GAS CHROMATOGRAPHIC DETERMINATION OF INSECTICIDE RESIDUES IN THE MILK OF TREATED DAIRY COWS

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PESTICIDE RESIDUES IN MILK

BOSTANIAN

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AN ABSTRACT OF THE THESIS OF

Noubar John Bostanian for M.S. in Biology (Entomology).

Title: Gas chromatographic determination of insecticide residues in the milk of treated dairy cows.

Extraction of the insecticides using the technique developed by Langlois *et al*. (1964) followed by a quantitative determination with gas-liquid chromatography indicated that treatment of cows with insecticides for ectoparasite control resulted in insecticidal contamination of the milk.

After three weeks from the date of application 0.016 and 0.006 ppm of BHC were found in the milk of cows sprayed with 0.06 percent and 0.03 percent BHC respectively. For the same period of time an initial application of 0.5 percent and 0.25 percent chlordane resulted in residues of 0.320 and 0.232 ppm respectively. An application of 0.75 percent and 0.38 percent ronnel resulted in undectable residue levels 72 hours after the date of application. Statistical analysis of fly counts indicated that all the three insecticides had comparable controlling abilities for 19 days.

As a result of this investigation the use of pesticides on milking cows should be stopped unless there are heavy infestations of ticks and blood sucking flies. The former are carriers of numerous diseases to cows whereas the latter might cause serious drops in milk production. In case of such pressures ronnel should be used in preference to BHC and chlordane.

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I. INTRODUCTION

It has been known for many years that heavy fly infestations on dairy cows cause reductions in milk production. Consequently it has become customary for cattlemen to control ectoparasites on cattle by various means.

The discovery of DDT as a potent contact insecticide during World War II paved the way for the syntheses of numerous chlorinated hydrocarbons, carbamates and organophosphate pesticides. With the appearance and widespread use of these compounds, scientists predicted that pesticides might appear in raw agricultural commodities. The development of accurate analytical techniques verified this prediction and underlined the need for further research to evaluate the hazards involved in the use of such materials.

The present work was undertaken at the Agricultural Research and Education Center (AREC), in the Beqa'a Plain, Lebanon in the summer of 1966 to determine the persistence of the most commonly used insecticides BHC and chlordane in the milk of treated dairy cows and compare them with that of ronnel, an insecticide still not commonly used in the Middle East. Another purpose of the work was to study the effectiveness of these insecticides for the control of ectoparasites, mainly ticks and face-flies.

II. REVIEW OF LITERATURE

Prior to the advent of gas liquid chromatography in residue work by Coulson (1960), residue analyses were accomplished either spectrophotometrically or chemically using complicated extensive extraction and cleanup procedures (Zweig 1964). Bioassay techniques which were also employed, though accurate, were often less quantitative and exhibited greater variability (Kawar 1963).

Coulson's pioneer instrument did not gain immediate popularity because of the complicated nature of the coulometric detector cell and its marginal sensitivity.

The answer for a rapid accurate analytical tool came about by the combination of the electron capture ionization detector, with gas liquid chromatography by Lovelock and Lipsky (1960).

As stated by Lovelock (1961), the electron capture detector was specifically designed to exploit recombination effects for the measurement of compounds having an affinity for free electrons. Consequently, the range of detection for chlorinated pesticides was extended from the microgram to the nanogram level. Since then, numerous publications have appeared in the literature based upon studies in which this technique was used.

Kiigemayi *et al.* (1958) studying the endrin content of milk in cows receiving endrin daily at 0.1 to 2.00 ppm total dietary concentration for twelve weeks, determined spectrophotometrically the presence of endrin after twelve weeks at 0.003 ppm.

Gannon et al. (1959 a) fed cows dieldrin for twelve weeks at 0.1, 0.25, 0.75 and 2.25 ppm and determined spectrophotometrically the presence of dieldrin residues at 0.02, 0.06, 0.11, 0.28 ppm respectively after a six weeks period of dieldrin free diet.

Helrick et al. (1958) and Cheng et al. (1958) found minute but detectable amounts of methoxychlor in the milk of treated cows, and demonstrated that the concentration diminished rapidly with successive samplings after spraying or dusting.

Gannon et al. (1959 b) attempting to learn the different rates of accumulation for various insecticides fed cows with different dosages of aldrin, DDT, heptachlor, and methoxychlor for twelve weeks. Analysis of milk samples revealed that the rates of accumulation were aldrin, DDT, heptachlor and methoxychlor. DDT and methoxychlor were excreted as such, whereas aldrin and heptachlor were excreted as their oxygen analogs.

Ware and Gilmore (1959) detected lindane residues seventeen days from the date of application in the milk of cows treated with 1.5 lb of 25 percent lindane wettable powder in 100 gallons of water.

Meyer et al. (1960) confirmed the findings of Gannon et al. (1959) and stated that heptachlor which is used extensively for the control of forage insects, was metabolized to its epoxide in cows and stored in the fatty tissue. Eventually it finds its way into the milk.

Cluet et al. (1960) using spectrophotometric and total organic chlorine determination techniques were unable to detect methoxychlor in the milk following dusting of dairy cows at the recommended level of 10 gm of 50 percent powder per animal. However spraying with aqueous suspensions or emulsions revealed a maximum of 0.10 ppm one day after treatment which eventually levelled off by the end of a week.

Eheart _et al_. (1962) using spectrophotometric methods for the determination of carbaryl (Sevin®) residues concluded that if a 0.5 percent spray or a 50 percent dust were made immediately after the morning or evening milking, no residues of the compound would be found in later milkings.

Zweig _et al_. (1963 a) fed cows low levels of toxaphene in order to find a level that would be undetectable by total chloride method. Extrapolating his residue data he found that 1.0 ppm of toxaphene in the daily ration of cows resulted in less than 0.03 ppm residue in the milk.

Zweig _et al_. (1963 b) were the first to study colorimetrically the excretion pattern of Kelthane®. They reported that 1.0 ppm Kelthane® added to the cows' daily feed produced insignificant residues in the milk.

Westlak _et al_. (1963) had two groups of dairy cows graze on pasture treated with granular chlordane at 0.25 and 0.50 pound per acre respectively. Analyses of milk samples using gas chromatography and spectrophotometry revealed the presence of heptachlor epoxide and chlordane at a little less than 0.1 ppm even after eight weeks past the date of chlordane application.

Langlois _et al_. (1964) described a rapid one step sample clean-up procedure for animal products prior to chlorinated insecticide residue analysis with electron capture gas chromatography. Very high recoveries of the insecticides were obtained with this method.

Stemp et al. (1964) elaborated on Langlois' clean-up method and included information on numerous factors that further improved the accuracy and precision of electron capture gas chromatography.

Williams et al. (1964) using electron capture gas chromatography in a study involving the feeding of cows with low levels of five chlorinated hydrocarbon insecticides, found that heptachlor epoxide and dieldrin transferred to the milk in greater concentrations than did endrin and lindane with DDT appearing in the lowest amounts.

Giuffrida and Ives (1964) reported the recent development of the sodium thermionic detector; a flame detector highly sensitive to phosphorous esters. Their study revealed recovery rates of 85 to 100 percent for malathion, parathion, diazinon and carbophenothion (Trithion®).

McKinley et al. (1964) emphasized the need for a thorough cleanup of samples before the use of gas liquid chromatography or spectrophotometry, since samples containing extraneous fats or wax adhered to the detector and caused a drop in sensitivity. Moreover the background level was altered resulting in responses that complicated the interpretation of results. With spectrophotometric methods the presence of the extraneous materials resulted in high blanks, because these substances interfered either by absorbing light of the wavelength used for the quantitative measurement, or by reacting with the reagents to produce a product which absorbed at that wavelength.

Bruce et al. (1965) reported that heptachlor and chlordane were excreted in the milk of dairy cows as their epoxides up to 45 days after an initial intake of 0.2 ppm was discontinued. They also underlined that heptachlor epoxide has the highest propensity for storage of any of the chlorinated hydrocarbon insecticides.

Adkins et al. (1965) reported the absence of residues in milk after dimethoate and malathion were applied with back rubbers to dairy cattle. The results of the investigation indicated that all the milk samples contained less than 0.005 ppm residue of dimethoate and less than 0.02 ppm residue of malathion. The first insecticide was analyzed by gas chromatography whereas the second colorimetrically.

Treece and Ware (1965) using colorimetric methods reported that green alfalfa containing 1.33 ppm lindane on a dry weight basis resulted in the presence of 0.53 and 0.46 ppm lindane in the milk during the first and second feeding periods.

Claborn et al. (1965) using electron capture gas chromatography for determining ronnel residues in milk found less residue in the milk of cows treated with a multiple mist sprayer than with a conventional sprayer. Fourteen days after treatment he found 0.005 ppm of the insecticide with a single spray as compared with 0.003 ppm with a multiple mist spray.

