ADSORPTION OF EXTERNALLY APPLIED INSECTICIDES BY ANIMAL TISSUE AND SECRETION IN MILK

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By

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PREFACE

The widespread use of the chlorinated hydrocarbons (DDT, methoxy DDT, chlordan, chlorinated camphene and hexachlorocyclohexane) as sprays for external parasite control has made it desirable to determine whether or not these compounds are adsorbed in the tissues and secreted in the milk of treated animals. Before the danger to humans consuming such contaminated food products can be evaluated, it is necessary to know how much of the insecticide may be stored following routine spraying for external parasite control. Possibly one or more of the materials should not be used on animals producing food for man.

To obtain further information on this subject, cooperative work involving the departments of Agricultural Chemistry Research, Animal Husbandry, Dairying and Entomology was undertaken. Groups of range and dairy animals were sprayed with two quarts each of one of the materials mentioned above. Each material was suspended in water and diluted to 0.5 % by weight. Different groups were sprayed from 1 to 4 times. All animals were handled in the usual manner and no attempt was made to segregate sprayed groups to prevent licking. Milk from dairy animals was collected weekly and preserved in an equal volume of alcohol and tissues from beef animals were obtained at slaughter and frozen until analyzed.

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ACKNOWLEDGMENT

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INTRODUCTION

Shortly after our entry into the recent war, studies were initiated by the Bureau of Entomology and Plant Quarantine for the purpose of developing control measures for certain insects of importance to the Armed Forces. Of the numerous compounds tested chlorinated hydrocarbons proved to be of value, particularly, DDT (2,2 bis(p-chlorophenyl)-1,1,1-trichloroethane), chlordan (1,2,4,5,6,7,8,8-Octachloro-4,7-methane-3a,4,7,7a-tetrahydroindane), chlorinated camphene (2,3,3,trimethyl 2,4,5,5,6,6,7,7, octachlorobicylicpentane, methoxy DDT (2,2,-bis(p-methoxyphenyl) 1,1,1-trichloroethane), and hexachlorocyclohexane (1,2,3,4,5,6-hexachlorocyclohexane) were outstanding in performance (8). Because of their effectiveness against a large number of insects, extensive effort was made to explore their possibilities and to develop formulations suitable for their practical use in insect control.

More publicity has been accorded DDT and the other chlorinated hydrocarbons in the past five years than had been given to any other group of insecticides in a comparable length of time. Their remarkable effectiveness in controlling such disease-carrying insects as the body louse, mosquito, and fly (6) has attracted world-wide attention. In addition to their value in preventive medicine, DDT and the other chlorinated hydrocarbon insecticides are among the most effective agents against a wide variety of agricultural pests.

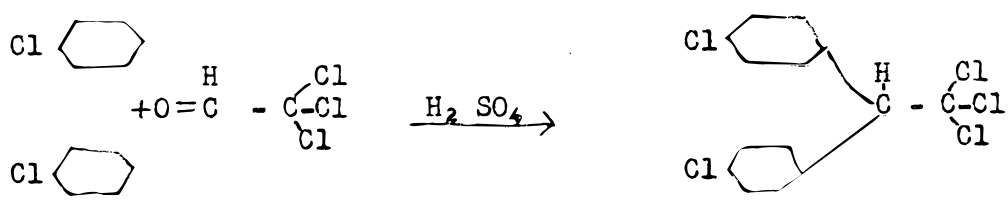
Insects affecting livestock are of major economic importance in Oklahoma. It is estimated that during the past year more than five million cattle in Kansas, Oklahoma and Texas were sprayed one or more times (17) with DDT for the control of external parasites. Additional numbers were sprayed with hexachlorocyclohexane, chlorinated camphene, chlordan and methoxy DDT. Such large scale utilization of materials of known or potential toxicity to warm-blooded animals made of interest the determination of the absorption rate by the sprayed animals. Since the flesh of the animal and the milk secreted were to be used as food for humans, data on tissue storage and elimination in milk was needed to evaluate the possible hazard from such extensive use of these toxic materials. The work of Telford (33), Hackman (13), and Heller (18) showed that these insecticides were present in the milk from cows sprayed with these insecticides. Much additional information was needed, however, to determine the extent of contamination of milk and tissues following routine spraying for external parasite control. Possibly, one or more of the materials should not be used on animals producing food for human consumption. To obtain such further information on this subject, a cooperative project was instituted involving the departments of Agricultural Chemistry Research, Animal Husbandry, Dairying, and Entomology of the Oklahoma Agricultural Experiment Station. This report presents a portion of this study.

DDT

The symbol DDT is a contraction for dichloro-diphenyl-trichloroethane, the generic name of the active insecticidal principle (6). Theoretically, there are forty-five possible dichloro-diphenyl-trichloroethanes, excluding stereoisomeric forms. However, the term DDT has been confined to the product obtained on condensation of chloral with chlorobenzene in the presence of sulphuric acid according to the process originally described by Zeider (8) in 1874. The major constituent in the product so obtained is about seventy-five per cent of the other isomers of DDT, the chief one being ortho-DDT. This product has been referred to as technical DDT and is of somewhat variable composition depending upon the temperature and other conditions during its synthesis. A refined grade of DDT which con-consists of ninety-five percent or more of 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane has been designated as pure DDT (26).

Following Zeidler's description of DDT's synthesis in 1874, no further mention of this compound is found in the literature until 1942. In that year a British patent assigned to J. R. Geigy A-G., was issued which states that p,p'-dichloro-diphenyltrichloroethane may be produced as follows (26):

> "225 parts of chlorobenzene are mixed with 147 parts of chloral or the corresponding amount of chloral hydrate and then 1,000 parts of sulphuric acid monohydrate are added. While stirring well the temperature rises to 60 degrees C and then sinks slowly down to room temperature, the mass then containing solid parts. It is poured into a great deal of water, whereupon the product separates in solid form. It is well washed and crystallized from ethyl alcohol forming fine white crystals, having a weak fruit-like odour."



Monochlorobenzene Chloral DDT

DDT is nearly insoluble in water, moderately soluble in petroleum and vegetable oils, and readily soluble in many common organic solvents. Solubility values in a large number of solvents have been determined for once-recrystallized DDT (m.p. 107.5 - 108° C.). The following tabulation gives the approximate solubilities in a few of the more useful solvents at 27-30 degrees. Most of these solvents are inflammable, some are highly inflammable and when used as sprays, without emulsifying in water, create highly explosive concentrations in the air when used indoors or in enclosed places (8):

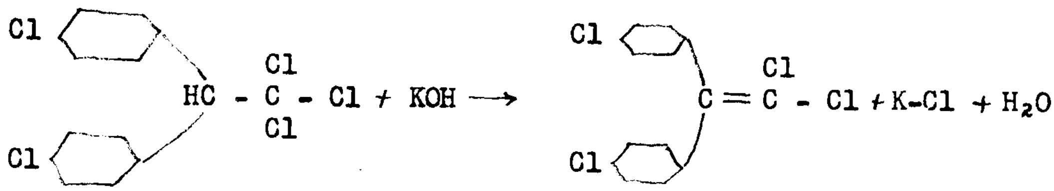
Solvent	Grams per 100 ml.
Cyclohexanone	116
Benzene	78
Isophorone	74
Trichloroethylene	64
Tetrahydronaphthalene	61
Acetone	58

Because DDT is a stable organic compound, soluble in many solvents, it is extremely versatile and has been prepared for use in a number of different ways, for example as dusts, solutions, emulsions, suspensions in aqueous and non-aqueous solvents and aerosols.

