fat were more persistent in the low-energy group.

Residues in whole milk did not change when the fat content of milk was drastically reduced by feeding pelleted hay. This indicates that HEOD excretion via the mammary gland is related to factors other than the total amount of fat synthesized in the gland. Since HEOD levels in blood did not parallel those in milk fat it is suggested that a change occurred in the partition coefficient or rate of transfer by which HEOD was removed from the blood by the mammary gland. Also it is possible that the mammary gland performed selective withdrawal of certain lipid or lipoprotein fractions which transport a disproportionate amount of HEOD in the blood.

As the pesticide was eliminated from the body via secretion in the milk, residue levels in shoulder fat of the heifers contaminated prepartum declined exponentially at rates of 1.16 to 1.82% per day (8.1 to 12.7% per week). Limited evidence for differential storage and mobilization of HEOD was obtained. Thyroprotein-feeding appeared to affect mobilization of fat without stimulating release of stored HEOD since the residual HEOD was more concentrated in the adipose tissue of cows fed thyroprotein.

About 2.5% of the daily intake was excreted as unchanged HEOD in the feces during contamination. Only trace amounts were found in the urine. The appearance of HEOD in feces 30 days after contamination ceased

indicates a metabolic origin and recycling of the pesticide. Bile, pancreatic juice, and saliva were probable pathways through which HEOD appeared in the feces of heifers during decontamination.

Placental transfer of dieldrin occurred in each of 33 dams, resulting in contamination of all newborn calves. Calculated recoveries of HEOD in the male calves averaged 0.9% of that ingested by the dams. The similar residue levels found in all calves, regardless of stage and degree of contamination of the dams, suggests that fetal residues were a function of both concentration and length of time that the dam's blood containing the pesticide was in contact with the developing fetus. HEOD levels in omental fat and blood of calves contaminated in utero declined with age and at 52 weeks negligible residues were detectable in only one of five female calves. This would indicate that at the dieldrin levels used in these studies, female calves contaminated in utero will not contain sufficient HEOD at parturition for detection in milk.

Based on limited data it appears that whole milk having up to 0.5 ppm can be fed to growing calves contaminated in utero without increasing body fat residues. However, feedstuffs contaminated with chlorinated hydrocarbon pesticides should not be fed to pregnant dairy heifers as has been previously recommended in several states.

LITERATURE CITED

LITERATURE CITED

1. Ambrose, A. M., Christensen, H. C., and Robbins, D. J. 1953. Pharmacological observations on chlordane. Fed. Proc., 12:298.

2. Ambrose, A. M., Christensen, H. C., Robbins, D. J., and Rather, L. J. 1953. Toxicological and pharmacological studies on chlordane. Arch. Ind. Hyg. Occupational Med., 7:197.

3. Aronovsky, E., Levari, R., Kornblueth, W., and Wertheimer, E. 1963. Comparison of metabolic activities of orbital fat with those of other adipose tissue. Investigative Opthalmology, 2:259.

4. Bache, C. A., Gyrisco, G. G., Fertig, S. N., Huddleston, E. W., Lisk, D. J., Fox, F. H., Trimberger, G. W., and Holland, R. F. 1960. Effects of feeding low levels of heptachlor epoxide to dairy cows on residues and off-flavors in milk. J. Agr. Food Chem., 8:408.

5. Baker, A. H., Whitney, G. F. H., and Worden, A. N. 1958. Insecticidal aerosols. Lancet, 2:1177.

6. Bann, J. M., DeCino, T. J., Earle, N. W., and Sun, Y. P. 1956. The fate of aldrin and dieldrin in the animal body. J. Agr. Food Chem., 4:937.

7. Barnes, J. M., and Heath, D. F. 1964. Some toxic effects of dieldrin in rats. Brit. J. Industr. Med., 21:280.

8. Bishop, J. L., and Huber, J. T. 1964. Secretion of Telodrin in the milk of cows fed varying levels of Telodrin. J. Dairy Sci., 47:552.

9. Bleiberg, M. J., and Larson, P. S. 1957. Studies on derivatives of 2,2-bis(p-chlorophenyl)-1, 1-dichloroethane (DDD, TDE) with special reference to their effects on the adrenal cortex. J. Pharmacol. Exptl. Therap., 121:421.

10. Bovard, K. P., Priode, B. M., Whitmore, G. E., and Ackerman, A. J. 1961. DDT residues in the internal fat of beef cattle fed contaminated apple pomace. J. Animal Sci., 20:824.

11. Brambell, F. W. R., and Hemmings, W. A. 1960. The transmission of antibodies from mother to fetus. In: The Placenta and Fetal Membranes, edited by C. A. Villee. The Williams and Wilkins Co., New York.