Loeffler et al. (1966) reported that using fluorometric methods no detectable residues of Guthion® were found in the milk samples regardless of feeding rates. However he found some residues of the oxygen analog of Guthion® and other benzazimide containing moieties, which appeared 24 hours after treatment and disappeared within three days after the discontinuation of the treatment.

Burke and Malone (1966 a) reported the effect of calcination temperature and time on the retentive properties of florisil used for pesticide residue analyses. They found that a three-hour calcination at 1250^{o}F gave the best activation of florisil prior to use in a single step-cleanup of animal products.

Moats and Kotula (1966) reported that high elution rates of 250 ml per minute for partially inactivated florisil in a single step-cleanup did not affect results. On the contrary the recovery of endrin and other insecticides were improved. This method has reduced the time for cleanup from 45 to 2 minutes.

Yauger et al. (1966) reported the development of a novel electron capture detector using Ni^{63} as its radioactive source, whose design permitted continual operation for six months at 300^{o}C with no variations in its response. Its predecessor, has tritium occluded in titanum, coated on a stainless steel foil, and cannot be used above 200^{o}C. Furthermore the emittance of radioactive material reduces the sensitivity and necessitates frequent recalibration. Burke et al. (1966 b) reported that the least loss of pesticides during the process of concentration, could be achieved by attaching a micro Synder column to a Kuderna-Danish collection tube and evaporating on a steam bath.

The past few years have witnessed the gradual shift of residue chemists from spectrophotometric methods to electron-capture gas chromatography. The present trend is to develop ever finer techniques in electron capture gas chromatography in order to insure precision and accuracy.

However Burke recently (1965) sounded a very important warning. He stated that though gas chromatography with specific or selective detectors, provides qualitative and quantitative analyses of numerous pesticides present simultaneously in a single sample it does not provide an unequivocal identification and totally false responses are possible. He underlined the need of another column which would elute the pesticide chemicals in an entirely different order so that a second gas liquid chromatography would confirm the findings of the first identification.

Fortunately Burke and Holswade (1966 c) have just reported the development of such a column as emphasized by Burke (1965). The column packing consists of equal portions of the previously coated 80/100 mesh Gas Chrom Q with 15 percent QF-1 and another with 10 percent DC-200. The two columns are maintained in a dual column single oven instrumentation operating at 200^{o}C and the residue analyses are carried out in duplicate using two different columns.

III. MATERIALS AND METHODS

Procedure

The research was divided into two phases. A pilot experiment using the insecticide BHC and the main project using BHC, chlordane and ronnel. The pilot experiment was undertaken to determine the frequency of milk sampling, required for the second phase of the project.

Four Friesian Holstein cows were used in the pilot experiment. Using a Hardy power sprayer, two cows were treated with 0.12 percent BHC and the other two with 0.06 percent.

The main experiment involved 21 Friesian Holstein cows, which were divided into seven similar groups of three cows each. The animals were selected on the basis that each group produced the same quantity of milk daily, had the same physical appearance, and were of comparable age.

Three insecticides were applied at two levels each, a high concentration as recommended by the manufacturer, and a lower concentration at half the recommended level (Table I).

The two chlorinated hydrocarbons were applied as a water based emulsion, whereas the organic phosphate as a wettable powder. A "Solo" motor powered knapsack sprayer was used for the application of the insecticides (Figure 1). While spraying, care was taken to avoid direct application of the insecticides on the udders, and faces of the cows.

Table I. Insecticides and Their Concentrations Used in Spraying Cows for Ectoparasite Control

Common Designation	Chemical Name	Commercial Brand	Manufacturer	Concentration High	Concentration Low	Experiment
BHC	1,2,3,4,5,6-hexachlorocyclohexane	Gammatox 20% γ-isomer emulsifiable concentrate	Cooper, Mc-Dougall & Robertson,Ltd. Berkhamsted, Herts. U.K.	0.06%	0.03%	Main project
				0.12%	0.06%	Pilot experiment
Chlordane	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	Engo 40% W/W technical chlordane emulsifiable concentrate	Fort Dodge Lab. Inc., Ford Dodge Iowa, U.S.A.	0.50%	0.25%	Main project
Ronnel	0,0-Dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate	Nankor 25% wettable powder	Dow Chemical Co., Midland, Michigan, U.S.A.	0.75%	0.38%	Main project

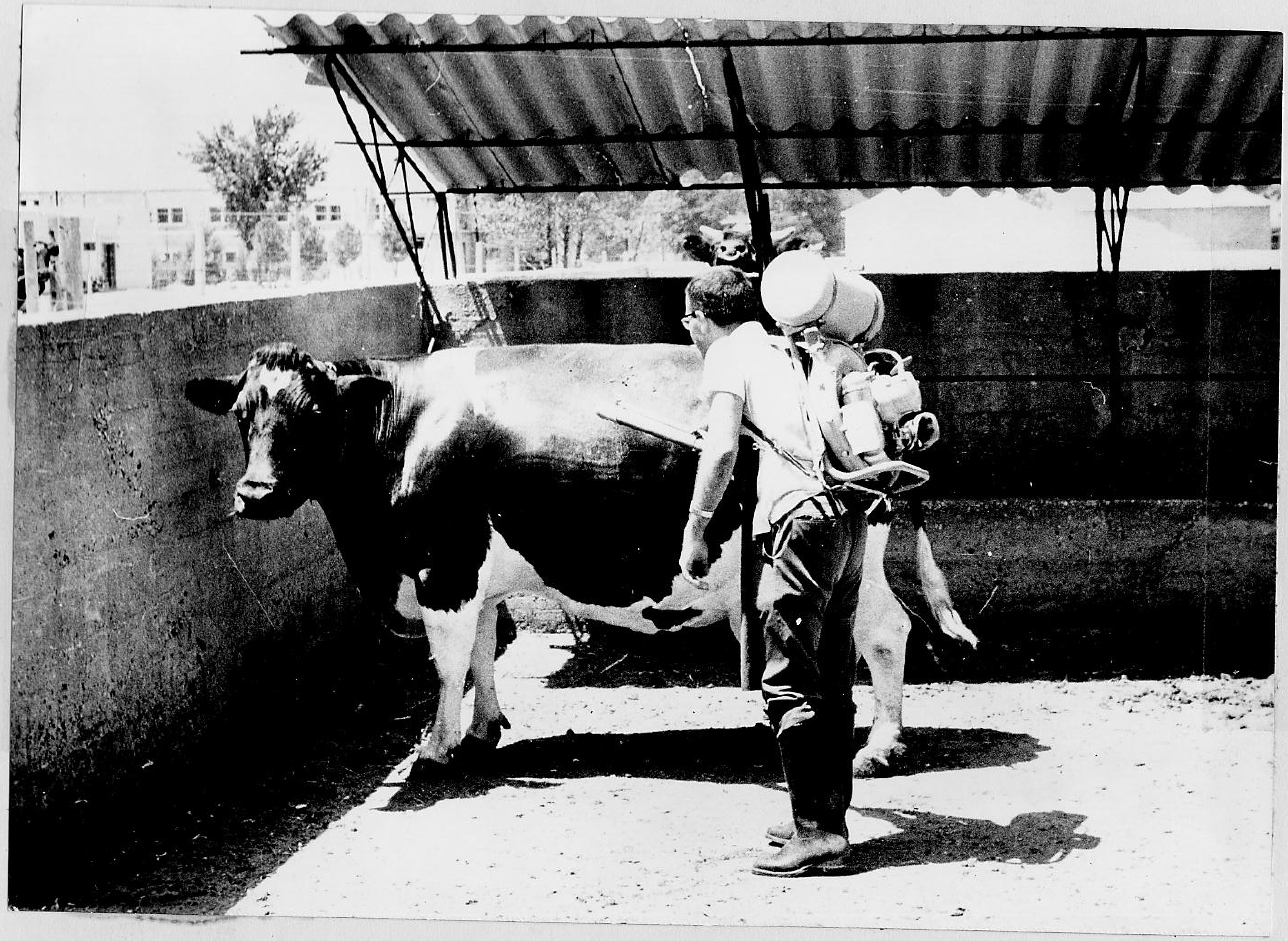

Figure 1. Spraying a cow with a "Solo" sprayer.

The cows were milked each day at 5 AM and a 250 ml milk sample was taken immediately for the investigation.

In the pilot experiment samples were taken every other day following the treatment. Whereas in the main experiment samples were taken on the first and second day following the treatment and then every third day; for three consecutive weeks. The samples were kept frozen until the time of analyses.

The experimental technique for residue determination consisted of two phases. An extraction and purification phase, followed by a quantitative determination of the insecticide by electron capture gas chromatography.

The method described by Langlois _et al._ (1964) was adopted. The insecticides were extracted directly from the whole milk rather than from the extracted butterfat. This method yields cleaner extracts and offers a truer picture for the semi-polar organic phosphates which are appreciably soluble in the non-fatty component of the milk and might be lost when extracted from the butterfat only.