No one questions the lethal effect of DDT upon many insects, but its toxicity to man and warm-blooded animals is less certain. There is no doubt that DDT is toxic; however, the toxicity of DDT to humans is of a sufficiently low order to permit the use of DDT as an insecticide without danger to personnel if reasonable precautions are taken. In spite of the extensive employment of this insecticidal ingredient to date, there has been no established case of poisoning. Many reports have been received of symptoms of toxicity attributed to DDT. In many instances it has not been clearly established that the observed symptoms may not have been caused by the carrier rather than the insecticide itself. In many cases the public and medical profession is not aware of the symptoms of toxicity of commonly used carriers with the resulting erroneous conclusions (3).

There is little doubt, however, that DDT itself is a toxic substance. The effect of DDT on man and other warm-blooded animals has been investigated by three principal groups, the U. S. Food and Drug Administration, the U. S. Public Health Service, and the Kettering Laboratory of Applied Physiology of the University of Cincinnati. These investigations have shown that DDT is stored as well as transmitted in animal tissue and excretions. Furman (1) has shown that hexachlorocyclohexane was absorbed by dairy animals and transmitted through in their milk. The actual amount of hexachlorocyclohexane present in the milk of the treated animals was probably too low to present actual health hazards to the consumers of the milk. Since milk containing a detectable quantity of hexachlorocyclohexane is classified as an adulterated food under the Federal Food, Drug and Cosmetic Act, and since very small quantities in the cream produce a musty odor, it was deemed inadvisable to spray or dip dairy cattle with aqueous suspensions of hexachlorocyclohexane unless the milk obtained for at least one week following treatment is used for other than human consumption. Heller (18) showed that DDT was transmitted through the milk from sprayed animals, but no attempt was made to show the effect of the milk containing the DDT on other warm-blooded animals consuming the milk. Telford (33) showed that the milk from goats sprayed with hexachlorocyclohexane was not only toxic but lethal to white mice.

Several methods are now available for the determination of DDT in biological material. Several of these attempt to relate organic chloride content to DDT content. The organic chlorides are determined by combustion, followed by the determination of the released chlorides by one of the conventional methods (14). The total organic chlorine may also be determined by refluxing with metallic sodium in isopropyl alcohol or other soluble solvent and subsequently determining the chloride removed (34). Another analytical method consists of refluxing DDT with alcoholic potassium hydroxide (13). Under this latter condition DDT is hydrolyzed as follows:



The chloride found by this procedure may then be determined. Since results with technical DDT by this latter method are higher than the actual content of the p,p'-isomer, a blank must also be determined.

A newly developed colorimetric method gives promise of being very useful (27). In this method a sample is nitrated, the tetranitrate derivative formed is extracted with ether, and the ether extract is washed with alkali, dried, and evaporated. The residue is dissolved in benzene and treated with a methyl alcohol solution of sodium methylate. p,p'-DDT gives a blue color, o-p'-DDT, violet red color. By use of suitable spectrophotometric procedure, the exact content of p,p'-DDT may be measured. This method is sensitive to about ten micrograms of the p,p' isomer. Another colorimetric method involves treatment of dry DDT or a dry extract of the sample with xanthydrol-potassium hydroxide-pyridine reagent (32). DDT gives a red color with this reagent. This method is equally sensitive.

Hexachlorocyclohexane

Recently 1,2,3,4,5,6,-hexachlorocyclohexane, commonly called benzene hexachloride, has been made available as an insecticide and has proved to be of considerable agricultural importance. The present technical material is a mixture of at least five of the sixteen possible sterо-isomers, of which the so-called gamma isomer is by far the most biologically active. The activity of the gamma isomer is so marked and so superior to that of the other isomers that the evaluation of hexachlorocyclohexane is usually expressed in terms of the per cent gamma content, and the latter is regarded as an index of the biological activity (7). In March, 1945, the outstanding insecticidal properties of the gamma isomer of benzene hexachloride were made public by Slade (29) in England when delivering the Hurter Lecture. Hexachlorocyclohexane owes its discovery as an insecticide to the war. In 1941 the need for a substitute for derris (rotenone) was acute. Chemists of the Imperial Chemical Industries reviewed their tests of thousands of synthetic organic compounds and selected about forty for further evaluation. Among them were the hexachlorides of mono- and o-dichlorobenzenes, both chosen primarily because of their bad odor, the thought being that they might act as repellents. When called upon for samples of these compounds, one of the laboratories of the Imperial Chemical Industries suggested that the parent compound, hexachlorocyclohexane, be included. The technical samples furnished proved more effective as an insecticide than the original laboratory preparations. After considerable investigation, the toxicity was traced to the gamma isomer.

The gamma isomer of hexachlorocyclohexane (Gammexane in England), is one of five isomers obtained when chlorine reacts with benzene in the presence of light. The gamma isomer comprises about ten to twelve per cent of the product and can be separated by fractional crystallization from organic solvents.

Development work in both the manufacturing and the biological fields has been hampered by a lack of rapid analytical methods. Analyses by biological assay has been employed and should probably be considered as a final test for any insecticidal material. However, this method does not lend itself to high accuracy and is further limited by the requirements of a rather specialized technique and the fact that it is time-consuming. Daasch (7) has recently described an infrared absorption method which should prove valuable, particularly since it permits the simultaneous determination of five isomers.

The discovery of the fifth isomer, designated as "Epsilon", has been announced by Kauer, DuVall and Alquist (20). The exact structure of the Beta isomer has been established by the use of X-ray studies of the crystal. The exact structure of the other isomers is still questionable. The possible structures with chlorine and hydrogen atoms arranged in different combinations with relation to the carbon atoms account for the fifteen isomeric forms. Because of the close proximity of the chlorine atoms in some forms, Slade (29) states that there are only five isomeric forms which can exist without molecular strain.

Chlordan

A material produced by the chlorination of hydrocarbons derived from coal tar has been used as an insecticide. The active insecticidal component of this material has the empirical formula $C_{10}H_6Cl_8$ and has recently been assigned the following structure: 1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindan (1). In order to provide a more convenient designation, the chlordan (9) was coined. Technical chlordan as is currently produced is a viscous liquid containing between sixty and seventy per cent of 1,2,3,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindan. Also present are several related compounds which possess some insecticidal properties. Technical chlordan is readily soluble in most organic solvents, including the petroleum derivatives but insoluble in water. It may be formulated in solutions to be applied directly or to form emulsions in water, or as a dust. Like DDT, chlordan is dehydrohalogenated rather rapidly in the presence of alkali forming compounds of low or no toxicity to insects.

