THE INVESTIGATION OF THE OCCURRENCE AND LEVEL OF PESTICIDE RESIDUES IN THE UNIVERSITY FARM, SAMARU-ZARIA

BY

Eyitemi Emmanuel Akporhonor
B.Sc. Biochemistry (Hons) Uniben
Department of Chemistry
Ahmadu Bello University,
Zaria.

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Eyitemi Emmanuel Akporhonor

Department of Chemistry,
Faculty of Science,
Ahmadu Bello University, Zaria.

A thesis submitted to the Postgraduate School, A.B.U, in partial fulfilment of the requirements for the degree of M.Sc. (Analytical Chemistry)

1982.

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other University or Institution. Information derived from the published or unpublished work of others has been acknowledged in the text.

'Temi Akporhonor

Date:.....

DEDICATION

This Thesis Is Dedicated To The Magnificient

Seven, Founding Fathers Of The Pyrates Confraternity.

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In every enterprise there is a moment of truth, and this is it. Life is full of struggles. A well deserved success has never been easy to achieve because the frustrating thing about success is that it does not always fit our ignition. When an action is conceived, it has already begun, so also is the fact that anything that goes up must surely come down someday.

At long last the course has come to an end. The journey was longger and rougher than expected, but then when rain falls on a leopard, does it wash off its spots?. The future looked bleak but to resign oneself to it was to be crippled fast. The mechanics of getting this thesis ready was much more complicated than the research itself, they were more tiresome. The difficulties were dealt with conscientiously with courage, vigour and encouragement from various individuals/group of individuals whom I would very much want to acknolwedge.

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Ovemi Akporhonor

1982.

ABSTRACT

Soil samples from the University Farm, Samaru-Zaria were analysed for the level and occurrence of pesticide residues (γ-BHC, α-Endosulfan, β-Endosulfan, o,p'-DDT and p,p'-DDT). γ-BHC was detected and quantified in all the six samples analysed with concentrations of 4.05, 3.04, 2.31, 1.78, 1.11 and 2.06 ppm respectively while α-Endosulfan had concentrations of 13.95 and 6.16 ppm in two samples, detectable and unquantifiable residues in three and no residue at all in one of the samples. Neither of the isomers of DDT (o,p'-DDT/p,p'-DDT) nor β-Endosulfan was detectable.

Prior to the analyses of the soil samples optimisation of the instrument parameters was carried out and the following operational conditions were found to be most appropriate: Column temperature 200^{o}C; injector temperature 220^{o}C, Detector temperature 250^{o}C; Gas flow rate 30ml/min, column pressure 10550'; Cylinder head pressure 60lbC/in^{2}; Attenuation 64; Detector:- Ni-63 Electron Capture Detector, Column:- 10% OV-1 on chromosorb W60/80, 5'x1/8" stainless steel (GC No. 08529); Recorder:- Gow Mac Model 70-700, Chart Speed 2min/cm, Instrument - Varian Model 3700 Gas Chromatograph.

Chapter 1

1.0.0 INTRODUCTION

Man's basic needs include, food, water, and shelter. The problem of population growth has created the need for increased food production. However, with the dangers posed by pests to food production, crop protection against insects and other pests by means of pesticidal chemicals became necessary. These chemicals are selected because of specific toxic properties which allow them to be used in pest control. They are classified into groups primarily on the basis of the group of organisms they control. For example, pesticides controlling insects are known as insecticides. Similarly, we have fungicides - against fungi, herbicides against weeds, bactericides - against bacteria, molluscicides against molluscs, nematocides - against nematodes, and so on.

Each individual group is again subdivided into smaller sub-groups depending on their chemistry, mode of action, etc. These sub-groups include the chlorinated hydrocarbons, organophosphates, and carbamates for the insecticides. Fungicides include metallic salts, non-metallic salts, organomercurials, thiocarbamates and dithiocarbamates while herbicides include acetic acid derivatives, amide derivatives, arsonate derivatives, pyridine derivatives and

propionic acid derivatives. There is also an important group of pesticides which are derived from plants.

Another factor used in pesticide classification is selectivity which depends on the mode of action of the pesticides. In this respect, there are:-

i. Systematic pesticides - ability to kill pests without harming the host.

ii. Contact pesticides - hit pests directly and kill on contact.

iii. Stomach poisons - affect pests directly when eaten.

As a result of widespread use, pesticides have proliferated intensely in the biosphere and thus have significantly contributed to problems of environmental pollution due to their persistent nature [1,2,27,28]. The persistence and ultimate fate of pesticides in food, soil, water and air of man's environment is affected by weather conditions eg humidity, wind, solar UV radiation level and the physical properties of the pesticides such as volatility and chemical stability[3]. Due to the persistence of pesticides and the dangers they pose in terms of environmental pollution, the necessity arose for continous monitoring to safeguard the quality of the environment. The attention which has been focussed in recent years on the occurrence of the residues and their metabolites in the environment is demonstrated by the numerous reports of various official and unofficial studies[4,5] which have reported

the results of a large number of assays of pesticide residues in human and animal tissues, food, plant, water, soil and air.

1.1.0 OBJECTIVE OF INVESTIGATION

In Nigeria, the organochlorine pesticides have been used entensively for insect control,[21,22,23,24] and are still being imported in large amounts for agricultural and health purposes. For example, according to the Nigerian Trade Summaries, there was an upsurge in the amount of pesticides imported between 1966 and 1972. Insecticides also increased from 5,000 to 10,000 long tons (5080 - 10,160 metric tons) while fungicides rose from 1000 to 2000 long tons (1,016 - 2,032 metric tons)[6]. Their usage is increasing yearly and with the "Operation Feed the Nation" programme of the Military regime under General Obasanjo and the present Federal Government's acceleration of "Green Revolution" which seeks to revolutionise Nigeria's agriculture, it is anticipated that a lot more pesticides will be used in order to improve the yeild of farm crops.

Inspite of these increasing demands for, and usage of, pesticides all these years, Nigeria has not established an Environmental Protection Agency (EPA). It was only in 1981 that in its effort to find effective ways and means of maintaining a healthy, decent and clean Nigerian environment, that the Federal

Ministry of Housing and Environment, then under Dr. Wahab Dosumu sent the "Federal Environmental Protection Agency Bill, 1981" to the National Assembly for consideration and passage into law. However not much has been done in this respect. It was only recently[25] that the Committee on Housing, Community and Environment called for memoranda, from the public, on the bill.

In the northern part of Nigeria, there is practically no worthwhile information on work on pesticides, their residues and their environmental effects. For more than fifty years now, the Institute for Agricultural Research (IAR) of the Ahmadu Bello University, has been involved in improving farm output through research. The improvement of indegenous crops through breeding and selection, evaluation of various levels of fertilizers and pesticides and various planting densities are among the research areas. Until recently there has been little or no research on pesticide residues especially in soils.

This study (which is being carried out at the Institute of Agricultural Research farm where pesticide trials have been carried out for several years [21,22,23,24] involves the relative level of pesticide residues e.g. DDT, γ-HHC, Carbaryl, Endosulfan, in soils. The main steps of the trace analysis are summarized thus.

a. Establishing conditions for optimum resolution and limits of detection of pesticide residues.

b. Extraction of samples with suitable solvents.

c. Clean-up of sample extracts

d. Identification and quantitative estimation of pesticide residues in extracts, using the Gas Chematograph.