Reagents

Technical grade methylene chloride and reagent grade petroleum ether, bp 40^{0} to $60^{0}C$, were redistilled before use. The standards and unknown samples were prepared in n-hexane, bp 68^{0} to $69^{0}C$, and stored at $4^{0}C$. Florisil, a synthetic magnesium silicate, 60/100 mesh activated by the supplier at $650^{0}C$, was obtained from the Floridin Company, Tallahassee, Florida.

The florisil was reactivated by heating at $140^{0}C$ for 12 to 14 hours. Five percent water was next added and the mixture shaken thoroughly and kept in an air tight container for 48 hours before use. This time was necessary for the water-florisil mixture to gain equilibrium. The eluant was a mixture of 20 percent methylene chloride in petroleum ether.

Equipment

The chromatographic columns were 20 X 550 mm Pyrex tubes plugged at one end with glass wool and a stopcock size number two. The other end had a glass tank with a capacity of 300 ml (Figure 2).

The analytical instrument was a Varian Aerograph Hi-Fi model 600 C gas chromatograph with an electron capture detector cell containing a 250 mc tritium occluded on a titanum stainless steel ribbon (Figure 3). The instrument was operated with a 90 volt potential across the detector. The analytical column was a coiled Pyrex glass 1/8 inch o.d. and 5 feet long packed with 5.0 percent Dow 11 Silicone on 60/80 mesh hexamethyldisilazane (HMDS) treated Chromosorb W. Both compounds were obtained from Varian Aerograph, the supplier of the gas chromatograph. The recorder used was a 1 mv Leeds and Northrup model H with a disc integrator unit.

Cleanup

Ten grams were taken from each milk sample and ground with 25 g of florisil in a porcelain mortar to form a free flowing powder.

The column was then packed with 25 g of florisil forming the bottom layer. This was prewashed with 50 ml of an equal mixture of

Figure 2. A battery of chromatographic tubes used for the extraction and cleanup of the insecticides from the milk samples.

Figure 3. Aerograph model 600 C gas chromatograph equipped with an electron capture detector.

methylene chloride and petroleum ether to remove any impurities from the florisil. The washings were discarded. Next the sample-florisil mixture was poured into the chromatographic column to form the top layer. Two hundred milliliters of the eluant mixture was used to extract the insecticide. The eluant was collected in an Erlenmeyer flask evaporated to approximately 0.5 ml in a water bath set at 65^{0}C. The residues were then dissolved in chromatographic grade n-hexane and made up to a total volume of 10 ml each.

Analysis

The column in the gas chromatograph was operated at 200^{0}C with prepurified dry nitrogen gas at a flow rate of 50 ml per minute.

Every day the column was activated with 100 nanograms of pure insecticide. Standard insecticide solutions were analyzed for the establishment of a standard curve followed by the samples. Depending upon the insecticide concentration, either 4 or 6 of unknown sample were analyzed.

In determining the concentration of the insecticides from the graphs, BHC was measured by comparing the size of the γ isomer (lindane) peak to the size of a peak from a known quantity of lindane. This method was preferred to the measurement of peak area, as lindane has a short retention time with a very sharp peak and a narrow base making the triangulation method rather inaccurate. In case of the other two insecticides the areas under the curves were measured either by triangulation or by a disc integrator. Both methods were used according to convenience, since they yielded comparable results. In plotting the disappearance curves of the various insecticides the mean of the three replicates was used.

It is worth noting that all milk samples were extracted in duplicate and analyzed in duplicate in order to minimize experimental error.

Fly Counts

Fly counts were taken at intervals of three days. Counting was done by standing about a meter from the cow and calculating the number of flies per unit time. This was repeated a number of times and an average calculated. Care was taken not to scare the cow as its movements might affect the number of flies on its back. All counts were taken between 8.00 and 9.00 AM as the fly population fluctuated with the time of the day.

IV. RESULTS AND DISCUSSION

Examination of the results of this investigation clearly indicate that all the insecticides appear in the milk shortly after the treatment of the cows. However there are marked differences between the insecticides as to the levels at which they appear as well as their persistence in the milk.

The data of Tables II and III and Figures 4 and 5 indicate that BHC applied as a high volume spray formulated at 0.12 percent and 0.06 percent persists at detectable levels for the entire period of the pilot experiment. A sharp drop of BHC residue occurs during the first three days after application. Then the rate of disappearance decreases and the insecticide persists at appreciable levels for the entire experimental period of forty days.

The data of Tables IV and V and Figures 6 and 7 again exhibit the presence of BHC residue in the milk of treated cows. However closer examination of Table IV reveals that the level of BHC appearing 24 hours after treatment is three times its corresponding value in Table II. This difference is quite high especially when it is seen that the cows of Table II are treated with 0.12 percent BHC and those of Table IV are treated with only 0.06 percent. Comparing the initial levels in Table III with that of Table IV were both groups had received 0.06 percent BHC, it is clearly noted that the latter result is four times that of the former. Moreover almost the same

Table II. Gas Chromatographic Determination of BHC (Lindane ppm) in the Milk of Treated Dairy Cows. Pilot Experiment

Concentration Sprayed	Days After Treatment	Replicates I	Replicates II	Mean
0.12%	1	0.332	0.286	0.306
	2	0.200	0.126	0.163
	4	0.062	0.074	0.068
	6	0.047	0.051	0.049
	8	0.033	0.031	0.032
	10	0.025	0.022	0.024
	12	0.023	0.019	0.021
	14	0.016	0.017	0.016
	16	0.014	0.016	0.015
	18	0.013	0.015	0.014
	20	0.011	0.012	0.011
	22	0.010	0.010	0.010
	24	0.010	0.010	0.010
	40	0.007	0.008	0.007

Each figure under the replicates is the result of a single determination.

Table III. Gas Chromatographic Determination of BHC (Lindane ppm) in the Milk of Treated Dairy Cows. Pilot Experiment

Concentration Sprayed	Days After Treatment	Replicates I	Replicates II	Mean
0.06%	1	0.285	0.253	0.269
	2	0.067	0.046	0.057
	4	0.051	0.041	0.046
	6	0.042	0.030	0.036
	8	0.033	0.025	0.029
	10	0.025	0.020	0.023
	12	0.020	0.018	0.019
	14	0.017	0.016	0.016
	16	0.015	0.015	0.015
	18	0.014	0.014	0.014
	20	0.014	0.012	0.013
	22	0.013	0.011	0.012
	24	0.011	0.008	0.009
	40	0.004	0.003	0.003

Each figure under the replicates is the result of a single determination.