Kerns, Ingle and Metcalf (21) have described the general insecticidal properties of chlordan. It appears to function as an insecticide in a manner somewhat similar to DDT. It possesses considerable residual toxicity.

Ingle (19) has reported that, weight for weight, chlordan and DDT appear to be of the same order of toxicity to white rats. Rats receiving either chlordan or DDT showed a wide range of individual susceptibility, making it difficult to establish an absolute minimum lethal dose.

Chlorinated Camphene

Another polychlorinated organic compound, designated by the trade name Toxaphene (Hercules Powder Company), has received some attention as an insecticide. Chemically, this material is a mixture containing polychlorobicyclic terpens. The approximate empirical formula for this compound is $C_{10}H_{10}Cl_8$, (2,3,3 trimethyl 2,4,5,5,6,6,7,7, octochlorobicyclopentane) and the commercial product is a yellow, waxy solid, melting over a broad range from sixty-five to ninety degrees C.

Stearns *et al*. (31) found that this material behaved somewhat like DDT as an insecticide, although against several insects Toxaphene was less toxic than DDT. Toxaphene is readily soluble in most commercial solvents, but is more soluble in the aromatics than in the aliphatic hydrocarbons. It is not soluble in water. Like the other chlorinated hydrocarbons of this type, including DDT, Toxaphene slowly evolves HCl upon heating, the rate depending upon the temperature and the presence of catalytic impurities, such as iron compounds. High alkaline conditions cause dehydrohalogenation.

Toxicological investigations of Toxaphene have shown that it is a poisonous substance which has an irregular toxicity range. In solvent form it is readily absorbed through the skin (16). It can cause toxic effects on the nervous system by ingestion, inhalation, or absorption through the skin into the body. In solid form it is not readily absorbed through the skin of animals.

Methoxy DDT

Methoxy DDT is a slightly different derivative of the parent compound, DDT, is less toxic to warm-blooded animals and more effective on certain insects than DDT itself. In addition, many plants are less subject to injury from methoxy DDT. Methoxy DDT has the additional insecticidal advantage of a "knock-down" effect on such insects as flies and mosquitoes. It may be used either as a spray or a dust (10).

Methoxy DDT is the approved generic name being applied to 2,2-bis (p-methoxyphenyl) 1,1,1-trichloroethane, and supplies such designations as methoxychlor, methoxy analog of DDT, and dianisyl trichloroethane. Technical methoxy DDT is eighty-eight per cent 2,2-bis(p-methoxyphenyl) 1,1,1-trichloroethane and twelve per cent other relating compounds (2).

In acute toxicity tests, methoxy DDT has been shown to be far less toxic to warm-blooded animals than DDT; in some cases having approximately one thirty-fifth the toxicity of DDT (23). Methoxy DDT may be considered to have about the same acute toxicity as rotenone (30).

The Haskell Laboratory of Industrial Toxicology (Du Pont) is making a thorough study of the toxicity of methoxy DDT (10). Results to date indicate that in addition to being less toxic than DDT, there is a lesser tendency (one-fifth or less) for methoxy DDT to be stored in the body fat of animals when methoxy DDT is eaten or applied to the skin in oil solutions (11).

EXPERIMENTAL

Insecticide content of milk following external application.

In order to determine the amount of DDT, hexachlorocyclohexane, chlorinated camphene, methoxy DDT and chlordan absorbed by dairy cattle and secreted in the milk following topical application for routine insect control, a group of twenty-five grade dairy cows from the college herd were selected. These were divided into five lots of five cows each without regard for breed differences or stage of lactation. These animals were fed a standard milk-producing ration, supplemented by access to a limited amount of summer pasture.

The insecticides used and the method and time of application were essentially as follows: The DDT used on the dairy animals was Du Pont's Deenate, 50% wettable powder. The chlordan used was Velsicol, 50% wettable powder, produced by the Velsicol Corporation of Chicago, Illinois. The hexachlorocyclohexane used was a 20% gamma isomer, oil emulsion, (this was diluted to a 6% emulsion before application) produced by the California Spray Chemical Company, Richmond, California. Toxaphene, produced by the Hercules Powder Company, Wilmington, Delaware, was the chlorinated camphene used. The methoxy DDT used was a 25% methoxy DDT Du Pont test material, oil base emulsion, described as follows: "Test No. 1085-166 as of letter 5-11-48".

The materials were applied externally in accordance with the usual commercial methods. A three nozzle fog spray apparatus was employed using 200 pounds pressure. Each animal received approximately two quarts of a 0.5% solution or suspension of the insecticide, sprayed in such a manner as to completely drench the entire animal. These dairy cows were sprayed on May 4, July 14, and September 17, 1948. These spraying dates were determined by the actual need for the application of an insecticide for the control of flies.

Samples of milk were collected as follows: All samples were secured at the milking shed to preclude any errors or contamination by the milking crew. Milk from the evening milking was used throughout the test. Since the milk obtained by hand stripping contains more fat than that secured by the machine alone, each animal was hand-stripped and the milk thus obtained combined with that from the machine milking. The composite was thoroughly mixed and a 200 ml. aliquot removed. The aliquots from each cow in the group were then pooled, an equal volume of alcohol added, and the samples stored until analyzed.

Storage of insecticide in tissues following external application.

In order to determine the degree of storage of DDT in the fat and tissues of animals receiving topical application of commercial insecticides for external parasite control, a test was devised to determine the concentration in the fat and tissues of range beef cattle treated according to the usual commercial practice.

The test animals consisted of two groups of sixty-seven long-two-year-old grade Hereford range steers. The feeder steers were long-yearling grade Herefords. The range steers received native grass pasture supplemented with cottonseed cake or soybean oil meal. The feeder steers were on a standard fattening ration. Each animal was placed in a small pen and sprayed with approximately two quarts of a 0.5% concentration of the insecticide. The source and type of insecticide was the same as that previously described. All materials used were applied at 200 pounds pressure with a three nozzle fog spray and sprayed in such a manner to completely drench the entire animal. Four groups of feeder steers were sprayed one, two, three and four times, respectively, to determine the effect of repeated application on the accumulation of insecticide in the tissues.

An attempt was made to determine the effect on a calf sprayed with a chlorinated hydrocarbon, and at the same time receiving milk from a cow that was also sprayed with a chlorinated hydrocarbon. To accomplish this, a dairy suckling calf and a range suckling calf were selected and samples of their fat, liver, kidney, and lean meat were secured at time of slaughter and frozen until analyzed.

