STUDIES ON THE OCCURRENCE AND SOURCES OF ORGANO-CHLORINE PESTICIDE RESIDUES IN EGGS FROM EMBU AND MERU DISTRICTS

JOHN MUTUA MUGAMBI, B.V.M.,
University of Nairobi

A thesis submitted in fulfilment for the degree of Master of Science in the University of Nairobi.

Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi.

1986

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

.....

J.M. MUGAMBI

This thesis has been submitted for examination with our approval as University supervisors.

.....

PROF. P. LØKKEN, D.D.S, M.Sc., Ph.D.

.....

DR. T.E. MAITHO, B.V.M., M.Sc., Ph.D.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACKNOWLEDGEMENTS

I am very grateful to my supervisors, Professor P. Løkken and Dr. T.E. Maitho for their continued guidance, useful suggestions and positive criticisms. I am also thankful to Dr. J.U. Skaare of the Veterinary College, Norway, for the help she has rendered especially with the counter-checking of some of my samples and in ordering some laboratory equipment.

My sincere thanks also go to Mrs. L. Kanja and Dr. J.K. Kahunyo for their help during the initial stages of this project and to Dr. A.C. Maritim for her assistance with the drawing of figures.

I am grateful to Mr. C.K. Baranya of the Ministry of Agriculture, Embu and some of his staff members for introducing me to farmers during my initial sample collection ventures.

I thank the technical staff of the Department, particularly C.M. Kamau, F.K. Gitau, K.S.N. Nyang'au and B.R. Ndubai for their assistance during this study.

I cannot forget Mr. N.O. Ooko of the Kenya Polytechnic, Nairobi, for his assistance with some statistical work. Not least are all those friends and relatives, who have been a constant source of encouragement to me.

The excellent work done by Mr. J.K. Mbugua, of typing this thesis is gratefully acknowledged.

I extend my thanks to Professor J.M. Gathuma, Chairman of the Department of Public Health, Pharmacology and Toxicology, University of Nairobi, for allowing me to do the research in the Department.

I am greatly indebted to the Norwegian Agency for International Development (NORAD) for the financial support and research grant which enabled this project to be carried out.

ABSTRACT

Studies on the Occurrence and Sources of Organochlorine Pesticide Residues in Eggs from Embu and Meru Districts

Pesticides play an important role in the control of vector-transmitted diseases of man and animals. Their importance is perhaps even greater in the protection of agricultural crops and products. It has been estimated that without their use, our food supply would be reduced by 30 to 50%. However, because of potential health hazards and certain ecological adverse effects, many countries have banned or restricted the use of some pesticides. This applies particularly to organochlorine pesticides such as DDT, which are stable lipophilic compounds that persist and accumulate in food chains. Whether or not a pesticide should be allowed in a particular situation requires a consideration of benefits versus risks. It is important that such decisions are based on sound scientific information rather than on emotional bias. For the evaluation of benefits and risks, it is essential to know whether a population is exposed to unacceptable amounts. If so, the relevant authorities should take steps to reduce the exposure.

In a recent study on organochlorine pesticide residues in 145 eggs and 105 chicken fat samples from 11 districts in Kenya, 12 different residues were

detected (Kahunyo, M.Sc. thesis, University of Nairobi, 1983). The DDT-group showed both the highest incidence and the highest levels. Particularly high were the concentrations in 15 eggs collected in Embu.

The present study was initiated as a more extensive follow-up study centred on Embu and the neighbouring district Meru, which have similar living conditions and agricultural activities. It was aimed at verifying to what extent the findings of Kahunyo were representative. A further intention was to trace the sources of pesticide residues in the eggs, as well as to evaluate the potential health hazards for man and to discuss ways of reducing the exposure.

In the first experimental chapter (Chapter 3) a commercial mixture of 13 pesticides was used to test the ability of the investigator to correctly identify and quantitate the pesticides using gas liquid chromatography. By comparing the elution patterns obtained to those supplied by the manufacturer of the pesticide standard and those of the U.S. Environmental Protection Agency, it was assured that all 13 compounds were adequately resolved. It was further confirmed that the sensitivity was acceptable and that the compounds were adequately quantitated within the relevant range of concentrations.

In Chapter 4 two different extraction and clean-up procedures were compared with regard to recoveries

of pesticides from eggs spiked with the pesticide mixture standard. One was the "old" method so far used in our laboratory (a modification of the method described by de Faubert Maunder *et al.*, *Analyst, 89 :* 168 - 174, 1964). Since this method was rather expensive and time-consuming, it was decided to investigate whether an alternative method might offer advantages in terms of recoveries, purification, time consumption and costs. This "new" method was originally developed for aquatic organisms (Bjerk and Sundby, *Norsk Vet. Tidsskr., 82 :* 241 - 246, 1970).

At a "low" spiking level the recoveries of all 13 compounds were significantly better with the "new" method. For 5 of them the recoveries were excellent, for 3 good and for 5 acceptable, while the recoveries with the "old" method were acceptable for 4, poor for 3 and unacceptable for 6 compounds. At a "high" spiking level both methods gave improved recoveries. The "new" method still gave the best results, although the differences in favour of the "new" method were not as marked as at the "low" spiking level. Experiments to identify where in the clean-up procedure of the "old" method the losses, especially of aldrin occurred, revealed that the recoveries could be improved by changing the ratio of dimethylformamide to hexane from 1:4 to 1:2 at a partition step. Other factors were also found to be critical : For example, the recovery of

dieldrin ranged from 0 to 42% when Florisil was deactivated and the compounds eluted as previously done in the laboratory. An increase in the concentration of deactivating water from 2 to 3%, raised the recovery to 60 to 73%.

Comparison of the overall costs of chemicals, revealed a cost of Ksh. 36 per sample with the "new" method compared to Ksh. 258 with the "old" method. The "new" method also proved to be less time-consuming and it was adopted for the subsequent egg analyses.

It was decided to test whether the "new" method could also be used for extraction and clean-up of pesticides from poultry feed (Chapter 5). The main conclusion was that it is also adequate for this purpose.

Between May and October 1984 a total of 367 eggs and 42 feed samples were collected from 61 farms in Embu and Meru. On each occasion a questionnaire was filled in to obtain information about the use of pesticides on the farms. The results and implications of these findings are presented in Chapter 6.

p,p'-DDT and/or its major metabolite p,p'-DDE, were found in all the eggs. 27% of the eggs had residues at or above the Extraneous Residue Limit (ERL) of 0.5 mg total DDT/kg eggs. The mean DDT concentration 1.15 mg/kg (Range 0.02 - 10.25) of the 156 eggs collected from free-range chickens, was significantly ($p < 0.01$)

higher than the corresponding value, 0.36 mg/kg (0.01 - 2.62) for 211 eggs from enclosed chickens. While the mean ratio of DDT to DDE was 0.53 (0 - 2.03) in the eggs from free-range chickens, it was significantly ($p < 0.01$) higher, 0.97 (0 - 3.00) in the eggs from enclosed chickens. This indicated that the enclosed chickens had a more direct exposure to DDT, while free-range chickens obtained DDT from sources where more of the compound had undergone environmental transformation to DDE. Feed samples from farms with enclosed chickens had a higher mean total DDT-concentration, 0.12 (< 0.01 - 0.71), than the mean concentration 0.03 (< 0.01 - 0.20) in the feed samples from farms with free-range chickens. When the accumulation ratios (levels in eggs to those in corresponding feed) were compared to those reported in the literature, the residue levels in the feed from most of the farms with enclosed chickens were likely to account for the levels in the corresponding eggs. In none of the farms with high concentration in the eggs, however, was the concentration in the feed high enough to account for the egg levels. These chickens must have been exposed to other sources which were not analysed, for example, earth-worms capable of accumulating chemicals such as the DDT-complex or supplementary feed such as cabbages. When correlated to the main agricultural activities in the areas of collection, the highest mean concentration 2.93 mg/kg (0.21 - 10.25) was found in eggs from a

rice-growing area; the mean was lower, 1.39 mg/kg (0.04 - 6.07) in the cotton-tobacco areas; and lowest, 0.34 mg/kg (0.01 - 2.40) in the coffee-tea areas. It was difficult to obtain reliable information on the pesticide usage, especially from the farmers in the cotton-tobacco and rice areas. In the coffee-tea areas there seemed to be more frequent use of the more expensive but less persistent organophosphate and carbamate pesticides. This represents a possible explanation for the relatively low DDT-content in most eggs from these areas. In contrast to p,p'-DDT and p,p'-DDE, the two other members of the DDT-complex, o,p'-DDT and p,p'-DDD, were mostly found in low concentrations if at all detected.

The cyclodiene group includes aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide. Dieldrin was found in all the eggs from enclosed chickens, with a mean concentration of 0.16 mg/kg (0.01 - 1.44) which exceeds the ERL of 0.1 mg dieldrin/kg eggs. Although 12% of the eggs from free-range chickens had no detectable amounts of dieldrin, the mean value was as high as 0.61 (< 0.01 - 14.90). While the present results agreed well with Kahunyo's findings with regard to DDT, the high dieldrin values contrasted sharply with his findings, since he found most of the eggs negative for dieldrin. The method previously used in our laboratory might have failed to detect the compound as a

result of the compound's retention in the Florisil column. Another possible explanation is that the use of dieldrin and/or aldrin has started recently. The mean dieldrin concentration was highest in the eggs from the rice-growing area 1.12 mg/kg (< 0.01 - 14.90), 0.31 (< 0.01 - 9.74) in the coffee-tea areas and 0.15 (< 0.01 - 0.69) in the cotton-tobacco areas. Not a single farmer reported use of dieldrin, although it was found in most of the eggs. Aldrin is a possible source, since dieldrin is a metabolic product of aldrin. Aldrin was detected in only two eggs, and it is noticeable that these eggs also had the highest dieldrin concentrations. The two compounds occurred in 55% of the feed samples, but since the egg-to-feed accumulation ratio was low, the feed levels could not account for the amounts in most of the corresponding eggs. Endrin occurred in 14 eggs, while neither heptachlor nor heptachlor epoxide were detected in any of the eggs.

Technical grade hexachlorocyclohexane (HCH) consists of a number of stereo-isomers. Lindane contains about 99% of the γ-isomer. Detectable amounts of γ-HCH occurred in 66% of the eggs. The mean γ-HCH concentration in the eggs from enclosed chickens was 0.01 mg/kg eggs (< 0.01 - 0.04), compared to 0.03 mg/kg (< 0.01 - 0.53) in eggs from free-range chickens and 8% of these were at or above the ERL of 0.1 mg lindane/kg eggs. As with DDT and dieldrin, the highest concentra-

tions were found in eggs from the rice-growing area. Lindane was reported to be widely used, especially on cabbages, and was also frequently detected in feed samples. Generally, the feed levels agreed well with the concentrations in eggs from enclosed chickens, but the relationship was less evident with eggs from free-range chickens. The α-isomer was quantifiable in 5% of the eggs and the β-isomer in 9% of the eggs.

PCBs (polychlorinated biphenyls) and HCB (hexachlorobenzene), could be detected with the analytical method used, but all the samples were negative for these compounds. PCBs are industrial chemicals which during the last 10 to 15 years have been recognized as environmental contaminants in industrialized countries, and so has also the fungicide and industrial product HCB. So far they do not seem to represent a pollution problem in the developing countries, and the present results support this view.

The most noticeable findings in the present study were with respect to the DDT-complex and dieldrin. The egg levels of the DDT complex found in the present and in Kahunyo's study, are much higher than those reported from most other countries, and only these two Kenyan studies have given mean values exceeding the ERL. Even more alarming is the finding of high levels of dieldrin in the present study.

Evaluation of the potential health hazards is difficult, however, since it is uncertain to what extent levels such as those reported here, can cause chronic toxicity in mammals, and if so, which manifestation may occur. Practically all these compounds are acutely neurotoxic. Whether or not exposure to these compounds can impair the normal development and functioning of the central nervous system, is one of the uncertainties. Our methods are at present very imprecise for detecting minor or even moderate disturbances in many of the numerous brain functions. In any case, special attention should be paid to the amounts to which the younger age groups are exposed.

Based on an overall evaluation of the available information, the joint FAO/WHO Codex Alimentarius Commission has drafted standards intended to allow proper use of pesticides without endangering the health of the public; as well as Extraneous Residue Limits (ERLs) for pesticide residues in foodstuffs, and limits for the Acceptable Daily Intake (ADI). The ADI for man, expressed on a body-weight basis, is the amount of a pesticide that can be taken daily without appreciable risk, and it should not be exceeded over a prolonged time.

Although the approach might be somewhat arbitrary, the present findings were related to the ADIs, to give a certain illustration of the exposure. The ADI of DDT is 0.005 mg/kg. On an average the DDT-content

of each egg was one-tenth of the ADI for an adult (70 kg) and half of the ADI for a 3 year-old child (15 kg). The amount of DDT in the egg with the highest concentration was 1.5 times the ADI for an adult and 7.5 times the ADI for the child. The ADI of dieldrin is as low as 0.0001 mg/kg. On the average the dieldrin content of each egg was 2.7 times the ADI for an adult and 13 times the ADI for a 3 year old child. The amount of dieldrin in the egg with the highest concentration was 117 times the ADI for an adult and 547 times the ADI for the child. It is of course likely that they might get additional residues from other sources.

Accordingly, the present findings indicate that there is a need to ensure improved practices in the use of some chlorinated hydrocarbon pesticides, especially aldrin/dieldrin, at least in parts of Kenya. The answer to the question of whether or not these compounds should be banned is less obvious.

CHAPTER ONE

INTRODUCTION

Pesticides are important in the control of vector-borne diseases of man and animals. They are perhaps even more important in the protection of crop and crop products. Without the use of pesticides it is estimated that up to 50% of our agricultural food supply would be lost (Tschirley, 1979).

On the other hand, the pesticides, especially the persistent fat-soluble chlorinated hydrocarbon insecticides, leave residues in crop and animal products. Concern about these residues arises because the chemicals are poisonous in nature and hence pose some potential health hazards. Since these compounds tend to accumulate along food-chains, the threat is greatest to the individuals at the top of contaminated ecosystems. In addition the chemicals present some ecological adverse effects, for instance, an increase in the target pests following the destruction of their natural enemies by the pesticides (Hill, 1983).

Accordingly, many countries have restricted or banned the use of pesticides. In Western Europe, the use of DDT was discontinued in the 1970's (Fimreite *et al.*, 1982). The U.S. Environmental Protection Agency (EPA) allows the use of DDT only in special

situations and has placed similar restrictions on other chlorinated hydrocarbon insecticides (EPA, 1985). Most developed countries have also set up national laboratories to monitor the residue levels in crop and animal products. Further, they have set the maximum allowable levels in various foodstuffs to protect the consumers. Such decisions on whether to restrict or ban a pesticide in a given situation should be based only on scientific evidence as should the setting of safety limits.

The Kenya Bureau of Standards has not come up with any limits (Olielo, 1985. Personal communication), but as in several other countries which have not yet established their own limits, the standards set by the joint FAO/WHO Codex Alimentarius Commission are used as guidelines. However, the Government has created a Pest Control Products Board under the Pest Control Products Act of 1982 to control pesticides during manufacture, distribution and application. The Board's main objectives are (Omamo, 1983):

i) To assist in the national goal of self-reliance in food production without endangering the environment.

ii) To educate people on the safe use of pest control products without risking their lives.

Although there has been widespread use of organochlorine pesticides in Kenya, only a few publications have dealt with the residue situation. Fish-eating birds of Lake Victoria were analysed in 1968 (Koeman and Pennings, 1970). In the early 1970's human adipose tissues obtained at autopsy were examined (Wassermann *et al.*, 1972). At about the same time and extending up to mid 1970's studies involving wild birds, plants and water were carried out (Koeman *et al.*, 1972; Lincer *et al.*, 1981; Frank *et al.*, 1977; Greichus *et al.*, 1978).

Some foodstuffs have also been analysed: non-fatty foods and feeds (Muinamia, 1976) and cow's milk and body fat (Maitho, 1978). Several residues notably DDE, DDT and dieldrin were found in these studies, but generally the concentrations were within acceptable limits.

A more recent study on pesticide residues in chicken fat and eggs (Kahunyo, 1983) gave somewhat more alarming results. In 145 eggs and 105 chicken fat samples from 11 districts in Kenya, 12 different residues were detected. In chicken fat, there were a few individual high values of lindane, dieldrin and DDT, but the mean values were low. In eggs, the DDT-group showed both the highest incidence and the highest levels, of the compounds detected. The average concentration was 0.61 mg total DDT/kg eggs, which exceeds the

international Extraneous Residue Limit (ERL) of 0.5 mg/kg. Particularly high were the concentrations in eggs from Embu, where the mean concentration of total DDT in the 15 eggs collected was 3.52 mg/kg.

The present work was initiated as a more extensive follow-up study centred on Embu and the neighbouring district Meru which have similar living conditions and agricultural activities.

The main objectives were:

- to verify to what extent Kahunyo's findings were representative,
- to attempt to establish the sources of contamination by analysing feed samples and interviewing farmers,
- to evaluate potential health hazards for man and ways of reducing overexposure,
- to investigate whether an alternative extraction and clean-up procedure of pesticides from eggs could be advantageous to the method so far used in our laboratory,
- to select an appropriate method for extraction and clean up of pesticides in poultry feed,
- to obtain quality assurance for the investigator's ability to correctly identify and quantitate pesticides by means of gas liquid chromatography.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Pesticides are used to control crop pests and thus improve food production for the increasing world population. They also improve man's health by controlling vectors of diseases like malaria, bubonic plague and typhus. Further, they are useful in controlling some diseases of domestic animals such as East Coast Fever, anaplasmosis and mange.

Several chemicals have in the past, been used on a limited scale to control pests. Sulphur was used for the prophylaxis of plant diseases as early as 1000 B.C., and by the 16th century the Chinese were employing moderate amounts of arsenic as an insecticide (Tschirley, 1979; Cremlyn, 1980). Prior to World War II, pesticides were of two types, the inorganic ones like arsenicals and fluoride compounds and the organic ones of plant origin such as rotenone and the poisonous nicotine products (Spindler, 1983).

Large scale application of pesticides dates from the time of the War, when the organochlorine pesticides were introduced. This class of pesticides is broad and it includes the chlorinated hydrocarbon insecticides, which are divided into the DDT, hexachloro-

cylohexane (HCH) and cyclodiene groups, and they were introduced in that order. DDT is usually taken as the prototype of the chlorinated hydrocarbon insecticides. Although both this compound and HCH were synthesized in the 1800's, it took long before their insecticidal properties were recognized.

The first studies of the acute and subacute oral and percutaneous toxicity of DDT in laboratory animals and humans demonstrated that the compound was only slightly toxic to mammals and man (Domenjoz, 1944). The compound was shown to be very poorly absorbed through the skin and it is on this fact that the compound's safety record mainly rests. The low acute mammalian toxicity, broad spectrum of action and long residual activity made DDT unique. The compound owes its excellent insecticidal activity, particularly its residual effect, to its low vapour pressure (1.5×10^{-7} mm Hg at $20^{\circ}C$), its high fat solubility (about 100,000 ppm), its extremely low water solubility (0.0012 ppm at $25^{\circ}C$) and its stability against photo-oxidation (Spindler, 1983).

During and immediately after World War II, conditions were particularly favourable for the DDT insecticides. The insecticides were effectively used to control various pests of crops and crop products. They also proved invaluable for the prophylaxis and

control of vector-borne diseases, for example, the unique success obtained with DDT in the control of malaria vectors. Following the global malaria eradication programme initiated by WHO in 1957, malaria was eradicated by 1970 in several countries such as China, Australia, U.S.S.R. and U.S.A. 800 to 1000 million people living in these originally malaria infested areas were thus freed from the risk of the disease (WHO, 1971).

In the course of time and with the development of sensitive analytical methods the persistence and wide distribution of these chemicals in the environment was finally perceived. The very factors that made DDT such an effective insecticide were responsible for this persistence (Metcalf, 1973). It was established that the compounds were ubiquitously distributed in the environment, stored in animal tissues, excreted in milk, retained as residues on food crops and could damage the livers of rodents (Radeleff, 1970).

During the 1950's and 1960's, the organophosphate and the carbamate pesticides were introduced (McEwen and Stephenson, 1979). The organophosphates (and the carbamates) are more susceptible to enzymatic attack than organochlorines and therefore leave little or no residues on or in living organisms (Huang, 1973). These two classes of pesticides are increasingly replacing the chlorinated pesticides.

2.2 CHLORINATED HYDROCARBON INSECTICIDES

2.2.1 DDT group

The most important insecticide in this group is p,p'-DDT. The technical DDT product also contains o,p'-DDT. Apart from these isomers, the DDT group includes p,p'-DDE and p,p'-DDD. Both compounds are p,p'-DDT metabolites. The major metabolite is p,p'-DDE. p,p'-DDE is as persistent as p,p'-DDT, but has practically no insecticidal activity. In contrast, p,p'-DDD is also commercially available as an insecticide.

There are other compounds that are chemically and structurally related to DDT such as dicofol.

Common name - DDT

Chemical name - 1,1,1-trichloro-2,2-di-(p-chlorophenyl)ethane.

Uses* - control of vector-borne diseases like malaria and anaplasmosis.
- control of ectoparasites like mites and lice.
- control of agricultural and forest pests.

Common name - DDD (TDE)

Chemical name - 1,1-dichloro-2,2-di-(p-chlorophenyl) ethane.

Uses - as for DDT
- control of different forms of adrenal over-production of corticoids.

Common name - DDE

Chemical name - 1,1-dichloro-2,2-di-(p-chlorophenyl) ethylene.

* information on the uses of DDT compounds and other chlorinated hydrocarbon insecticides was obtained from Soulsby (1978) and Hayes (1982).

2.2.2 *Cyclodiene group*

The main cyclodiene compounds are aldrin, dieldrin, heptachlor and isodrin. They are highly insecticidal cyclic hydrocarbons which are formed by the Diels-Alder reaction (O'Brien, 1967). Some of these compounds can be epoxidised *in vivo* or *in vitro* to give analogues which are also insecticidal. The epoxides of aldrin, isodrin and heptachlor are dieldrin, endrin and heptachlor epoxide respectively.