1.2.0 BRIEF LITERATURE SURVEY

A lot of studies have been carried out on organochlorine pesticides recently[7,30]. Some of these, however, are mainly degradation[8,9,10] and contamination studies[11]; development, modification and progress in extraction and clean-up procedures[12,13,21,32,33,34,35] determination of traces of residues in vegetables, fruits and animals[14,15,17] and toxicological studies on some vertebrates[16]. Specific studies on soils are very limited. This could be because it is thought that pesticide residues are translocated in a chainlike manner, i.e. from soils to plants which are then taken up by animals consumed by man or washed by rain into a lake or stream (from which fish or cattle drink) and then consumed by man or used for irrigation purposes where they are again transferred to crops. Therefore at any stage where residues are found whether in vegetables, fruits, milk, beef etc., it is assumed to have had the origin mainly in soil which were sprayed with pesticides during cultivation.

Lichtensten and Schulz[17] have reported on residues of aldrin, dieldrin etc., on cucumbers that were grown on soils

previously treated with the pesticides. The fact that aerial spraying had been applied to these crops indicates that these pesti-cides were taken up by cucumber to a very little extent. Decker et al[2] reported that the residues in the soils never exceeded 13.2% of the total applied when tested one year after the last application. Lichtenstein and Schulz[1] have also reported that for a loamy soil under existing weather conditions, aldrin, DDT and dieldrin residues were present at a level approximately 20% of the total applied insecticidal dosage over a five-year period.

In translocation studies carried out by Terriers and Ingalsbe[18] residues were found in crops planted in a pretreated soil after the second and third seasons and the residues of BHC, DDT, aldrin and dieldrin were shown to persist in the soil, a sandy loam, for at least three years.

In pesticide residue analysis, the need for the sample to be representative of the plot from which it is taken[19] and the necessity for the participation of the residue chemist in sampling[2 0] cannot be overemphasized. These needs are so great that it is surprising that more experimental work has not been reported on the comparison of sampling proceedures for residues analysis especially for soils. The sampling techniques include stratified sampling whereby the plots are divided into strata and random sampling whereby the soil representative samples collected in bags are further reduced by random sampling to limit the number required for analysis.

Chapter 2

OPTIMIZATION OF INSTRUMENT PARAMETERS AND CALIBRATION CURVES

2.1.0 OPTIMIZATION OF INSTRUMENT PARAMETERS

2.1.1 INTRODUCTION

This being the first research work on pesticide residues in the department of Chemistry it was necessary to determine the optimum working conditions of the Gas chromatograph interms of resolution, sensitivity and minimum detectable quantities of pesticide residues.

2.1.2 MATERIALS

n-Hexane and Methanol were BDH Analytical Grade Reagents - for chromatographic analysis.

The columns were: 10% OV-1 on chromosorb W60/80, 5'x1 8", stainless steel (SC No. 08529) and Carbowax 20m on chromosorb W60/80, 5'x1/8" stainless steel (SC No. 08529).

Pesticide standards (δ-BHC, Carbaryl, Technical DDT, α-Endosulfan and β-Endosulfan) were kindly supplied by Dr. G. H. Dromilo of Chemical Liaison Unit, Rothamsted Experimental Station, Habenden, England through Mr. S. Nwokocha of Crop Protection, Institute for Agricultural Research (IAR), Samaru, Zaria.

Detectors were Flamelonisation and Election Capture Detectors.

2.1.3 METHOD

In order to determine the optimum working conditions in terms of resolution, sensitivity and minimum detectable quantities of pesticides residues, the Varian Model 37000 Gas Chromatograph was operated under different conditions of column, oven temperatures, carrier gas flow rate, and detector. Some preliminary analyses were carried out in stages using the pesticide standards (δ-BHC, Carbaryl, α-Endosulfan, β-Endosulfan and Technical DDT.

A 1% solution of each of δ-BHC, α-Endosulfan, β-Endosulfan and Technical DDT was made in n-hexane, while carbaryl, which was insoluble in n-hexane, was dissolved in (2:1) hexane-methanol mixture, for injection into the Gas Chromatograph.

Throughout the analysis parameters like injector temperature, ion-detector temperature, column pressure, carrier gas flow rate, chart speed, cylinder head pressure, attenuation were left constant while varying the column temperatures, detectors and columns.

A. Flame Ionisation Detector (FID).

Constant Parameters:

Injector temperature:	220°C
Ion detector temperature	250°C
Carrier Gas	N_2
Column Pressure	10PSIG
Chart Speed	2cm/min
Recorder Input	1mv
Gas Flow Rate	25ml/min
Cylinder head pressure	60lbf/in^2
Attenuation	1024,128

Varied Parameters:

i. Column temperatures, 160°C, 180°C,; 200°C
and programming between 120°C -- 200°C

ii. Columns: a. 10% OV--1 on chromosorb W60/80, 5'x1/8" stainless steel (SO No. 03529) b. 10% Carbowax 20M on chromosorb W60/80, 5'x1/8" stainless steel (SO No. 03529)

1µl of each of the 1% pesticide solutions was injected. Retention times for the various standards were measured. Peak heights were also observed. The effects of the variation of parameters on the resolution and positions of the peaks were also noted.

Similarly, 1µl of a solution containing 1% of each of the pesticides was injected.

D. Electron Capture Detector (ECD)

The Flame Ionisation Detector was replaced with a Nickel-63 Electron Capture Detector for further analyses. Operating conditions were same as for the FID.

1µl of each of the 1% pesticide solutions was injected separately, in same solution and retention times measured. The peak heights and effect of the varied parameters and detector on resolution were also noted. Then 1ppm pesticide solutions were prepared and injected separately and retention times and peak heights recorded.

2.1.4 RESULTS AND DISCUSSION

A. Flame Ionisation Detector: The results obtained with the FID are given in Table 1 below.

TABLE 1

RETENTION TIMES FOR THE INDIVIDUAL PESTICIDE STANDARDS AT 200°C COLUMN TEMPERATURE WITH FID.

PESTICIDE	RETENTION TIME (SEC)
Carbaryl	72
γ-BHC	96
α- Endosulfan	336
β - Endosulfan	480
o,p' - DDT	540
p,p' - DDT	696

Colum a - 10% OV-1: From the table it can be seen that retention times were Carbaryl 72 sec, γ-BHC 96, α-Endosulfan 336, β-Endosulfan 480 sec while the technical DDT gave two well resolved

peaks O,p' - DDT and p,p' - DDT with retention times 540 sec and 696sec respectively at 200°C column temperature. The shapes and relative positions of signals were consistent with those in the literature i.e. O,p'-DDT having a smaller peak height and being eluted before p,p' - DDT with a bigger peak height. At a lower column temperature of 160°C none of the pesticides was eluted but at a higher column temperature of 130°C the different pesticides were eluted with O,p'-and p,p'-DDT still being well resolved with some retention times as when column temperature was 200°C. However, the other pesticides - carbaryl, α-end β-Endosulfan, γ-BHC were not well resolved. It should be noted that carbaryl solution was made up in 1:2 methanol -hexane mixture as it did not dissolve in n-hexane alone, and the change of attenuation from 1024 to 128 only affected (increased) the observed peak heights and not the retention times.