Samples of fat and various tissues were obtained from each animal at the time of slaughter, placed in sterile jars, rapidly frozen, and maintained in the frozen state until analyzed. The fat tissue was mesentery fat. The muscle tissue was obtained from the diaphram muscle. Liver and kidney tissue was a representative portion of these two organs.

Analytical Methods.

Since no colorimetric method for hexachlorocyclohexane, chlorinated camphene, chlordan, and methoxy DDT in biological materials was available, the method used was based upon the determination of total organic chloride. The method employed was essentially that described by Carter

(4). Organically bound chlorides were converted to sodium chloride by the action of metallic sodium in isopropyl alcohol. The chloride thus obtained was determined titrametrically by the Volhard-Arnold method. The only modification made in this method was the utilization of Nessler tubes to aid in determining the end-point. One drop of ammonium thiocyanate solution was placed in a Nessler tube and 100 cc. of distilled water was added. To this ferric alum indicator was added in the same amount used in the unknowns. The red color produced in this tube was used as a standard end-point and all the unknowns were titrated to this point. In order to eliminate the interference to the color by the suspended silver chloride, the solution was filtered into the Nessler tube before the color was read. In the event the end-point had not been reached, the solution was returned to the flask and titration continued.

Table I compares the results obtained in samples of milk using the Schecter colorimetric method (28) and the chloride procedure of Carter. It will be seen that excellent agreement between the two methods was obtained.

Table I

Comparison of the Schecter Method and the Carter Method for the Recovery of DDT

Sample No.	Schecter Method DDT p.p.m.	Carter Method Chlorine X 2 DDT p.p.m.
1	3.4	1.7 X 2 = 3.4
2	5.2	2.6 X 2 = 5.2
3	5.0	2.5 X 2 = 5.0
4	9.6	4.8 X 2 = 9.6
5	2.2	1.1 X 2 = 2.2
6	4.6	2.3 X 2 = 4.6

In order to determine the per cent recovery of DDT, known quantities of DDT and the other insecticides used were added to milk and the amount present determined using the chloride method. The results are shown in Table II.

Table II

Per Cent Recovery From Known Concentrations of the Insecticides Used

Insecticide Used Mg.	Mg. Chlorine Found	Percent Insecticide Recovered
DDT		
10	4.9	98.0
10	5.1	102.0
10	4.8	96.0
15	7.3	98.6
15	7.2	96.0
Hexachlorocyclohexane		
10	7.1	97.0
10	7.1	97.7
15	10.9	99.8
15	10.8	98.7
20	14.5	99.1
Chlordan		
10	6.8	98.9
10	6.9	99.7
15	10.3	99.0
20	13.8	99.7
20	13.7	99.5
Chlorinated Camphene		
5	3.2	93.9
10	6.6	97.0
10	6.6	96.3
20	13.5	98.4
25	16.8	98.3
Methoxy DDT		
10	3.0	97.4
10	2.9	94.8
15	4.5	98.5
15	4.5	97.4
20	6.0	97.4

The DDT content of the fat, liver, kidney and lean meat was determined by the Schecter and Pogorelskin (28) method using the Coleman Spectrophotometer to determine the density of the resulting color. The concentration of DDT was determined by comparison with a standard curve prepared by using known amounts of technical DDT.

RESULTS

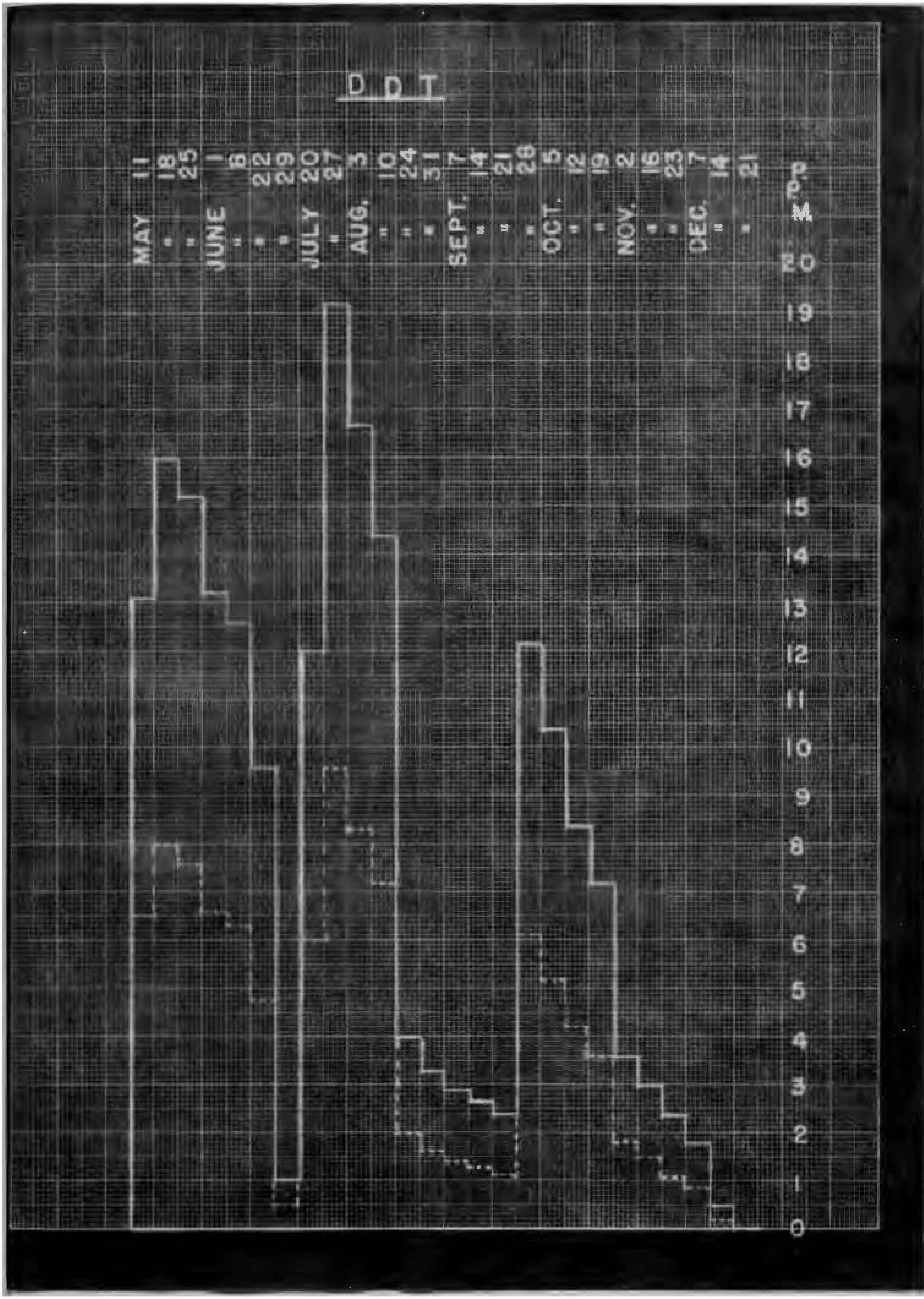
Graphs 1, 2, 3, 4, and 5 show the concentration of chlorine in parts per million and the calculated amount of insecticide present on the basis of per cent chloride present in the original compound. These graphs cover the period ranging from May 11, 1948 to December 21, 1948 for DDT, methoxy DDT, hexachlorocyclohexane, chlordan and chlorinated camphene found in the milk.