Common name - Aldrin

Chemical name - 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene

Uses - control of ectoparasites like bed bugs.
- seed dressing.
- mixed with fertilizer for control of soil pests.
- control of insects of forage, vegetable and fruit crops.

Common name - Dieldrin

Chemical name - 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene.

Uses - control of ectoparasites like bed bugs.
- control of vector-borne diseases like malaria.
- control of soil pests.

Common name - Endrin

Chemical name - 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4 - *endo*, *endo*-5,8-dimethanonaphthalene

Uses - control of crop pests.

Common name - Heptachlor

Chemical name - 1,4,5,6,7,8,8-heptachloro-3a, 4,7, 7a-tetrahydro-4,7-methanoindene

Uses - control of soil and cotton pests.
- control of grasshoppers

Common name - Heptachlor epoxide

Chemical name - 1,4,5,6,7,8 ,8-heptachloro-2,3-epoxy-3a, 4,7,7a-tetrahydro-4,7-methanoindene

2.2.3 Hexachlorocyclohexane (HCH) group

HCH can theoretically exist as at least 8 different isomers, but the technical product has only 5 of these (2 optical α- isomers, β-, γ- and δ), with the α-, β- and γ- isomers being the most common. A specific steric configuration is necessary for insecticidal activity, since only the γ-isomer (lindane) is a powerful insecticide.

Common name - Lindane

Chemical name - gamma-1,2,3,4,5,6-hexachlorocyclohexane

Uses - control of ectoparasites such as fleas and mites.

- control of soil and crop pests and wire worms.
- control of grasshoppers.

Common name - α-HCH

Chemical name - alpha-1,2,3,4,5,6-hexachlorocyclohexane.

Common name - β-HCH

Chemical name - beta-1,2,3,4,5,6-hexachlorocyclohexane.

2.3 ENVIRONMENTAL ASPECTS

Organochlorine pesticide residues are ubiquitous - they occur everywhere in the global environment. They accumulate in the fat of fish, birds and terrestrial mammals.

The pesticides enter the environment from various sources and are transported mainly by air and water. They are picked up by biological and other systems and transformed into other compounds (Matsumura, 1975).

Great concern for the implications of pesticide residues in the environment was initiated by the publication of Carson's book the *Silent Spring* in 1962. Much of this concern has centred on aquatic ecosystems and wild birds. In several cases when used in aquatic or forested areas, these chemicals

killed fish and accumulated in some predatory birds, in concentrations detrimental to the reproduction of the birds (McEwen and Stephenson, 1979).

The greater part of the research on these chemicals has been performed in temperate climates. In tropical conditions, early disappearance of DDT from the environment is favoured by more rapid degradation and volatilization (El Zorgani, 1976). Photo-chemical degradation, a process by which DDT absorbs ultra-violet light from the sun and is transformed to DDE, DDD, di-(p-chlorophenyl)acetic acid (DDA), 2,2-dichloro-diphenyl-1-monochlorinated unsaturated ethane (DDMU) and other compounds is one way of changing the compound in the environment (Crosby and Moilamen, 1977). Volatilization of persistent pesticides may influence the contamination levels in the environment, far from the initial place of use (Perfect, 1980).

2.4 TOXIC AND OTHER EFFECTS

The majority of insecticides exert their toxicity primarily on the nervous system. They reach the animal tissues percutaneously, by inhalation when the animals are in contaminated environments, or by gastrointestinal absorption when contaminated feed is consumed.

2.4.1 Insects

Toxicity - Insects have a well developed nervous system, almost comparable in organization to that of mammals. Generally, the success of an insecticidal compound depends on the degree of nerve development of the target insect (Matsumura, 1975). DDT is an efficient contact insecticide because it readily penetrates the chitinous insect cuticle. It is less toxic to mammals since the skin and mucous membranes are relatively impermeable to the pesticide (Clarke *et al.*, 1981). In some cases, differences in susceptibility to nerve poisons between insects and mammals is partly attributable to morphological differences in the pattern of nerve distribution. For instance, insects possess several nerve endings that are exposed without protection, while mammals have relatively few of these (Matsumura, 1975).

Chlorinated hydrocarbon insecticides are non-specific stimulants of the central nervous system (CNS). They have no stereo-selective receptor that has yet been identified and therefore there are no known antagonists (Hatch, 1982). The manner in which these compounds exert their toxic effects is not known either. Probably they do so by binding to the nerve membrane and interfering with sodium and potassium ion balances across the membrane. In this way the compounds disrupt the normal transmission of nervous

impulses, resulting in tremors, incoordination, convulsions, prostration and death. Although the mode of action of these chemicals appears to be the same, there is evidence indicating that while HCH and cyclodiene compounds probably act on the same site, DDT acts on a different site (Cremlyn, 1980).

Resistance - Resistance is the ability of a given strain of insects to tolerate doses of an insecticide which would kill the majority of a normal population of the same insect species. Resistance to a particular insecticide is more widespread if the toxicant is persistent and the insect has a short-life cycle. Several factors have been proposed to account for the phenomenon, two of which are:

(a) Morphological characteristics like the development of thick impermeable insect cuticles.

(b) Increased detoxification. This is the more important factor. In DDT resistant insect strains, tolerance is often due to an abnormally high concentration of the enzyme, DDT-dehydrochlorinase which converts DDT to the non-insecticidal DDE. Another detoxification mechanism is the conversion to dicofol, a compound used commercially as an acaricide, but without general insecticidal activity (Cremlyn, 1980).

2.4.2 Fish

Chlorinated hydrocarbon insecticides are also very toxic to fish, their lethality being comparable only to that of rotenone. The acute oral LD_{50}'s of DDT, dieldrin or lindane established by various investigators for several fish species such as rainbow trout and blue gill are far less than 1 mg/kg (Matsumura, 1975; McEwen and Stephenson, 1979). Fish exhibit a high bioaccumulation ratio of the chemicals present in water. In case of DDT, this ratio ranges from 2,500:1 to 3000:1 (El Zorgani, 1980). They are also known to have inefficient mixed-function oxidase systems to detoxify these insecticides (Matsumura, 1975).

The pesticides may also affect the behaviour of fish. For instance, brooktrout placed in water contaminated with DDT failed to distinguish between the dark and light sides of the water tank (Anderson and Peterson, 1969). Other species of fish such as sheepshead minnows have inherent abilities to detect and avoid some pesticides like DDT and endrin (Hansen, 1969).

The compounds also cause hatchery losses of fry lake trout when the fry absorb contaminated yolk-sacs (McEwen and Stephenson, 1979).

2.4.3 Laboratory animals

Intoxications with chlorinated hydrocarbon pesticides elicit several symptoms such as hyperirritability, muscular spasms, convulsions, salivation, abnormal postures and ruffled fur. In DDT intoxications convulsions usually appear terminally, while in intoxications with HCH and cyclodiene compounds, convulsions come first.

The pesticides might also cause marked changes in the livers of rodents such as induction of microsomal enzymes and an increase in smooth endoplasmic reticulum of hepatocytes, with a resultant liver hypertrophy. Some of the changes may also lead to tumour formation, especially in the mouse (Hayes, 1982).

2.4.4 Domestic mammals

Domestic mammals are usually poisoned owing to man's careless handling of the chemicals. Occasionally, poisoning occurs when farm animals are fed with heavily contaminated plants (McEwen and Stephenson, 1979). In normal cases, it is unlikely that there will be a residue build-up large enough to cause intoxication.

The signs elicited in poisoned animals are the same as for laboratory animals. The order in which the signs occur may, however, vary with the insecticide

and with the species of animal (Radeleff, 1970).

2.4.5 Wild mammals

There is little evidence of DDT's toxicity to wild mammals except bats. In contrast some cyclodiene compounds often cause extensive mortalities to the mammals. For instance, some species like ground-squirrels were eliminated and others such as fox-squirrels and wood chucks killed in large numbers, when dieldrin was used to control the Japanese beetle in Illinois, U.S.A. (Scott *et al.*, 1959).

The effect of dieldrin, added at 5 and 25 ppm in the diet for 3 years, on the physiology and growth of the white-tailed deer has been reported. Fawns from dieldrin-fed does were smaller at birth and had greater post-partum mortalities than fawns fed the untreated feed, but the fertility of the male progeny was unaffected (Murphy and Korschgen, 1970).

2.4.6 Man

Acute toxicity

As illustrated in Table 1, p. 19, pesticides such as DDT appear to be surprisingly non-toxic to man. Nevertheless, the available records indicate that pesticides represent a major health concern

Table 1 Dosage-effects of DDT in man (WHO, 1979. Slightly modified by Spindler, 1983).

Single dose (mg/kg)	Observations and remarks, respectively
Unknown	fatal
16-286	prompt vomiting at higher doses (all poisoned, convulsions in some).
10	moderate poisoning in some.
6	moderate poisoning in one person.
Repeated doses and exposure, respectively, tolerated without any adverse effects.	
mg/kg/day	
1.5	orally administered as therapy for 6 months.
0.73*	from breast-fed infants in Guatemala, 1971 (average intake with human milk).
0.5	from volunteers for 21 months.
0.5	from workers for 6.5 years.
0.25	from workers for 25 years.
0.02*	from breast-fed infants in Basle, 1978 (average intake with human milk).
0.0025	from the general population in the U.S.A.,1953/54.
0.0004-0.00007	from the general population in England, Canada and U.S.A., 1969-1977.

*Exact duration of the lactation period not known (approx. 3 - 6 months.

(McEwen and Stephenson, 1979). Estimates that are not very recent show that accidental cases of poisoning by pesticides are about half a million per annum on a global basis. This figure has been associated with a mortality rate of 1% in those countries where medical treatment and antidotes are readily available, but the rate is possibly higher in other countries (Copplestone, 1977).

By far the majority of poisonings are due to organophosphorus insecticides and as the use of these is on the increase with the continued withdrawal of the organochlorines, the number of poisonings is also increasing. Signs of poisoning may vary from mild ones such as headache, nausea and dizziness to complete incapacity and convulsions (McEwen and Stephenson, 1979). Parathion has been documented to cause acute but reversible injury to human kidney tubules (Morgan and Roan, 1969).

Intoxications with chlorinated hydrocarbon insecticides give similar signs to those observed with organophosphates. An insecticide may, however, have a particular order in which the signs occur. The earliest sign of DDT poisoning is hyperaesthesia of the mouth and lower part of the face. Thereafter others, such as dizziness, disturbances of equilibrium, tremor of the extremities, confusion, headache and vomiting

follow. Convulsions occur only in severe poisoning. Intoxication with lindane is shown as malaise and dizziness followed by collapse and convulsions. Several case studies of dieldrin poisoning indicate that convulsions almost always precede other signs. As in epilepsy, non-continuous fits occur in people with high dieldrin levels. This compound may also cause temporary behavioural changes (Hayes, 1982). Illness after over-exposure to endrin, heptachlor and other chlorinated insecticides have been reported in applying, mixing and formulating the chemicals.

The carbamates are the most recent widely used group of insecticides and exhibit a wide range of mammalian toxicities. Illnesses have occurred with their use but not to the same extent noted for organophosphates.

With organochlorine insecticides, diagnosis of poisoning is not easy and recovery is sometimes protracted. In contrast, with poisoning by organophosphate and carbamate insecticides, recovery is fairly rapid (McEwen and Stephenson, 1979).

Chronic toxicity

Chronic effects on humans have been the subject of an immense amount of speculation with little documentation. Speculation has mostly centred on DDT (McEwen and Stephenson, 1979).

Neurological effects - In acute toxicity, the CNS is mainly affected and it can also be affected in chronic toxicity. Practically all insecticidal organochlorine compounds are neurotoxic. Their build up in the CNS begins very early in its complex ontogeny. Whether this can impair the normal CNS development and functioning is not known because of deficient methods of measuring these sophisticated functions (Berlin *et al.*, 1979).

Although the exact mechanism of toxicity is unknown, the available evidence indicates that DDT and possibly other chlorinated hydrocarbon insecticides act by changing the electrophysiological and associated enzymatic properties of nerve cell-membranes, especially axonal ones. It is clear that changes in the concentrations of biogenic amines (noradrenaline, serotonin, etc) parallel the toxicity of the insecticides. Dieldrin is known to alter the electroencephalogram (Hayes, 1982). Of the HCH compounds, β-HCH is the most stable and persistent and has the highest chronic toxicity (Swedish National Food Administration, 1983).

Enzyme-induction - DDT can induce microsomal enzymes in man's liver (O'Brien, 1967) and in a number of other organs (Berlin *et al.*, 1979). Such induction can hasten the metabolism of steroids and thus exert effects,

for instance, on reproductive performance. In addition, the metabolism of some drugs can be affected. In contrast to DDT, aldrin and dieldrin are very poor enzyme inducers in man (Hayes, 1982).

Miscellaneous effects - HCB is known to impair haem synthesis (Berlin *et al.*, 1979). The possibility that chlorinated hydrocarbon insecticides are carcinogenic, teratogenic or mutagenic in man has been advanced but there is no firm evidence (Barnes, 1976; McEwen and Stephenson, 1979). Attempts have also been made to implicate pesticides in some human diseases such as aplastic anaemia and asthma. However, the limited epidemiological evidence available neither incriminates nor exonerates them (McEwen and Stephenson, 1979).

Benefits versus risks

Although the chemicals are potentially toxic, an extensive review by Spindler (1983) portrayed DDT as one of the safest and least hazardous insecticides in handling and application. Clinical studies in humans as well as examinations of production workers and spraying personnel exposed to DDT for up to 25 years revealed no ill-effects. McEwen and Stephenson (1979)

estimated that a 70 kg man would have to consume 14 g of DDT for a lethal dose, on the basis of the calculated LD_{50} of the rat of 200 mg/kg body weight. More direct studies on volunteers have shown that the oral dose of DDT necessary to produce any sign or symptom is at least 10 mg/kg. Even the more toxic cyclodiene compounds such as dieldrin require massive doses to cause death, and when handled with care are unlikely to be hazardous to man (Hayes, 1982).

Carelessness in handling the chemicals is responsible for most poisonings. Children are particularly at risk when opened and empty containers are accessible to them (Copplestone, 1977).

Without the use of pesticides, it is estimated that there would be a 50% loss in food production per annum (Tschirley, 1979). Brooks (1974a) concluded that DDT was by then still essential in the control of vectors of diseases like malaria, bubonic plague and typhus, especially in the Far East, because of its effectiveness and cheapness. This conclusion might still be valid (Spindler, 1983).

In relation to the quantities used and the benefits derived from it, DDT's safety record for man is outstanding (Brooks, 1974b). This is probably true also for other chlorinated hydrocarbon insecticides.

Medical applications - Apart from the use of DDT and other insecticides in the control of insect-borne diseases like malaria, there are other potential uses. Thompson *et al.* (1969) used DDT as an enzyme inducer, at a daily oral dose of 1.5 mg/kg body weight to treat a boy suffering from unconjugated jaundice. No side effects were noted during the 6 months of treatment and plasma bilirubin remained low for at least 7 months after cessation of medication. DDD has been used to control adrenal over-production of corticoids (Hayes, 1982).

2.5 RESIDUE STUDIES IN KENYA

Only a few studies have been published. It is possible that there are other studies which are unpublished or unavailable. Muinamia (1976) studied the organochlorine and organophosphorus pesticide residues in non-fatty foodstuffs and feedstuffs. Maitho (1978) studied the two kinds of residues in cow's milk and bovine fat in some parts of the country. In both studies low levels were reported. Kahunyo (1983) carried out a study on eggs and chicken fat in 11 districts. A few individual values of lindane, dieldrin and DDT were high in chicken fat, but the mean values were low. In eggs, however, the mean DDT value, 0.61 mg/kg eggs, was above the ERL of 0.5 mg/kg.

In 1972, Lincer *et al.* (1981) examined the flora and fauna of Lakes Nakuru, Naivasha, Elmenteita and Baringo in the Rift Valley. The vegetation, aquatic insects and plankton of Lake Elmenteita contained higher DDE levels than in the other lakes. Of the other lakes, Lake Nakuru had the highest DDE residues particularly in fish and frogs. Other compounds like toxaphene were suspected but the concentrations were too low for accurate determination. In another study on Lake Nakuru, the lesser flamingo, white pelican and cichlid fish, were examined for insecticides and metals (Koeman *et al.*, 1972). DDE, DDT and dieldrin were detected, with dieldrin having the highest value recorded, 0.091 mg/kg, in the liver of a white pelican. In yet another study on the lake, bottom sediments, algae and fauna were sampled and analysed (Greichus *et al.*, 1978). p,p'-DDE was the most prevalent residue. The other residues found were DDD, DDT and dieldrin. In all these studies, the levels found indicated that the chlorinated compounds were not important pollutants at the time.

An earlier study on fish-eating birds collected along the shores of Lake Victoria, a non-Rift Valley lake, revealed higher levels of dieldrin and DDE. At that time, dieldrin was in use against tse-tse flies, and DDT was used on cotton (Koeman and Pennings, 1970).

Frank *et al.* (1977) studied the residues in raptors from agricultural and non-agricultural areas. Most raptors from the agricultural areas contained residues of DDT metabolites, and several had dieldrin. Raptors from non-agricultural areas contained very low levels of the pesticides.

In man, Wassermann *et al.* (1972) analysed 83 adipose tissue samples obtained at autopsy. DDT and its derivatives, β-HCH, dieldrin and heptachlor were detected. There was a positive association between the concentration of DDT derived residues and increasing age of those investigated.

There is no published data on the residue situation in human milk in Kenya, but there is a study that is currently in progress (Kanja, 1986. Personal communication).

2.6 PESTICIDES AND WILD BIRDS

2.6.1 Acute toxicity

Like in other species, the acute toxicity of pesticides to avians is mainly reflected on the nervous system. Death may occur when involuntary processes such as heart-beat and respiration are no longer controlled as a result of intoxication.

2.6.2 Chronic toxicity

It was not until after several years' use of chlorinated hydrocarbon insecticides that population declines in predatory birds became obvious. It took even more years before the cause-effect relationship could be verified. It was only then that a correlation between pesticide levels in birds and thin egg-shells was shown, and by working backwards, it has been possible to date when the problem first appeared (McEwen and Stephenson, 1979). Chronic toxicity of these compounds to birds is primarily reflected as egg-shell thinning, egg breakage and poor reproductive success, principally involving carnivorous birds. The egg-shell thinning parameter has received the greatest prominence.

Egg-shell thinning - Ratcliffe (1967, 1970) reported that since 1950 egg shell breakage occurred with unprecedented frequency in the nests of some British birds. A decrease in egg-shell thickness or weight paralleled the regional pattern of contamination with DDT and lindane during this period when the compounds were introduced for veterinary and agricultural use (Ratcliffe, 1970). The pesticides were implicated in the decline of wild bird populations in North America during the same period (Hickey and Anderson,

1968). The introduction of cyclodiene insecticides after 1955 caused further contamination and probably increased the initial egg-shell change. The increased use of PCBs during the post-war period contaminated the environment further, and these compounds probably also contributed to the change (Ratcliffe, 1970).

Poor reproductive success of peregrine falcons in California U.S.A is still occurring. This is because of the continued heavy usage of DDT-contaminated dicofol on citrus crops. As a result there are elevated DDE levels that have remained so since 1969, which cause egg-shell thinning and hence poor nesting success (Interior department of the office of endangered species, fish and wild life service, 1986). The mechanism of egg-shell thinning is yet unknown, but there has been a number of postulates.

2.6.3 Factors affecting accumulation

Dietary habits, agricultural practices and proximity of pesticide manufacturing plants to birds' habitat are important factors that affect accumulation in wild birds. Henny *et al.* (1977) examined eggs from two bird species one year after application of DDT in an area in U.S.A. Eggs from one of the species had 10 times the residues in eggs from the other. The

difference was attributed to the differing dietary habits. The same explanation was given by Niethammer *et al.* (1984) for residue levels observed among three heron species from Lousiana, U.S.A. Residues of DDE, DDD, toxaphene and PCBs in green-backed herons were significantly higher than those in the other two species. This species fed primarily on small fishes that normally have higher residue levels than cray fish on which the species with the least residues mainly fed.

Wild birds' eggs collected from areas of intensive agriculture are likely to contain higher residue levels than eggs from areas with extensive or no agriculture. This has been shown for birds in Kenya and Zimbabwe (Frank *et al.*, 1977; Tannock *et al.*,1983). p,p'-DDE was the most prevalent residue.

The proximity of birds to pesticide manufacturing plants also influences the residue levels in the birds. The nearer to the plant the birds live, the higher the residue levels (Fleming and Cromartie, 1981; Yadav *et al.*, 1981).

Since chlorinated hydrocarbon pesticides were banned in the industrialised countries, the residue levels in various samples have been exhibiting a downward trend. Cain (1981) found a significant decrease of DDT residues in wings of adult mallards

and black ducks in U.S.A. analysed during 1979/80 period compared to the levels reported in 1976. In a similar study in Norway, significantly lower values of HCB, p,p'-DDE and total PCBs were reported in puffin livers and brains in 1983 than in 1982 (Ingebrigtsen *et al.*, 1984).

2.7 PESTICIDES AND POULTRY

2.7.1. Toxicity

There is no available information on the acute or chronic toxicity effects of pesticides in poultry under natural conditions, although it can be assumed that what applies to wild birds can also apply to domestic birds. It has, however, been suggested that domestic birds may be less sensitive to sublethal effects such as egg-shell thinning, than wild birds. It has further been suggested that stressful conditions such as poor weather and fasting may make wild birds more susceptible to pesticide toxicity than domestic birds (Koeman *et al.*, 1967; Ecobichon and Saschenbrecker, 1969).

2.7.2. Chicken eggs and poultry feed

This aspect is more fully covered in Ch. 6, p. 158.