To study the possible interference between the individual pesticides, a mixture of the four pesticides was injected. With this injection a little shift in retention times from values got with separate injections was observed. However, o,p-DDT and β-Endosulfan were eluted at approximately the same retention times giving an increased overlapping peak. This was not surprising because the individual retention times of O,p'-DDT, 540sec and β-Endosulfan, 430sec were close enough as to give superimposed peaks. The resolution of the carbaryl in the mixture was very poor.

In order to determine the effect of column temperature on the resolutions of the peaks, temperature programming was carried out. On programming the column temperature between 120°C and 200°C, the seperation (resolution) of peaks from the injected mixture remained same, retention times increased slightly but the relative retention times remained unchanged. In short apart from the slight increase in retention times, the resolution was not improved beyond that obtained at 200°C column temperature. A change in column was then effected to see its effect on resolution of peaks, retention times and its sensitivity to pesticides.

Colum b - 10% Carbowax 20m:

With all operational conditions remaining unchanged, none of the pesticides injected separately or in a mixture was eluted at column temperatures of 160°C, 180°C and 200°C. However, on programming the column temperatures betwoen 120°C and 200°C, only DDT was eluted as a single, irregular trailing peak. No signal was obtained even when the attentuation was changed from 1024 to 128. This meant that the colum was not appropriate for this particular work under the various operational conditions

B. Electron Capture Detector (ECD)

The detector was changed from FID to ECD using the more efficient (already determined) OV-1 column, in order to determine the sensitivity and minimum detectable quantity for the

detectors. The change was also necessitated by the well known fact that the ECD is the most sensitive of all detectors for pesticide work, especially chlorinated insecticides.

The ECD was operated under the following conditions:

Injector temperature:	220°C
Detector temperature	250°C
N_2Carrier Gas flow rate	30ml/min
Chart speed	2min/cm
Recorder imput	1mv
Attenuation	128, 1024

The results in Table 2 were obtained at a column temperature 200°C. The resolution at column temperatures 160°C, 180°C and programming between 120 and 200°C were not as good as that obtained at 200°C although they were better than those obtained with FID.

The ECD enhanced the signal level of both pesticides and background signals. Nevertheless the positions of the peaks of the respective pesticides were identical with the retention times obtained earlier with FID.

TABLE 2

RESULTS OBTAINED WITH ECD AT COLUMN TEMPERATURE 200°C

PESTICIDE	CONCENTRATION (PPM)	RETENTION TIME (SEC)	PEAK HEIGHT (mm)	ATTENUATION
α-Endosulfan	1	342	69	1024
α-Endosulfan	1	342	92	128
β-Endosulfan	1	540	10	1024
β-Endosulfan	1	540	77	128
γ-BHC	1	108	110	1024
Carbaryl	1	60	67	128
Technical DDT:	1			
o,p'-DDT		408	86	128
p,p'-DDT		510	216	128

From the table, the retention times are α-Endosulfan 342 seconds, β-Endosulfan 540, γ-BHC 108 Carbaryl 60, o,p'-DDT 408 and p,p'-DDT 510 seconds respectively. The peak heights varied with change in attentuation with 69 mm, for α-Endosulfan 10mm, for β-Endosulfan and 110mm for γ-BHC at attenuation 1028 and 92mm, 77, 67, 88, 216mm

for ∝-Endosulfan, β-Endosulfan, Carbaryl, o,p'-DDT and p,p'-DDT respectively at attenuation 128. In all, p,p'-DDT had the highest peak and carbaryl the lowest at 67mm.

Comparing the retention times obtained with the different detectors a shift can be noted. The retention times of carbaryl 72secs, o,p'-DDT - 540 and p,p'-DDT 69sec with FID were decreased to 60, 400 and 510 seconds respectively with ECD while the retention times of γ-BHC 96, ∝-Endosulfan 336, β-Endosulfan 480 were increased to 100, 342, and 540 seconds respectively. This showed that apart from enhancing the resolution of the peaks, the interference between o,p'-DDT and β-Endosulfan hitherto obtained with FID had been corrected.

From the foregoing it can be concluded that the better detector for this work is the Nickel-63 electron capture detector using the 10% OV-1 on chromosorb W60/80, 5'x1/8" stainless steel (SS No. 08529) column under the following operating conditions: recorder attenuation 1mv; column temperature 200°C; injector temperature 220°C; Detector temperature 250°C; Gas (H_2) flow rate 30ml/min, chart speed 2min/cm, column pressure 10PSIG, cylinder pressure 60lb/in^2.

2.2.0 DETERMINATION OF CALIBRATION CURVES

2.2.1 INTRODUCTION

Having optimized the instrument parameters, the calibration curves were determined for the various pesticides with the Varian Model 37000 Gas Chromatograph under the following operating conditions

OPERATING CONDITIONS:

Column temperature	200^{o}C
Injector temperature	220^{o}C
Detector temperature	250^{o}C
Gas (N_2) flow rate	30ml/min
Column	OV-1
Attenuation	64
Column pressure	10PSIG
Cylinder head pressure	60lbf/in^2
Chart speed	2min/cm
Detector	ECD
Recorder attenuation	10mv

2.2.2 METHOD

Standard solutions of the different pesticides were prepared. γ-BHC standard solutions contained 0.5, 1, 1.5, 2, 2.5, 5, 8, and 10 nanograms respectively; technical DDT - 20, 30, 35, 40, 43, 50, and 100 nanograms respectively while β-Endosulfan standard solution contained 5, 10, 12.5, 25, 50 and 100 nanograms respectively. For α-Endosulfan it was 1, 1.5, 2, 2.5, 3.5, 4, 5 and 10 nanogram standard solution respectively.

1µl each of the pesticide solutions was injected seperately and peak heights were measured. A plot of peak height in millimetres against amount of pesticide was drawn for each pesticide.

2.2.3 RESULTS AND DISCUSSION

The attenuation was changed from 128 to 64 for the gas chromatograph and 1mv to 10mv for the recorder, in order to enhance sensitivity as the amount of pesticides in solution was in the nanogram range

TABLE 3

DATA FOR CALIBRATION CURVES

α-Endosulfan		β-Endosulfan		γ-BHC		Technical DDT		
Amount (nano-gram)	Peak Height (mm)	Amount (nano-gram)	Peak Height (mm)	Amount (nano-gram)	Peak Height (mm)	Amount (nano-gram)	Peak Height (mm)	
1.0	4	5	14	0.5	7		OP' DDT	PP DDT
1.5	40	10	22	1	20	20	8	26
2	52	12.5	33	1.5	30	30	14	41
2.5	54	25	58	2	40	35	19	49
3.5	67	50	77	2.5	50	40	22	52
4	73	100	120	5	65	43	24	56
5	70			8	72	50	32	65
10	96			10	78	100	53	85

Figures 1-4 show calibration curves for γ-BHC, Technical DDT, α-Endosulfan and β-Endosulfan respectively.

FIG. 1 CALIBRATION CURVE FOR γ-BHC.