Table III shows spraying dates, the date of slaughter and the concentration of DDT in parts per million for the fat as determined by the method of Schecter (28), and the concentration of methoxy DDT as determined by the determination of total organic chlorides (4) assuming that the methoxy DDT contained 30.79% chlorine.

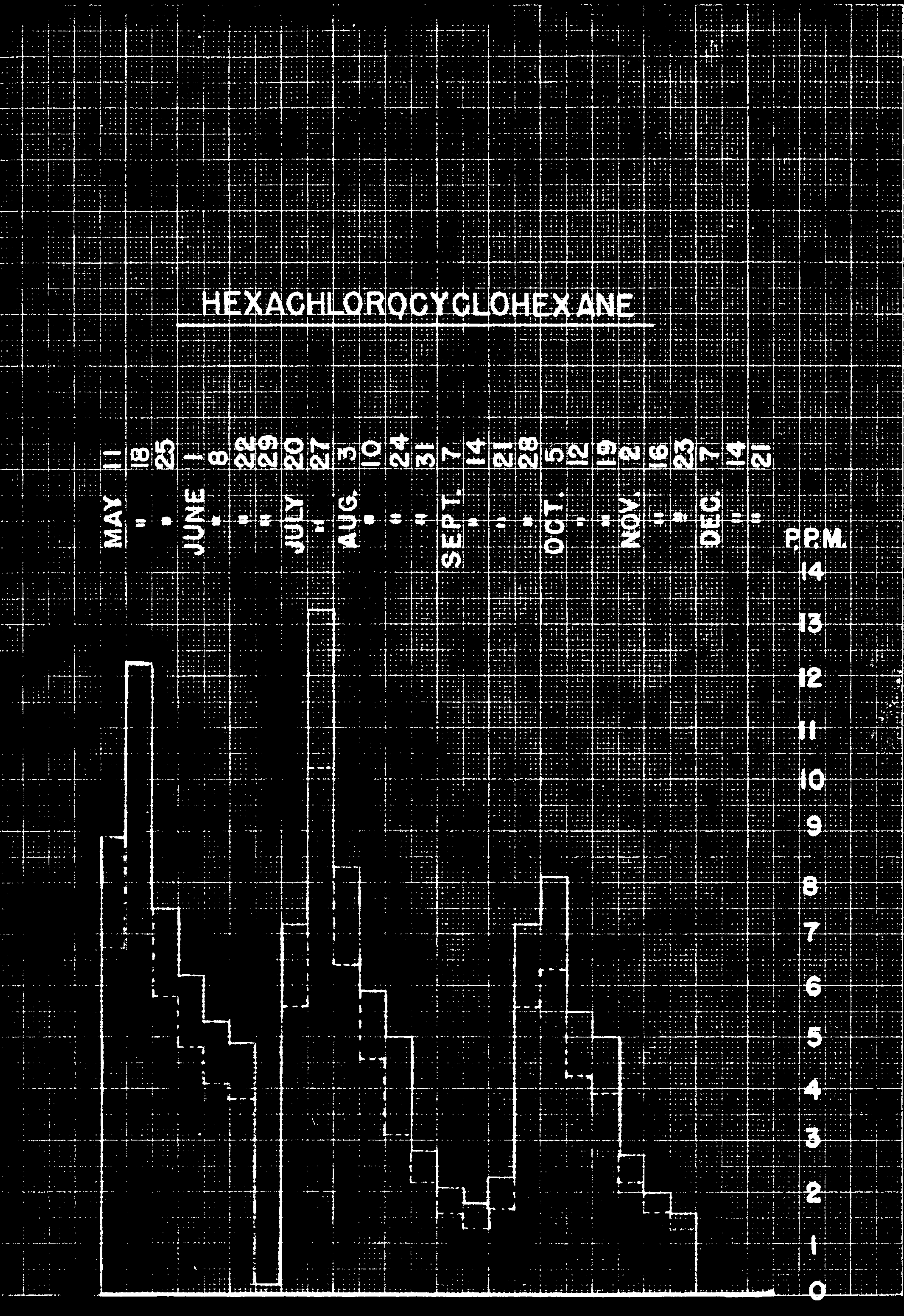
Table III

The Concentration of DDT and Methoxy DDT in the Fat of Range Steers Sprayed with these Insecticides.

Type Animal	Number of Animals in Test	Number of Times Sprayed	Date of Spraying	Date of Slaughter	Insecticide Content Found in Parts Per Million	
					DDT	Methoxy DDT
Range Steers	67	3	June 11 June 29 August 12	August 24	13.3	30.3



D D T
MAY 11
" 18
" 25
JUNE 1
" 8
" 22
" 29
JULY 20
" 27
AUG. 3
" 10
" 24
" 31
SEPT. 7
" 14
" 21
" 28
OCT. 5
" 12
" 19
NOV. 2
" 16
" 23
DEC. 7
" 14
" 21
P.P.M.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20