Pesticide residue data in eggs and poultry feed from various countries is given in Tables 20, p. 177 ; 21, p. 178 ;22, p. 179;23, p. 180 and 24, p. 181, and a comparison between levels in Kenyan samples and those from other countries presented.

2.8 FEEDING EXPERIMENTS

2.8.1 Egg-shell thinning

The experimental results on pesticide exposure and egg-shell thinning are conflicting. Smith *et al.*, (1970a) found a decrease in shell thickness after feeding 10 ppm of technical DDT in the diet for 2 months, to 2 year old laying hens. The same observation was made with hens of similar age, when fed a diet containing 10 or 50 ppm of p,p'-DDT and with pullets fed 10 ppm of either DDT or lindane in the diet for 10 weeks (Cecil *et al.*, 1973; Sauter and Steele, 1972). Other researchers have reported pesticide-related decreases in shell thicknesses of eggs from various species. Bitman *et al.* (1969), working with pullets of Japanese quail found that o,p'-DDT or p,p'-DDT, added at 100 ppm in the diet were equally effective in causing the egg-shell change. This was also demonstrated for p,p'-DDE, when fed to mallards at 10 or 40 ppm in the ration for over a year (Heath *et al.*, 1969).

A decrease in shell thickness has also been reported with dieldrin, when a ration containing 4 ppm of the compound was fed for 90 days to mallards (Muller and Lockman, 1972).

In contrast to these reports, Cecil *et al.* (1973) found an increase in egg-shell thickness when pullets were fed a diet containing 10 or 50 ppm of technical DDT or 50 ppm of p,p'-DDT for 40 weeks.

Yet other studies showed the pesticides to have no effect. Feeding 5 to 30 ppm of o,p'-DDT, p,p'-DDT or p,p'-DDE in the diet for 28 weeks to pullets and then at levels of 50, 150 and 300 ppm for a further 12 weeks had no effect on egg-shell thickness (Cecil *et al.*, 1972). Other investigators have found certain levels of heptachlor, DDT and PCBs to have no effect on egg-shell thickness (Wagstaff *et al.*, 1981; McBlain *et al.*, 1974; Scott *et al.*, 1975). Scott *et al.* (1975) used both domestic chicken and Japanese quail in their study. Up to 100 ppm technical DDT and 20 ppm PCBs in separate diets were fed to birds. The authors also studied the effects of organic and inorganic mercury in the diet, on chicken eggs and showed that organic mercury caused marked egg-shell thinning. Accordingly, it was concluded that the egg-shell quality that had declined since about 1946 was due to organic mercury compounds, but there could

have been a synergistic relationship between the mercury compounds and the organochlorine compounds.

Several suggestions have been advanced to account for the observed differences in the experimental results (Kan and Tuinstra, 1976a):

(a) Different strains and lines of birds may differ considerably in their susceptibility to chemicals in their diets.

(b) Nutritional and environmental factors may affect the susceptibility of birds to the chemicals.

(c) Owing to biological variability, the absence or presence of an effect cannot always be proven statistically as a result of an insufficient number of observations.

Age differences and grades of the pesticides have also been implicated (Cecil *et al.*, 1972).

Thus, although pesticides are often blamed, there may be an interplay of various factors in bringing about an observable effect. Indeed, Edwards (1973) noted that although pesticides are implicated for the decline in numbers of wildlife, changes in land and water development and usage probably exert a much greater influence.

2.8.2 Mortalities, hatchability and egg production

Embryonic mortality and egg production - Feeding 10 and 40 ppm of p,p'-DDE in the diet to mallards for over a year resulted in shell-cracking and marked embryonic mortality (Heath *et al.*, 1969). p,p'-DDE was found more toxic in this respect than either p,p'-DDT or technical DDD. In another experiment, DDT, diazinon, lindane and malathion were added to feed at levels of 0.1 to 10 ppm and fed to pullets for 10 weeks (Sauter and Steele, 1972). The 4 compounds reduced egg production and hatchability significantly. Hens have also been reported to show decreased egg production after feeding on 10 ppm DDT in the diet for 2 months (Smith *et al.*, 1970a).

In contrast to these reports, no significant decrease in egg production was observed when subtoxic levels of dieldrin or parathion, added at 4.0 and 10 ppm in the diet respectively, were fed to mallards for 90 days (Muller and Lockman, 1972). Feeding 300 ppm of DDT in the diet of hens for 30 days had no effect also, on embryos (Waibel *et al.*, 1972). Even direct injection of dieldrin or DDT analogues into yolks had no appreciable toxicity to the embryos. The embryo mortalities, corrected for infertile eggs, were 38% and 14% for 257 mg dieldrin/kg eggs and 385 mg p,p'-DDT/kg eggs respectively (Guthrie and Donaldson, 1970). Smith *et al.* (1970b) conducted

similar injection studies on eggs and concluded that high doses of the pesticides were needed to produce embryotoxicity.

Studies on the residue levels or effects in eggs after external application of pesticides have also been reported. Naber and Ware (1961) found that chicken eggs from a house treated annually with 25% HCH dust to control mites and lice had a musty odour and the HCH concentrations in the yolks ranged from 0.01 to 11.30 mg/kg. In another study, an aerial application of 0.5 kg malathion in 40 litres diesel oil/acre was found to cause abnormalities such as oedema, cyanosis and limb malformations to chicken embryos (Abott *et al.*, 1964). More recently toxaphene was reported to cause embryonic mortality at half the dosage recommended for field applications, when applied to mallard eggs. In the same experiment, lindane was teratogenic only at doses at least 5 times the field level of application (Hoffman and Eastin, 1982).

Distribution of the pesticides in embryos - Guthrie and Donaldson (1970) injected isotope-labelled DDT and dieldrin into the yolks of chicken eggs before incubation. A rapid distribution of radioactivity from the yolk to the albumen and embryo was observed

and the concentrations were dependent on the lipid content of the tissues. Metabolism of both compounds was evident by day 12 of incubation. DDT's metabolites (DDD and DDE) accounted for 5%, while a water-soluble metabolite of dieldrin accounted for 3% of the radioactivity.

Mortality of hatched chicks - Eggs can contain pesticides like DDT at levels large enough to cause high mortality to hatchlings (Jones and Summers, 1968). However, Driver *et al.* (1976) found 0.1 ppm of DDT, dieldrin, heptachlor or mirex in feed to have no effect on hatchlings when fed to white leg horn layers.

Injection studies have also shown that pesticides have no appreciable effect on hatchlings (Guthrie and Donaldson, 1970). In contrast Koeman *et al.* (1967) reported that injection of 1 mg and 10 mg of dieldrin into eggs, the same doses adopted later by Guthrie and Donaldson (1970) caused severe nervous signs and death to hatchlings after a few days of life. The authors concluded that stress aggravated the situation.

Possible reasons for the observed differences - The type and levels of chemical in feed, and the length of the feeding period may be responsible for the differences reported in this section.

2.8.3 Accumulation of pesticides

Tendency to accumulate - Cummings *et al.* (1966) found that dieldrin and heptachlor epoxide had the highest propensity for storage in eggs, followed by endrin, p,p'-DDT and lindane. Other studies have tended to array the storage potential of the different compounds in the same sequence. For example, dieldrin, heptachlor, DDT and lindane, fed separately to different groups of hens accumulated at levels corresponding to the order in which they are listed (Stadelman *et al.*, 1965). Endrin residues in poultry fat fall rapidly after cessation of exposure (Brooks, 1974b).

Cecil *et al.* (1972) noted that when p,p'-DDT was fed in the diet to chickens, the proportion of p,p'-DDE in eggs increased until 12 weeks and then levelled off to constitute 25% of total DDT. A similar figure, 20.5%,was reported in the eggs of Japanese quail (Bitman *et al.*, 1971).

A low calcium (1.5%) diet has been found to increase the conversion of p,p'-DDT to p,p'-DDE leading to a greater retention of DDT in form of DDE in the fat of pullets (Cecil *et al.*, 1973).

Distribution of pesticides between egg yolk and egg white - Cummings *et al.* (1966) reported the following

ratios of pesticides in yolk to egg white : lindane, 90:10; p,p'-DDT, 93:7; p,p'-DDE, 95:5; heptachlor epoxide, 99:1; dieldrin, 99:1 and endrin, 100:0. Ware and Naber (1961) found that lindane concentrated only in the yolk. This differential distribution of pesticides is due to the fact that egg white has no fat, while fat constitutes 33% of the yolk.

Pesticides in abdominal fat versus eggs - Cecil *et al.* (1972) found that the concentration of pesticide residues in chicken abdominal fat was about 13 times the concentration in eggs. Fat accounts for about 10% of a chicken's shell-free egg and therefore the residue concentration in body fat is about the same as in egg fat.

Residue plateaus - Cummings *et al.* (1966, 1967) showed that prolonged feeding of the same concentration of pesticides to layers resulted in relatively constant levels of the residues in eggs and fat. The finding has been confirmed by others (Cecil *et al.*, 1972; Wright *et al.*, 1972; McBlain *et al.*, 1974). Plateau levels of DDT in the tissues of domestic fowl occur within 5 to 16 weeks (Cecil *et al.*, 1973; Driver *et al.*, 1976). In broilers, however, plateau levels in

tissues occur after 2 to 4 weeks of feeding DDT contaminated feed (de Vos *et al.*, 1972). Plateau levels are attained in the fat and eggs of Japanese quail within 6 weeks (McBlain *et al.*, 1974). The magnitude of the level and the time taken to attain it varies directly with the dose (Cummings *et al.*, 1966; Avrahami and Steele, 1972; McBlain *et al.*, 1974).

The attainment of a plateau level indicates an equilibrium between uptake and excretion. The level allows the calculation of the accumulation ratio (the concentration of a pesticide residue in animal fat or product to its concentration in feed). Kan (1978) has reviewed this aspect of accumulation ratios for a number of pesticide residues in eggs and chicken fat. The accumulation ratio is apparently independent of the concentration in feed over a fairly wide concentration range (Kan and Tuinstra, 1976b). Male bird tissues have significantly higher accumulation ratios than female bird tissues (Wright *et al.*, 1972; Kan *et al.*, 1978).

2.8.4 Metabolism

DDT - The principal routes of DDT metabolism in various organisms include (O'Brien, 1967):

(a) Oxidation to DDA, dicofol or dichlorobenzophenone

(b) Dehydrochlorination to DDE

(c) Reductive dechlorination to DDD

The reductive dechlorination pathway which forms DDD was first shown to occur in yeast by Kallman and Andrews (1963). They also showed that the process did not require p,p'-DDE as an intermediate. The process also occurs in pigeon liver *in vitro* under anaerobic conditions, in avian liver homogenates, in rat liver and in bovine rumen (Bunyan *et al.*, 1966; Jefferies and Walker, 1966; Klein *et al.*, 1964; McCully *et al.*, 1966).

Pinto *et al.* (1965) suggested that detoxification of DDT to DDA in mammals is more effective than formation of DDE because of DDA's relatively lower toxicity. DDA is the principal water-soluble metabolite in many mammals and is also a metabolite in chickens, in additon to DDE, DDD and DDMU (Matsumura, 1975).

Hydroxylated derivatives of p,p'-DDE in the faeces of rats fed the compound have been demonstrated (Sundström *et al.*, 1975).

o,p'-DDT undergoes ring-hydroxylation in rats and chickens (Feil *et al.*, 1973; 1975). This reaction has not been reported with either p,p'-DDT or p,p'-DDD and is, at least in part, responsible for the faster elimination of o,p'-DDT than of p,p'-DDT or p,p'-DDD (Hayes, 1982).

Aldrin and dieldrin - Aldrin is rapidly metabolised to dieldrin by many organisms including man (Hayes, 1982). In chickens the metabolism is gradual (Waldron and Naber, 1974b).

Dieldrin is more slowly metabolised. In rats the compound is metabolised to water-soluble compounds which are excreted in faeces and urine (Hayes, 1982). This probably also occurs in other organisms.

Endrin - Endrin is converted to hydroxylated derivatives in the rabbit and to fairly water-soluble compounds in the rat (Hayes, 1982). This possibly also occurs in birds.

Heptachlor - Heptachlor is epoxidised in organisms to heptachlor epoxide, which is more toxic, and to other more rapidly excretable metabolites (Hayes, 1982).

HCH - Several researchers suggest that HCH is metabolised by dechlorination, dehydrogenation, hydroxylation and dehydrochlorination. γ-pentachlorocyclohexane (γ-PCCH) is believed to be central in the degradation of lindane by warm-blooded organisms, but is not a major metabolite.

A high proportion of α-HCH is metabolised by

conjugation with glutathione in the final step (Hayes, 1982).

2.8.5 Excretion from birds

Via eggs - In a study on the excretion of HCB, α-HCH, β-HCH, lindane, heptachlor, p,p'-DDT and dieldrin by broiler breeder hens, 35% of the ingested HCB and β-HCH was excreted via eggs. 25% of total DDT and dieldrin, and 10% of heptachlor in form of heptachlor epoxide, were excreted (Kan and Tuinstra, 1976b).

In other studies, 34% and 42% of ingested p,p'-DDT and p,p'-DDE were excreted in eggs of white leghorn layers, while 75% of radiolabelled HCB was excreted through the same route in Japanese quail (Cecil *et al.*, 1972; Ingebrigtsen *et al.*, 1983).

Via faeces - 10% of HCB and β-HCH were excreted in the faeces of broiler breeder hens (Kan and Tuinstra, 1976b). HCB in faeces can in fact be used for diagnosis of HCB intoxication in chicken (Avrahami and Steele, 1972). Very small amounts of α-HCH and lindane, and only 2% of heptachlor (in form of epoxide) were excreted via this route. For DDT and dieldrin, 5% and 7% respectively were excreted via faeces.

Similar values were reported later for broilers

but the excretion of α-HCH and lindane was 10 times, while dieldrin was 2 times the values reported above (Kan *et al.*, 1978).

25% of radio-labelled HCB ingested by Japanese quail was lost in faeces (Ingebrigtsen *et al.*, 1983). The difference between the values obtained with hens and with Japanese quail are probably due to species difference.

Via miscellaneous routes - The remaining portions of the ingested pesticides are disposed of through the skin, feathers and lungs (Kan and Tuinstra, 1976b). The uropygial gland also plays a part in HCB excretion (Ingebrigtsen *et al.*, 1983).

Increase in residue depletion from birds - This aspect has been studied by manipulating factors that affect pesticide metabolism and excretion. Some of the methods investigated include fat depletion by starvation (Donaldson *et al.*, 1968; Ecobichon and Saschenbrecker, 1969), force-molting by feed restriction (Smith *et al.*, 1970a; Lillard and Noles, 1973) and thyroxine-induced hyperthyroidism (Lillard and Noles, 1973). Others are, feeding charcoal-containing feed (Waibel *et al.*, 1972; Foster *et al.*, 1972) and a combination of starvation, androgen feeding and dietary protein changes (Wesley *et al.*, 1966, 1969). Most of the methods, however,

proved uneconomical since the small gains in insecticidal depletion rates were accompanied by unfavourable physiological effects such as weight loss and decreased production.

Half-life values of residues - Depletion of residues from birds follow the kinetics of a first-order reaction (Lillard and Noles, 1973; Kan and Jonker-den Rooyen, 1978). It is therefore possible to calculate half-life times and depletion rate constants. Lillard and Noles (1973) obtained half-life values of DDT of 7.3 and 7.6 weeks in fat and eggs after feeding layers 20, 30 and 40 ppm by capsule daily for 5 days. Force-molting reduced the values to 5.0 and 5.9 weeks. Kan and Jonker-den Rooyen (1978) obtained half-life times of 6.0 and 6.7 weeks for fat and eggs of layers with no molting. In a review, lindane was reported to have half-life times of 1.5 to 2 weeks in eggs and fat, β-HCH, 7 weeks and dieldrin, 6 weeks (Kan, 1978). The reported half-life time for HCB in chicken eggs is 11 weeks (Avrahami and Steele, 1972; Kan and Tuinstra, 1976b).

2.9 ENZYME INDUCTION AND OESTROGENIC ACTIVITY

2.9.1 Enzyme induction

The chlorinated hydrocarbon insecticides are known to

induce microsomal enzymes in mammals. With rats and Japanese quail representing mammals and birds respectively, enzyme induction was tested, using o,p'- and p,p'-isomers of DDT, DDE and DDD in the diet (Bitman *et al.*, 1971). Pentobarbitone sleeping times in males and females of each species were used as indices of induction.

In rats, there was a clear induction of the enzymes. The order of decreasing effectiveness was DDE, DDT and DDD and only the o,p'-isomer of DDT was not as effective as the p,p'-isomer in lowering the sleeping time.

In complete contrast to the shortening of sleeping time in rats, the compounds prolonged sleeping time in quail. There was, however, a decline in sleeping time after an early increase, suggesting that an adaptation to the stimulus was taking place. Livers of ring-doves fed 10 ppm of DDT for 3 weeks have been shown to double the metabolism of oestradiol *in vitro* (Peakall, 1969). This contrast indicates that there is either a species difference among avians or the enzymes responsible for metabolising oestradiol are different from those for pentobarbitone and that induction can be selective on the enzymes.

Pesticide interactions - The interaction mechanisms

are related to enzyme-induction of the pesticides. Street (1964) reported that the retention of dieldrin in fatty tissues of rats was reduced when DDT was fed together with the dieldrin. A similar finding was reported by Smith *et al.* (1970a), with layers. However, de Vos *et al.* (1972) found no such interaction between the 2 compounds in broilers. In rats, dieldrin's accumulation was reduced because DDT enhanced dieldrin elimination (Street and Blau, 1966; Street *et al.*, 1966). Examples of interactions between other pesticides include HCB versus dieldrin in rats, in which case a HCB-enhanced elimination of dieldrin was suggested and DDE versus dieldrin in Japanese quail, in which DDE storage was increased (Avrahami and Gernert, 1972; Ludke, 1974).

Chlorinated hydrocarbon insecticides also interact with several organophosphorus compounds and thus increase or decrease the toxicities of the compounds, but the effects are usually minimal (Menzer, 1970).

The interaction mechanisms are complex, the results being opposite in some species compared to others. Detailed studies on the effects of the compounds on the microsomal enzymes of different species may eventually unveil the nature of the complex relationships (Hayes, 1982).

2.9.2 Oestrogenic activity of DDT

Since the configuration of DDT is similar to that of the synthetic oestrogen, diethylstilboestrol, DDT is thought to have oestrogenic activity. An extensive review on this aspect is given by Foster (1974) and the results are conflicting.

Bitman and Cecil (1970) found that halide and alkyl groups in the p,p'-positions of the DDT compounds rendered them oestrogenically inactive. This means that p,p'-DDT, p,p'-DDE and p,p'-DDD are inactive. The authors also found that when p, or p' positions were either unoccupied or were occupied by a hydroxy or methoxy group, the compounds were active. This implies that o,p'-DDT, o,p'-DDE, o,p'-DDD and methoxychlor have oestrogenic activity.

PCBs are also oestrogenically active.

2.10 POSSIBILITIES OF REDUCING RESIDUES IN FOODS

In a review by Duggan and Weatherwax (1967) on the dietary intake of pesticide chemicals by man, meat, fish and poultry products were identified as the major sources of chlorinated hydrocarbon pesticide residues. Together with dairy products, these commodities accounted for more than half of the intake of the

residues. Hence, there is a need to reduce the residues in eggs and in other commodities.

Some high residue levels, especially of dieldrin and DDT, were found in the present study (Ch. 6) and there are various methods of reducing such levels.

2.10.1 Improved practices in pest control

On growing crops - The most practical measure is the use of less persistent chemicals such as organophosphates and carbamates. Their use was actually widespread in the coffee-tea areas. Malathion, an organophosphorus compound of very low mammalian toxicity was the commonest. Many of the organophosphate insecticides, such as parathion, however, have high acute mammalian toxicities. Carbamates are generally less toxic than the organophosphates. These two newer groups of chemicals are more expensive than the chlorinated hydrocarbon insecticides, which is another disadvantage.

Non-chemical methods of pest control can also be used. They include utilization of natural enemies (predators, parasites and disease causing pathogens), growing of resistant crop varieties and cultural practices such as crop rotation. In Kenya, the lady bird beetle has been used successfully against the

citrus blackfly and experimentally dusts or suspensions containing *Bacillus thuringiensis* have been used against insects. However, although such biological agents may occur in their natural habitat in a controlled form, care must be taken so that they do not create other adverse ecological effects, when used for control operations (Copplestone, 1977).

New techniques of biological pest control are coming up. For instance, manipulation of pheromones, a complex mixture of volatile chemical compounds released by courting insects, has resulted in reduced insect reproduction. Slower release formulations which maintain pheromone concentrations long enough to disrupt the mating behaviour of insects have been developed for use on cotton. This technique is reportedly ready for commercial application in the Middle East.

Pheromones can also be utilized in pest population surveys to determine the presence of a particular insect species so that control measures can be instituted in time (Hill, 1983).

Use of biological control methods reduces the necessity of employing chemical poisons and in its most successful cases gives long-term control (Hill, 1983).

On stored agricultural products - Several ways of controlling pests of stored products (wheat, rice, maize, millet, beans, peas, etc.) are available, each with its advantages and disadvantages. The advantages of chemical methods are their effectiveness, simplicity, low cost and versatility, while the main disadvantage of non-chemical methods include the high capital cost and lack of versatility (FAO/WHO, 1982).

FAO/WHO (1982) has listed a number of non-chemical pest control measures:

(a) Biological methods - the potential for these on stored products is very limited.

(b) Physical methods - these include cold and heat applications, air-tight storage, controlled atmosphere storage and irradiation.

At present most of these methods appear to have little practicability in a country like Kenya. Chemical control methods will therefore have to continue for quite some time.

On livestock - The less persistent chemicals are mainly used in Kenya today especially for dipping and spraying to control ectoparasites. Dioxathion (Delnav$^{(R)}$), coumaphos (Asuntol$^{(R)}$) and carbaryl (Sevin$^{(R)}$) are examples of chemicals in wide usage. In and around

poultry pens, however, and also on small animals, the use of some chlorinated hydrocarbon insecticides such as DDT is still practiced.