12. Braund, D. G. 1967. Dieldrin residue carryover from young to lactating dairy animals and feeding practices affecting residue persistence in lactating cows. Final Report, USDA Agricultural Research Service, Michigan State University Pesticide Residue Contract No. 12-14-100-8165 (44). October.

13. Braund, D. G. 1967. Effect of heptabarbital on storage of dieldrin in rats. Unpublished data.

14. Braund, D. G., Brown, L. D., Huber, J. T., Leeling, N. C., and Zabik, M. J. 1967. Placental transfer of dieldrin in dairy heifers contaminated during three stages of gestation. J. Dairy Sci., in press.

15. Braund, D. G., Brown, L. D., Leeling, N. C., and Zabik, M. J. 1966. Effects of dietary energy level and thyroprotein on excretion and storage of dieldrin in dairy cows. J. Dairy Sci., 49:740.

16. Braund, D. G., Brown, L. D., Leeling, N. C., Zabik, M. J., and Huber, J. T. 1967. Storage, excretion and placental transfer of dieldrin by dairy heifers contaminated during three stages of gestation. J. Dairy Sci., 50:991.

17. Brodie, B. B., and Maickel, R. P. 1961. Comparative biochemistry of drug metabolism. In: Metabolic factors controlling duration of drug action. Proc. 1st Internat. Pharmacol. Meeting., 6:299.

18. Brooks, G. T. 1967. Perspectives of cylodiene metabolism. Paper presented at Shell Symposium on the Science and Technology of Residual Insecticides with Special Reference to Aldrin and Dieldrin. St. Charles, Illinois, November.

19. Brown, V. K. H., Hunter, C. G., and Richardson, A. 1964. A blood test diagnostic of exposure to aldrin and dieldrin. Brit. J. Industr. Med., 21:283.

20. Brown, W. H., Witt, J. M., Whiting, F. M., and Stull, J. W. 1966. Secretion of DDT in milk by fresh cows. Bull. Environ. Contam. and Toxicol., 1:21.

21. Bruce, W. N., Link, R. P., and Decker, G. C. 1965. Storage of heptachlor epoxide in the body fat and its excretion in milk of dairy cows fed heptachlor in their diets. J. Agr. Food Chem., 13:63.

22. Claborn, H. V., Bowers, J. W., Wells, R. W., Radeleff, R. D., and Nickerson, W. J. 1953. Meat contamination from pesticides. Agr. Chem., 8:37.

23. Claborn, H. V., Radeleff, R. D., and Bushland, R. C. 1960. Pesticide residues in meat and milk--a research report. ARS-33-63, Agr. Res. Serv., USDA.

24. Clark, R. D. 1965. Personal Communication.

25. Christensen, H. E. 1965. The distribution of aldrin and dieldrin in the tissues and body fluids of experimental animals. Diss. Abstr., 25:4087.

26. Cluett, M. L., Lowen, W. H., Pease, H. L., and Woodhouse, C. A. 1960. Determination of methoxychlor and/or metabolites in milk following topical application to dairy cows. J. Agr. Food Chem., 8:277.

27. Conney, A. H., Welch, R. M., Kuntzman, R., and Burns, J. J. 1967. Effects of pesticides on drug and steroid metabolism. Clinical Pharm. and Therap., 8:2.

28. Cook, R. M., Thomas, J. W., Emery, R. S., and Huber, J. T. 1967. Dieldrin recycling from the blood to the gut in ruminants. J. Animal Sci., in press.

29. Crosby, D. G., and Archer, T. E. 1966. A rapid analytical method for persistent pesticides in proteinaceous samples. Bull. Environ. Contam. and Toxicol., 1:16.

30. Crosby, D. G., Archer, T. E., and Laben, R. C. 1966. DDT contamination in milk following a single feeding exposure. J. Dairy Sci., 50:40.

31. Cueto, C., Jr., and Hayes, W. J., Jr., 1962. The detection of dieldrin metabolites in human urine. J. Agr. Food Chem., 10:366.

32. Dale, W. E., Curley, A., and Cueto, C., Jr. 1966. Hexane extractable chlorinated insecticides in human blood. Life Sci., 5:47.

33. Dale, W. E., Gaines, T. B., and Hayes, W. J., Jr. 1962. Storage and excretion of DDT in starved rats. Toxicol. Appl. Pharmacol., 4:89.

34. Dale, W. E., Gaines, T. B., Hayes, W. J., Jr., and Pearce, G. W. 1963. Poisoning by DDT: relation between clinical signs and concentration in rat brain. Science, 142:1474.