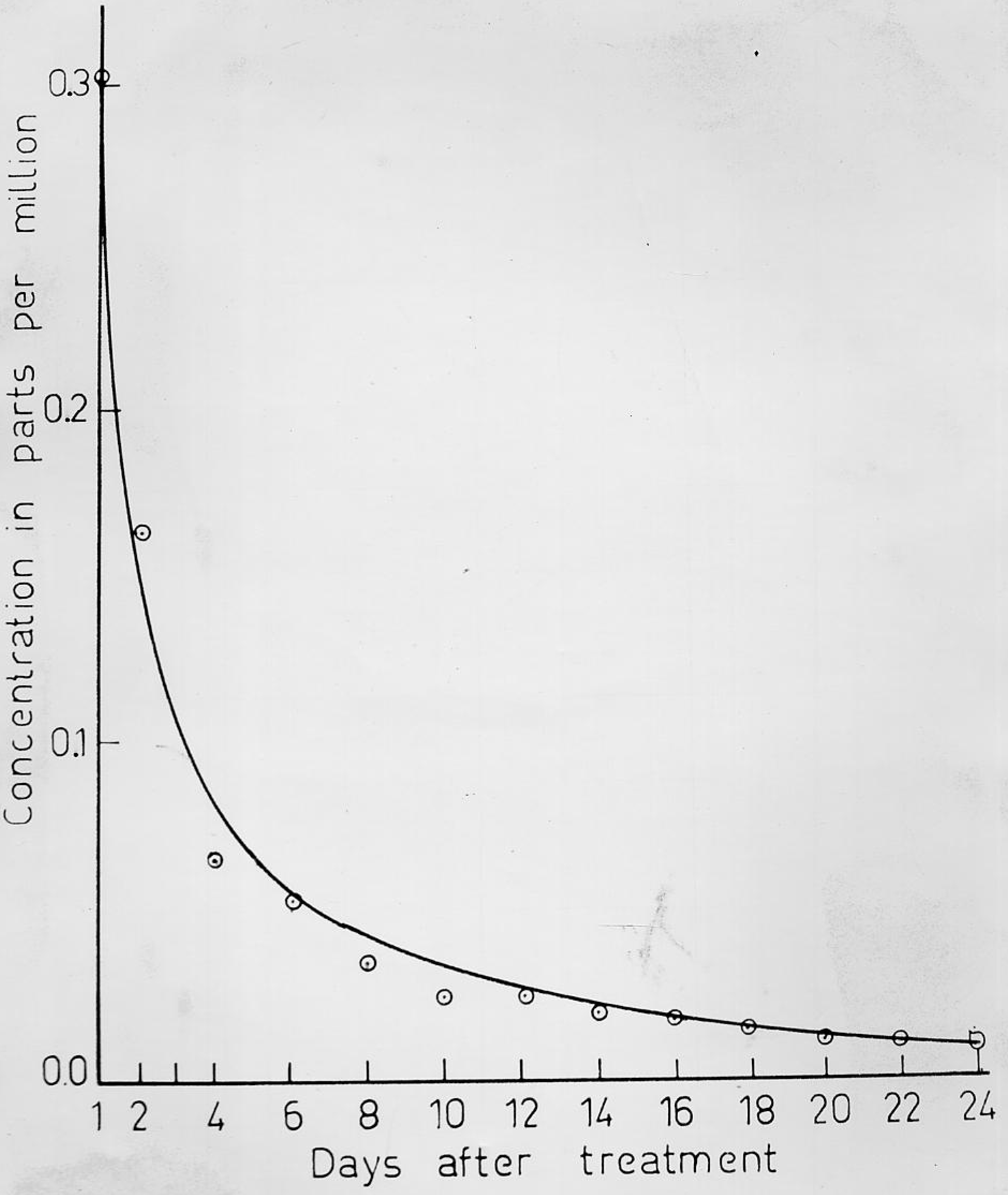

Fig. 4 Disappearance of BHC from the milk of cows treated at the 0.12% level.

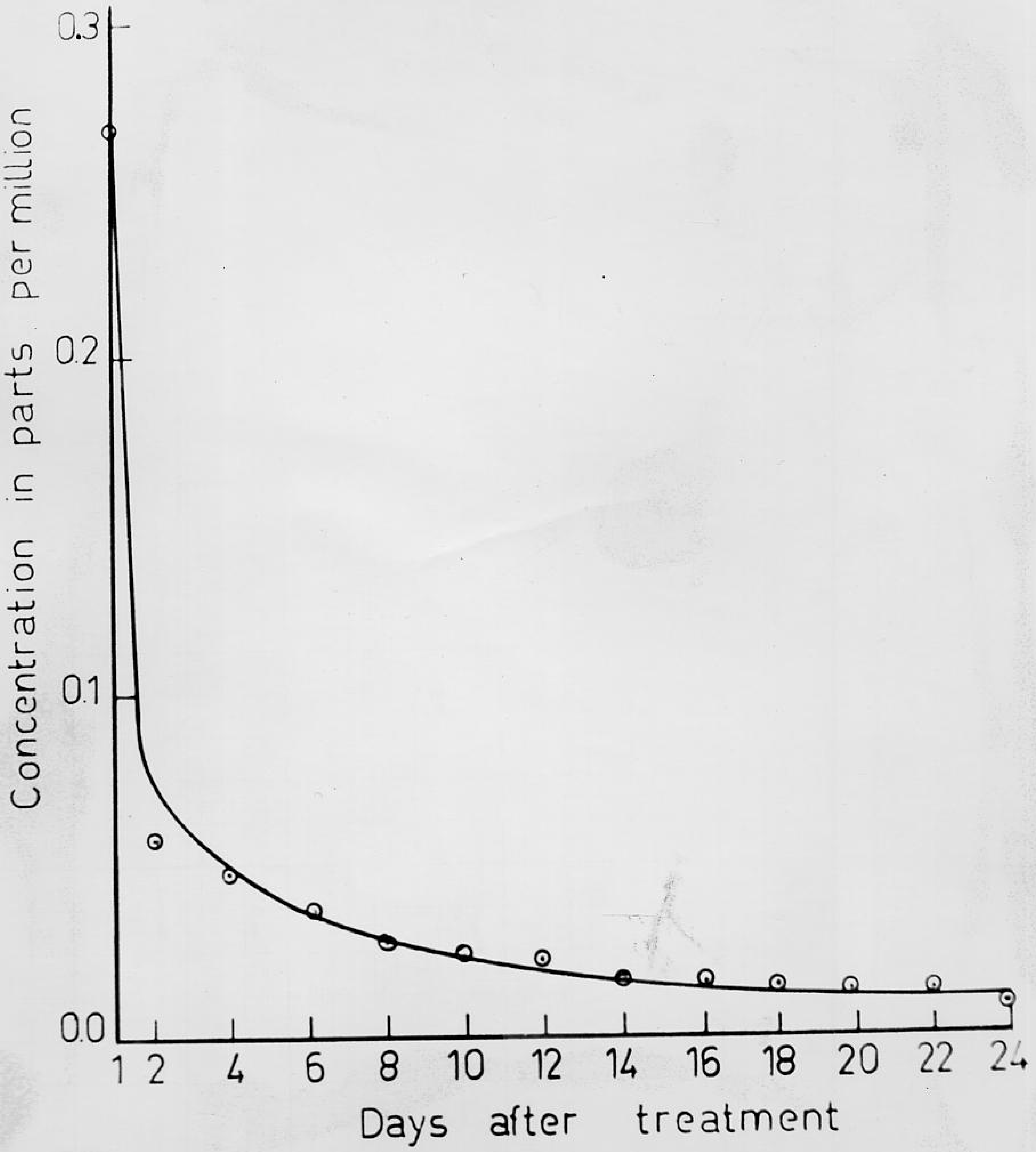

Fig. 5 Disappearance of BHC from the milk of cows treated at the 0.06 % level

Table IV. Gas Chromatographic Determination of BHC (Lindane ppm) in the Milk of Treated Dairy Cows. Main Project

Concentration Sprayed	Days After Treatment	Replicates I	II	III	Mean
0.06%	1	1.161	0.970	0.600	0.985
	2	0.400	0.380	0.300	0.378
	4	0.095	0.150	0.028[x]	0.122
	7	0.081	0.100	0.013[x]	0.090
	10	0.055	0.052	0.160[x]	0.054
	13	0.034	0.042	0.088[x]	0.038
	16	0.024	0.025	0.021	0.025
	19	0.020	0.018	0.018	0.018
	22	0.016	0.018	0.014	0.016

Each figure under the replicates is the mean of four determinations.

[x] These figures are not included in the mean, since the cow suffered from mastitis and milk production dropped drastically.

Table V. Gas Chromatographic Determination of BHC (Lindane ppm) in the Milk of Treated Dairy Cows. Main Project

Concentration Sprayed	Days after Treatment	Replicates I	II	III	Mean
0.03%	1	0.595	0.511	0.468	0.525
	2	0.208	0.150	0.130	0.163
	4	0.059	0.053	0.038	0.050
	7	0.031	0.025	0.034	0.032
	10	0.023	0.016	0.018	0.018
	13	0.013	0.012	0.014	0.013
	16	0.013	0.012	0.011	0.012
	19	0.010	0.007	0.006	0.007
	22	0.010	0.005	0.004	0.006

Each figure under the replicates is the mean of four determinations.