2.10.2 Reduction of residues by cooking

de Vos *et al.* (1972) found a decrease in the residues of dieldrin, endrin, heptachlor epoxide, HCB, p,p'-DDT and lindane after frying broiler meat. The residues were decreased through losses of some subcutaneous fat during preparation and by leaching with the fat during frying. Loss by the latter method was also reported by Ritchey *et al.* (1972) who found that residues in chicken tissues were lost mainly by leaching with fat and water, although there was some lindane destruction. Autoclaving of heptachlor- and endrin-containing chicken tissues also resulted in some destruction of the pesticides, when 10 to 90% of the residues were destroyed within 3 hours (Stemp *et al.*, 1965). Pressure cooking and simmering were also found to reduce residues of dieldrin, lindane, DDT, DDE and DDD in chicken meat. The levels of pesticide residues consumed could be reduced appreciably by discarding the broth and fat drippings because they contained considerable quantities of the pesticide residues initially present in tissues (Morgan *et al.*, 1972; McCaskey *et al.*, 1968). Kroger (1968) found that severe freeze-drying and deodorization

could considerably reduce heptachlor and dieldrin residues occurring in milk.

2.11 ANALYTICAL METHODS

Prior to 1960, most pesticide residue analyses were performed colorimetrically (Burchfield and Wheeler, 1966). Such early methods required rigorous sample preparation for accurate and sensitive results and each method was usually specific for one compound (Langlois *et al.*, 1964; Stemp *et al.*, 1964). In the late 1950's Mills (1959) devised a method that could detect several residues simultaneously, but it still required rigorous sample preparation.

Gas chromatography, an efficient and rapid method of residue analysis was introduced in 1952 by James and Martin, but at that time clean-up of tissue extracts was so demanding that the method was impractical (Burchfield and Wheeler, 1966). In the late 1950's, Coulson *et al.* (1960) and Lovelock and Lipsky (1960) developed two detectors, the micro-coulometric cell and the electron capture detector which gave way to the modern gas chromatographic analysis of pesticide residues. The detectors are highly selective for elements like chlorine, sulphur and phosphorus, commonly present in pesticides.

There are five main steps of pesticide residue analysis (Sherma, 1979; Juszkiewicz, 1983):

(a) Sampling
(b) Extraction of the residue from the sample matrix
(c) Removal (clean-up) of interfering co-extractives
(d) Identification and estimation of the quantity of residues in the clean extract
(e) Confirmation of the presence and identity of the residues.

2.11.1 Sampling and storage

Sampling enables part of a population to be handled in the laboratory and therefore the collected samples must be representative for meaningful results to be obtained (Mcleod, 1973; Sherma, 1979).

Proper storage of samples prevents them from being contaminated and avoids degradation of the already present residues. Tissue samples for extraction within 24 hours are kept at refrigeration temperatures (+2 to +4^{o}C). For longer periods of storage, the samples are kept frozen at -12 to -18^{o}C (EPA, 1980).

2.11.2 Extraction

Recoveries - Optimum extraction conditions, such as

solvent polarity, time and manner of contact between sample and solvent are established by recovery studies (Sherma, 1979). Recovery studies are done whenever a sample is examined for the first time with a particular method (Mcleod, 1973). A small known volume of a standard pesticide solution, prepared in the same solvent used for subsequent extraction is added to the sample, mixed and left for several hours to equilibrate before extraction. It is not possible, however, to artificially add a pesticide to a sample in a manner that completely simulates the natural way, unless the sample is a liquid or melts at a low temperature (de Faubert Maunder *et al.*, 1964).

Recovery studies are also helpful in establishing whether there are interfering substances or degradation products in the final extract.

Residue analysis involves the assay of nanogram or smaller quantities of pesticides and the sample is passed through many stages. Hence, procedures are considered adequately quantitative when values ± 15% to ± 20% or better are obtained with samples spiked at ppm levels or ± 30%, at ppb levels (Sherma, 1979).

Extraction solvents - Hexane or hexane-acetone mixture is suitable for the extraction of non-polar fat

soluble organochlorine pesticides. The solvents have been used on eggs (Onley and Mills, 1962; de Faubert Maunder *et al.*, 1964). Acetone has been used on crops and animal tissues (Goodwin *et al.*, 1961; Taylor *et al.*, 1964).

Chloroform, dichloromethane and acetonitrile are suitable for the more polar compounds such as organophosphates and the carbamates. However, they are also used for the chlorinated pesticides. For instance, the compounds can be extracted from eggs and animal feeds with acetonitrile (Sawyer, 1966; Wells, 1967). Solvent mixtures are also used, for example, 20% dichloromethane in petroleum ether on eggs (Langlois *et al.*, 1964; McCaskey *et al.*, 1968).

Examples of other solvents employed include diethylether on eggs (Ingebrigtsen *et al.*, 1984) and petroleum ether on chicken feed (Waldron and Naber, 1974a).

2.11.3 Clean -up

Clean-up removes co-extractives which interfere with residue determination and instrument performance.

Most conventional methods involve either liquid-liquid partitioning followed by column-

chromatography or column-chromatography alone (e.g. de Faubert Maunder *et al.*, 1964; Holden and Marsden, 1969; Wood, 1969; Ferry *et al.*, 1973). Hydrolysis of fat and protein with aqueous sodium hydroxide followed by column-chromatography can also be used (Ahmad, 1979).

Solvents commonly used for liquid-liquid partitioning are acetonitrile, dimethylformamide and dimethylsulphoxide. There are several adsorbents for column-chromatography. They include alumina, Florisil, silica gel and Celite 545. Used with the adsorbents in column-chromatography are several elution solvents such as mixtures of diethylether in petroleum ether, dichloromethane in hexane and hexane alone.

Other clean-up procedures include acid and base treatment (Bjerk and Sundby, 1970), gel-permeation chromatography (Johnson *et al.*, 1976) and microprecipitation techniques (McCully and McKinley, 1964).

2.11.4 Detection of residues

Picogram quantities of residues are detectable in gas liquid chromatography, with the highly sensitive electron capture detector. Other less sensitive methods include paper, thin-layer and liquid-liquid chromatographic techniques.

2.11.5 Confirmation of residues

The electron capture detector is not selective for organochlorine compounds. Some other electron-capturing substances are detected and their retention times may coincide with those of pesticides. Hence, further identification of residues in done by:

(a) Using an alternative column with different packing material from the analytical one (e.g. Mes *et al.*, 1974; Hashemy-Tonkabony and Mosstofian, 1979).

(b) Thin layer chromatography (e.g. Maitho, 1978; Kaphalia *et al.*, 1981; Kaphalia and Seth, 1981).

(c) Chemical derivatization (e.g. Reeves *et al.*, 1977).

(d) Mass spectrometry (e.g. Cain, 1981; Niethammer *et al.*, 1984).

Acid and base treatment is another simple confirmatory technique (Sericano and Pucci, 1984).

CHAPTER THREE

IDENTIFICATION AND QUANTITATION OF ORGANOCHLORINE PESTICIDE RESIDUES BY GAS LIQUID CHROMATOGRAPHY

3.1 INTRODUCTION

Gas chromatography is a method of separating volatile components by distribution between a stationary and a mobile gaseous phase (Simpson, 1970). In gas liquid chromatography, the stationary phase is a non-volatile liquid adsorbed on an inert support usually of diatomaceous earth. For pesticide residue analysis silicone stationary phases such as SE-30$^{(R)}$, OV-17$^{(R)}$ and SP 2401$^{(R)}$ are commonly used, while the inert gas nitrogen or a mixture of argon - methane are often used as mobile phases.

The separated components are identified by means of a selective detector. The microcoulometric detector was originally used for the organochlorine pesticide residues, but has now been largely superseded by the more sensitive electron capture detector. The microcoulometric detector has a detection limit of 1-3 ng (Sherma, 1979), while the electron capture detector, detects residues even in the picogram range. It is, however, less specific for these residues, than the former detector.

The principle of electron capture detection lies on the ability of certain compounds to capture free electrons. The detector has a radioactive source which emits β-particles to ionise the carrier gas molecules. The resulting electrons migrate between two electrodes in the ionisation chamber and thus produce a baseline ("standing") current of about 10^{-9} to 10^{-8} amperes. When a component with an electron affinity enters the ionisation chamber, it takes up electrons and the "standing" current is reduced. This is indicated as a peak on the recorder trace (Dickes and Nicholas, 1976).

The time of emergence of each component, referred to as retention time, is characteristic of the component and serves to identify it. The area under the peak is used for quantitation. However, since accurate measurement of the peak area is time-consuming, the peak height is commonly used as it gives an acceptable quantitative value.

The aim of the study presented in this Chapter was to obtain quality assurance for the investigator's ability to correctly identify and adequately quantitate pesticide residues by means of gas liquid chromatography.

3.2 MATERIALS AND METHODS

3.2.1 Equipment and chemicals

Equipment

Equipment	Description	Supplier
Gas chromatograph	Series 204	("Pye Unicam Ltd." Cambridge, England)
Detector	^{63}Ni, electron capture	("Pye Unicam Ltd." Cambridge, England)
Recorder	Philips PM 8251	("Philips", Eindhoven, Holland)
Glass columns	1.7 M long, 4 mm i.d., coiled	("Pye Unicam Ltd." Cambridge, England)
Filters	Oxygen and moisture removing	("Chromopack Co." Middelburg, Holland)
Pump	Water-operated	-
Packing kit		("Mettler Instruments" Zurich, Switzerland)
Microsyringe	10 µl, with 7 cm long needle	("Scientific Glass Engineering Ltd." North Melbourne, Australia)
Carlsburg's pipettes	1000 µl	("John Poulten Ltd." Essex, England)
Volumetric flasks	10 ml	

Equipment	Description	Supplier
Septa	Silicone	("Scientific Glass Engineering Ltd." North Melbourne, Australia)
Glass wool	Silane-treated	("Supelco, SA" Gland, Switzerland)

Chemicals

Chemical	Brand name/Grade	Supplier
Packing materials:		
1.95% trifluoro-propyl silicone + 1.5% 50:50 methyl: phenyl silicone on AW, DMCS treated diatomite 100/120 mesh	1.95% SP 2401 + 1.5% SP 2250 on Supelcoport 100/120 mesh	("Supelco, SA" Gland, Switzerland)
4% methylsilicone + 6% trifluoropro-pyl silicone on AW, DMCS treated diatomite 100/120 mesh	4% SE 30+6% SP 2401 on Supelco-port 100/120 mesh	("Supelco, SA" Gland, Switzerland)
Silylating-agent:		
5% dimethyldichloro-silane in toulene	Sylon-CT	("Supelco, SA" Gland, Switzerland)

Chemical	Brand name/Grade	Supplier
Isooctane	Analytical	("Merck" Darmstadt, West Germany)
Acetone	Laboratory	("May and Baker", Dagenham, England)
Methanol	Laboratory	("Merck", Darmstadt, West Germany)
Nitrogen gas	Purified	("East Africa Oxygen Co., Ltd." Nairobi, Kenya)

3.2.2 Gas chromatograph - operating conditions

A gas chromatograph series 204 was used with a ^{63}Ni electron capture detector. The stationary phase used for routine work was 1.95% SP 2401 + 1.5% SP 2250 while for confirmatory purposes, 4% SE 30 + 6% SP 2401 was employed. Both mixed phases were coated on Supelcoport 100/120 mesh and packed in coiled glass columns. Purified nitrogen was used as the mobile phase at flow rates of 60 to 70 ml/min, measured by means of a bubble flow-meter. The gas was passed through oxygen and moisture removing filters before use. The operating temperatures were:

injector : 250°C

column : 200°C

detector : 250°C

The recorder was operated at 10 mv, with a chart speed of 10 mm/min and an attenuation setting of 64 or 128. The same attenuation setting was maintained throughout a day's analyses.

Injection technique - Ten µl S.G.E. syringes fitted with 7 cm long needles were used to manually inject 3 to 5 µl of samples and standards. Prior to each injection, the syringe was rinsed several times with redistilled hexane.

Standards were injected before, during and at the end of a day's analyses. The initial injections were for "priming" the column as well as for checking the instrument's performance. The other injections were for quantitation purposes and for indicating any changes in the performance.

3.2.3 Pesticide standards

A high quality chlorinated pesticides mixture (CPM) of 13 compounds dissolved in 1 ml isooctane in sealed ampoules, was obtained from "Supelco, S.A.", Gland, Switzerland, with the following compounds and concentrations:

Compound	Concentration (µg/µl)
p,p'-DDE	0.100
p,p'-DDT	0.260
p,p'-DDD	0.190
o,p'-DDT	0.225
o,p'-DDD	0.200
Lindane (Y-HCH)	0.025
α-HCH	0.025
β-HCH	0.100
Aldrin	0.050
Dieldrin	0.120
Endrin	0.200
Heptachlor	0.025
Heptachlor epoxide	0.080

Dilution - The 1 ml content of each ampoule was transferred into a 10 ml volumetric flask with a 1000 µl Carlsburg's pipette. The ampoule was rinsed six times with 1 ml volumes of isooctane and the rinsings transferred to the volumetric flask. Isooctane was then added to give a dilution of 1:10. From this dilution further serial dilutions of 1:100, 1:1000, 1:2000 and 1:4000 were made. Dilutions of 1:10, 1:100 and 1:1000 were kept as stock solutions, while CPM 1:2000 and 1:4000 were used as working standards. Isooctane was selected as solvent because

of its high boiling point and hence less tendency to evaporate. All dilutions were made at room temperature.

Storage - Concentrated and intermediate chlorinated pesticide standards should maintain uniform strength for one year at -10 to -15°C (EPA, 1980). All standard dilutions were therefore stored in a deep freezer. During analyses, the working standards were kept in a refrigerator, together with the sample extracts to be injected on the same day. Although working standards can be stored for a period of one year without chemical decomposition, frequent replacements were made because of possible solvent evaporation.

3.2.4 Columns

Preparation - Since glass contains silanol groups which may cause decomposition of endrin and p,p'-DDT (Sherma, 1979), the columns were treated with 5% dimethyldichlorosilane in toluene, after washing them with acetone followed by methanol. The silylating agent was thoroughly washed out with fresh methanol after one min in the column. A water pump was used to draw solvents and silylating agent into the columns, which were finally oven-dried at 100°C for 2 hours in a stream of nitrogen.

Packing - A packing kit was used to fill the dried columns with mixed phase materials. The outlet end of each column was plugged with a small wad of silane-treated glass-wool and a vacuum source connected, to aid in filling. A funnel was placed at the inlet end of the column and the packing material added in small amounts, while gently vibrating it to ensure compact packing. Finally, a wad of silane-treated glass-wool was placed at the inlet end of the filled column.

Conditioning - This involves heating the packed column for a period of at least 16 hours at a temperature which is slightly above the normal operating one, with carrier gas flowing through. The process rids the material of volatile impurities, which would give rise to column "bleed".

Each column was conditioned after packing or when it remained unused for more than 2 weeks. The column was connected at the inlet end and a nitrogen flow of 80 ml/min passed through. The column oven temperature was raised to 50^{o}C after 15 to 30 min, then to 230 to 245^{o}C after a further 30 to 60 min. The column was maintained at this temperature for at least 16 hours and then cooled. The detector end was connected, normal operating parameters set and after

an equilibration period of 1 to 2 hours, "priming" standard solutions were injected to assess the column's performance.

3.2.5 Resolution and linearity

A check on resolution and linearity was done prior to analyses of samples. Resolution was checked by injecting the CPM standard into the gas chromatograph and the resulting elution pattern compared to those supplied by the CPM manufacturer and EPA (1980).

Linearity was checked by injecting equal volumes of diluted CPM standards into the gas chromatograph and calibration graphs drawn. The best fitting line was determined by the method of least squares.

3.2.6 Pesticide identification, quantitation and confirmation in samples

Identification - This was achieved by measuring and comparing retention times of the components in the sample with those of the standard solutions on the analytical column.

Quantitation - This involved comparing the peak heights of sample components with those of corresponding

standards of known concentrations. The amount of each identified component present in one gram of wet sample was calculated by taking into account the final volume of extract, the sample size and any dilutions carried out during analysis. Values were expressed as mg/kg or parts per million.

Confirmation - To confirm the identity of a pesticide, a different column from the analytical one was used. Confirmation is necessary because the retention time of a pesticide peak and that of a contaminant may coincide on one column. Aliquots of representative sample extracts and of standards were injected into the confirmatory column and retention times compared.

3.3 RESULTS AND DISCUSSION

Fig. 1 p. 71 shows a typical chromatogram obtained when 3 µl of CPM 1:4000 were injected into the gas chromatograph on the analytical column. All the 13 compounds were adequately resolved. The column for confirmatory purposes gave eleven peaks under the given operating conditions - lindane and β-HCH eluted as one peak, as did endrin and o,p'-DDT (Fig. 2, p. 72). Both chromatograms are similar to those presented by EPA (1980).

Calibration graphs were linear for the dilutions tested (Fig. 3, p. 73 ; Fig. 4, p. 74 ; and Fig. 5, p. 75). This section of the linear range was used for quantitation.

1. α-HCH
2. Lindane
3. β-HCH
4. Heptachlor
5. Aldrin
6. Heptachlor epoxide
7. p,p'-DDE
8. Dieldrin
9. o,p'-DDD
10. Endrin
11. o,p'-DDT
12. p,p'-DDD
13. p,p'-DDT

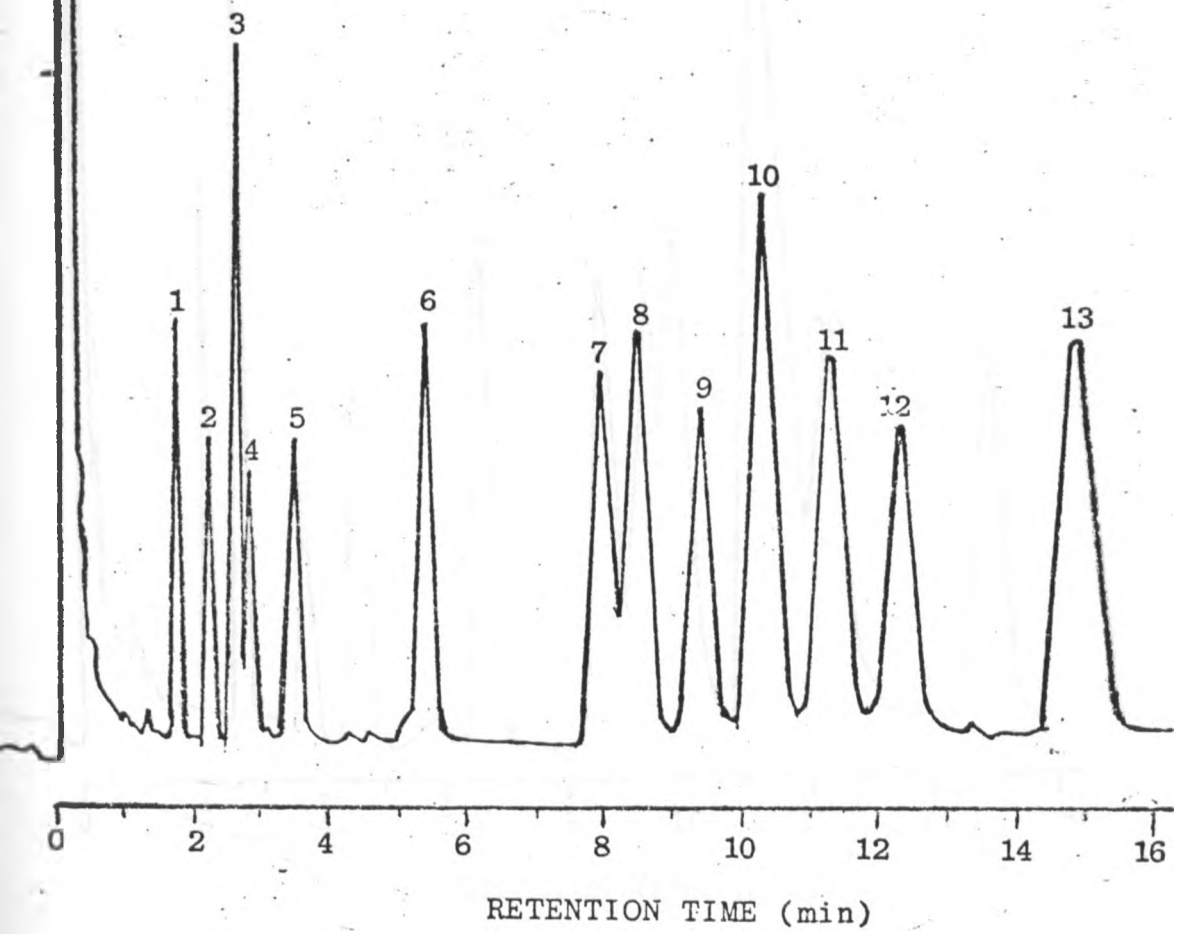
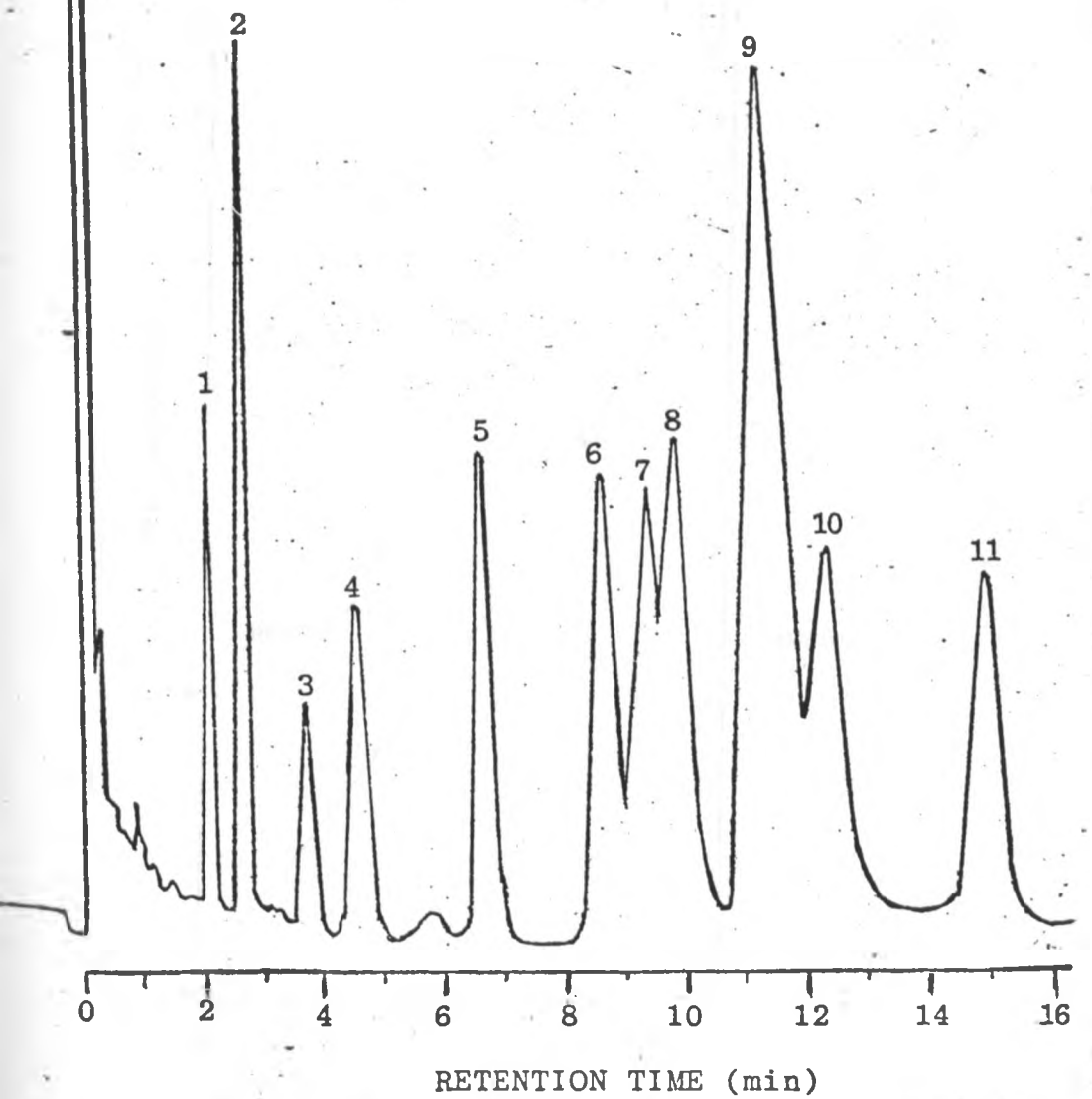


Fig. 1 Resolution of the CPM compounds on a 1.7 M x 4 mm i.d. analytical column with 1.5% SP 2250 + 1.95% SP 2401 on 100/120 Supelcoport, at detector and column temperatures of 250°C and 200°C and a nitrogen flow rate of 70 ml/min.