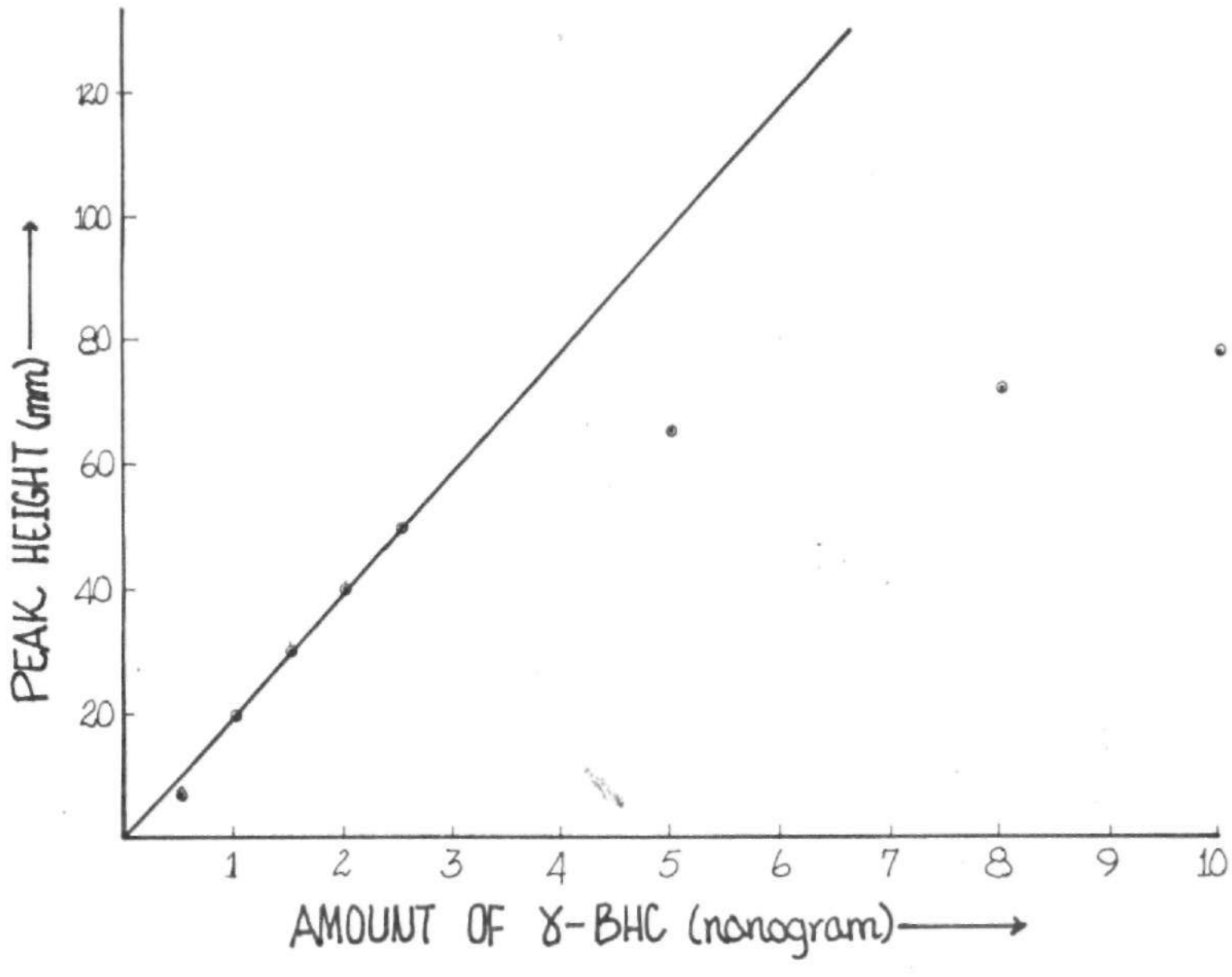


FIG. 2 CALIBRATION CURVE FOR TECHNICAL DDT.