HEXACHLOROCYCLOHEXANE
MAY 11
" 18
" 25
JUNE 1
" 8
" 22
" 29
JULY 20
" 27
AUG. 3
" 10
" 24
" 31
SEPT. 7
" 14
" 21
" 28
OCT. 5
" 12
" 19
NOV. 2
" 16
" 23
DEC. 7
" 14
" 21
P.P.M.
14
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7
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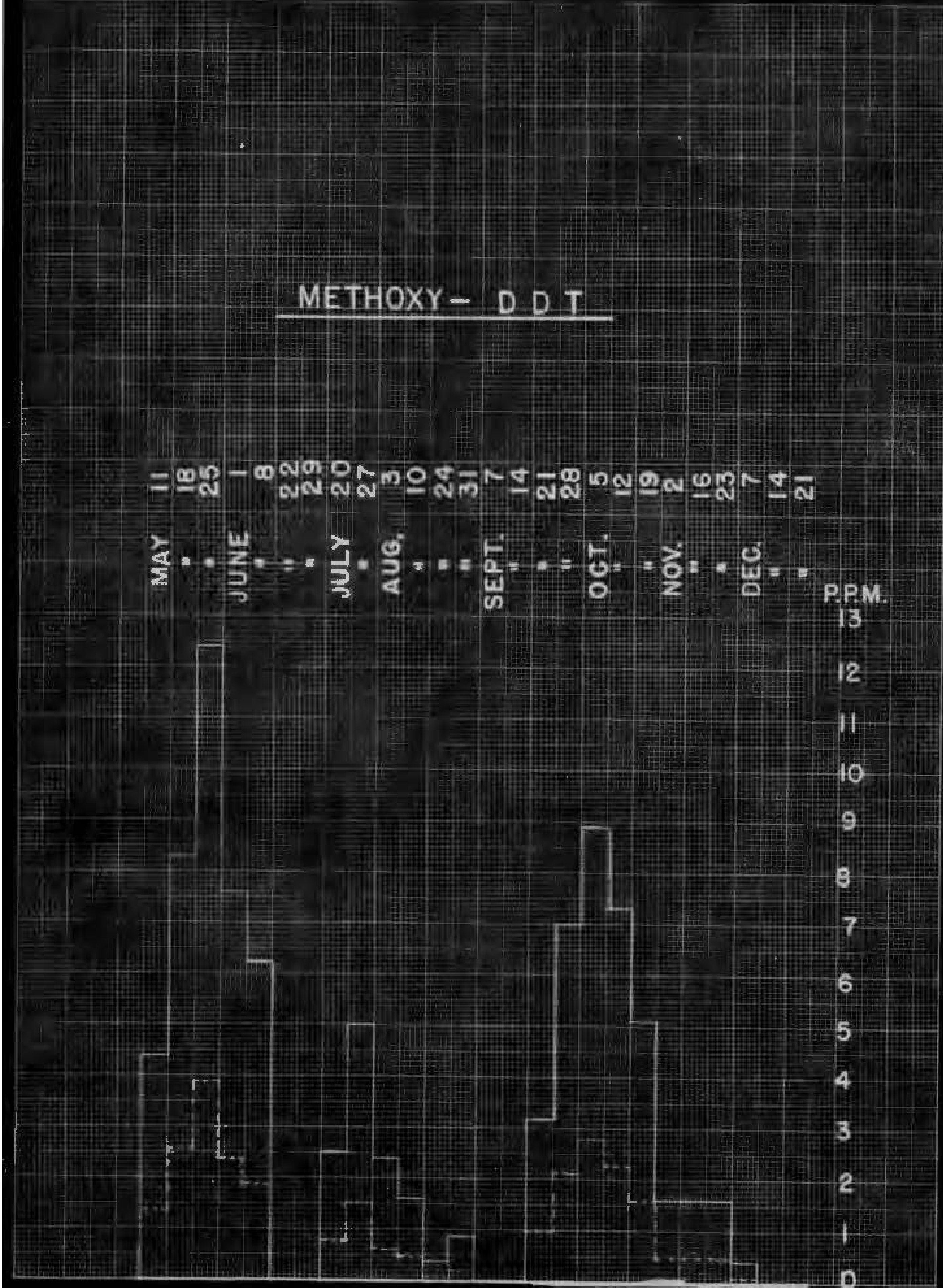
5
4
3
2
1
0


METHOXY- DDT
MAY 11 18 25
JUNE 1 8 22 29
JULY 20 27
AUG. 3 10 24 31
SEPT. 7 14 21 28
OCT. 5 12 19
NOV. 2 16 23
DEC. 7 14 21
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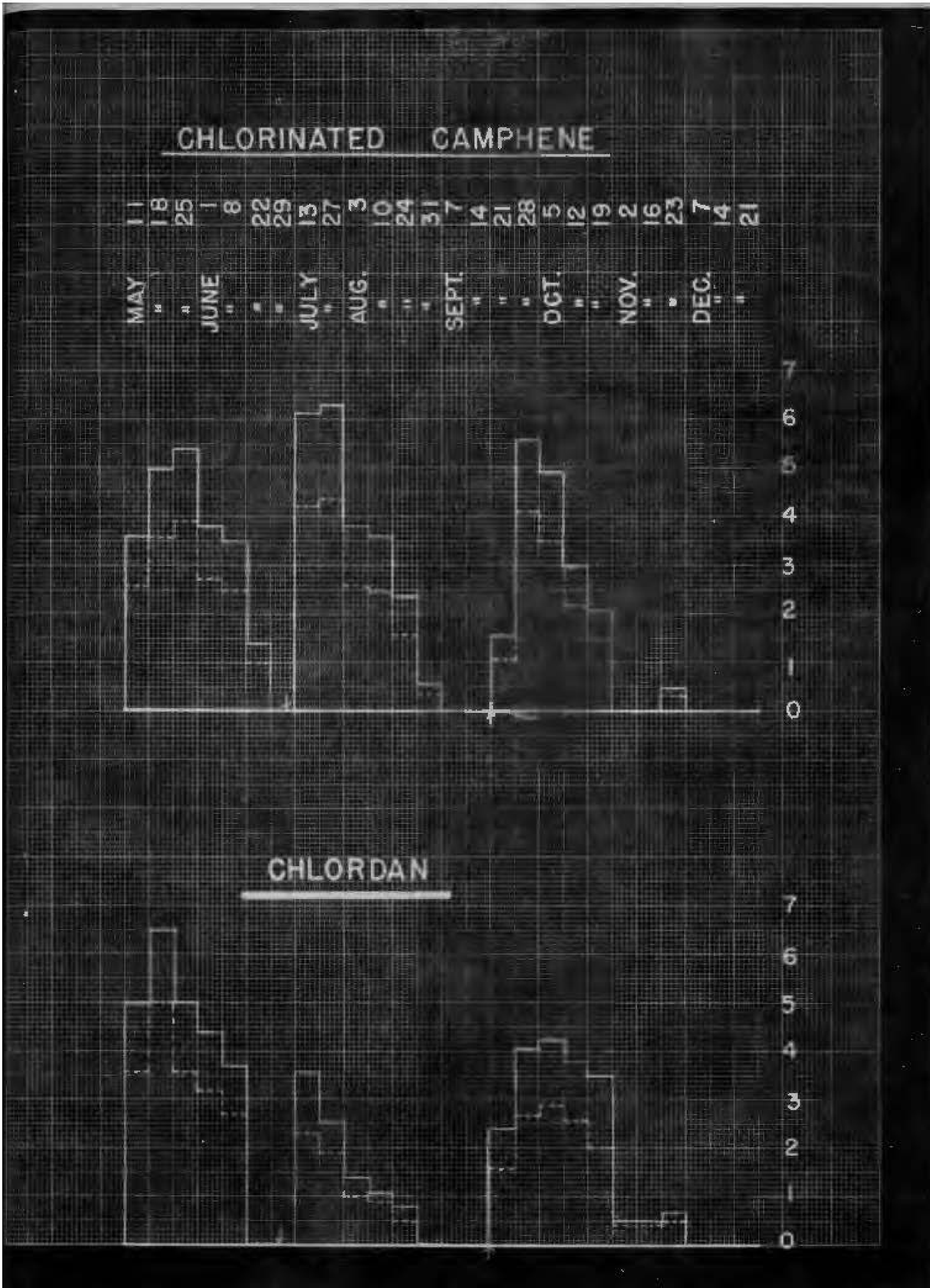