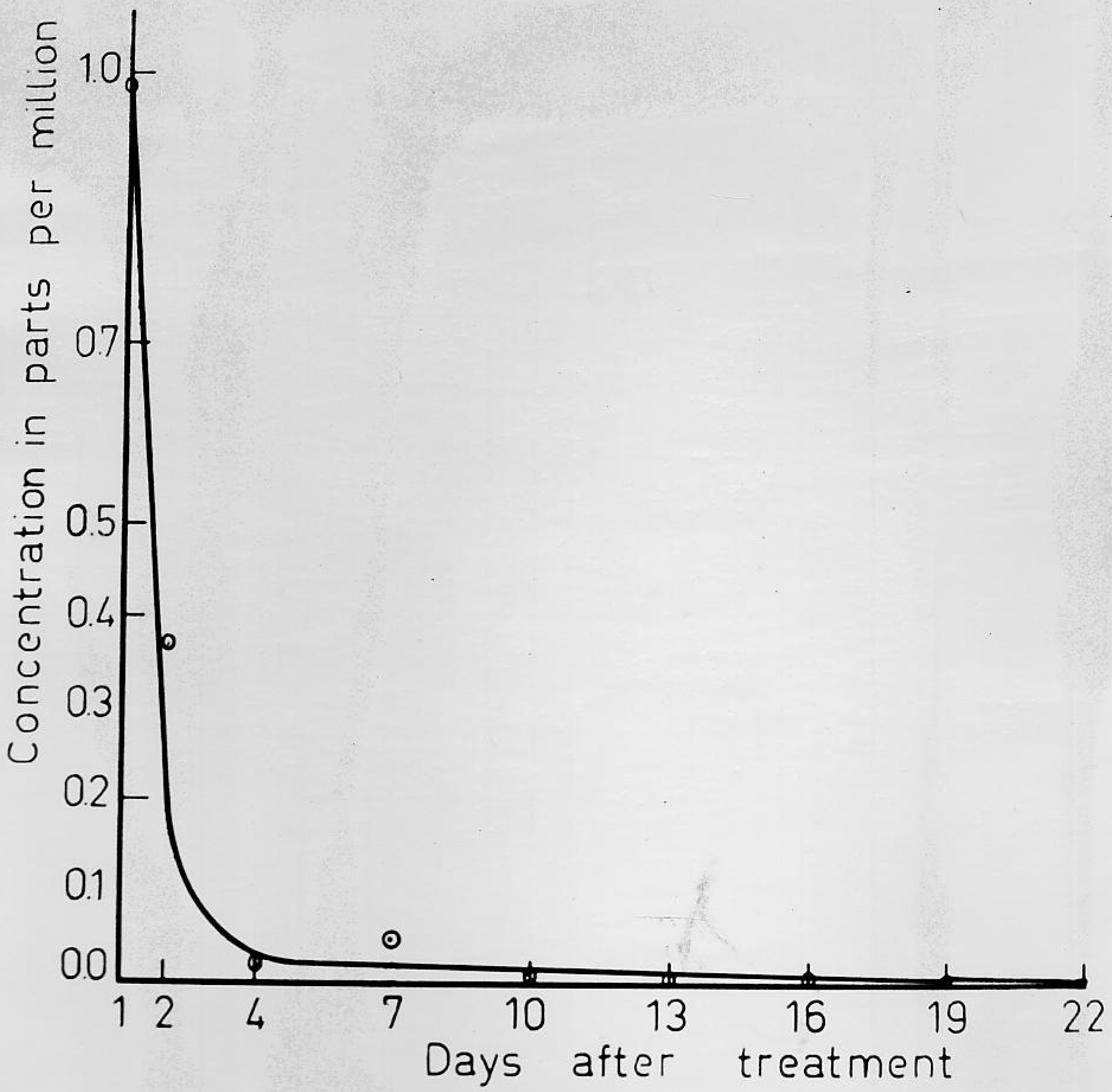

Fig. 6 Disappearance of BHC from the milk of cows treated at the 0.06 % level.

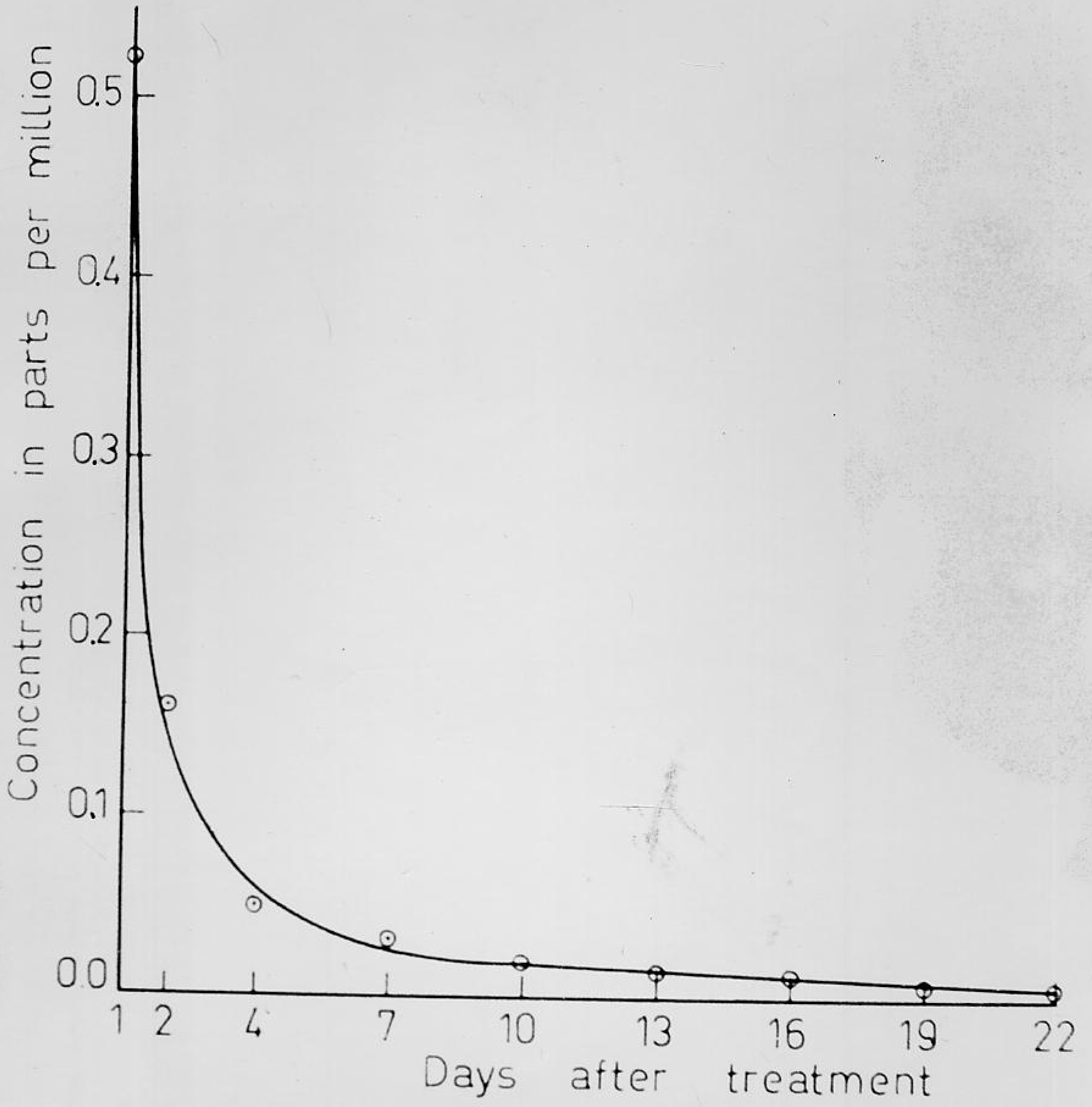

Fig. 7 Disappearance of BHC from the milk of cows treated at the 0.03 % level.

behavior is exhibited throughout the duration of the experiment. This wide discrepancy is probably due to the method of application of the insecticides. In the pilot experiment a high volume sprayer was used, whereas in the main project BHC was applied as a low volume spray. It is believed that low volume spraying has a higher and longer residual effect than high volume. Further examination of Table IV indicates that the residue values of the third replicate between the fourth and thirteenth days after treatment do not agree with the values of the other two. The reason for this difference is that the cow suffered from mastitis resulting in a drop of milk production which influenced the BHC levels.

Examining Table VI and VII as well as the disappearance curves (Figures 8 and 9), it is readily seen that the presence of chlordane residues throughout the experiment is unquestionable. The pattern of persistence parallels that of BHC, except that it is initiated and persists at much higher levels even after three weeks from the date of application. It is worth noting that chlordane is one of the cyclodiene insecticides which are known for their high stability and long residual effect.

The third insecticide, ronnel, behaves almost like the other two with the characteristic sharp drop 48 hours after treatment. However it differs in that the next sample taken four days after application reveals no detectable residues. This is true for both levels of treatment (Table VII and Figures 10 and 11).

This investigation indicates that ronnel, which is an organic phosphate disappears from the milk at a much faster rate then BHC and

Table VI. Gas Chromatographic Determination of Chlordane (ppm) in the Milk of Treated Dairy Cows. Main Project

Concentration Sprayed	Days After Treatment	Replicates I	Replicates II	Replicates III	Mean
0.50%	1	2.133	2.515	2.605	2.417
	2	1.513	1.800	2.000	1.771
	4	0.406	0.465	0.506	0.459
	7	0.380	0.445	0.460	0.408
	10	0.360	0.421	0.430	0.404
	13	0.321	0.410	0.410	0.380
	16	0.315	0.369	0.379	0.354
	19	0.298	0.328	0.334	0.320
	22	0.294	0.320	0.345	0.319

Each figure under the replicates is the mean of four determinations.

Table VII. Gas Chromatographic Determination of Chlordane (ppm) in the Milk of Treated Dairy Cows. Main Project

Concentration Sprayed	Days After Treatment	Replicates I	II	III	Mean
0.25%	1	1.200	2.025	1.566	1.597
	2	0.500	1.911	0.583	0.758
	4	0.388	0.625	0.450	0.488
	7	0.370	0.438	0.426	0.411
	10	0.315	0.368	0.366	0.363
	13	0.280	0.325	0.315	0.307
	16	0.257	0.280	0.267	0.268
	19	0.233	0.250	0.242	0.242
	22	0.230	0.238	0.234	0.232

Each figure under the replicates is the mean of four determinations.