35. Datta, P. R., Laug, E. P., Watts, J. O., Klein, A. K., and Nelson, M. J. 1965. Metabolites in urine of rats on diets containing aldrin or dieldrin. Nature, 208:289.

36. Davidow, B., and Frawley, J. P. 1951. Tissue distribution, accumulation, and elimination of the isomers of benzene hexachloride. Proc. Soc. Exptl. Biol. Med., 76:780.

37. Davidow, B., Radomski, J. L., and Ely, R. 1953. Excretion of heptachlor epoxide in milk of a dairy cow fed heptachlor. Science, 118:383.

38. Demott, B. J., Miles, J. T., Hinton, S. A., and Hardin, L. J. 1966. Secretion of heptachlor epoxide in milk. J. Dairy Sci., 49:1495.

39. Dénes, A. 1962. Lebensmittelchemische Probleme von Rückständen chlorierter Kohlenwasserstoffe. Nahrung, 6:48.

40. Dubois, K. R. (Chairman). 1966. Report of the Food and Drug Administration's Advisory Committee Appointed to Review the Petition of the California State Departments of Public Health and Agriculture to establish a tolerance for DDT and related compounds (DDD and DDE) in milk and milk products. October 26.

41. Durham, W. F. 1963. Pesticide residues in foods in relation to human health. Residue Reviews, 4:3.

42. Durham, W. F. 1967. The interaction of pesticides with other factors. Residue Reviews, 18:21.

43. Durham, W. F., Cueto, C., Jr., and Hayes, W. J., Jr. 1956. Hormonal influences on DDT metabolism in the white rat. Am. J. Physiol., 187:373.

44. Durham, W. F., Ortega, P., and Hayes, W. J., Jr. 1963. The effect of various dietary levels of DDT on liver function, cell morphology, and DDT storage in the Rhesus monkey. Arch. Intern. Pharmacodyn., 141:111.

45. Egan, H. 1966. Pesticide residues in fat-containing foods and in human fat. Proc. Nutr. Soc., 25:44.

46. Ely, R. E., Moore, L. A., Carter, R. H., Mann, H. D., and Poos, F. W. 1952. The effect of dosage level and various methods of administration on the concentration of DDT in milk. J. Dairy Sci., 35:266.

47. Erb, R. E., Randel, R. D., and Estergreen, V. L. 1967. Urinary estrogen excretion and levels of progesterone in blood plasma of the cow during pregnancy. J. Dairy Sci., 50:1001.

48. Finnegan, J. K., Haag, H. B., and Larson, P. S. 1949. Tissue distribution and elimination of DDD and DDT following oral administration to dogs and rats. Proc. Soc. Exptl. Biol. Med., 72:357.

49. Fries, G. F., Flatt, W. P., and Moore, L. A. 1967. Body fat loss and the secretion of DDT and its metabolites into milk. J. Dairy Sci., 50:991.

50. Fries, G. F., and Kane, E. A. 1967. Retention of DDT and DDE by the bovine. J. Dairy Sci., 50:1512.

51. Gannon, N., and Decker, G. C. 1960. The excretion of dieldrin, DDT, and heptachlor epoxide in milk of dairy cows fed on pastures treated with dieldrin, DDT, and heptachlor. J. Econ. Ent., 53:411.

52. Gannon, N., Link, R. P., and Decker, G. C. 1959. Storage of dieldrin in tissues and its excretion in milk of dairy cows fed dieldrin in their diets. J. Agr. Food Chem., 7:824.

53. Gannon, N., Link, R. P., and Decker, G. C. 1959. Storage of dieldrin in tissues of steers, hogs, lambs, and poultry fed dieldrin in their diets. J. Agr. Food Chem., 7:826.

54. Gannon, N., Link, R. P., and Decker, G. C. 1959. Insecticide residues in the milk of dairy cows fed insecticides in their daily ration. J. Agr. Food Chem., 7:829.

55. Gillette, J. R. 1962. Oxidation and reduction by microsomal enzymes. Proc. 1st Internat. Pharmacol. Meeting, 6:13.

56. Gowdey, C. W., Graham, A. R., Seguin, J. J., and Stravraky, G. W. 1954. The pharmacological properties of the insecticide dieldrin. Can. J. Biochem. and Physiol., 32:498.

57. Hardee, D. D., Gutenmann, W. H., Keenan, G. I., Gyrisco, G. G., Lisk, D. J., Fox, F. H., Trimberger, G. W., and Holland, R. F. 1964. Residues of heptachlor epoxide and Telodrin in milk from cows fed at part per billion insecticide levels. J. Econ. Ent., 57:404.