1. α-HCH
2. Lindane and β-HCH
3. Heptachlor
4. Aldrin
5. Heptachlor epoxide
6. p,p'-DDE
7. o,p'-DDD
8. Dieldrin
9. o,p'-DDT and endrin
10. p,p'-DDD
11. p,p'-DDT



Eig. 2 Resolution of the CPM compounds on a 1.7 M x 4 mm i.d. confirmatory column with 4% SE 30 + 6% SP 2401 on 100/120 Supelcoport, at detector and column temperatures of 250°C and 200°C and a nitrogen flow rate of 70 ml/min.

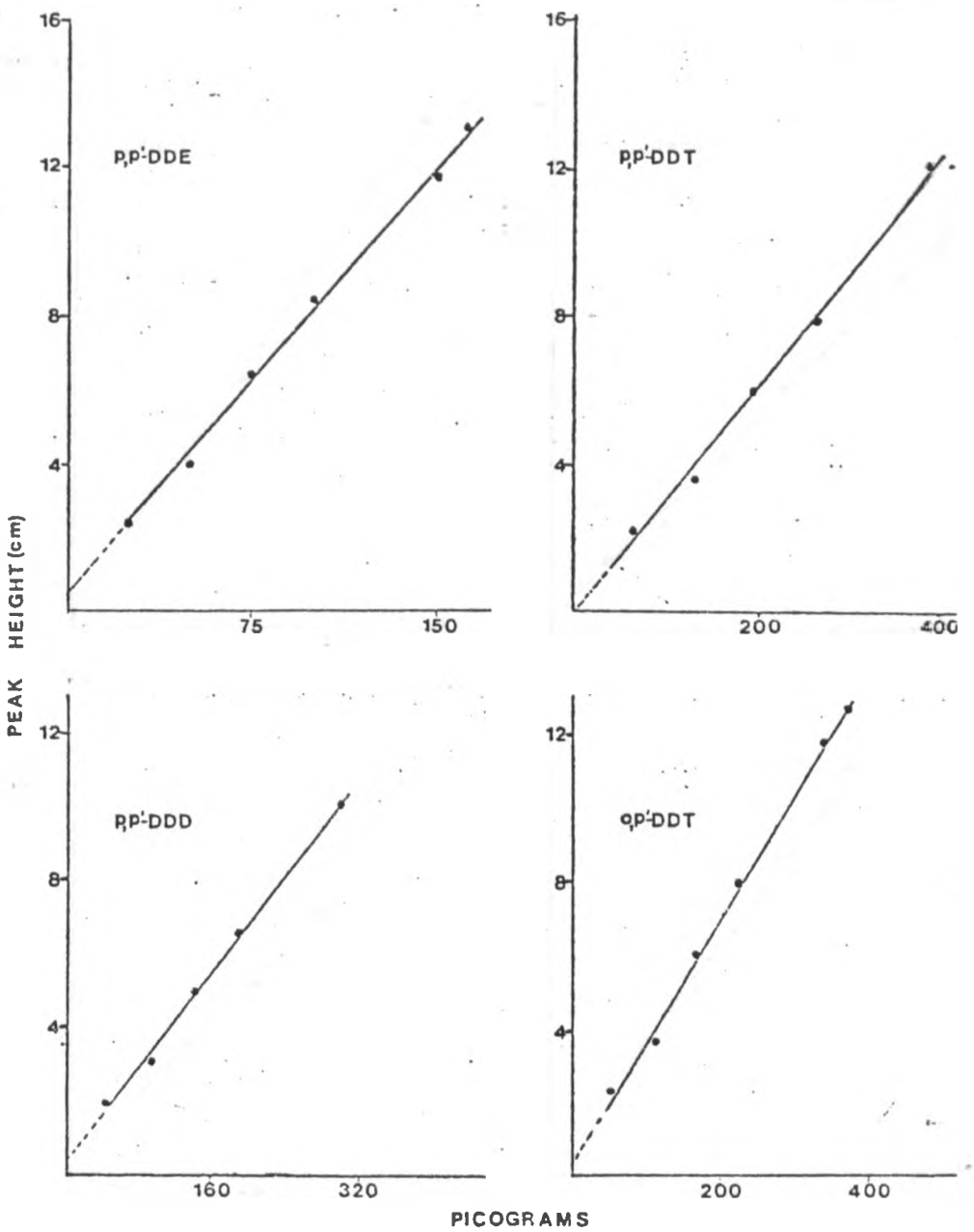


Fig. 3 Linearity of the gas chromatograph's detector response after injecting duplicates of selected amounts of p,p'-DDE, p,p'-DDT p,p'-DDD and o,p'-DDT in 3 µl volumes of isooctane on the analytical column.

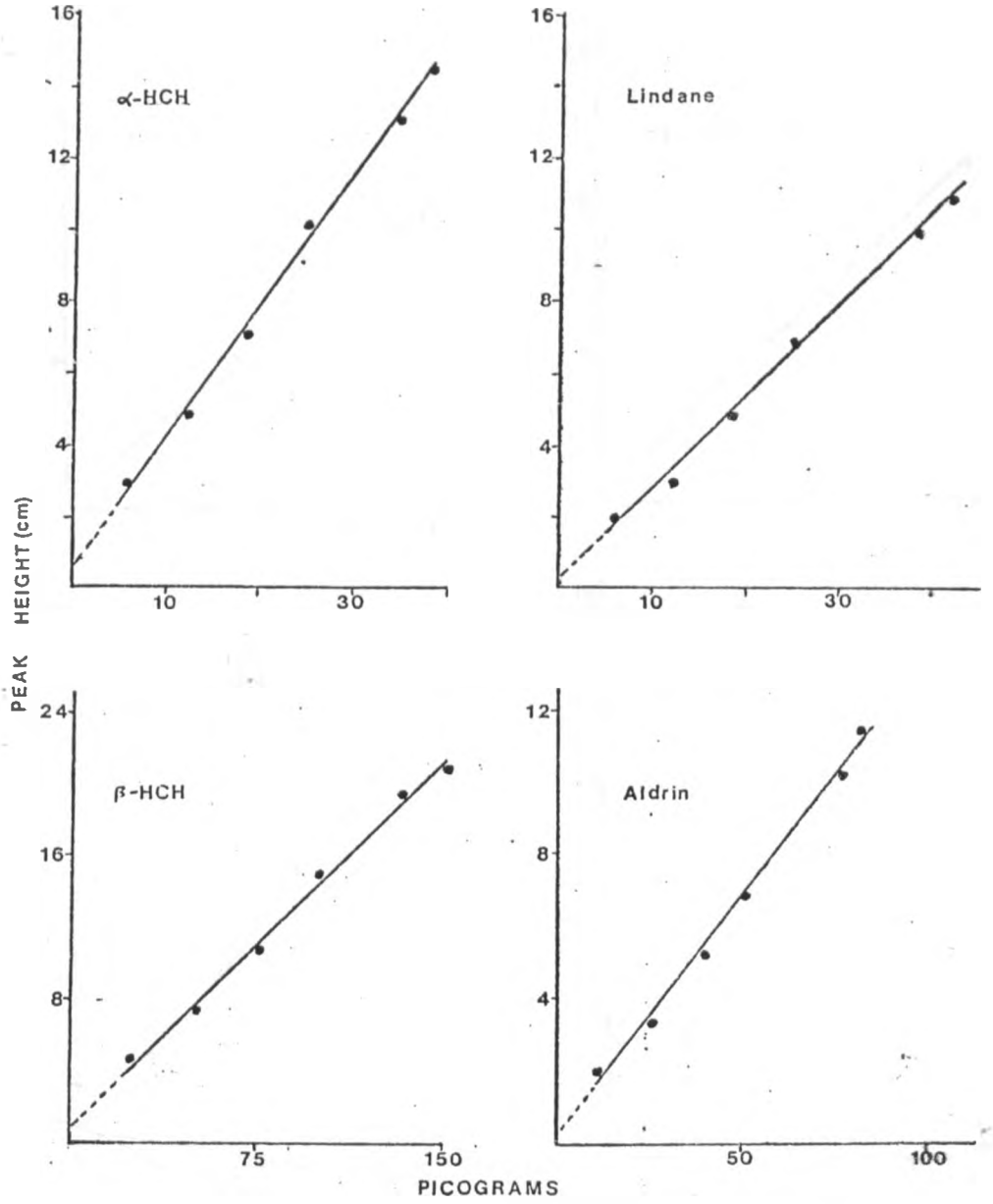


Fig. 4 Linearity of the gas chromatograph's detector response after injecting duplicates of selected amounts of α-HCH , lindane, β-HCH and aldrin in 3 µl volumes of isooctane on the analytical column.

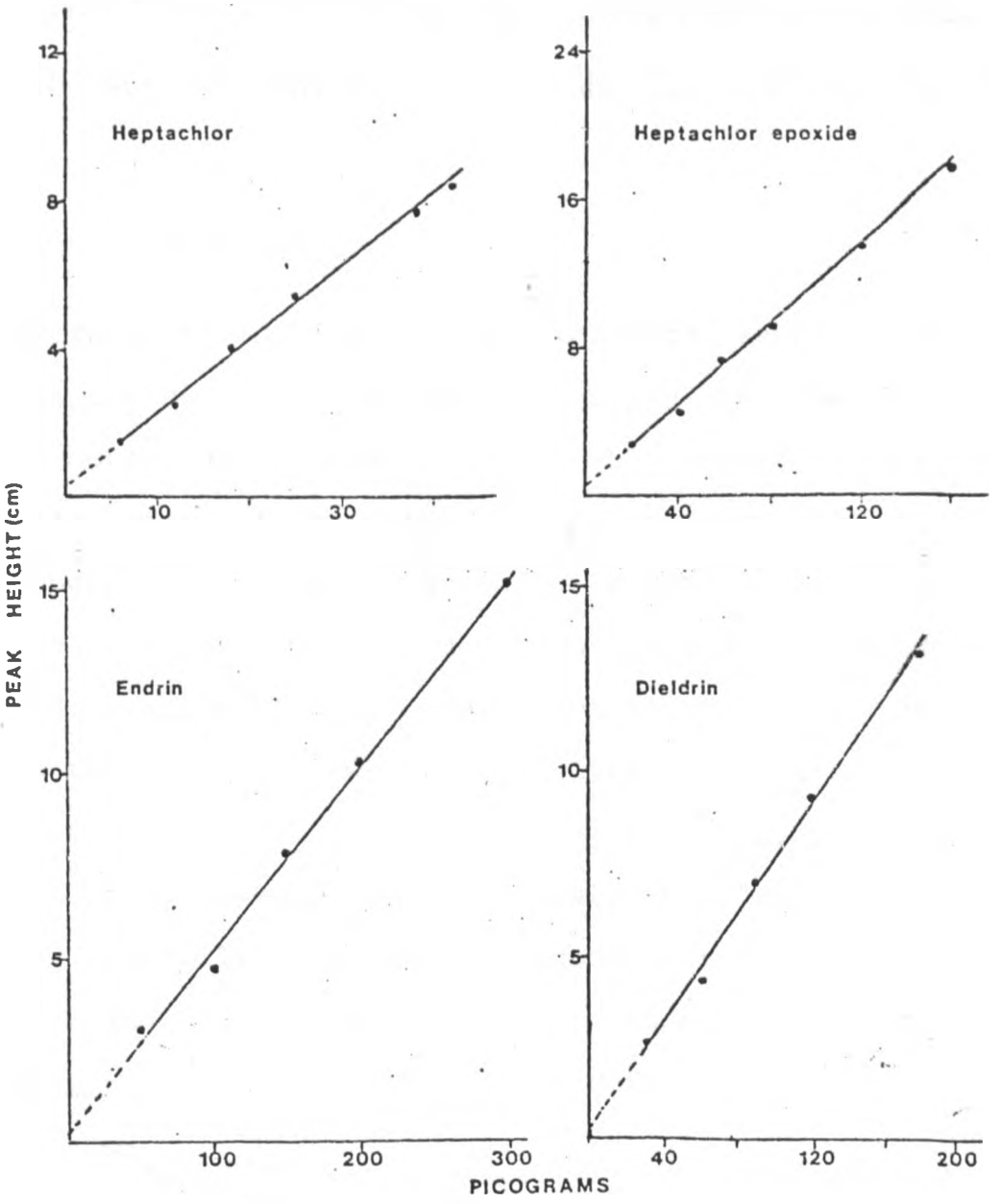


Fig. 5 Linearity of the gas chromatograph's detector response after injecting duplicates of selected amounts of heptachlor, heptachlor epoxide, endrin and dieldrin in 3 µl volumes of isooctane on the analytical column.

CHAPTER FOUR

A COMPARISON OF PESTICIDE RECOVERIES FROM SPIKED EGGS WITH TWO DIFFERENT EXTRACTION AND CLEAN-UP PROCEDURES

4.1 INTRODUCTION

Before pesticide residues in biological samples are analysed by gas liquid chromatography, they have to be extracted and cleaned up by suitable procedures. A modification of the method described by de Faubert Maunder *et al.* (1964), hereafter referred to as the "old" method has been used in our laboratory (Maitho, 1978; Kahunyo, 1983). The aim of the present study was to investigate whether an alternative method might offer advantages with regard to recoveries, purification, time consumption and costs. This "new" method was originally developed for aquatic organisms by Bjerk and Sundby (1970) and later adopted for eggs (Skaare, 1983. Personal communication).

Aliquots of eggs, spiked with known amounts of 13 pesticides were carried through both procedures. The cleaned extracts were then analysed to compare the pesticide recoveries with the spiked amounts.

The extraction principle of the "old" method

involves refluxing the homogenized dried sample with hexane using a soxhlet apparatus. Clean-up is by liquid-liquid partitioning with hexane-dimethyl-formamide, followed by column-chromatography.

Extraction by the "new" method entails passing diethylether through a column packed with dried sample homogenate and collecting the eluate. The clean-up involves treating portions of the extract with an acid and/or a base. The four alkali-stable compounds that appear in the base treated portion are aldrin, dieldrin, endrin and heptachlor epoxide. The remaining acid-stable compounds, including the DDT group, the HCH group and heptachlor, appear in the acid treated portion.

4.2 MATERIALS AND METHODS

4.2.1 Equipment and chemicals

Equipment

Non-glass equipment	Description/Supplier
Gas chromatograph	See Ch. 3, p. 61.
Balances	("Sartorius-Werke GMBH", Goettingen, West Germany)

Non-glass equipment	Description/Supplier
Rotary evaporator	("Buchi Laboratoriums-Technik AG", Flawil, Switzerland)
Vacuum pump	("Corning Ltd.", Halstead, England)
Centrifuge	("Joh. Achelis and Johne", Bremen, West Germany)
Waring blendor	("Moulinex", France)
Water-bath	("Techne Ltd.", Cambridge, England)
Whirl mixer	("Lab-line Instruments Inc.", Melrose Park, U.S.A.)
Rotary mixer	
Pestle and mortars	
Cellulose extraction thimbles	Whatman, 80 x 30 mm (o.d)
Filter papers	Whatman no. 3, 15 cm diameter.
Cotton wool	Hexane-washed.

Glassware equipment	Description/Supplier
Beakers	150 ml
Rods	15 cm x 5 mm
Volumetric flasks	10 ml
Round bottomed flasks	250 ml, 500 ml
Filter funnels	8 or 10 cm mouth diameter
Measuring cylinders	50 ml, 100 ml, 250 ml
Dimpled flasks	500 ml
Chromatographic columns	60 cm x 2.5 cm (o.d.)
Separatory funnels	250 ml, 500 ml
Centrifuge tubes	10 ml, 15 ml, with stoppers
Carlsburgs pipettes	500 µl, 1000 µl
Pipettes	1 ml, 2 ml, 5 ml
Pasteur pipettes	
Bottles	7.4 ml
Glass-wool	Pyrex(R), hexane-washed
Extraction columns	Disposable

The extraction columns were made by heating soft glass test tubes at the middle and pulling to give columns with tapering sealed ends, 10 cm long. The wide upper part was at least 5 cm long.

Chemicals

Chemical	Grade/Supplier
Chlorinated pesticides mixture (CPM)	See Ch. 3, p. 64
n-Hexane*	Laboratory ("May and Baker", Dagenham, England)
Acetone*	Laboratory ("May and Baker", Dagenham, England)
Dimethylformamide	Laboratory ("May and Baker", Dagenham, England)
Dichloromethane	Laboratory ("May and Baker", Dagenham, England)
Sodium sulphate decahydrate	Laboratory ("May and Baker", Dagenham, England)
Sodium hydroxide pellets	Laboratory ("May and Baker", Dagenham, England)
Methanol*	Laboratory ("Riedel-De Haen AG", Hannover, West Germany)
Concentrated sulphuric acid**	("East Anglia Chemicals", Hadleigh, England)

Chemical	Grade/Supplier
Diethylether	Analytical ("May and Baker", Dagenham, England/"BDH Chemicals Ltd.", Poole, England)
Anhydrous Sodium sulphate	Analytical ("Hopkins and Williams", Chadwell Heath, England)
Sodium chloride	Analytical ("Dubuit Kenya Ltd.", Nairobi, Kenya)
Florisil***	PR,60-100 mesh ("Merck", Darmstadt, West Germany/ "Supelco S.A.", Gland, Switzerland)
Distilled water	
Nitrogen gas	Ordinary (See p. 63)

* Redistilled as described on p. 82.

** Concentrated sulphuric acid was washed 3 times as follows: volumes of the acid and hexane (2+1) were transferred into a 500 ml separatory funnel. The funnel was stoppered and shaken vigorously, then left for about 10 min for the liquids to separate.

The lower acid layer was drained into another 500 ml separatory funnel and the process repeated 2 more times. Finally, the acid was drained into a suitable container with stopper, ready for use.

*** Deactivated as described on p. 90 .

Cleaning of glassware - Each piece was rinsed with tap-water and then cleaned with a brush and warm water containing a liquid detergent. Thereafter it was rinsed again with tap-water, distilled water and finally with re-distilled acetone before drying it in an oven at 150°C. Just before use, each piece was rinsed with hexane.

Distillation of solvents - Two and half litre volumes of hexane, acetone and methanol were distilled once in an all-glass fractionating column equipped with a water cooled condenser. Boiling chips were used in the distillation flask to prevent the solvents from super-heating. The solvents were distilled slowly to avoid co-distillation of impurities. The first 200 ml of the distillate and the last 400 ml of solvent in the distillation flask were discarded as recommended by Maitho (1978).

Solvent purity check - Hexane's purity was checked by concentrating an aliquot 30 times using a rotary evaporator and then injecting the concentrate into the gas chromatograph at the normal operating conditions. The detector response was then observed for 20 min. Some peaks were observed just close to the solvent front. These peaks emerged before the ∝-HCH peak, the earliest CPM peak and were therefore not regarded as important interferences. The purity of acetone, methanol and analytical grade solvents such as diethylether was ascertained as follows : a volume was evaporated to dryness in a quick fit flask using a rotary evaporator . The flask was rinsed with 1 to 2 ml of re-distilled hexane and the rinsing injected into the gas chromatograph. The detector response was then observed as described above. Most analytical grade solvents were free of interfering peaks and the laboratory grade solvents needed no more than the first distillation.