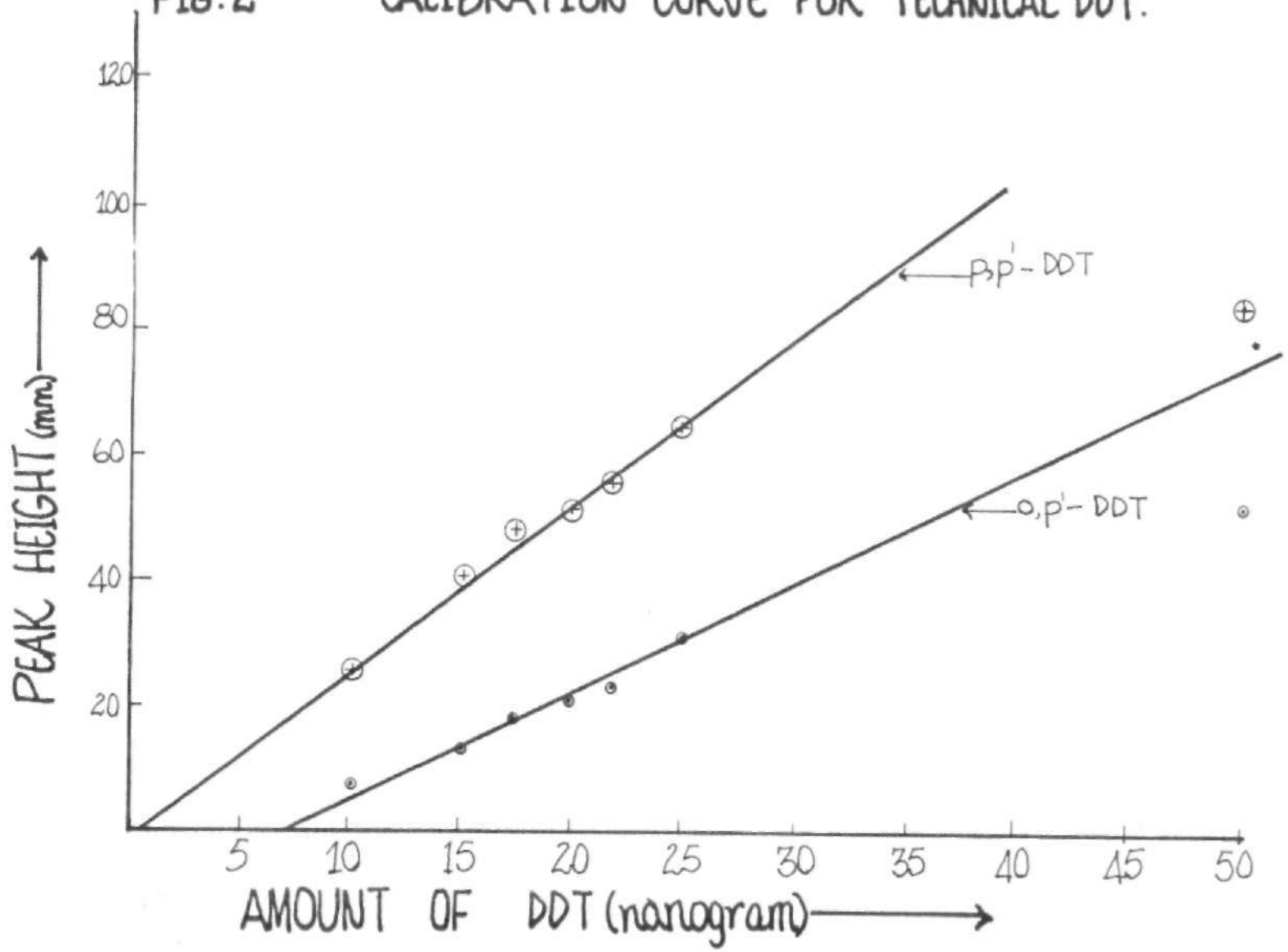


FIG. 3 CALIBRATION CURVE FOR ∝- ENDOSULFAN

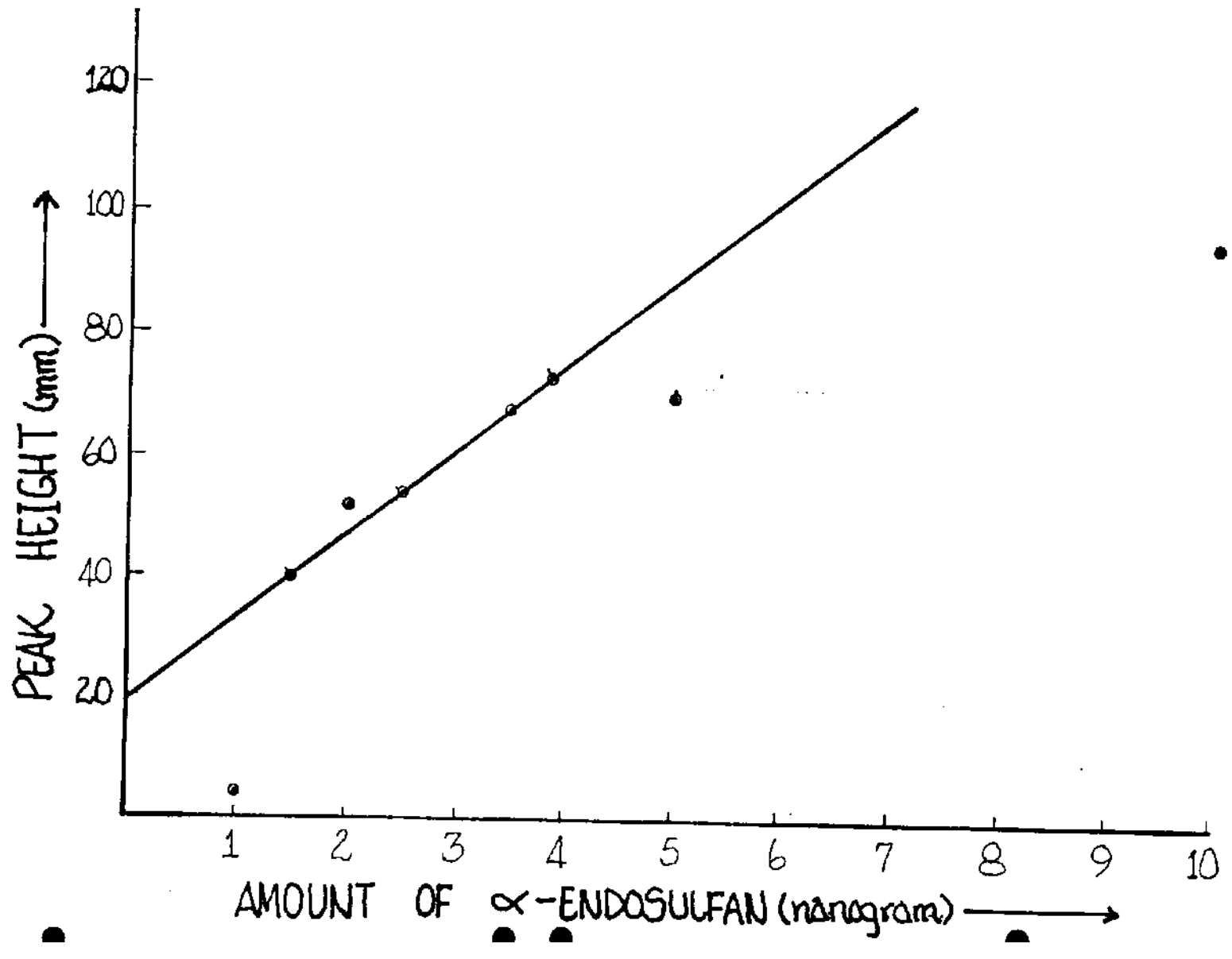
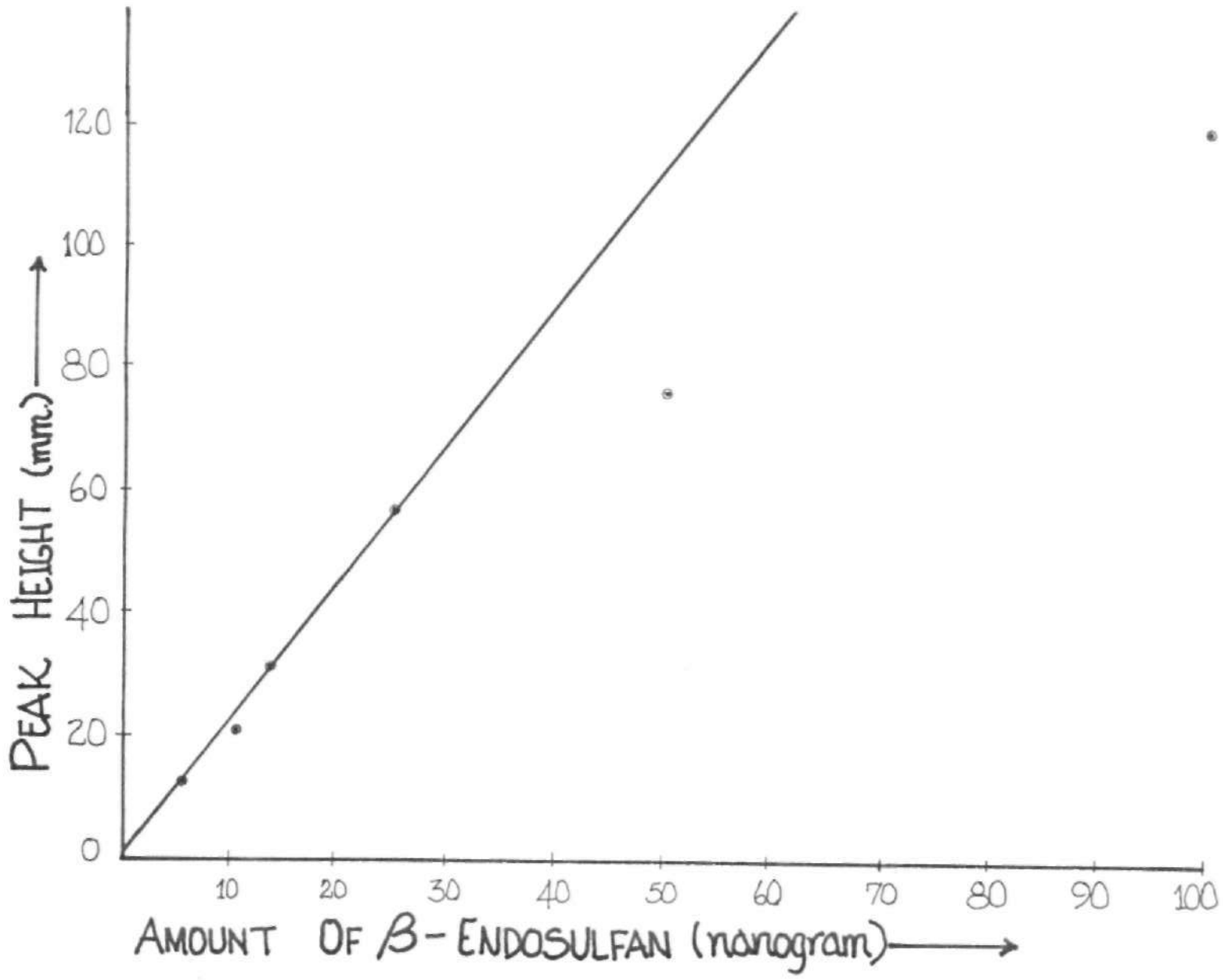


FIG.4 CALIBRATION CURVE FOR β-ENDOSULFAN



MINIMUM DETECTABLE QUANTITY MDQ:

MDQ = Twice Noise signal.