58. Harris, L. E., Harris, J. R., Mangelson, F. L., Greengood, D. A., Biddulph, C., Binns, W., and Miner, M. L. 1953. Effect of feeding DDT-treated alfalfa hay to swine and of feeding the swine tissue to rats. J. Nutr., 51:491.

59. Hayes, W. J., Jr. 1959. In: DDT, The Insecticide Dichlorodiphenyltrichloroethane and Its Significance, 2:9. Edited by P. Miller, Birkhauser Verlag, Basel.

60. Hayes, W. J., Jr. 1965. Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals, pp. 27-52. In: Annual Review of Pharmacology. Annual Reviews, Inc., Palo Alto, Calif.

61. Hayes, W. J., Jr., Durham, W. F., and Cueto, C., Jr. 1956. The effect of known repeated oral doses of chlorophenothane (DDT). J. Am. Med. Assoc., 162:890.

62. Hayes, W. J., Jr., Ferguson, F. F., and Cass, J. S. The toxicology of dieldrin. I. Dermal toxicity of various formulations to laboratory animals. Report from Technical Development Services, Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia (undated).

63. Hayes, W. J., Jr., Ferguson, F. F., and Cass, J. S. 1951. The toxicology of dieldrin and its bearing on field use of the compound. Am. J. Trop. Med., 31:519.

64. Hayes, W. J., Jr., Quinby, G. E., Walker, K. C., Elliot, J. W., and Upholt, W. M. 1958. Storage of DDT and DDE in people with different degrees of exposure to DDT. Arch. Ind. Health, 18:398.

65. Hathway, D. E. 1965. The biochemistry of dieldrin and Telodrin. Arch. Environ. Health, 11:380.

66. Hathway, D. E., Moss, J. A., and Rose, J. A. 1966. Transport of dieldrin from mother to blastocyst and from mother to foetus in pregnant rabbits. Shell Research Ltd., Tunstall Laboratory, Sittingbourne, Kent, England. Unpublished.

67. Heath, D. F., and Vandekar, M. 1964. Toxicity and metabolism of dieldrin in rats. Brit. J. Industr. Med., 21:269.

68. Heineman, H. E. O., Jaynes, H. O., and Heflin, J. L. 1966. Pesticides--a dairy industry problem. J. Dairy Sci., 49:509.

69. Heineman, H. E. O., and Miller, C. B. 1961. Pesticide residues and the dairy industry. J. Dairy Sci., 44:1775.

70. Hisaw, F. L., and Meyer, R. K. 1929. The oestrous hormone in the urine of pregnant cows. Proc. Soc. Exptl. Biol. Med., 26:586.

71. Huber, J. T., and Bishop, J. L. 1962. Secretion of heptachlor epoxide in the milk of cows fed field-cured hay from soils treated with heptachlor. J. Dairy Sci., 45:79.

72. Ivey, M. C., Claborn, M. V., Mann, H. D., Radeleff, R. D., and Woodward, G. T. 1961. Aldrin and dieldrin content of body tissues of livestock receiving aldrin in their diets. J. Agr. Food Chem., 9:374.

73. Jensen, J. A., Cueto, C., Dale, W. E., Rothe, C. F., Pearce, G. W., and Mattson, A. M. 1957. DDT metabolites in feces and bile of rats. J. Agr. Food Chem., 5:919.

74. Kadis, V. W., and Jonasson, O. J. 1965. The detection and persistence of chlorinated insecticides in human and animal blood. Can. J. Public Health, 56:433.

75. King, R. L., Hemken, R. W., Bush, M. B., Heinle, E., and Lester, J. W. 1966. Current status of pesticide residue work at Maryland. Proc. Maryland Nutr. Conf., March.

76. Koransky, W., Portig, J., Vohland, H. W., and Klempau, I. 1964. Die Elimination von α-und γ hexachlorcyclohexan und ihre Beeinflussung durch Enzyme der Lebermikrosomen. Arch. Exptl. Pathol. Pharmakol., 247:49.

77. Korte, F., and Arent, H. 1965. Metabolism of insecticides, IX (1) Isolation and identification of dieldrin metabolites from urine of rabbits after oral administration of dieldrin-^{14}C. Life Sci., 4:2017.

78. Korte, F., Ludwig, G., Vogel, J., Stiasni, M., Rechmeier, G., and Kochem, W. 1963. Metabolic studies with ^{14}C-labelled drin-insecticides. Fifth Int. Pest. Congr., London. July.