4.2.2 Spiking of eggs

Three eggs were broken using a spatula and the contents transferred into a waring blendor. After homogenization, 100 g were weighed into another jar of the blendor and a volume of isooctane containing

CPM added. For every 10 g sample, 1 ml or 2 ml of CPM 1:100 was added by means of a Carlsburgs pipette to give a "low" or "high" spiking level, with the following concentrations in eggs:

Compound	Concentration (µg/g of egg)	
	"low"	"high"
p,p'-DDE	0.100	0.200
p,p'-DDT	0.260	0.520
p,p'-DDD	0.190	0.380
o,p'-DDT	0.225	0.450
o,p'-DDD	0.200	0.400
Lindane	0.025	0.050
α-HCH	0.025	0.050
β-HCH	0.100	0.200
Aldrin	0.050	0.100
Dieldrin	0.120	0.240
Endrin	0.200	0.400
Heptachlor	0.025	0.050
Heptachlor epoxide	0.080	0.160

The spiked egg homogenate was left on the bench for the solvent to evaporate. Thereafter it was re-homogenized, transferred into a glass bottle which was stoppered and kept in a deep freezer. The remaining

unspiked egg homogenate was also kept in the deep freezer in a stoppered bottle, for use as a blank.

4.2.3 "New" method

An outline of the method is shown in Fig. 6, p. 95.

Extraction - The thawed spiked egg homogenate was shaken thoroughly. Six 3 g samples were weighed into separate mortars and to each, 4.5 g anhydrous sodium sulphate and an equal amount of acid-washed sand were added. A pestle was used to crush and mix the three ingredients until a freely flowing powder was obtained. A 4 g portion of the powder was transferred onto a small wad of hexane-washed cotton wool in a disposable extraction column. The column was mounted on a stand and the sides tapped gently with a wooden rod to obtain a uniform packing. Diethylether was added to the columns and the set-up left for 15 min following which the column's tapering end was broken and the ether allowed to run into a pre-weighed 10 ml or 15 ml centrifuge tube. Small volumes of ether were added to the column until 10 to 15 ml were collected in the tube. When anhydrous magnesium sulphate was used, sizeable amounts of sediment passed past the wad

of cotton-wool, while with anhydrous sodium sulphate negligible amounts passed.

The ether extracts were completely evaporated in a water-bath at 40°C under a stream of nitrogen. The tubes with the extracts were left on the bench for at least 20 min after which they were re-weighed and the difference was taken as the weight of the extracted fat. From the proportions of egg, sand and anhydrous sodium sulphate used, the fat obtained in each tube was that of 1 g egg.

Clean-up - The fat obtained from the previous step was dissolved in hexane with the aid of a whirl mixer, to give a concentration of not more than 0.05 mg fat/ml hexane. A uniform volume of 4 ml was used in this study, and from it, two 1 ml portions were pipetted into two labelled centrifuge tubes. The remaining extract was discarded.

Acid clean-up - To one of the 1 ml portions, 1.5 ml of hexane-washed concentrated sulphuric acid was added. The tube was stoppered and the contents mixed on a whirlmixer, then left for 1 hour. Thereafter, the tube was centrifuged at 3,500 r.p.m. for 2 to 3 min

and the upper hexane layer pipetted into a small bottle using a Pasteur pipette. The bottle was capped and kept at -20°C. The extract was now ready for gas liquid chromatography.

Base clean-up - To the other 1 ml portion, 1.5 ml of 15% (W/V) sodium hydroxide in methanol were added. The tube was stoppered and the mixture kept in a water-bath at 40°C overnight. The tube was then removed from the bath and 3 ml of 2% aqueous sodium chloride solution followed by 2 ml of hexane, added. The mixture was shaken thoroughly on a whirlmixer and centrifuged at 3,500 r.p.m. for 2 min. The upper hexane layer was pipetted into a small bottle, which was then capped and kept in a deep freezer. The extract was ready for analysis.

Two blanks were also run through the entire method.

Modifications

(a) Instead of 2.4 g of egg, 3 g were used. The amounts of sand and drying agent used were increased proportionately.

(b) Anhydrous sodium sulphate was used as the drying

agent instead of magnesium sulphate.

(c) When anhydrous sodium sulphate was used, centrifugation of the diethylether before the evaporation step was found unnecessary.

(d) A volume of 2 ml hexane instead of 1 ml was used for the base clean-up.

4.2.4 "Old" method

An outline of the method is shown in Fig. 7, p.96.

Extraction - Six 10 g samples of the spiked, and two of the unspiked egg homogenates were each weighed into separate 150 ml beakers. To each beaker, 23 g of anhydrous sodium sulphate were added and the mixture made granular with a glass-rod. The ensuing powder was transferred into an extraction thimble which was then plugged with hexane-washed cotton wool. The thimble was mounted onto a soxhlet apparatus and extraction done for 2 hours with 200 ml of hexane. Thereafter, the volume was reduced to approximately 30 ml on a rotary evaporator.

Clean-up - This was achieved by liquid-liquid partitioning and column-chromatography, carried out in succession.

Liquid-liquid partitioning - The extract from the previous step was quantitatively transferred into a 250 ml separatory funnel using hexane. Forty ml of hexane-saturated dimethylformamide (4:1) were added and the funnel stoppered. The mixture was shaken thoroughly and left for 10 min. The lower dimethylformamide phase was drained into a 500 ml separatory funnel. Any emulsion was left with the upper hexane layer. This partitioning process was repeated twice and the hexane portion discarded.

A 200 ml volume of 2% aqueous sodium sulphate solution was added to the combined dimethylformamide layers and the mixture extracted with 4 x 40 ml portions of hexane. In each case, 6 min were allowed for the layers to separate. The lower aqueous layer was drained into another 500 ml separatory funnel for further extraction. The combined hexane portions were washed with 2 x 20 ml of 2% aqueous sodium sulphate solution. Finally, they were passed over anhydrous sodium sulphate held on a filter paper in a funnel to remove traces of water. The volume was then reduced to about 10 ml on a rotary evaporator and kept ready for the next step.

Column-chromatography - Florisil was used as the adsorbent and was treated as follows before use: 200 g were weighed into a crucible and heated in a closed oven for 13 hours at 160^{o}C, then cooled to room temperature in a dessicator. An appropriate amount of the activated Florisil was transferred into a dimpled flask and distilled water added to form a 3% (W/W) water-Florisil mixture. The mixture was then tumbled for 2 hours with a rotary mixer. Three instead of 2% water in the mixture was preferred because in preliminary trials, little dieldrin and endrin eluted from the chromatographic column when 2% water was used to deactivate the Florisil. Finally, the partially deactivated adsorbent was kept in a desiccator for at least 5 hours for the Florisil and water to equilibrate.

A wad of hexane-washed glass wool was inserted into a chromatographic column to make a 1 cm length at the bottom. The column, which had a stop-cock was pre-wetted with 70 ml of hexane after which 25 g of Florisil were added, in small amounts. The sides of the column were tapped gently to ensure uniform and tight packing. A 2 cm layer of anhydrous sodium sulphate was added on top of the Florisil. The pre-wetting hexane was drained slowly until the meniscus

was just above the top end of the sodium sulphate layer. A 500 ml flask was placed below the column out let to collect the eluate. The extract was quantitatively transferred into the column and the inside of the column rinsed with 20 ml of 1:4 dichloromethane: hexane, the eluting mixture. The rest of the eluant (230 ml) was added to the column in small volumes. The top of the anhydrous sodium sulphate layer was kept covered with eluant until all of it had been added. Elution was at an approximate rate of 5 ml/min.

The volume of the eluate was reduced to about 4 ml on a rotary evaporator and then quantitatively transferred into a 10 ml volumetric flask, and the volume made to the 10 ml mark with hexane. The extract was ready for gas liquid chromatography. Ten instead of 25 ml volumetric flasks were used in this study.

Modifications by Maitho (1978) of the original de Faubert Maunder et al. (1964).method.

(a) In column-chromatography, Florisil was substituted for alumina.

(b) The elution was accordingly changed from hexane to dichloromethane-hexane mixture.

(c) Hexane instead of an acetone-hexane mixture was used for extraction.

4.2.5 "Old" method - attempts to improve recoveries

With the "old" method, used as described above, aldrin, heptachlor and sometimes p,p'-DDE were poorly recovered. Some experiments were conducted to find out where in the clean-up procedure the loss, especially of aldrin, occurred.

Direct partitioning of aldrin between dimethyl-formamide and hexane followed by column-chromatography - Aldrin (0.5 µg) and 3 µg of p,p'-DDT were dissolved in 1 g cooking oil, to simulate egg fat. The oil had been tested and found free of pesticides. The oil was quantitatively transferred into a 250 ml separatory funnel and subjected to the partitioning process and column-chromatography as described on pp. 89 to 91. The final concentrate was transferred into a 10 ml volumetric flask and filled up to the 10 ml mark with hexane, ready for gas chromatographic analysis.

Direct partitioning of aldrin between dimethylformamide and hexane and direct column-chromatography - Loss of aldrin could possibly occur at the partitioning step, the column chromatography step or at both steps. Each step was therefore tested separately. Aldrin was dissolved in hexane to give a concentration of 0.05 µg/ml. Two 10 ml volumes of this solution were passed through the partitioning process. Since it was suspected that some pesticides could still be present in the hexane normally discarded, the hexane was concentrated to 10 ml and injected into the gas chromatograph.

Another two 10 ml portions of the aldrin solution were added into 2 columns of partially deactivated Florisil and eluted (p. 90). The final concentrates were transferred into 10 ml volumetric flasks and made to the 10 ml mark using hexane. They were subsequently injected into the gas chromatograph.

Direct partitioning of CPM between dimethylformamide and hexane - Since the loss of aldrin was traced to occur at the partitioning step, it was found relevant to test the potential loss of other components of the CPM at this step. A CPM standard worked out to give a final concentration of 1:2,500

assuming 100% recoveries was partitioned between dimethylformamide and hexane (1:4 and 1:2), then injected into the gas chromatograph.

4.2.6 "Old" method - modification according to the results of Section 4.2.5

The results of Section 4.2.5 indicate that the dimethylformamide: hexane ratio of 1:4 used in this laboratory before, was not optimal especially with regard to aldrin. The recovery studies at the "low" spiking level were therefore repeated with a changed dimethylformamide to hexane ratio of 1:2. The total volume was about 40 ml, as before. There were six replicates.

4.2.7 Gas chromatographic analyses

Analyses of final extracts were done as described in Ch. 3, p.68 under the gas chromatograph's operating conditions given on pp. 63 and 64.

4.2.8 Statistics

t-values were computed as described for comparing two groups of equal size and equal standard deviations, but to compensate for possible differences in standard deviations, t-tables were used with n-1 instead of 2(n-1) degrees of freedom, as recommended by Snedecor and Cochran (1967).

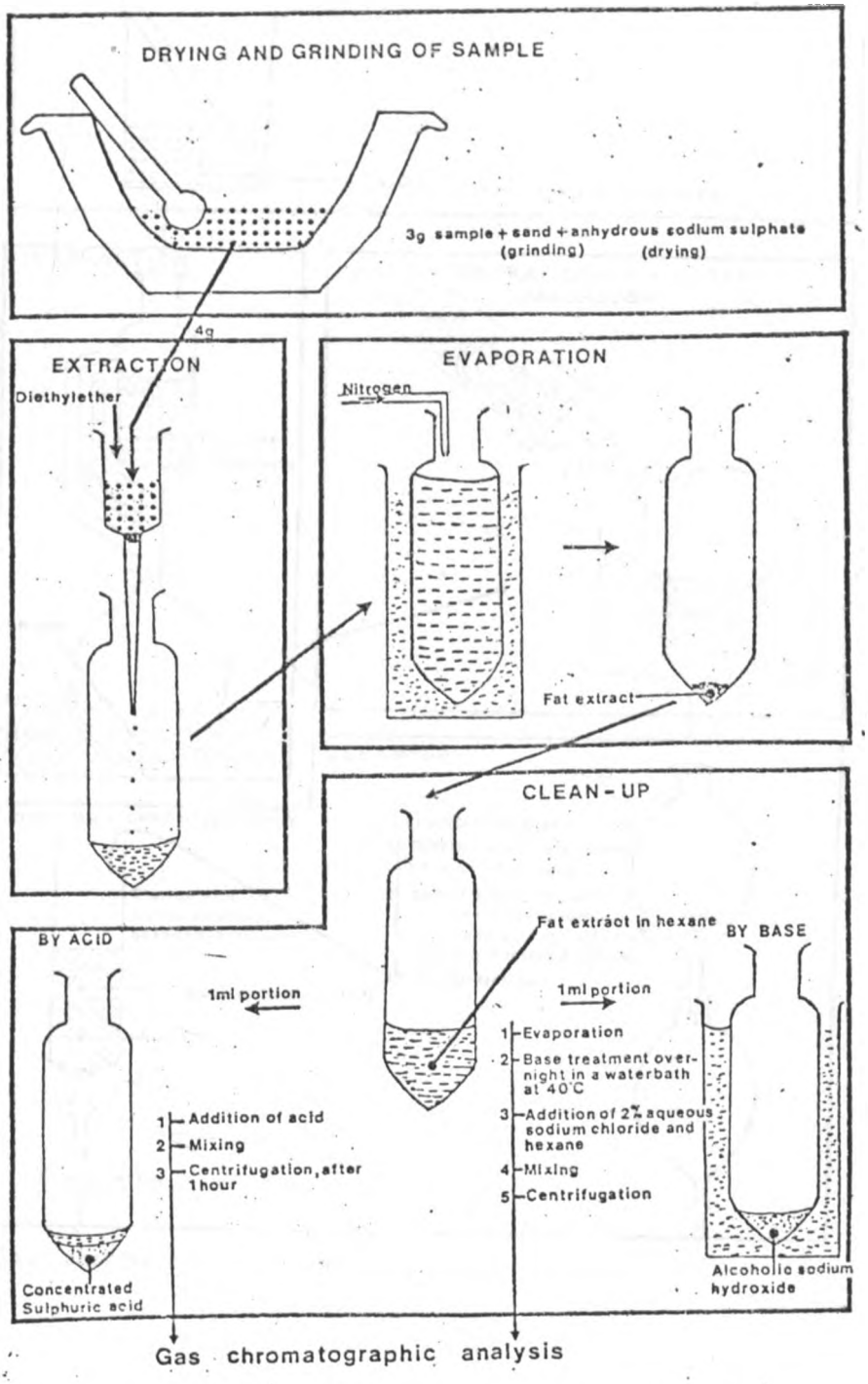


Fig. 6 Preparation of biological samples with the "new" method for gas chromatographic analysis.

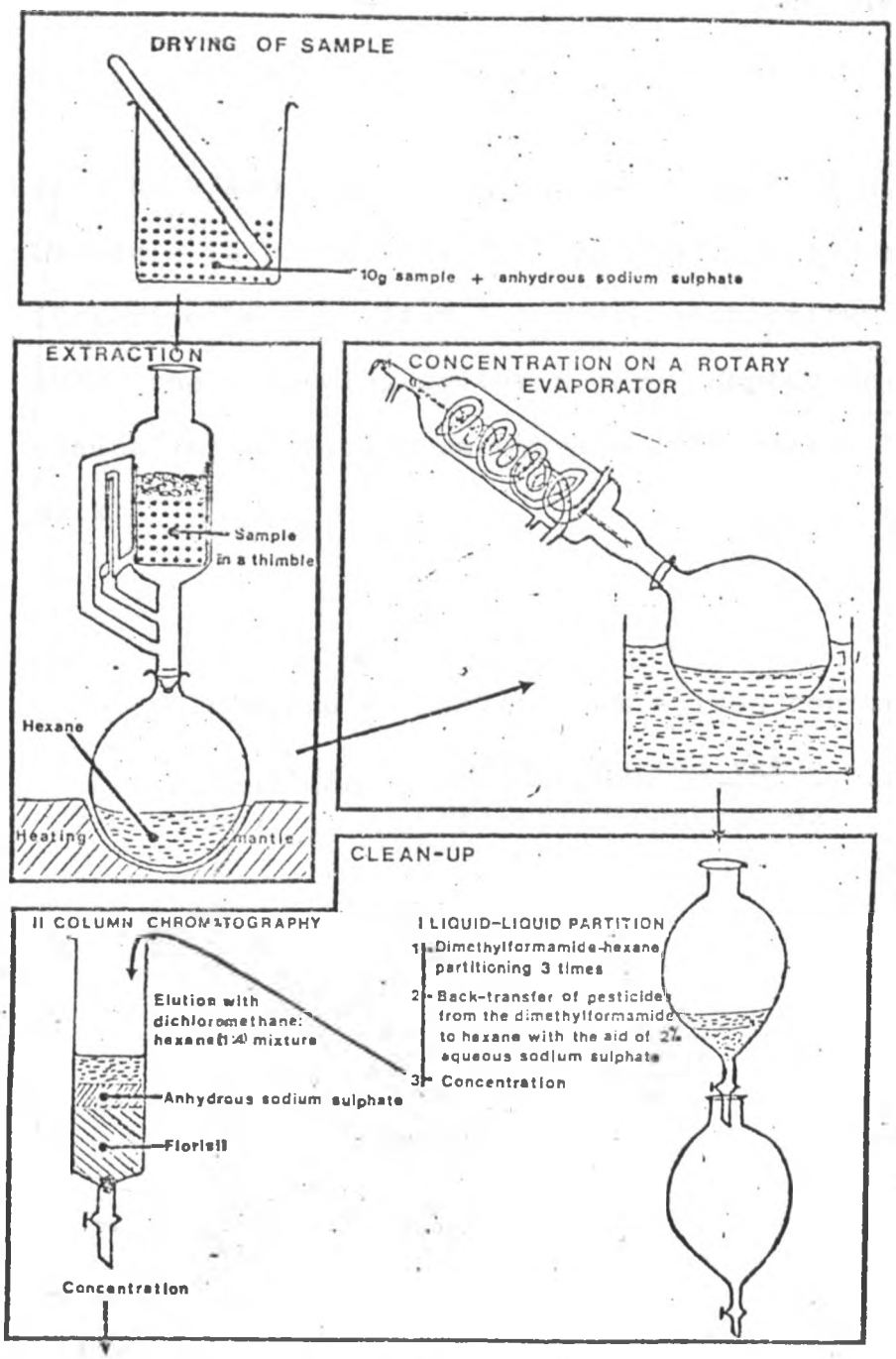


Fig. 7 Preparation of biological samples with the "old" method for gas chromatographic analysis.

4.3 RESULTS AND DISCUSSION

4.3.1 Criteria for evaluation of results

In a UNEP/WHO project on the assessment of Human Exposure to Pollutants Through Biological Monitoring (Report of a consultation on organochlorine compounds, 1980) the following criteria were adopted for evaluating pesticide recoveries from spiked human milk:

Δ = percentage deviation from spiked concentration

(calculated as $\frac{100 \text{ X reported value}}{\text{spiked concentration}} - 100$)

$\Delta = \pm 10$ excellent

$\Delta = \pm 20$ good

$\Delta = \pm 30$ acceptable

$\Delta = \pm 40$ poor

$\Delta = \pm 50$ unacceptable

Part of the criteria were also adopted in another UNEP/WHO project on the Assessment of Human Exposure

to Selected Organochlorine Compounds Through Biological Monitoring (Swedish National Food Administration, 1983).

The same criteria were adopted, in evaluating the results of the present study.

4.3.2 Pesticide recoveries - "new" versus "old" method

"Low" spiking level - A paired comparison of the recoveries is presented in Fig. 8, p.115. Individual values are given in Appendix 1, p.223.

With the "new" method, no compound had a recovery percentage below the acceptable level (Table 2, p. 111). Five compounds had excellent, 3 good and 5 acceptable recoveries. With the "old" method, no compound had a recovery percentage above the acceptable level. Four compounds had acceptable, 3 poor, and 6 unacceptable recoveries.

The "new" method gave better recoveries than the "old" one for all compounds. The differences in means were significant at the 0.1% level for p,p'-DDD, o,p'-DDT, lindane, aldrin and heptachlor; at 1% for p,p'-DDE, p,p'-DDT, o,p'-DDD, α-HCH, β-HCH and dieldrin and at 5% for heptachlor epoxide and endrin.

"High" spiking level - Both methods gave better recoveries than at the "low" spiking level (Fig. 8, p.115 and Appendix 2, p. 224). The "new" method gave excellent recoveries for 9 compounds while recoveries for the remaining 4 compounds were good (Table 2, p. 111). With the "old" method, 4 compounds had excellent and another 4, good recoveries. Acceptable, poor and unacceptable recoveries were obtained for 2, 1 and 2 compounds respectively.

At the "high" spiking level, the "new" method still proved better than the "old" one although not to the same extent as at the "low" level. For aldrin, the difference was still significant at the 0.1% level. Differences for p,p'-DDE, endrin and heptachlor were significant at the 1% level, and those for o,p'-DDT, o,p'-DDD, α-HCH and dieldrin, at the 5% level. For o,p'-DDD, the difference was in favour of the "old" method. There were no significant differences for the rest of the compounds.

4.3.3 "Old" method - loss of aldrin

(a) When aldrin and p,p'-DDT were dissolved in oil and cleaned-up with the "old" method, the mean recoveries were 34.2 $\pm$ 2.2% for aldrin and 74.2 $\pm$ 4.1 % for p,p'-DDT.

(b) The main loss of aldrin occurred at the liquid-liquid partitioning step with the hexane that is normally discarded, while there was no loss at the column-chromatography step (Table 3, p. 112). It is, however, difficult to explain this in view of the 92% recovery obtained by de Faubert Maunder *et al.* (1964).

(c) When CPM was passed directly through the liquid-liquid partitioning step with a dimethylformamide to hexane ratio of 1:4, there were substantial losses of some other pesticides, notably heptachlor and p,p'-DDE. Further, recovery of aldrin as well as recoveries of other compounds improved when the ratio was changed to 1:2 (Table 4, p. 113).