The height of the noise signal was 1mm as such the minimum detectable quantity for all the pesticides is equal to amount of the various pesticides that will correspond to 2mm on the calibration curve. This will however be true when the curve passes through the origin as it was for γ-BHC, or very close to the origin as was the case of β-Endosulfan. The MDQ for γ-BHC was 0.11 nanogram and β-Endosulfan ~0.011 nanogram.

From the calibration curve it can be seen that the γ-BHC standard curve passed through the origin and was linear to about 3 nanogram but non-linearity set in between 5 and 10 nanograms. This showed that above the 3 monogram no true value of an unknown can be estimated from the plot. Therefore all estimation of unknown pesticides from the plot should be done between 0 and 3 nanograms. This also applied to β-endosulfan where the linearity ended at 30 nanograms, α-Endosulfan 4 nanograms. 0,p'-DDT and p,p'-DDT 25 nanograms. As seen from the DDT plot the amount of pesticide plotted is half the amount of Technical DDT on the table. This was because the technical DDT contained 0,p'-DDT and p,p'-DDT isomers in an unknown proportion. It was therefore assumed that both isomers were present in equal amounts.

The ∝-Endosulfan plot cut the peak height axis at 20mm signifying that without any pesticide the instrument could give an internal signal equal to that amount. For β- Endosulfan, the internal signal was 2mm same as the HCC. As for the technical DDT, the 0,p' isomer gave a linear plot that passed through the quantity axis at 6.65 nanograms, an indication that below this amount no response or signal would be obtained.

Chapter 3

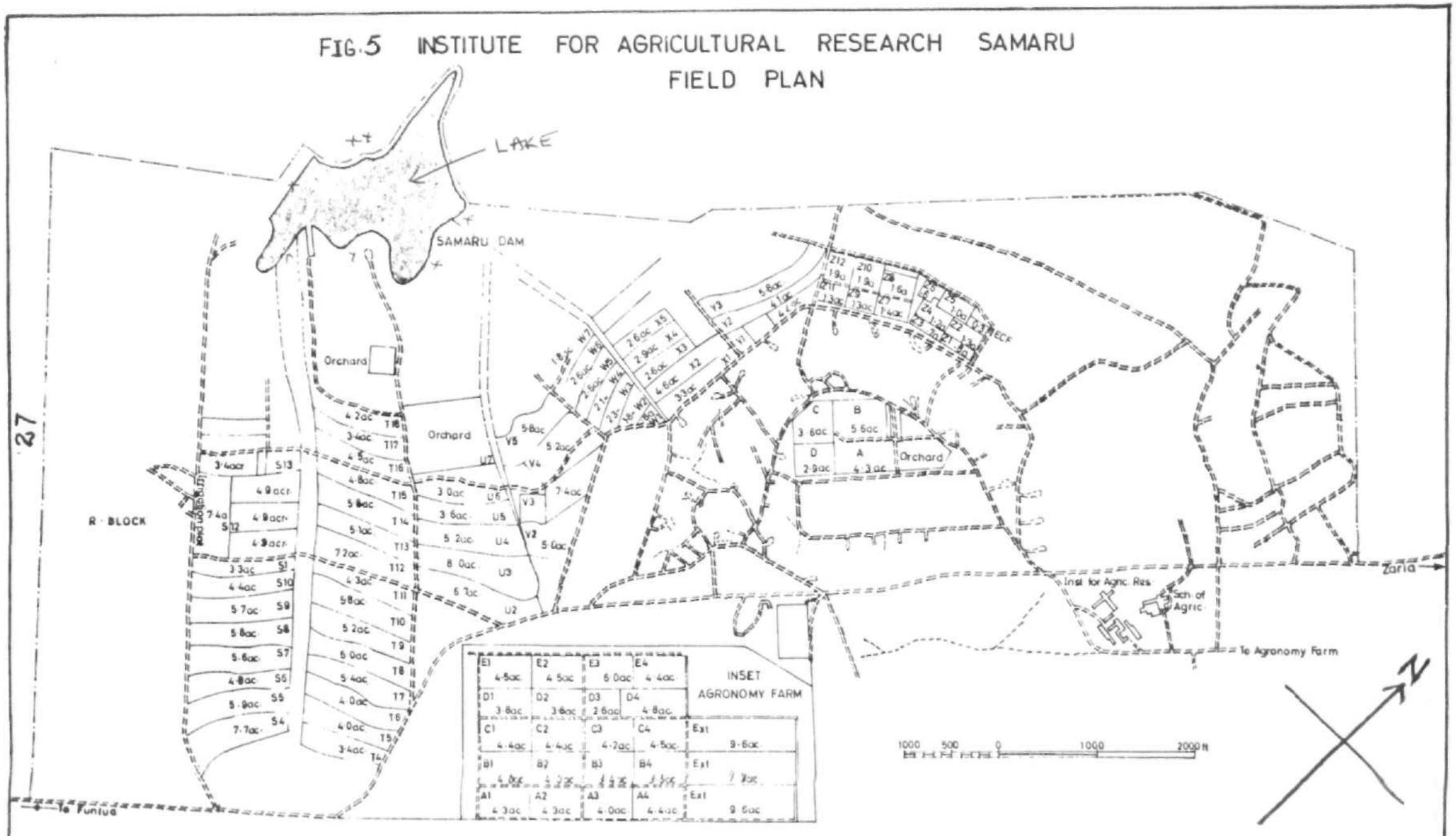
ANALYSES OF SOIL SAMPLES

3.1.0 INTRODUCTION

The Institute for Agricultural Research (IAR) Farm is shown in figure 5. The samples were collected by the Soil Survey Section of IAR from the K Block. The block is usually divided into plots of various sizes for easy allocation to individuals, groups of individuals, departments or sections for the purpose of experimentation. Almost all the plots are cultivated annually except for some that are left to fallow. There is a man-made lake (dug during the construction of the dam) some distance from the Blocks. The lake which was constructed around 1961 serves as a source of water for irrigation, drinking water for cattle, and is also used by the villagers around for washing and other household purposes. The farm land slopes towards the lake. There are about three orchards to the farm.

The experimental crops include cotton, millet, sorghum, maize, cowpeas, kenaf, onions, pepper, tomatoes and vegetables while pests encountered include grasshoppers, beetles, termites, millipedes, Aphids etc.