79. Kügemagi, U., Sprowls, R. G., and Terriere, L. C. 1958. Endrin content of milk and body tissues of dairy cows receiving endrin daily in their diet. J. Agr. Food Chem., 6:518.

80. Kunze, F. M., and Laug, E. P. 1953. Toxicants in tissues of rats on diets containing dieldrin, aldrin, endrin, and isodrin. Fed. Proc., 12:339.

81. Kunze, F., Laug, E. P., and Prickett, C. S. 1950. Storage of methoxychlor in the fat of the rat. Fed. Proc., 9:293.

82. Kunze, F., Nelson, A. A., Fitzhugh, O. G., and Laug, E. P. 1949. Storage of DDT in the fat of the rat. Fed. Proc., 8:311.

83. Laben, R. C., Archer, T. E., Crosby, D. G., and Peoples, S. A. 1965. Lactational output of DDT fed prepartum to dairy cattle. J. Dairy Sci., 48:701.

84. Laben, R. C., Archer, T. E., Crosby, D. G., and Peoples, S. A. 1966. Milk contamination from low levels of DDT in dairy rations. J. Dairy Sci., 49:1488.

85. Laug, E. P., and Fitzhugh, O. G. 1946. 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane (DDT) in the tissues of the rat following oral ingestion for periods of six months to two years. J. Pharmacol. Exptl. Therap., 87:18.

86. Lee, M., Harris, K., and Trowbridge, H. 1964. Effect of the level of dietary protein on the toxicity of dieldrin for the laboratory rat. J. Nutr., 84:136.

87. Lehman, A. J. 1956. The minute residue problem. Assoc. Food Drug Officials U. S. Quart. Bull., 20:95.

88. Long, W. H., Newsom, L. D., and Mullins, A. M. 1961. Endrin residues in the fat of lambs grazed on endrin-treated pastures. J. Econ. Ent., 54:605.

89. Ludewig, S., and Chanutin, A. 1946. Distribution of 2,2 (p-chlorophenyl) 1,1,1-trichlorethane (DDT) in tissues of rats after its ingestion. Proc. Soc. Exptl. Biol. Med., 62:20.

90. Ludwig, G., and Korte, F. 1965. Metabolism of insecticides, X(1) Detection of dieldrin metabolite by GLC-analysis. Life Sci., 4:2027.

91. Ludwig, G., Weis, J., and Korte, F. 1964. Excretion and distribution of aldrin-^{14}C and its metabolites after oral administration for a long period of time. Life Sci., 3:123.

92. Marth, E. H. 1962. Chlorinated hydrocarbons deposited in biological material. II. Animal and animal products. J. Milk Food Tech., 25:72.

93. Matsumura, F., and Hayashi, M. 1966. Dieldrin: interaction with nerve components of cockroaches. Science, 153:757.

94. Mattson, A. M., Spillane, J. T., Baker, C., and Pearce, G. W. 1953. Determination of DDT and related substances in human fat. Anal. Chem., 25:1065.

95. McCollister, D. D., Beamer, W. H., Atchison, G. J., and Spencer, H. G. 1950. Studies with low vapor concentrations of carbon tetrachloride labeled with carbon 14. I. Absorption, distribution, and elimination upon inhalation by monkeys. Fed. Proc., 9:300.

96. McFarlane, A. S. 1957. Use of labelled plasma proteins in the study of nutritional problems. Progr. Biophys. biophys. Chem., 7:115.

97. Mellin, T. N., and Erb, R. E. 1965. Estrogen in the bovine--a review. J. Dairy Sci., 48:687.

98. Mendel, J. L., and Walton, M. S. 1966. Conversion of p,p'-DDT to p,p'-DDD by intestinal flora of the rat. Science, 151:1527.

99. Menzie, C. M. 1966. Metabolism of pesticides. Special Scientific Report--Wildlife No. 96. U. S. Dept. of Int., Washington, D. C.

100. Miller, D. D. 1967. Effect of thyroprotein and a low-energy ration on removal of DDT from lactating dairy cows. J. Dairy Sci., 50:1444.

101. Mörsdorf, K., Ludwig, G., Vogel, J., and Korte, F. 1963. The excretion of aldrin-C^{14} and dieldrin-C^{14} and their metabolites through the bile. Med. Exper., 8:90.

102. Moss, J. A., and Hathway, D. E. 1964. Transport of Organic Compounds in the Mammal: Partition of dieldrin and Telodrin between the cellular components and soluble proteins of blood. Biochem. J., 91:384.

103. Mumma, R. O., Wheeler, W. B., Frear, D. E. H., and Hamilton, R. H. 1966. Dieldrin: Extraction of accumulations by root uptake. Science, 152:530.