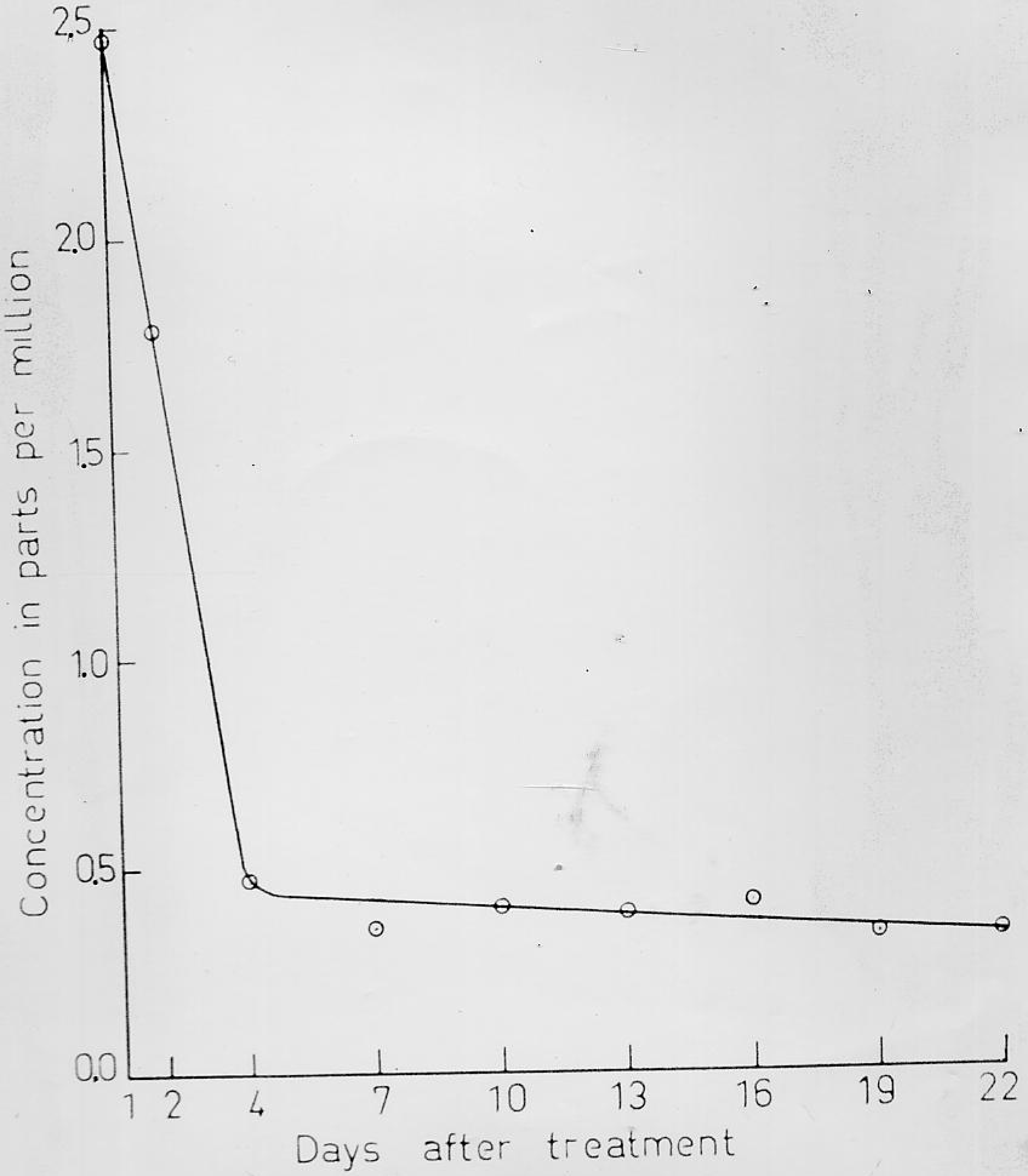

Fig. 8 Disappearance of chlordane from the milk of cows treated at the 0.5 % level.

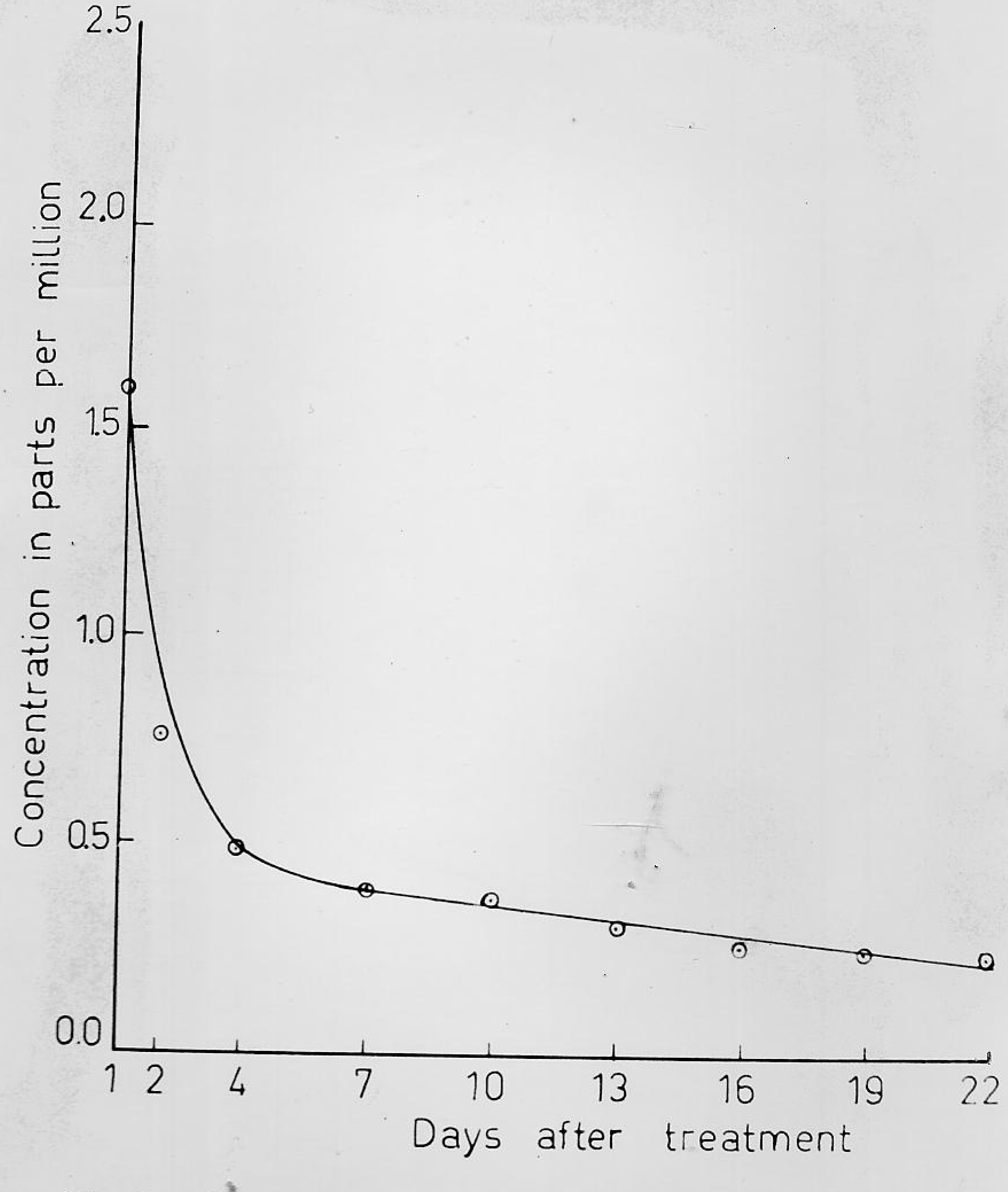

Fig. 9 Disappearance of chlordane from the milk of cows treated at the 0.25 % level.

Table VIII. Gas Chromatographic Determination of Ronnel (ppm) in the Milk of Treated Dairy Cows. Main Project

Concentration Sprayed	Days After Treatment	Replicates I	II	III	Mean
0.75%	1	1.80	1.73	1.75	1.76
	2	0.120	0.161	0.125	0.138
	4	0.000	0.000	0.000	0.000
	7	0.000	0.000	0.000	0.000
0.38%	1	0.850	0.808	0.772	0.810
	2	0.115	0.081	0.090	0.095
	4	0.000	0.000	0.000	0.000
	7	0.000	0.000	0.000	0.000

Each figure under the replicates is the mean of four determinations.