The liquid-liquid partitioning step, with or without column-chromatography gave unacceptable aldrin recoveries, similar to the ones obtained with spiked eggs.

4.3.4 Recoveries from eggs spiked at the "low" level using a dimethylformamide to hexane ratio of 1:2.

Except for lindane, the recoveries of all the other compounds were markedly improved (Fig. 9, p.116 and Appendix 3, p.225). With a dimethylformamide to

hexane ratio of 1:4 the recoveries at the "low" spiking level were not above the acceptable level. With the new ratio, however, 1 and 4 compounds had excellent and good recoveries respectively, while 5 compounds had acceptable recoveries. Poor and unacceptable recoveries were obtained for 1 compound each (Table 2, p.111). Although aldrin's recovery had improved significantly, it was still unacceptable.

The recoveries of heptachlor and p,p'-DDE had improved from unacceptable to the acceptable range. However, lindane's recovery dropped from the acceptable to the poor range.

The differences in mean recovery with the two ratios were significant at the 0.1% level for aldrin, heptachlor and β-HCH, and at 1% level for p,p'-DDE, o,p'-DDT, α-HCH, endrin and heptachlor epoxide. At the 5% level, the differences were significant for p,p'-DDD and dieldrin. The improvements in the mean recoveries of p,p'-DDT and o,p'-DDD were not significant, while the recovery for lindane was lower with the 1:2 ratio, but the difference was not significant.

With the dimethylformamide to hexane ratio of 1:2, the differences in recoveries between the "old" and "new" methods had narrowed. However, the "new" method was still superior for some compounds.

At the 0.1% level, differences were significant for p,p'-DDD, aldrin, lindane and β-HCH. In this case, the recovery for β-HCH was better with the "old" method. At the 1% level, differences were significant for p,p'-DDE, p,p'-DDT, o,p'-DDT and o,p'-DDD. The difference for dieldrin was significant at the 5% level, while the differences for the remaining 4 compounds were not significant.

The results indicate that even better recoveries might be obtained by manipulating the dimethylformamide to hexane ratio. Lindane's recovery with the dimethylformamide to hexane ratio of 1:2, was 105% in the absence of egg (Table 4, p. 113), but only 62% (Appendix 3, p. 225) when extracted from a spiked egg. It might therefore appear that the egg may have affected the compound's partition coefficient at the liquid-liquid partitioning step. However, according to Beroza and Bowman (1965) this is unlikely because the coefficient of an insecticide in a given binary solvent system is characteristic and not only remains constant in the presence of food extractives but can also be used to identify and/or confirm the identity of the insecticide. The loss of lindane is therefore unlikely to have occurred at the partitioning step.

Preliminary methodological trials on the "old" method showed that endrin and dieldrin were poorly

recovered (with a recovery range of 0 to 42%) when Florisil, partially deactivated with 2% water was used in the chromatographic column. Each batch of Florisil should be standardized by verifying the pesticide elution pattern in the absence of substrate (Cummings *et al.*, 1966). This is necessary because its adsorptive activity varies (Hall, 1971; EPA, 1980; Todoroki *et al.*, 1983). Accordingly, Florisil, deactivated to a higher degree with 3% instead of 2% water, was used and found to give better recoveries of the 2 compounds from the Florisil column, than before. However, the volume of eluant can also affect the recoveries. Especially large volumes of eluant are required to elute the 2 compounds from a Florisil column. 400 ml of a 15% mixture (V/V) of diethylether in petroleum ether were still required to elute the compounds even after 200 ml of 6% diethylether in petroleum ether were run through the chromatographic column of activated Florisil (EPA, 1980). In another study, Osadchuk *et al.* (1971) found that 300 ml of 30% dichloromethane in hexane were necessary to elute the 2 compounds from a column of Florisil, that had been partially deactivated with 2% water. The 300 ml were required although 1,500 ml of the eluting solvent mixture in varying proportions had already been passed through the column. It is therefore evident that the 250 ml of 25% dichloromethane

in hexane used in the "old" method was inadequate especially at the lower degree of deactivation of Florisil.

Al-Omar *et al.* (1985) also reported poor recoveries (55%) of dieldrin from spiked lamb meat and beef with an established procedure that involves Florisil column-chromatography. They noted that the elution process of the compound was poor.

While aldrin got lost at the liquid-liquid partitioning step, dieldrin and endrin were lost in the column-chromatographic step. Both are clean-up steps. Slight modifications of the steps improved the recoveries of the compounds. Ahmad (1979) modified the de Faubert Maunder *et al.* (1964) method at the liquid-liquid partitioning step and obtained better recoveries of p,p'-DDT from spiked fat compared to recoveries reported earlier by other workers. The modification involved employing a U-shaped partitioning column instead of separatory funnels. It is therefore apparent that the most uncertain aspects of residue analysis reside at the clean-up step as Beroza and Bowman (1965) observed.

Poor recovery of aldrin from spiked eggs with the de Faubert Maunder *et al.* (1964) method has also been reported by Smart *et al.* (1974). With 3 different analytical columns, they reported aldrin recoveries of 22, 24 and 1 to 60%. Their study also illustrates that the selection of column packing may

affect recoveries. For instance, with one column a recovery of 51% and a S.D. of 23 was obtained for p,p'-DDE, while on another column the value was 94 ± 19.

Also tested were 3 different methods on a variety of spiked foodstuffs. Since the results with spiked samples check the losses in clean-up and determitive steps, giving little or no information on extraction efficiency, the methods were, in addition, tested on feedstuffs with incurred residues. The method giving the highest values of the incurred residues was assumed the best. The de Faubert Maunder *et al.* (1964) method gave the highest values for eggs with incurred residues.

With regard to the loss at the partitioning step, Johnson *et al.* (1976) observed that recovery problems could be caused by unfavourable partition coefficients. Also, the partitioning involves many extraction and washing processes with consequent loss of pesticides (Holden and Marsden, 1969), and sometimes emulsions form which are difficult to deal with (Wood, 1969).

In the original method by de Faubert Maunder *et al.* (1964) there is no mention of the dimethylformamide to hexane ratio used. All is stated is that the

dimethylformamide has to be saturated with hexane. It is however, reasonable to assume that an excess of hexane saturates the dimethylformamide.

At both spiking levels used in this study, the "new" method gave better recoveries than the "old" method. The results are however, not completely conclusive because only two dimethylformamide to hexane ratios were tested and the use of 10 instead of 25 ml volumetric flasks for the final extract may have had an effect. Although not thoroughly investigated, the use of 2 ml rather than 1 ml of hexane during base clean-up appeared to achieve better transfer of pesticides from the methanol to hexane.

4.3.5 Effectiveness of the clean-up procedures

The elution patterns of the gas chromatograph's analytical column were satisfactory for samples cleaned with both the "new" and "old" methods (Fig. 10, p. 117 and Fig. 11, p. 118), although the base cleaned sub-samples gave characteristic "negative" peaks, and pigment removal was not complete. However, the "negative" peaks did not interfere with peak height measurements except for occasional cases with regard to the aldrin peak. Alkaline dechlorination of p,p'-DDT contributed to part of the p,p'-DDE of peak 14. The base also acted on some of the DDT compounds to result in a compound represented by peak 13 (Fig. 10).

4.3.6 Costs and time consumption

Chemicals - An exact comparison of the use of chemicals between the two methods cannot be made since the amounts of solvents used for certain purposes such as rinsing of glassware can only be approximated. However, larger and more glassware were used with the "old" method, requiring more solvent(s) for rinsing. Table 5, p. 114 shows that the "old" method required about 30 times more hexane than the "new" one. One reason for the smaller chemical usage with the "new" method is the smaller sample size, amounting to only 30% that of the "old" method, and of which only a third is actually run through the procedure. Loss of solvents during distillation contributes considerably to the total usage. An estimated 806 ml of hexane are used per sample, with the "old" method. Since it is recommended that 600 ml be discarded at each distillation (Mcleod, 1973; Maitho, 1978) for a 2,500 ml bottle of hexane, 1,900 ml remain for use. Taking account of the hexane discarded after one distillation, the total hexane used per sample is about 1,056 ml (Table 5, p. 114). The loss is even bigger if more than one distillation is carried out. With the same amount of hexane, 73 samples can be extracted by the "new" method. Similar calculations can be made for acetone and methanol.

The overall cost of chemicals per sample was about 7 times more with the "old" than with the "new" method (Ksh.258 vs. Ksh.36, Table 5, p. 114), based on prices of chemicals supplied by the Kenya Laboratory Supply Centre (1985).

Time consumption - As with chemicals it is difficult to give a precise comparison between the two methods. In our laboratory 6 or more extractions are possible with the "old" method in a day. However, clean-up stages require much attention and so a single person cannot clean more than 4 samples per day. Smart *et al.* (1974) made a similar observation, that the method allows 2 to 5 determinations in a day by one worker. About 6 hours are required per sample. The time required for each sample when several are run simultaneously is expectedly more than 6 hours because one cannot attend to all of them at exactly the same time.

With the "new" method, an estimated 3 hours are required up to the time the acid-cleaned sub-sample is ready for analysis. Ten samples per person can be extracted upto the acid cleaning stage in one day. Since base cleaning entails leaving the mixture of sample and alcoholic sodium hydroxide in a water-bath

overnight, the samples cannot be ready for gas chromatography until the following morning.

While the "new" method requires two differently cleaned sub-samples to be separately injected into the gas chromatograph, the "old" method requires only one injection. If the time required for the CPM to elute is taken to be 15 min, for each sample run by the "new" method, about 30 min are required for the elution of the acid and base cleaned extracts. About 15 min are required for each extract by the "old" method. The overall time spent from extraction to gas chromatographic analysis inclusive is, however, shorter with the "new" method when many samples are run simultaneously.

Labour accounts for a significant portion of the cost of an analysis (Johnson *et al.*, 1976). The "new" method is advantageous because in a 24 hour period, it can allow 10 samples to be extracted and cleaned by one worker compared with only 4 by the "old" method. A major disadvantage of the "new" method is that it requires two injections per every sample and this is likely to result in faster column deterioration and detector contamination than with the "old" method.

A panel for the determination of organochlorine pesticide residues in foodstuffs of animal origin (1979) observed that cost, safety and speed of an analysis have influenced recent development of methods for determining pesticide residues. Further, Wood (1969) noted that a suitable method should be applicable to a wide range of samples and should not require large amounts of solvent and adsorbent. The "new" method fulfilled several of the above requirements better than the "old" one.

Table 2 Evaluation of the pesticide recoveries by the "new" and "old" methods at "low" and "high" spiking levels. E = excellent (± 10% of spiked amount), G = good (± 20%), A = acceptable (± 30%), P = poor (± 40%), U = unacceptable (± 50%)

Compound	"LOW" SPIKING LEVEL		"HIGH" SPIKING LEVEL		"LOW" SPIKING LEVEL
	"New" method	"Old" method	"New" method	"Old" method	"Own" modification of "old" method*
p,p'-DDE	E	U	E	P	A
p,p'-DDT	E	A	E	E	A
p,p'-DDD	E	A	E	E	G
o,p'-DDT	E	U	E	G	G
o,p'-DDD	E	A	E	E	G
Lindane	G	A	G	G	P
α-HCH	A	U	E	G	A
β-HCH	A	P	E	E	E
Aldrin	A	U	G	U	U
Dieldrin	G	P	E	A	A
Endrin	A	U	E	A	A
Heptachlor	A	U	G	U	A
Heptachlor epoxide	G	P	G	G	G
Summary of scores					
Excellent (E)	5	-	9	4	1
Good (G)	3	-	4	4	4
Acceptable (A)	5	4	-	2	6
Poor (P)	-	3	-	1	1
Unacceptable (U)	-	6	-	2	1
	13	13	13	13	13

*Dimethylformamide : hexane ratio of 1:2 was used instead of 1:4

Table 3 Mean percentage recovery of aldrin at various steps of the clean-up procedure of the "old" method, following duplicate determinations. The average of the two peak height measurements is given in parentheses.*

Step	Per cent recovery	Peak height (mm)
After dimethylformamide: hexane (1:4) partitioning	41.9	78, 82 (80)
Hexane discarded after partitioning	52.9	97, 104 (101)
Direct column-chromatography	99.5	188, 191 (190)

*100% recovery corresponded to the standard's concentration of 0.05 µg/ml. This concentration gave a mean peak height of 191 mm.

Table 4 Recovery percentages of CPM components passed through the liquid-liquid partitioning step with the dimethylformamide to hexane ratios of 1:4 and 1:2. Only one determination was made with each ratio.

Compound	PERCENT RECOVERY	
	1:4	1:2
p,p'-DDE	66.7	92.5
p,p'-DDT	86.5	94.6
o,p'-DDD	85.2	92.0
o,p'-DDT	71.4	90.5
o,p'-DDD	89.6	92.7
Lindane	85.7	105.2
α-HCH	93.4	101.9
β-HCH	91.4	89.3
Aldrin	37.0	60.3
Dieldrin	76.3	100.0
Endrin	70.7	93.2
Heptachlor	54.2	88.1
Heptachlor epoxide	79.1	100.9

Table 5 Quantities and costs of reagents used per sample during sample preparation by the "new" and "old" methods for gas chromatographic analysis.

Step / Reagent	Drying		Extraction		Clean-up		Rinsing		Distillation		*Total*		*Cost (KSh.)*	
	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"
Hexane	-	-	-	200 ml	6 ml	526 ml	20 ml	80 ml	8 ml	250 ml	34 ml	1056 ml	6.50	200.65
Acetone	-	-	-	-	-	-	30 ml	80 ml	9.5 ml	25 ml	39.5 ml	105 ml	4.85	12.95
Dimethyl-formamide	-	-	-	-	-	24 ml	-	-	-	-	-	24 ml	-	6.40
Dichloro-methane	-	-	-	-	-	50 ml	-	-	-	-	-	50 ml	-	6.00
Diethylether	-	-	20 ml	-	-	-	-	-	-	-	20 ml	-	2.75	-
Methanol	-	-	-	-	1.5 ml	-	-	-	0.5 ml	-	2 ml	-	0.05	-
Conc. sulphuric acid	-	-	-	-	1.5 ml	-	-	-	-	-	1.5 ml	-	0.12	-
Florisil	-	-	-	-	-	25 g	-	-	-	-	-	25 g	-	22.50
Anhyd. sodium sulphate	4.5 g	23 g	-	-	-	23 g	-	-	-	-	4.5 g	46 g	0.85	8.45
Sodium sulphate	-	-	-	-	-	4 g	-	-	-	-	-	4 g	-	0.80
Sodium chloride	-	-	-	-	0.1 g	-	-	-	-	-	0.1 g	-	0.03	-
Sodium hydroxide	-	-	-	-	0.2 g	-	-	-	-	-	0.2 g	-	0.02	-
Sand	4.5 g	-	-	-	-	-	-	-	-	-	4.5 g	-	0.60	-
Nitrogen gas	-	-	-	-	-	-	-	-	-	-		-	20.00	-
											Total cost (KSh.)		35.80	257.75

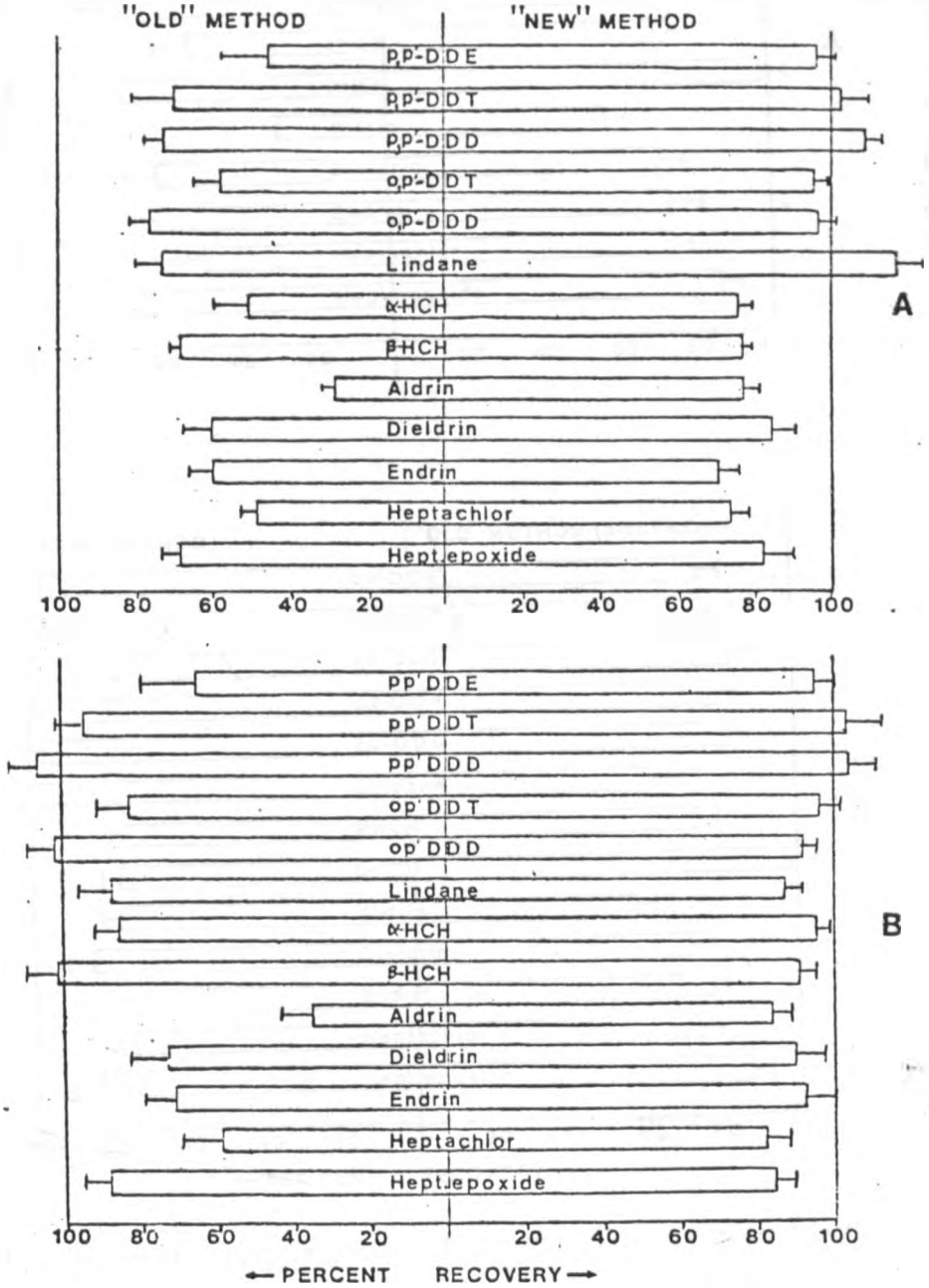


Fig. 8 A paired comparison of pesticide recoveries from spiked eggs (mean, S.D.) using two different extraction and clean-up methods at a "low" (A) and a high (B) spiking level.