104. Nalbandov, A. V. 1964. Reproductive Physiology. W. H. Freeman and Company, San Francisco, p. 196.

105. National Academy of Sciences - National Research Council. 1966. Nutrient Requirements of Dairy Cattle. Publication 1349.

106. O'Brien, R. D., and Dannelley, C. E. 1965. Penetration of insecticides through rat skin. J. Agr. Food Chem., 13:245.

107. Phillips, W. E. J., and Hidiroglou, M. 1965. Carotenoid and vitamin A concentrations in serum and liver of steers fed forages treated with DDT or MCPA. J. Agr. Food Chem., 13:254.

108. Radeleff, R. D., Claborn, H. V., Wells, R. W., and Nickerson, W. J. 1952. Effects on beef cattle of prolonged treatment with a DDT spray. Vet. Med., 47:94.

109. Radeleff, R. D., and Polen, P. B. 1963. Residues in beef fat following heptachlor feeding. J. Econ. Ent., 56:71.

110. Radomski, J. L., and Davidow, B. 1953. The metabolite of heptachlor, its estimation, storage, and toxicity. J. Pharmacol. Exptl. Therap., 107:266.

111. Richardson, L. A., Lane, J. R., Gardner, W. S., Peeler, J. T., and Campbell, J. E. 1967. Relationship of dietary intake to concentration of dieldrin and endrin in dogs. Bull. Environ. Contam. and Toxicol., 2:207.

112. Rusiecki, W., and Ochynski, J. 1964. Dieldrin metabolism. Zeszyty Problemowe Postepow Nauk Rolniczych., 51:63.

113. Rusoff, L. L., Waters, W. H., Gholson, J. H., Frye, J. B., Jr., Newsom, L. D., and Burns, E. C. 1962. Residues of heptachlor epoxide in butterfat of dairy cows grazing pastures treated with granular heptachlor. J. Agr. Food Chem., 10:377.

114. Salisbury, G. W., and Van Demark, N. L. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman and Company, San Francisco.

115. Schechter, M. S. 1963. Comments on the pesticide residue situation. J. Assoc. Offic. Agr. Chemists, 47:1063.

116. Schechter, M. S., and Westlake, W. E. 1962. Chemical residues and the analytical chemist. Analytical Chemistry, 34:25.

117. Shepherd, J. B., Moore, L. A., Carter, R. H., and Poos, F. W. 1949. The effect of feeding alfalfa hay containing DDT residue on the DDT content of cow's milk. J. Dairy Sci., 32:549.

118. Short, R. V. 1958. Progesterone in blood. II. Progesterone in the peripheral blood of pregnant cows. J. Endocrin., 16:426.

119. Smith, R. F., Hoskins, W. M., and Fullmer, O. H. 1948. Secretion of DDT in milk of dairy cows fed low-residue alfalfa hay. J. Econ. Ent., 41:759.

120. Stanley, R. W., and Morita, K. 1967. Effect of feeding thyroprotein to dairy cattle in a subtropical environment on milk composition and production, rumen metabolism, and fatty acid composition of milk fat. J. Dairy Sci., 50:1097.

121. Stohlman, E. F., Thorp, W. T. S., and Smith, M. I. 1949. Pharmacologic action of chlordane. Fed. Proc., 8:334.

122. Street, J. C. 1964. DDT antagonism to dieldrin storage in adipose tissue of rats. Science, 146:1580.

123. Street, J. C., and Blau, A. D. 1966. Insecticide interactions affecting residue accumulation in animal tissues. Toxicol. Appl. Pharmacol., 8:497.

124. Street, J. C., Chadwick, R. W., Wang, M., and Phillips, R. L. 1966. Insecticide interactions affecting residue storage in animal tissues. J. Agr. Food Chem., 14:545.

125. Street, J. C., Wang, M., and Blau, A. D. 1966. Drug effects on dieldrin storage in rat tissue. Bull. Environ. Contam. and Toxicol., 1:6.

126. Tauber, O. E., and Hughes, A. B. 1950. Effect of DDT ingestion on total cholesterol content of ovaries of white rat. Proc. Soc. Exptl. Biol. Med., 75:420.

127. Tinsley, I. J. 1966. Nutritional interactions in dieldrin toxicity. J. Agr. Food Chem., 14:563.

128. Wasserman, M., and Wasserman, D. 1966. Recognition of a new group of toxic substances as current constituents in the human body: the organochlorine insecticides. XV International Congress on Occupational Health, Vienna, Sept.

129. Weikel, J. H., Jr. 1956. The role of biliary excretion in the metabolism of methoxychlor (1,1,1-trichloro 2,2-bis (p-methoxyphenyl) ethane) in the rat. J. Pharmacol. Exptl. Therap., 116:60.