FIG. 5 INSTITUTE FOR AGRICULTURAL RESEARCH SAMARU
FIELD PLAN



The history and type of pesticides used on this plot is not known, but it is known that DDT, γ-BHC, Carbaryl, α-Endosulfan and β-Endosulfan have been used in the IAR farm from one time to another on experimental crops[21,22,23,24].

3.2.0 MATERIALS AND METHOD

3.2.1 MATERIALS

Florisil about 60-100 U.S. Mesh (BDH Analytical Grade Reagent) for Chromatographic Analysis.

Acetone and n-Hexane were BDH Analytical Grade Reagent - for chromatographic analysis.

Anhydrous Sodium Sulphate - AnalaR

All solvents used were dried, redistilled and then purity checked by gas chromatography. Florisil, glass wool and anhydrous sodium sulphate were extracted with 20% acetone in n-hexane (mixture for soil extraction) for a minimum of 60 cycles and purity checked by gas chromatography.

Soil samples were kindly supplied by Dr. Ibanga of Soil Survey Section of I.A.R.

3.2.2 METHOD

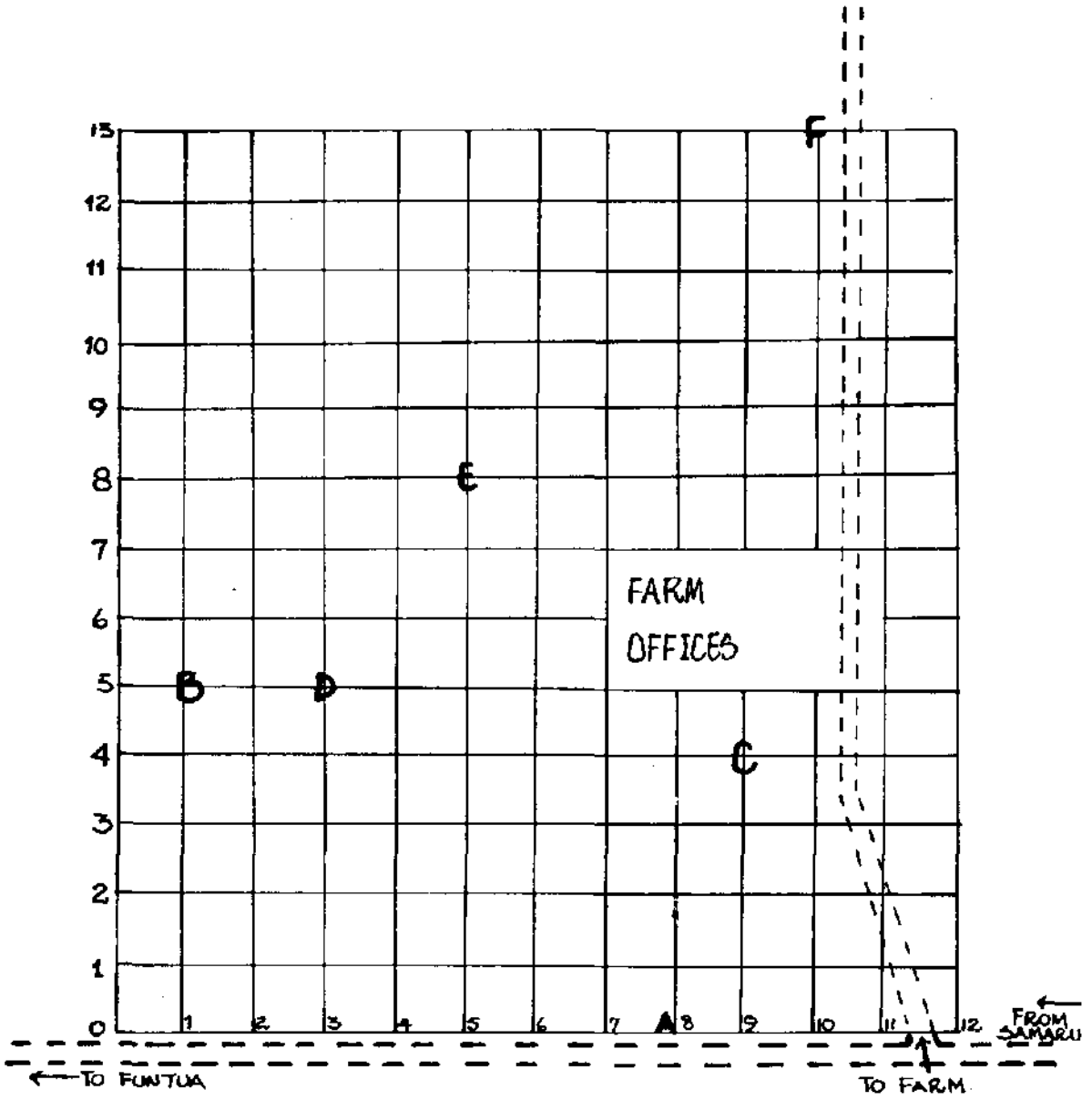
3.2.2.1 SAMPLE COLLECTION AND SAMPLING

Sampling was carried out by Mr. Vallete of the Soil Survey Section of IAR as follows:

The R Block was divided along two axes, the vertical axes or base lines and the horizontal axes or transverse lines running parallel to the road to Muntua. The base was divided into thirteen and transverses into twelve giving a total of 156 points with small squared plots of 50 x 50m each (see fig. 6). Augering was carried out at the various points to get representative soil profiles. Auger points were 7cm in radius. After augering, 32 auger points representing the soil profiles of the R-block were located. Pits of 4 x 6 x 6m were dug for sample collection. The soil samples were collected according to soil types and labelled. For example, Sample 1: Transverse 0, point 3(A), bag No. SS1, 0-14cm depth and stored in the Freezer prior to analyses. Only the first layers were collected for pesticide analysis.

The principles of random sampling was applied to reduce the soil samples from 32 to 6 samples. The labels were written on thirty two pieces of papers (each representing a sample) rolled into balls and put in a polyethylene bag. After shaking, 10 balls were picked, one at a time and the samples were recorded.

FIG.6 SCHEMATIC DIAGRAM OF R-BLOCK.
SHOWING SAMPLING POINTS



HORIZONTAL AXES CORRESPOND TO TRAVERSE LINES
VERTICAL AXES CORRESPOND TO BASE LINES
POINTS OF INTERSECTION CORRESPOND TO AUGUR POINTS
LETTERS A to F CORRESPOND TO SAMPLES I to VI RESPECT'LY

EACH SQUARE = 50m X 50m

Next, six balls were picked out of the ten following the same procedure, before three balls were further picked out of the six. Letters A to F on fig. 6 represents collection points of soil samples one to six respectively which were analysed.

3.2.2.2 EXTRACTION AND CLEAN-UP PROCEDURES

The extraction of soil samples and clean-up of sample extract were carried out according to the method outlined by Beyond and Elgar[25] with some modifications. The modifications were the extraction of 100g of soil samples with 20% acetone in n-hexane in a large soxhlet extractor for sixteen hours and collection and concentration of extract in a Buchi Rotavapor-R instead of a Kuderna-Danish evaporator.