CHLORINATED CAMPHENE
MAY 11
" 18
" 25
JUNE 1
" 8
" 22
" 29
JULY 13
" 27
AUG. 3
" 10
" 24
" 31
SEPT. 7
" 14
" 21
" 28
OCT. 5
" 12
" 19
NOV. 2
" 16
" 23
DEC. 7
" 14
" 21
7
6
5
4
3
2
1
0
CHLORDAN
7
6
5
4
3
2
1
0

The concentration of the other insecticides, chlordan, hexachlorocyclohexane and Toxaphene, in the fat and tissues of feeder steers sprayed for external parasite control is shown in Table IV. Both total organic chloride and insecticide content are expressed, the conversion being made on the basis of the chlorine content of the insecticide. It is recognized that this conversion is somewhat empirical, since there is no evidence as to the exact chemical nature of the toxicant as it is stored in the animal body and the percentage of chlorine may, therefore, differ from the parent compound which was applied. The expression of these data in terms of relative amounts of insecticide does, however, permit a more direct comparison of the degree of storage of different forms of widely differing chlorine content. For these calculations, the following percentage chlorine composition was assumed: hexachlorocyclohexane, 73.16; chlordan, 69.23; chlorinated camphene, 68.56.

DISCUSSION

As shown in the graphs, the concentration of the insecticide excreted in the milk reaches a maximum concentration point at a period from eleven to eighteen days after date of spraying and then rapidly diminishes. With the exception of DDT, the concentration of the other insecticides returned to zero shortly after the maximum point was reached. These data suggest that any of the five insecticides might be safely used providing that milk from the sprayed animals was not used for human consumption until several days after the period of maximum concentration in the milk had been reached. The concentration of DDT in the milk did not return to a low level, however, until it was found necessary to spray again in order to maintain effective control. The use of DDT,

Table IV

Concentration of Insecticide Found in Fat, Liver, Kidney, and Lean Meat of Feeder Steers and Calves Sprayed for External Parasite Control

Type Animal	No. of Animals in Test	Date of Spraying	Toxicant Used	Date of Slaughter	Insecticide Content: Fat Ins.	Fat Cl.	Liver Ins.	Liver Cl.	Kidney Ins.	Kidney Cl.	Lean Meat Ins.	Lean Meat Cl.
					parts per million							
Feeder Steers	2	June 10 July 21 August 17 Sept. 25	DDT	October 11	17.0		5.2		4.8		4.6	
Feeder Steers	1	July 21	DDT	October 11	14.6		5.0		4.6		4.5	
Feeder Steers	1	June 10 July 21 August 17 Sept. 25	Methoxy DDT	October 11	33.2	13.5	18.4	5.7	11.1	3.4	8.4	2.6
Feeder Steers	1	June 10 August 17	Methoxy DDT	October 11	24.5	10.8						
Feeder Steers	1	July 21	Methoxy DDT	October 11	18.6	5.8	10.8	3.4	6.1	1.9	4.1	1.3
Feeder Steers	1	June 10 July 21 August 17 Sept. 25	Chlordan	October 11	29.9	14.0	18.8	6.1	15.6	3.8	4.2	3.0
Feeder Steers	1	June 10	Chlordan	October 11	24.9	10.5						
Feeder Steers	1	July 21	Chlordan	October 11	10.2	7.3	3.0	2.1	1.8	1.3	1.8	1.3

Table IV (Cont'd)

Concentration of Insecticide Found in Fat, Liver, Kidney, and Lean Meat
of Feeder Steers and Calves Sprayed for External Parasite Control

Type Animal	No. of Animals in Test	Date of Spraying	Toxicant Used	Date of Slaughter	Insecticide Content (parts per million): Fat Ins.	Fat Cl.	Liver Ins.	Liver Cl.	Kidney Ins.	Kidney Cl.	Lean Meat Ins.	Lean Meat Cl.
Feeder Steers	2	June 10 August 17	Hexachloro-cyclohexane	October 11	13.2	10.2						
Feeder Steers	1	July 21	"	October 11	9.8	7.5	2.8	2.1	2.4	1.9	2.0	1.5
Feeder Steers	1	June 10 July 21 August 17 Sept. 25	Toxaphene	October 11	18.3	13.0	7.0	5.0	5.0	3.6	3.8	2.7
Feeder Steers	1	June 10 August 17	"	October 11	11.9	8.5						
Feeder Steers	1	July 21	"	October 11	9.3	6.7	3.7	2.7	2.8	2.0	2.1	1.5
Dairy Suckling Calf	1	July 10 July 23 July 31 August 18 Sept. 17	DDT	October 11	17.0		9.0		10.0		8.2	
Range Suckling Calf	1	June 11 June 29 August 12	DDT	October 12	19.0		8.8		9.0		8.4	

therefore, presents a definite hazard in that milk used for human consumption will contain appreciable amounts of this insecticide. Of the five chlorinated hydrocarbons used, methoxy DDT showed the least amount excreted in the milk and was the first to decline after reaching the maximum subsequent to spraying.

Howell and Heller (18) showed that milk contained a maximum of two parts per million of DDT shortly after spraying, but no attempt was made to check the toxic effect of the DDT contained in the milk. Telford and Guthrie (33) in 1945 showed that DDT when sprayed on goats was transmitted to their milk and that this milk when fed to white rats was very toxic and in some instances caused death. With the exception of the Howell and Telford experiments, there has been little work done on the effect of external application of these insecticides on the content in milk and tissues. Considerable work has been done, however, with regard to the oral toxicity of DDT and hexachlorocyclohexane. Chlordan, chlorinated camphene and methoxy DDT have received less investigation. Smith and Lillie (30) show that methoxy DDT was one-twentieth as toxic as DDT itself to warm-blooded animals.

SUMMARY

The results obtained show that five of the most commonly used hydrocarbon insecticides (DDT, methoxy DDT, chlordan, chlorinated camphene and hexachlorocyclohexane) were adsorbed and retained in beef cattle receiving topical applications for external parasite control. These compounds were likewise secreted in the milk of dairy cows similarly sprayed.

Considerable variation was found in the degree of storage of the same insecticide in various tissues (e.g. 33.2 p.p.m. for methoxy DDT in fat to 9.3 p.p.m. for chlorinated camphene). In general, the highest concentration of all insecticides was contained in the fat, followed by liver, kidney and muscle tissue in that order. The various insecticides were stored in descending order of degree of storage generally as follows: methoxy DDT, chlordan, DDT, hexachlorocyclohexane and chlorinated camphene.

Of the five insecticides used, it was found that methoxy DDT was secreted in the milk at the lowest concentration and the DDT in the highest. Moreover, the methoxy DDT level fell more rapidly than any other insecticide and reached an undetectable concentration earlier following application of spray. Both of the compounds were essentially equally as effective for fly control. The highest concentration observed in the milk at any time during the study for each of the insecticides was as follows: DDT - 19.2 p.p.m., chlorinated camphene - 6.16 p.p.m., hexachlorocyclohexane - 13.2 p.p.m., methoxy DDT - 12.4 p.p.m., chlordan - 7.0 p.p.m.