130. Wells, G. H. 1940. Adipose tissue, a neglected subject. J. Am. Med. Assoc., 114:2177.

131. Wertheimer, H. E. 1965. Introduction--a perspective. In: Handbook of Physiology. Sect. 5 Adipose Tissue. American Physiological Society, Washington, D. C.

132. Wesley, R. L., Stemp, A. R., Liska, B. J., and Stadelman, W. J. 1965. Depletion of DDT from commercial layers. Poultry Sci., 45:321.

133. Wheeler, W. B., Frear, D. E. H., Mumma, R. O., Hamilton, R. H., and Cotner, R. C. 1967. Quantitative extraction of root-absorbed dieldrin from the aerial parts of forage crops. J. Agr. Food Chem., 15:227.

134. Whitacre, D. M., and Ware, G. W. 1967. Retention of vaporized lindane by plants and animals. J. Agr. Food Chem., 15:492.

135. Williams, S., Mills, P. A., McDowell, R. E. 1964. Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. J. Assoc. Offic. Agr. Chemists, 47:1124.

136. Wilson, H. F., Allen, N. N., Bohstedt, G., Betheil, J., and Lardy, H. 1946. Feeding experiments with DDT-treated pea vine silage with special reference to dairy cows, sheep, and laboratory animals. J. Econ. Ent., 39:801.

137. Witt, J. M., Brown, W. H., Shaw, G. I., Maynard, L. S., Sullivan, L. M., Whiting, F. M., and Stull, J. W. 1966. Rate of transfer of DDT from the blood compartment. Bull. Environ. Contam. and Toxicol., 1:187.

138. Witt, J. M., Whiting, F. M., Brown, W. H., and Stull, J. W. 1966. Contamination of milk from different routes of animal exposure to DDT. J. Dairy Sci., 49:370.

139. Wong, D. T., and Terriere, L. C. 1965. Epoxidation of aldrin, isodrin, and heptachlor by rat liver microsomes. Biochem. Pharm., 14:375.

140. Woodward, G., and Ofner, R. R. 1946. Accumulation of DDT in the fat of rats in relation to dietary level and length of feeding. Fed. Proc., 5:215.

141. Woodward, G., Ofner, R. R., and Montgomery, C. M. 1945. Accumulation of DDT in the body fat and its appearance in the milk of dogs. Science, 102:177.

142. Zabik, M. 1967. Mimeo, unpublished, February.

143. Zavon, M. R. 1963. The toxicology and pharmacology of aldrin and dieldrin. Report, Kettering Laboratory, University of Cincinnati.

144. Zweig, G., Smith, L. M., Peoples, S. A., and Cox, R. 1961. DDT residues in milk from dairy cows fed low levels of DDT in their daily rations. J. Agr. Food Chem., 9:481.

APPENDIX

TABLE I.--Data on individual cows contaminated with dieldrin during lactation.

Cow	Age	Calving Date	Days Since Calving to June 1, 1965	Avg. Daily Milk Prod. May 12-17 Incl.	Body Weight May 10 & 11
	yr-mo			---------kg--------	
286	1-10	1/8/65	144	23.5	431
727	3-1	12/23/64	160	22.2	590
248	3-2	3/1/65	92	31.9	552
188	5-10	4/28/65	34	45.5*	618
Avg.	3-6		107.5	30.8	548
759	1-9	12/31/64	152	18.7	414
717	3-5	1/9/65	143	25.4	499
723	3-4	1/28/65	124	32.2	559
701	4-4	3/28/65	65	38.9	524
Avg.	3-3		121.0	28.8	499
758	1-11	1/22/65	130	19.1	474
264	2-7	1/15/65	137	21.4	542
257	2-11	2/9/65	112	33.7	582
224	4-1	2/7/65	114	34.8*	545
Avg.	2-11		123.3	27.3	536
247	3-1	2/2/65	119	21.3	524
707	4-0	1/22/65	130	32.8	491
721	3-5	2/5/65	116	32.4	591
250	3-2	4/6/65	56	33.2	552
Avg.	3-5		105.3	29.9	540

*5 day average.

TABLE II.--Data on individual heifers contaminated with dieldrin during 60 to 0 days prepartum.