In the extraction step, the extract was washed with water several times to remove acetone in the acetone-n-hexane (1:4) mixture while florisil was used as column material to remove any polar interfering substances and n-hexane for eluting the pesticides which are non-polar.

3.2.2.3 GAS-LIQUID CHROMATOGRAPHY OF EXTRACT

Six soil samples were analysed and an aliquot of each of the six concentrated extracts was injected into the Gas Chromatograph

equipped with Nickel-63 Electron Capture Detector and a 10% OV-1 column impregnated with chromosorb W60/80. The chromatography was carried out under the following operational conditions: Column temp. 200°C, Detector temperature, 250°C; Injector temperature, 220°C; Column pressure, 10PSIG; Cylinder head pressure 60lbf/in^2; Attenuation 64; Nitrogen Gas flow rate, 30ml/min; Recorder was a Gow-MAC Model 70-700 recorder, with imput at 10mv and chart speed of 2cm/min.

Sample standards were run periodically to check on recovery. Each aliquot was injected five times and average peak height recorded in millimeters.

3.2.3 RESULTS AND DISCUSSION

Table 4, shows the results of the quantitative estimation of pesticide residues in the soil samples:

CALCULATIONS

SAMPLE I

Quantity of extract concentrate = 18mls

Quantity injected into GC = 2µl

(i) γ-BHC

Average peak height = 10mm and it corresponds to 0.45ng/2µl from the calibration curve

$= 0.225 mg/\mu l$

$= 0.225 \times 10^{-6} mg/\mu l$

$= 0.225 \times 10^{-6} \times 10^{3} mg/ml$

∴ Amount of γ-BHC in 18 ml extract

$= 0.225 \times 10^{-6} \times 10^{3} \times 18mg$

= Mass of residue in 100g soil.

$= 4.05 \times 10^{-3}$ mg residue/100g soil

∴ Concentration of γ-BHC in 100g soil

$= 4.05 \times 10^{-3} \times 10^{3}$ ppm

= 4.05 ppm

(ii) α-ENDOSULFAN

Average peak height = 41mm = $1.55ng/2\mu l$

$= 0.775 \times 10^{-3} mg/ml.$

Amount of α-Endosulfan in 18ml

$= 0.775 \times 10^{-3} \times 18mg$

= Mass of residue in 100g soil

∴ Concentration = 13.95 ppm

The concentration of pesticide residues in the other samples (II to IV) were also calculated as shown above. Of all the pesticide residues of interest (γ-BHC, α-Endosulfan, β-Endosulfan, 0,p'- and p,p'-DDT) only γ-BHC and α-Endosulfan were identifiable and quantifiable.

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TABLE 4: RESULTS OF QUANTITATIVE ESTIMATION OF PESTICIDE RESIDUES IN SOIL SAMPLES

PESTICIDE	δ-BHC						∝-ENDOSULFAN					
Traverse (line)	0	5	4	5	8	13	0	5	4	5	8	13
Auger point	8(A)	1(B)	9(C)	3(D)	5(E)	10(F)	8(A)	1(B)	9(C)	3(D)	5(E)	10(F)
Bag No.	SS1	SSM1	SS32	SS27	SS12	SS68	SS1	SSM1	SS32	SS27	SS12	SS68
Depth (cm)	0-14	0-15	0-7	0-10	0-6	0-20	0-14	0-15	0-7	0-10	0-6	0-20
Sample no.	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Average peak Height (mm)	10	8.5	6.5	16.4	14.33	23.?	41	20	NR	0	8.3	15
Quantity of Extract concentrate (ml)	10	16	14	6	6.5	8.5	10.	16	14	6	6.5	8.5
Quantity of concentrate injected (µl)	2	2	2	3	4	5	2	2	2	3	4	5
Estimated concentration of pesticide residues (ppm)	4.05	3.04	2.31	1.78	1.11	2.06	13.95	6.16	NR	NR[1]	NR[2]	NR[3]

NR : No Detectable Residue
NR[1] : Detectable but unquantifiable residue

The average peak heights for γ-BHC in soil samples I to VI were 10, 8.5, 6.5, 16.4, 14.33 and 23.2mm respectively, while those for α-Endosulfan were 41, 30, 8, 8.3 and 15mm for samples I, II, IV, V,, and VI respectively. Sample III had no detectable residue. The concentration of γ-BHC in the soil samples were all approximately 2ppm and above, except for sample V with a concentration of 1.11ppm. However, as for α-Endosulfan, samples I and II had 13.95 and 6.16ppm respectively while there was no detectable residue in sample III and detectable but unquantifiable residues in samples IV to VI. The α-Endosulfan residues whose concentration could not be estimated was as a result of the calibration curve passing through the height axis at about 20mm which was well above the peak heights recorded for the residues. The fact that there was no detectable α-Endosulfan residue in sample III did not necessarily mean that it was not present in the soil sample. It could be that the concentration was too small to be detected at attenuation 64 which was established for the G.C. analysis.

The presence of γ-BHC and α-Endosulfan in the soil samples was not supprising. This could be attributable to the general stability of organochlorine pesticide residues in the soil which also agrees with Starnes[26] who found that, apart from the stability of organochlorine pesticide residues in soil, they were translocated into certain crops grown in γ-BHC treated soil.

Terriers and Ingalsbe[13] further confirmed the residual action of pesticides in soil; residues of γ-BHC, DDT, aldrin, dieldrin were shown to persist in a sandy loam soil for at least three years after pre-treatment.

The absence of DDT in the soil samples did not agree with previous work. DDT is known to be one of the most persistant organochlorine residues in soil. Decker et al[2] reported a 13.2% residue level when soil was analysed one year after the last application. Lichetenstein and Schulz[1] have also reported that for a loamy soil under existing weather conditions aldrin, DDT and dieldrin residues were present at a level of approximately 20% of the total applied over a five-year period. The fact that DDT was not detected might be because it had been degraded into its metabolites DDD or DDE. These could have been enhanced by weather conditions eg humidity, temperature solar UV radiation level and the chemical characteristics of the soil, pH, organic matter content, microorganisms, etc., if one assumes that their individual and collective effect on pesticide residues in the tropics would be different from that in the temperate regions. As already stated, although the general history of pesticide trials in the farm is known, that of the specific trials on the block where the soil samples were collected is not known. As such, the non-detectability of DDT residues could be attributed to the possibility that DDT trials were not carried out on this portion of the farm.

Chapter 4

SUMMARY AND CONCLUSION

The results obtained give some insight to the fact that some pesticide residues are present in the soil. It is a step forward in the assemblage of basic information on pesticide residues in the northern zones of Nigeria. Although the presence of pesticide residues has already been established, the number of samples (six) was small, accordingly, a categorical and statistically valid statement on pesticide residues in the IAR Farm can not be made. It is therefore hoped that further work will be carried out on more samples from this block and later extended to other blocks in the farm.

The presence of pesticide residues in soil will not be of great concern if, they are not translocated into food for human consumption. It is therefore recommended that as a follow-up to the analyses carried out on soil samples, the lakewater, food crops, vegetables and even live-stock which feed on these plants should be analysed for the presence of pesticide residues.

The information thus gathered from all the individual/ collective researches on pesticide residues would help the Federal Government's proposed Environmental Protention Agency in solving some problems of environmental pollution.

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