Because of the toxic nature of these compounds and the potential hazard thus created by their extensive use, it appears advisable to exercise considerable caution in their employment. From the toxicological viewpoint, on the basis of these studies, chlorinated camphene appears to be the insecticide of choice for external parasite control when the animals are to be slaughtered and used for food. Methoxy DDT appears to be superior with respect to excretion in the milk.

BIBLIOGRAPHY

1. Annand, P. N., Memorandum, Bureau of Entomology and Plant Quarantine, U.S.D.A., Feb. 26, 1947.

2. Busvine, J. R., " Insecticidal Tests with Analogues of DDT". Journal of Society Chemical Industries, London, Vol. 65, No. 11, pp. 356-358, 1949.

3. Calvery, H. O.(Food and Drug Administration) "DDT is Poisonous", Food Packer, pp. 61-62, April, 1945.

4. Carter, R. H., "Estimation of DDT in Milk by Determination of Organic Chlorine". Analytical Chemistry, Vol. 19, No. 1, pp. 54, January, 1947.

5. Cristol, Stanley J., " A Kinetic Study of the Dehydrochlorination of Substituted 2,2-diphenyl-chloroethanes Related to DDT". Journal of American Chemical Society, Vol. 67, pp. 1494, 1945.

6. Cristol, Stanley J. and Haller, H. L., "The Chemistry of DDT - A Review". Chemical and Engineering News, Vol. 23, pp. 2070-2075, Nov. 25, 1945.

7. Daasch, L. W., "Infrared Spectroscopic Analysis of Five Isomers of 1,2,3,4,5,6-hexachlorocyclohexane". Analytical Chemistry, Vol. 19, No. 10, pp. 779-785, Oct., 1947.

8. "DDT and Other Insecticides and Repellents". Miscellaneous Publication No. 606, United States Department of Agriculture, August, 1946.

9. Frear, Donald E. H., Chemistry of Insecticides, Fungicides and Herbicides. New York: D. Van Nostrand Company, Inc., September, 1948.

10. Grasselli Chemicals Department, Du Pont Pest Control Guide, E. I. Du Pont De Nemours and Company (Inc.), Wilmington, Delaware.

11. Grasselli Chemicals Department, Summary of Test Results, E. I. Du Pont De Nemours and Company, Inc., pp. 1-3, March 30, 1948.

12. Gunther, F. A., " A Rapid Method for the Quantitative Estimation of DDT and of DDT Spray or Dust Deposits." California University Citrus Experiment Station, 4 pp., addenda, 1 p., 1944.

13. Hackman, R. H., "The Persistence of DDT on Cattle". Journal of Council for Science and Industry, Res. 20 (1): 56-65, 1947.

14. Hall, S. A., Schecter, M. S., and Fleck, E. E., "Chemical Determination of DDT". U. S. Bureau of Entomology and Plant Quarantine, ET211, 6 pp., 1944.

15. Haller, H. L., "Wartime Development of Insecticides." Industrial and Engineering Chemistry, Vol. 39, No. 4, pp. 467-473, 1947.

16. Hercules Powder Company, Naval Stores Department, Toxaphene Manual, Section II, Wilmington, Delaware, October, 1947.

17. Howell, D. E., Personal Communique, November 1, 1948.

18. Howell, D. E., Cave, H. W., Heller, V. G., and Gross, W. G. "The Amount of DDT Found in the Milk of Cows Following Spraying". Journal of Dairy Science, Vol. 30 (9), pp. 717-721, 1947.

19. Ingle, L. "Toxicity of Chlordan to White Rats". Journal of Economic Entomology, Vol. 40, pp. 264-268, 1947.

20. Kauer, K. C., DuVall, R. B., and Alquist, F. N., "The Epsilon of 1,2,3,4,5,6-Hexachlorocyclohexane". Paper presented at 110th Meeting of the American Chemical Society, September 12, 1946.

21. Kerns, C. W., Ingle, L. and Metcalf, R. L. "A New Chlorinated Hydrocarbon Insecticide". Journal of Economic Entomology, Vol. 38, pp. 661-668, 1945.

22. Kunze, Frieda, Nelson, A. A., Fitzhugh, O. G., and Laugh, Edwin P., "Storage of DDT in the Fat of the Rat". Federation Proceedings, Vol. 8, Part I, p. 311, March, 1949.

23. Lehman, A. J., "Toxicity of Insecticides to Plants and Animals Other Than Man". Paper presented at AAEE Meeting, Chicago, Illinois, December 29, 1947.

24. Palmer, W. G. (Continental Can Company) "DDT-Manufacturers of". February 16, 1945.

25. Peterson, H. E. and Palmer, W. G. (Continental Can Company) "DDT (dichloro-diphenyl-trichloroethane)". Bulletin No. 7, 1945.

26. Roark, R. C., and McIndoo, N. E. (U. S. Department of Agriculture) "A Digest of the Literature on DDT Through April 30, 1944." E-631, December, 1944.

27. Schecter, M. S. and Haller, H. L., "Colorimetric Tests for DDT and Related Compounds". American Chemical Society Journal, Vol. 66, pp. 2129-2130, 1944.

28. Schecter, Milton S., Pogorelskin, Milton A., and Haller, H. L. "Colorimetric Determination of DDT in Milk and Fatty Materials". Analytical Chemistry, Vol. 19, No. 1, pp. 51-53, January, 1947.

29. Slade, R. E., "The Gamma-Isomer of Hexachlorocyclohexane (Gammexane)". Chemistry and Industry, Vol. 64, pp. 314-319, 1945.

30. Smith, M. I., Bauer, H., Stochlman, E. F., and Lillie, R. D. "The Pharmacologic Action of Certain Analogues and Derivatives of DDT". Journal of Pharmacology and Experimental Therapeutics, Vol. 88, pp. 358-360, 1946.

31. Stearns, L. A., Parker, W. L., MacCreary, D., and Conell, W. A. "A Chlorinated Bicyclic Terpene Used to Control Fruit and Vegetable Insects". Journal of Economic Entomology, Vol. 40, pp. 79-83, 1947.

32. Still, H. A., Jr., and Castillo, J. C. "The Determination of 2,2-bis (p-chloro-phenyl)-1,1,1-Trichloroethane (DDT) in Organs and Body Fluids After Oral Administration". Journal Biological Chemistry, Vol. 159, pp. 545-548.

33. Telford, Horace S. and Guthrie, James E. "Transmission of the Toxicity of DDT Through the Milk of White Rats and Goats". Science, December 21, 1945, Vol. 102, No. 1660, pp. 647.

34. Umhoefer, R. R. "Determination of Halogens in Organic Compounds". Industrial and Engineering Chemistry, Analytical Edition 15:383.

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