Heifer Number	Avg. B.W. During Contamination	Calving Date	No. Days Contaminated	Total HEOD Fed	Mean Daily HEOD Dose	Sex of Calf	Birth Weight of Calf
	kg			mg	mg/kg		kg
			Group A				
H-300	590	11/3/65	61	3,412	0.095	M[a]	30.4
H-303	538	12/2/65	56	2,796	0.095	F	37.2
H-770	536	12/18/65	60	3,050	0.095	F	40.8
H-306	538	12/24/65	74	3,598	0.090	M	37.6
H-301	603	12/26/65	52	2,873	0.092	F	36.3
H-308	555	1/14/66	64	3,253	0.092	M	46.3
Mean	560		61.0	3,164	0.093		38.1
			Group B				
H-766	602	11/14/65	53	3,008	0.095	M	40.8
H-767	533	12/5/65	55	2,796	0.095	M	37.2
H-768	558	12/23/65	55	2,796	0.091	F	39.5
H-307	586	12/24/65	62	3,271	0.090	M	42.2
H-315	512	12/30/65	60	2,917	0.095	M	42.6
H-775	520	2/2/66	61	3,101	0.097	F	38.1
Mean	552		57.7	2,982	0.094		40.1
			Group C				
H-291	607	12/1/65	54	3,222	0.098	F[b]	37.2
H-771	520	12/5/65	55	2,796	0.098	M	41.7
H-312	542	12/23/65	52	2,643	0.094	F	35.4
H-311	547	12/28/65	61	3,101	0.093	M	42.2
H-290	566	1/9/66	66	3,592	0.098	F	41.7
H-761	713	4/15/66	57	3,779	0.093	F	45.4
Mean	582		57.3	3,189	0.095		40.6

[a]Born dead. [b]Died soon after birth.

TABLE III.--Data on individual heifers contaminated with dieldrin during 120 to 60 days prepartum.

Heifer No.	Avg. B.W. During Cont.	No. Days Cont.	Total HEOD Fed	Mean Daily HEOD Dose	Calving Date	No. Days Between End Cont. & Calving	Actual No. Days Contam. Started Prepartum	Sex of Calf	Birth Wt. of Calf
	kg		mg	mg/kg					kg
H-305	559	60	3,315	0.099	3/31/66	56	116	M	37.6
H-769	581	60	3,448	0.099	3/31/66	51	111	F	32.7
H-777	527	60	3,050	0.096	4/4/66	57	117	M	40.8
H-780	525	60	2,917	0.093	4/12/66	58	118	F	39.9
H-318	521	60	3,050	0.097	4/12/66	54	114	F[a]	29.9
								F[a]	32.7
H-313	576	60	3,315	0.096	5/10/66	53	113	F	36.3
H-773[b]	538	60	2,917	0.090	4/9/66	36[b]	96[b]	M	35.4
Mean	547	60	3,145	0.096		52.1	112.1		35.7

[a]Twins.

[b]H-773 calved to an earlier breeding date than the one used for calculating contamination period.

TABLE IV.--Data on individual heifers contaminated with dieldrin during 180 to 120 days prepartum.

Heifer No.	Avg. B.W. During Cont.	No. Days Cont.	Total HEOD Fed	Mean Daily HEOD Dose	Calving Date	No. Days Between End Cont. & Calving	Actual No. Days Contam. Started Prepartum	Sex of Calf	Birth Wt. of Calf
	kg		mg	mg/kg					kg
H-319	492	60	2,785	0.095	6/9/66	120	180	M	41.3
H-815	460	60	2,652	0.096	6/17/66	108	168	M	41.7
H-783	521	60	3,182	0.102	9/8/66	110	170	F	36.7
H-814	540	60	3,050	0.094	9/14/66	116	176	F	37.2
H-788[a]	487	60	2,917	0.100	9/22/66	111[a]	171[a]	M	36.3
H-786	515	60	3,050	0.099	10/12/66	140[a]	200[a]	M	38.6
Mean	503	60	2,939	0.097		117.5	177.5		38.6

[a]H-786 calved to a later breeding date than the one used for calculating contamination period.

TABLE V.--Physical characteristics of dieldrin.

Chemical Name:	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
Molecular weight:	380.93
Physical state at 77° F.:	Solid, dry flakes
Melting point:	175-176°C
Odor:	Odorless
Vapor Pressure (mm Hg at 20°C):	7.78×10^{-7}
Flammability:	Nonflammable
Bulk density, lb./cu. ft.:	47 to 51
Solubility:	Moderately soluble in aromatics, halogenated solvents, esters & ketones; sparingly soluble in aliphatic hydrocarbons & alcohols; insoluble in water
Solvent:	Solubility at 26°C (g/100 ml)
Methanol	4.9
Acetone	54.0
Benzene	75.0
Hexane	7.7
Base oil (std. oil 10)	4.3
Water	insoluble (0.05 ppm)