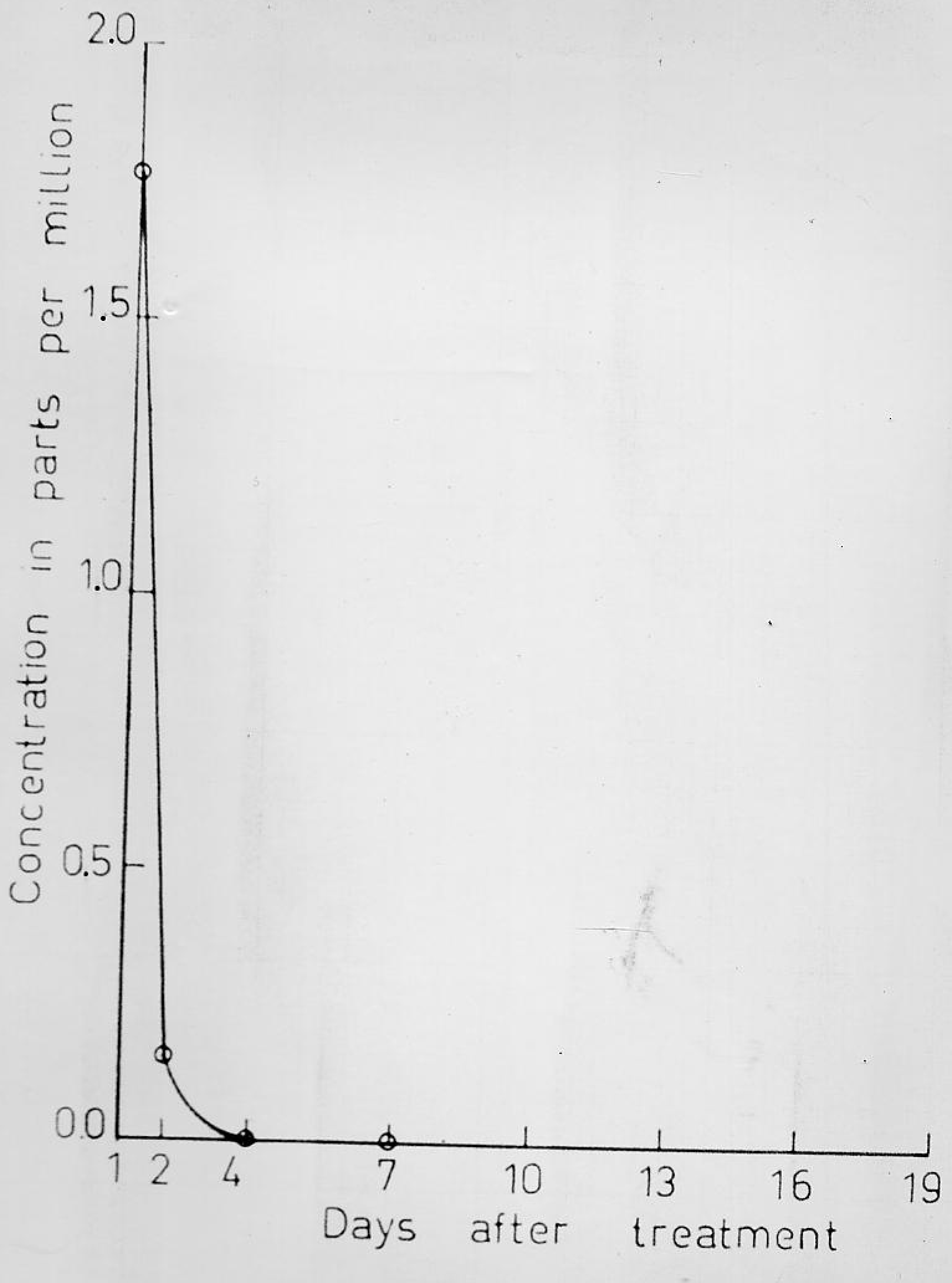

Fig.10 Disappearance of ronnel from the milk of cows treated at the 0.75% level.

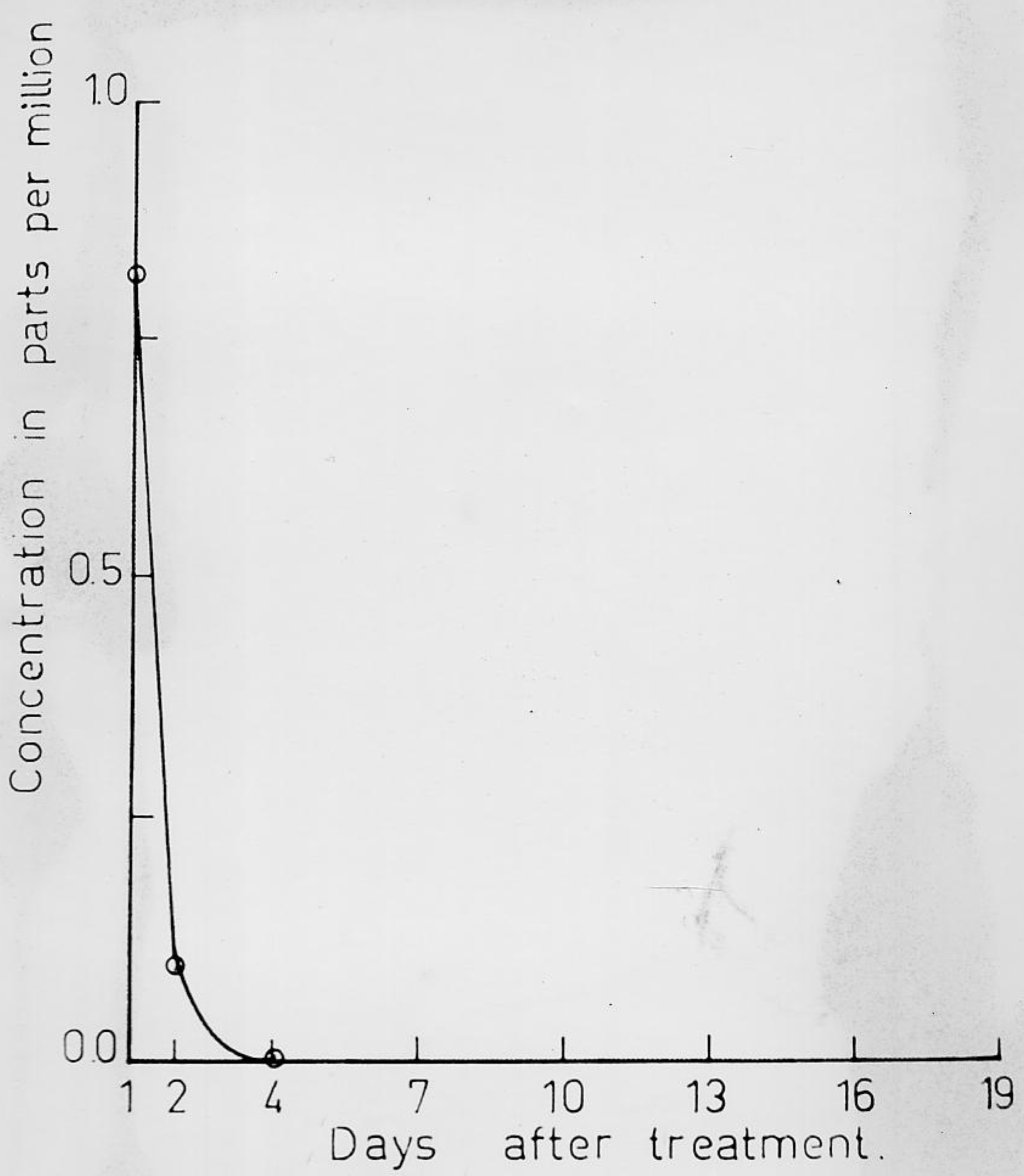

Fig. 11 Disappearance of ronnel from the milk of cows treated at the 0.38 % level.

chlordane. Moreover it maintains a comparable ectoparasite control as the two chlorinated hydrocarbons as will be seen from the fly counts. This is an obvious advantage of ronnel over the other two.

In the past decade the use of ronnel has become very popular in North America. Extensive research programmes are under way and the literature is being enriched continuously with publications of ronnel. Thus Drummond (1960) reports that ronnel was more effective in the control of the Lone Star ticks than the conventionally used insecticides lindane and malathion. McGregor et al. (1957) exploiting ronnel's systemic properties have successfully controlled cattle grubs by administering orally 100 mg per kg ronnel to heavily infested cows. On the other hand Radeleff and Woodard (1957) as well as Plapp and Casida (1958) have worked out the toxicology of ronnel in bovine metabolism.

The fly counts reported in Table IX from the three replicates are statistically analyzed. The analysis is designed as a split plot with the main plot as the factors between treatments and the sub-plot as the variation between dates. Examination of Table X reveals that, there is a highly significant difference in the treatments, but it does not indicate whether this difference is among the insecticide applications or between the applications and the control. The table also indicates that there is no significant difference between the replicates. Table XI is a summation of fly counts on the three replicates of each treatment, necessary for the calculation of the test of significance as reported in Table XII. There the Individual degree of freedom is used and comparisons of treatments between the

Table IX. Fly Counts Taken at Regular Intervals on Cows Used in the Experiment

Days After Treatment	BHC-0.06% Replicates			BHC-0.03% Replicates			Chlordane 0.50% Replicates			Chlordane 0.25% Replicates		
	I	II	III	I	II	III	I	II	III	I	II	III
0	4.0	4.0	3.9	4.0	6.8	6.0	4.1	4.2	3.7	3.7	3.4	3.6
1	5.5	6.0	4.0	5.0	4.3	6.0	4.2	4.3	3.8	4.3	4.0	4.4
5	5.7	5.0	4.9	8.05	7.8	8.9	6.4	9.6	7.01	9.0	8.9	10.0
8	6.0	7.0	8.0	9.0	8.0	10.0	7.0	9.8	7.2	9.1	6.8	7.4
12	6.5	11.0	12.5	9.6	10.0	11.0	8.5	10.0	8.5	9.5	7.3	8.2
15	7.0	12.0	11.6	10.0	10.1	11.0	8.9	11.0	9.0	9.8	8.3	10.0
19	7.9	12.0	11.0	10.8	10.9	12.1	9.7	11.3	9.8	10.0	11.0	12.0

Table IX (Cont'd)

Days After Treatment	Ronnel 0.75% Replicates			Ronnel 0.38% Replicates			Control		
	I	II	III	I	II	III	I	II	III
0	3.4	4.0	3.0	4.3	5.0	4.0	4.8	5.5	5.5
1	5.0	5.2	4.1	5.6	8.0	6.0	13.0	12.0	11.0
5	7.9	8.8	8.1	7.5	8.5	7.5	15.0	13.7	14.1
8	9.0	8.9	10.0	9.0	10.0	8.9	16.0	14.2	15.8
12	10.4	8.5	9.5	9.4	10.5	9.1	16.1	14.3	15.7
15	10.0	9.0	11.0	8.5	11.0	9.0	15.9	14.3	16.0
19	14.0	13.6	13.5	13.0	12.0	11.0	16.0	15.0	17.0

Each figure under the replicates is the mean of six fly counts.

Table X. Analysis of Variance

Source of Variation	Degrees of Freedom	Total Sum of Squares	Mean Sum of Squares	F Value	F Value 5%	F Value 1%
Replicates	2	4.9	2.5	0.75	19.41	99.42
Treatments	6	554.9	92.5	27.7 xx	3.0	4.8
Error a	12	40.0	3.34			
Dates	6	943.7	157.3	208.3 xx	2.2	3.04
Dates X Treatments	36	126.6	3.52	4.66 xx	1.54	1.84
Error b	84	63.4	0.76			
Total	146	1733.6	11.9			

Table XI. The Total Sum of Fly Counts on the Three Replicates of Each Treatment

Days After Treatment	Insecticides Used						
	BHC 0.06%	BHC 0.03%	Chlordane 0.50%	Chlordane 0.25%	Ronnel 0.75%	Ronnel 0.38%	Control
0	11.9	16.8	12.0	10.7	10.4	13.3	15.8
1	15.5	15.3	12.3	12.7	14.3	19.7	36.0
5	15.7	25.3	23.0	27.9	24.7	23.5	42.8
8	21.0	27.0	24.0	23.3	27.9	27.9	46.0
12	30.0	30.8	27.0	25.0	28.4	29.0	46.1
15	30.6	31.1	28.9	28.1	30.0	28.5	46.2
19	30.9	33.8	30.8	33.0	41.1	36.0	48.0

Table XII. Test of Significance, Individual Degree of Freedom Between the Treatments with the Highest and Lowest Fly Counts

Days After Treatment	Insecticide Level	F Value	F Value 5%	F Value 1%
0	BHC 0.03% minus BHC 0.06%	1.16	4.75	9.33
1	Ronnel 0.38% minus chlordane 0.50%	2.69		
5	Chlordane 0.25% minus BHC 0.06%	7.44[x]		
	Chlordane 0.25% minus chlordane 0.50%	3.97		
8	Ronnel 0.75% minus BHC 0.06%	2.37		
12	BHC 0.03% minus chlordane 0.25%	1.64		
15	BHC 0.03% minus chlordane 0.25%	0.04		
19	Ronnel 0.75% minus chlordane 0.50%	5.2[x]		
	Ronnel 0.75% minus BHC 0.06%	5.1[x]		
	Ronnel 0.75% minus chlordane 0.25%	3.2		

highest and lowest fly counts are made. Careful examination of Table XII reveals that there is no significant difference in the number of flies treated with the three insecticides at the two levels of significance. However BHC sprayed at the 0.06 percent concentration is slightly more effective in the control of flies than the other insecticides 5 and 19 days after treatment. The same behavior is exhibited by chlordane at the 0.50 percent concentration 19 days after treatment.

A second list of results of significance tests are reported in Table XIII where a comparison is made between the control and the treatments with the highest fly counts. Close examination of this table indicates that the difference between the control and treatments is significant at the 1 percent level in all cases except a few exceptions. The first fly counts taken a few hours after the treatment manifest a non-significant difference between the control and the treated cows. This is probably due to the high drop in the fly population in the barns following the application of insecticides on the cows. Also the last fly counts from replicates treated with 0.38 percent ronnel are significantly different from the control at the 5 percent level. However at the 0.75 percent concentration there is no difference from the control at the two levels of significance. This difference is probably due to an error in the fly counts. Consequently the three insecticides had a comparable fly controlling effect for the duration of the experiment.

It is worth mentioning that examination of the records of milk production indicate that the use of pesticides had no effect on the

Table XIII. Test of Significance - Individual Degree of Freedom Between the Control and the Treatments with the Highest Fly Counts

Days After Treatment	Insecticide Level	F Value	F Value 5%	F Value 1%
0	Control minus BHC 0.03%	0.05	4.75	9.33
1	Control minus ronnel 0.38%	13.34^{xx}		
5	Control minus chlordane 0.25%	11.00^{xx}		
8	Control minus ronnel 0.75%	16.38^{xx}		
12	Control minus BHC 0.03%	11.76^{xx}		
15	Control minus BHC 0.03%	11.45^{xx}		
19	Control minus ronnel 0.75%	2.39		
	Control minus ronnel 0.38%	7.19^{x}		
	Control minus BHC 0.06%	14.59^{xx}		
	Control minus BHC 0.03%	10.13^{xx}		
	Control minus chlordane 0.50%	14.75^{xx}		
	Control minus chlordane 0.25%	11.23^{xx}		

milking capacity of the cows. This is not surprising as blood-sucking dipterous pests notably the Tabanidae are few in number and do not seriously irritate the cows.

V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Treating domestic animals with insecticides for the control of external parasites is a common practice in many countries, including Lebanon. This presents a public hazard in the case of dairy cows since many insecticides are known to be transmitted into the milk. However no residue studies have been conducted previously on this subject in Lebanon.

The objective of the present study was to determine the persistence of the two commonly used insecticides, BHC and chlordane, and compare the results with those of ronnel, a new insecticide not used yet in Lebanon. Another purpose of the work was to study the effectiveness of these insecticides for the control of ectoparasites.

The work was conducted at the Agricultural Research and Education Center (AREC), in the Beqa'a Plain, Lebanon in the summer of 1966.

A pilot experiment using BHC at two levels of concentration was conducted in order to determine the frequency of sampling in the main project. The main project consisted of 21 Friesian Holstein cows which were divided into seven groups of three cows each. The insecticides were each applied at two levels and milk samples were taken on the first and second day following the treatment, and then every third day for three consecutive weeks. The insecticides were extracted from the milk by the column chromatographic method described by Langlois *et al*. (1964) and determined quantitatively by gas chromatography.

Examination of the data revealed that all three insecticides appeared in the milk shortly after treatment of the cows. However, ronnel disappeared within 72 hours whereas BHC and chlordane persisted for three weeks, with chlordane exhibiting the highest persistence level.

A statistical analysis of the fly counts with a Split-plot design revealed no significant difference among the treatments however a high significance between the control and the treated cows.

As a result of this investigation the use of insecticides should be discontinued. But in case of an outbreak of ticks then ronnel should substitute the chlorinated hydrocarbons, since it quickly disappeared from the milk; yet had the same controlling capabilities as BHC and chlordane.

Conclusions and Recommendations

The findings of this project support the view that the use of insecticides on milking cows should be discontinued, especially after it was found that in the absence of *Tabanidae*, *Musca* spp. did not present a real threat to the milking capacity of the cows. However in case of an outbreak of ticks, which are carriers of numerous diseases to cattle; ronnel 25 percent as a weltable powder applied at 0.38 percent should be used in preference to BHC and chlordane. This change is essential as it was shown that both of the chlorinated hydrocarbons persisted for prolonged periods in the milk whereas ronnel disappeared shortly after application. Moreover, it controlled flies as well as ticks as effectively as BHC and chlordane.

This project reminds entomologists that the use of chemical pest control agents constitutes a calculated risk. Nevertheless, the judicious use of pesticides results in benefits which, at the present time, far outweigh the known potential hazards. Considerable care must be exercised in the selection, as almost all pesticides are more or less toxic to man and cumulative and potentiation characteristics are not yet well understood. Alternative methods of pest control should be encouraged and until such methods become effective, chemical agents should be used cautiously.

Finally, it should be emphasized that in many countries where pesticide residue laws are enforced, the tolerance limit for insecticides in milk is zero. This is due to the fact that milk is the main food item for infants. Moreover, in the preparation of milk products, notably butter, the fat is concentrated, resulting in a subsequent concentration of any insecticide present.

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