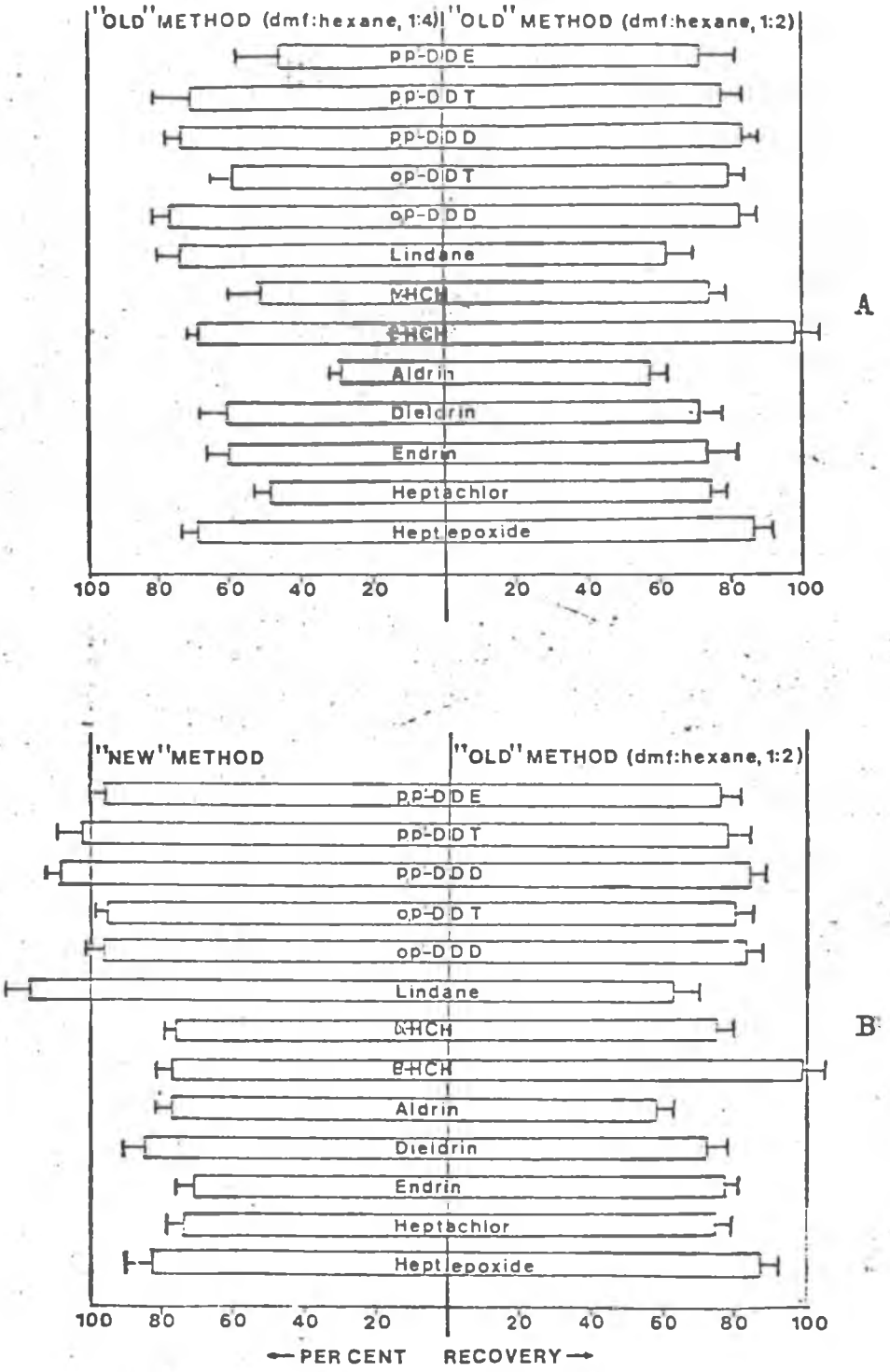
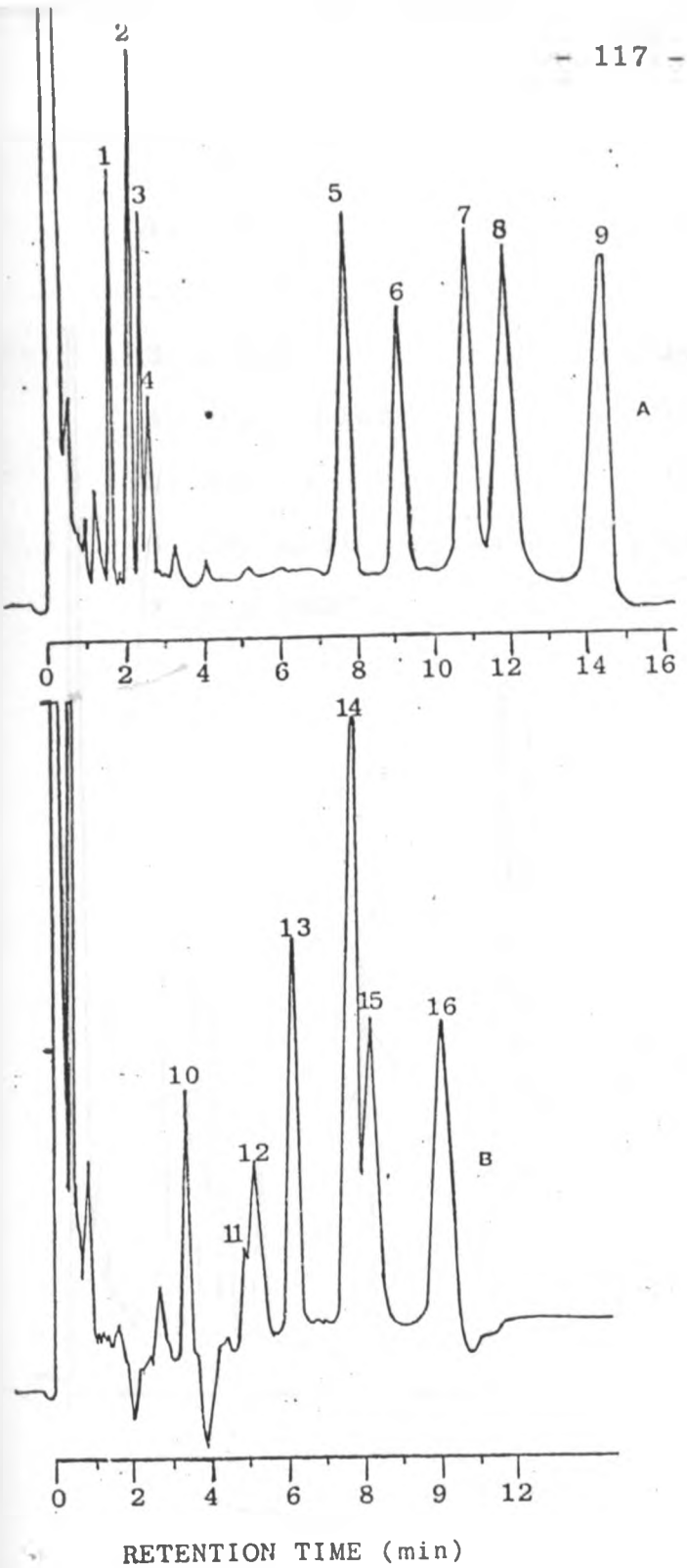


Fig. 9 A paired comparison of pesticide recoveries from spiked eggs (Mean, S.D.) at the "low" spiking level. (A) "old" method with dimethylformamide to hexane ratios of 1:4 versus 1:2; (B) "old" method with the ratio 1:2 versus the "new" method.



1. α-HCH
2. Lindane
3. β-HCH
4. Heptachlor
5. p,p'-DDE
6. o,p'-DDD
7. o,p'-DDT
8. p,p'-DDD
9. p,p'-DDT

10. Aldrin
11. ?
12. Heptachlor epoxide
13. Denaturation product of the DDT compounds
14. p,p'-DDE
15. Dieldrin
16. Endrin

Fig. 10 Chromatogram on the analytical column for eggs spiked with 13 chlorinated pesticide compounds, after extraction and clean-up with acid (A) and base (B) of the "new" method. The volumes injected were 3 µl at detector and column temperatures of 250°C and 200°C and a nitrogen flow rate of 65 ml/min.

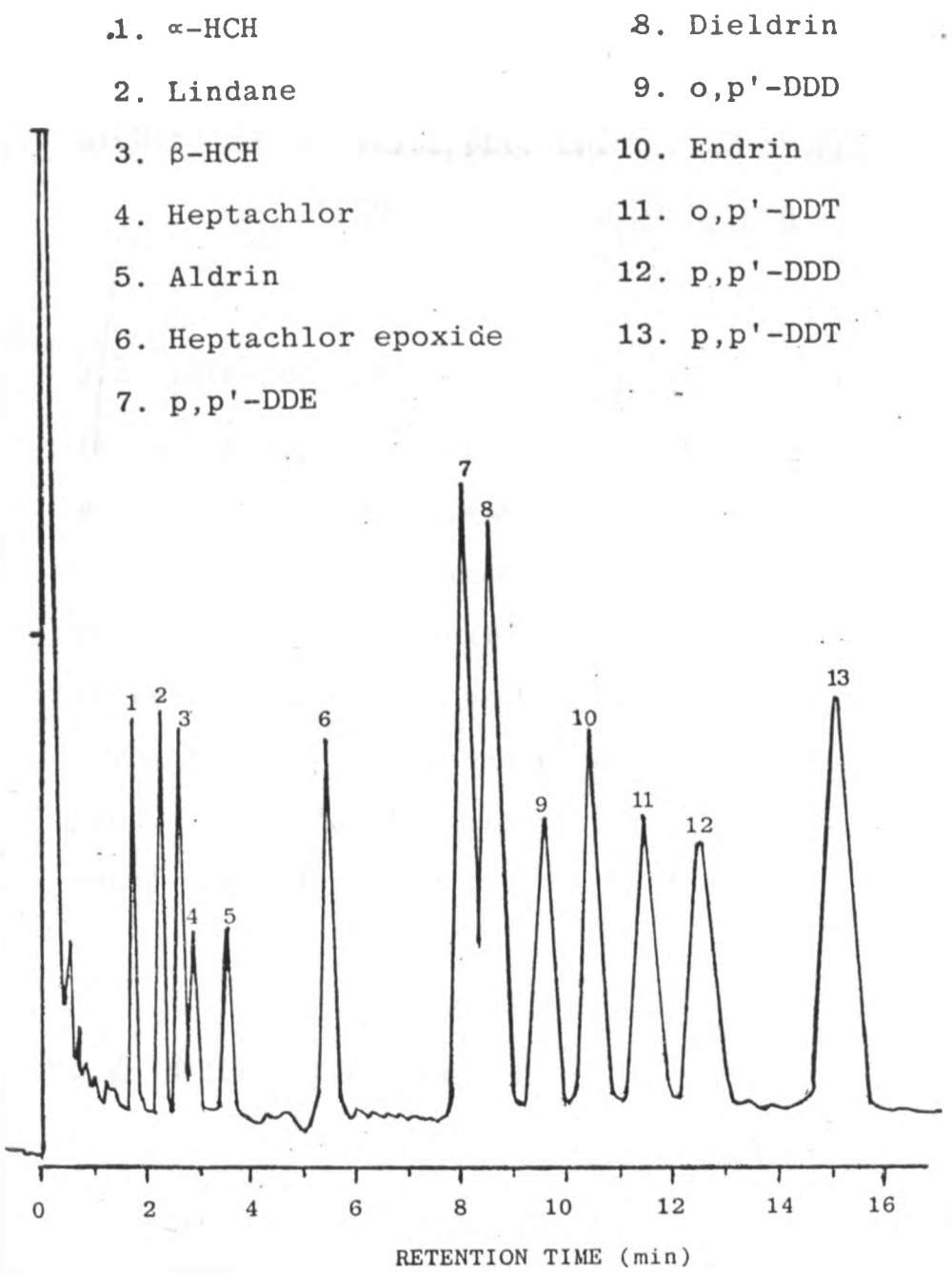


Fig. 11 Chromatogram on the analytical column for eggs spiked with 13 chlorinated pesticides compounds after extraction and clean-up with the "old" method. The volume injected was 3 µl at detector and column temperatures of 250°C and 200°C and a nitrogen flow rate of 65 ml/min.

CHAPTER FIVE

STUDIES ON PESTICIDE RECOVERIES FROM SPIKED POULTRY FEED WITH THE "NEW" METHOD

5.1 INTRODUCTION

In the attempts to trace the source of pesticide residues in eggs, it was decided to analyse poultry feed samples to supplement the information obtained through the questionnaires. It was also found relevant to test whether the "new" method could be suitable for extraction and clean-up of pesticides from poultry feed. To do this, commercial poultry feed (layers mash) and flour were first spiked with the CPM mixture.

5.2 MATERIALS AND METHODS

5.2.1 Equipment and chemicals

The equipment and chemicals were those applicable to the "new" method (Ch. 4, p. 77).

5.2.2 Spiking of feed

Commercial layers mash - The whole feed sample was transferred into a mortar and mixed thoroughly with a pestle. An amount of the mixed sample was transferred

into another mortar and using a Carlburg's pipette, 0.2 ml of isooctane, containing CPM 1:100 were added for every 10 g of the sample to give the "low" spiking level. Another portion of the mixed sample was spiked at the "high" level with 0.5 ml of CPM 1:100 for every 10 g sample. Re-homogenization was done using the pestles and the spiked portions kept in a refrigerator, in stoppered bottles, ready for extraction, clean-up and gas chromatographic analysis. The unspiked portion was similarly stored and was used as the blank.

Flour - The flour was obtained by grinding the collected grains using a waring blendor ("Dynamics Corporation of America", New Hartford, U.S.A.). Spiking was done as described for commercial feed except that for the "high" spiking, 0.4 ml of CPM 1:100 were added for every 10 g of flour.

5.2.3 Method

The "new" method (Ch. 4, p. 85) as described for eggs was employed. Unlike egg extracts, feed extracts were usually accompanied by some sediment. Therefore, to determine the weight of the feed extract the diethyl-ether of extraction had to be centrifuged first for the sediment to separate and thereafter the ether

transferred into another pre-weighed centrifuge tube, before evaporation. A 1 ml volume of hexane was used for the base cleaned portion.

5.3 RESULTS AND DISCUSSION

5.3.1 Pesticide recoveries

The criteria used for pesticide recoveries from spiked eggs (Ch. 4, p. 97) were also applied to recoveries from feed.

Commerical poultry feed

The recoveries at both spiking levels are shown in Table 6, p. 124.

"Low" spiking level - Excellent recoveries were obtained for 8 compounds, while good and acceptable recoveries were obtained for 2 and 3 compounds respectively.

"High" spiking level - Excellent recoveries were obtained for 6 and good ones for 5 compounds. Recovery

for 1 compound was acceptable, while another was poorly recovered.

Flour

The results are shown in Table 7, p. 125.

"Low" spiking level - As with commercial feed, 8 compounds were excellently recovered. Four compounds had good recoveries, while 1 compound was poorly recovered.

"High" spiking level - Ten compounds were excellently recovered. Good, acceptable and poor recoveries were obtained for 1 compound each.

General observations

The recoveries were satisfactory for both types of feed at the two spiking levels. However, β-HCH had recoveries of over 120% at the "low" spiking level with both feeds. Also, heptachlor epoxide was poorly recovered at the "high" spiking level with the two feeds. The observations were unexplainable.

5.3.2 Clean-up efficiency

The clean-up was satisfactory. There were no miscellaneous peaks and the chromatograms had stable base lines. As with eggs, however, the base cleaned portion gave "negative" peaks although the peaks did not interfere with peak height measurements. Pigment removal was also incomplete.

5.3.3 Conclusion

The "new" method was found suitable for the analysis of feed samples. Compared to the case with eggs, however, the ether of extraction eluted very slowly with feed samples. This was due to the very tight packing of the ground feed sample, in the extraction column.

Table 6 Mean recovery percentages (six replicates) ± S.D. of pesticides from commercial poultry feed spiked at "low" and "high" levels. E = excellent (± 10% of spiked value), G = good (± 20%), A = acceptable (± 30%), P = poor (± 40%), U = unacceptable (± 50%).

Compound	"LOW" SPIKING LEVEL				"HIGH" SPIKING LEVEL			
	ppm* added	per cent recovery	S.D.	Score	ppm added	per cent recovery	S.D.	Score
p,p'-DDE	0.020	110.0	8.9	E	0.050	96.0	8.6	E
p,p'-DDT	0.052	81.1	9.4	G	0.130	86.9	7.4	G
p,p'-DDD	0.038	105.3	5.5	E	0.095	98.8	9.1	E
o,p'-DDT	0.045	93.6	7.6	E	0.113	89.2	9.5	G
o,p'-DDD	0.040	104.6	5.6	E	0.100	90.8	10.4	E
Lindane	0.005	76.0	16.7	A	0.013	73.8	11.7	A
α-HCH	0.005	96.7	8.2	E	0.013	94.9	9.3	E
β-HCH	0.020	124.2	8.6	A	0.050	92.0	8.9	E
Aldrin	0.010	74.0	11.4	A	0.020	85.6	13.1	G
Dieldrin	0.024	101.2	10.1	E	0.060	87.3	10.0	G
Endrin	0.040	110.8	7.5	G	0.100	96.6	8.4	E
Heptachlor	0.005	90.0	11.0	E	0.013	81.9	9.3	G
Heptachlor epoxide	0.016	97.5	11.3	E	0.040	69.2	9.4	P
Summary of scores								
Excellent (E)				8				6
Good (G)				2				5
Acceptable (A)				3				1
Poor (P)				-				1
				13				13

* in µg of pesticide/g of feed.

Table 7 Mean recovery percentages (six replicates) ± S.D. of pesticides from flour spiked at "low" and "high" levels. E = excellent (± 10% of spiked value), G = good (± 20%), A = acceptable (± 30%), P = poor (± 40%), U = unacceptable (± 50%).

Compound	"LOW" SPIKING LEVEL				"HIGH" SPIKING LEVEL			
	ppm* added	per cent recovery	S.D.	Score	ppm added	per cent recovery	S.D.	Score
p,p'-DDE	0.020	93.3	12.5	E	0.040	107.0	8.9	E
p,p'-DDT	0.052	111.6	8.3	G	0.104	102.8	9.5	E
p,p'-DDD	0.038	113.1	10.4	G	0.076	109.9	10.8	E
o,p'-DDT	0.045	104.8	8.8	E	0.090	104.4	9.3	E
o,p'-DDD	0.040	106.3	9.7	E	0.080	102.5	9.7	E
Lindane	0.005	96.7	8.2	E	0.010	98.0	13.0	E
α-HCH	0.005	105.0	5.5	E	0.010	96.0	13.4	E
β-HCH	0.020	131.7	9.8	P	0.040	102.5	12.6	E
Aldrin	0.010	103.3	10.3	E	0.020	79.0	9.6	A
Dieldrin	0.024	88.2	11.4	G	0.048	94.6	6.8	E
Endrin	0.040	97.1	8.3	E	0.080	92.3	4.5	E
Heptachlor	0.005	104.7	12.3	E	0.010	82.0	8.6	G
Heptachlor epoxide	0.016	84.4	8.6	G	0.032	68.1	5.6	P

Summary of scores

Excellent (E)	8	10
Good (G)	4	1
Acceptable (A)	-	1
Poor (P)	1	1
	13	13

* In µg of pesticide/g of feed

CHAPTER SIX

ORGANOCHLORINE PESTICIDE RESIDUES IN EGGS FROM EMBU AND MERU .- OCCURRENCE, SOURCES AND HEALTH ASPECTS

6.1 INTRODUCTION

In a recent study on organochlorine pesticide residues in eggs and chicken fat from 11 districts in Kenya, 12 different residues were detected (Kahunyo, 1983). Particularly high levels of DDT were found in eggs from Embu, where 12 of the 15 eggs collected had total DDT residues exceeding the Extraneous Residue Limit (ERL) of 0.5 mg/kg. The present work was initiated as a more extensive follow-up study, and also aimed at tracing the sources of the residues.

There are several potential sources of such residues, including use of contaminated feedstuffs in poultry houses to control pests (Stadelman *et al.*, 1965; Whitehead, 1971; Lillard and Noles, 1973; Naber, 1977). Feedstuffs of plant origin may become contaminated by application of insecticides on crops, growing the crops in contaminated soils and using the chemicals on stored plant products (Naber, 1977). Therefore, feeds were analysed and questionnaires filled in to obtain supplementary information on possible sources of contamination in eggs.

6.2 MATERIALS AND METHODS

6.2.1. Sampling areas

Embu district - This district (Fig. 12, p. 135) shows the typical agro-ecological profile of the windward side of Mt. Kenya, from the cold and wet upper, to the hot and dry lower zones in the Tana River Basin. Farming changes with this profile from the tea-dairy to the livestock millet zones. Only a third of the district is productive, with small scale farming being the main practice. The other two-thirds are of very marginal agricultural potential (Jaetzold and Schimdt, 1983). The Runyenjes division constitutes most of the highly productive highlands, while the other two divisions, Gachoka and Siakago, constitute most of the less productive midland and lowland zones.

Meru district - This district (Fig. 13 , p. 136) lies on the south eastern slope of Mt. Kenya. The agro-ecological zones of Embu extend to Meru, but the contrast between wet and dry seasons is more marked. Less than 15% of the district, consisting of some parts of Nithi, Imenti, Mikinduri and Igembe divisions, is suitable for small scale farming. This area has one of the highest agricultural outputs in Kenya.

The sampled regions in both districts can be grouped into five main areas on the basis of cash-crop grown:

(1) coffee area

(2) coffee-tea area

(3) tea area

(4) tobacco and/or cotton area

(5) rice area

The location of these areas is roughly shown on the two maps (Fig. 12, p. 135 and Fig. 13, p. 136). Maize is grown in nearly all the areas, while vegetables, potatoes and garden peas are grown in the tea and coffee-tea areas. Dairy animals are also kept in these areas. Various types of beans and peas are grown in the more arid areas where cotton, tobbaco, millet and rice are grown.

Coffee and tea grow together in between areas growing either coffee or tea and these are grouped together in the results/discussion.

6.2.2 Sampling procedure

A total of 367 eggs and 42 feed samples were collected in 61 farms from the various localitites (Table 8, p. 134). No strict randomisation procedure was worked out but an

attempt was made to visit farms at random, without prior knowledge of their existence.

Eggs - The sampling took place between May and October 1984, at a time of widespread drought throughout the country. Therefore, no samples were obtained from the very dry areas of both districts.

Eggs were collected from both enclosed and free-range chicken farms. They were clearly labelled to identify them with the area and farm of sampling. In the laboratory, each egg was broken using a spatula, homogenized with a waring blendor and stored in a labelled glass bottle with screw-cap, in a deep freezer while awaiting analysis.

Feeds - Where chickens were kept enclosed, commercial poultry feeds were used. Small amounts of feed were taken from top, middle and bottom of the feed containers and the combined lot from one container put into a labelled sampling bottle with screw-cap. A similar procedure was also used for maize, millet, rice, sorghum and sunflower. In the laboratory, the feed samples were kept in a refrigerator until analysed.

Questionnaires - These were filled for each batch of eggs from each farm. They were designed to give such information as land and pesticide use, feeding and keeping methods of poultry (Appendix 4, p. 226) which could possibly account for the chicken's pesticide exposure.

6.2.3 Analysis of the samples and quality assurance

Extraction and clean-up of egg samples was carried out with the "new" method (Ch. 4, p. 85). The final extracts were analysed by gas liquid chromatography as described in Ch. 3 p. 68 under the operating conditions given on pp. 63 and 64. No corrections were made for recoveries because most compounds had good or excellent recoveries (Table 2, p. 111).

In addition to the recovery studies (Ch. 4) a further quality assurance test was done by extracting and cleaning the first 12 egg samples in duplicate. The values of each duplicate agreed closely (Appendix 11, p. 233). Reagent blanks were run through the method after every 30 to 40 samples to check for reagent contamination by the pesticides or other electron capturing substances. Pesticide recoveries were checked after every 50 samples by running spiked samples. Nine eggs were sent for parallel analyses

to the Department of Pharmacology and Toxicology, Norwegian College of Veterinary Medicine. The results agreed well with ours, except for the dieldrin concentration in two of the eggs (Appendix 5, p. 227).

The feeds were also extracted and cleaned-up with the "new" method.

6.2.4 Statistics

Where it was desirable to find out whether or not an observed difference between two sample means could be attributed to chance, the following formula was used (Freund, 1973):

$$z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the means of two random samples of sizes n_1 and n_2 and standard deviations s_1 and s_2. A standard normal distribution table was then used to obtain the probabilities.

6.2.5 Extraneous residue and detection limits

The results of egg analyses were evaluated against the Extraneous Residue Limits (ERLs), formerly known as the Practical Residue Limits, set by the Codex Alimentarius Commission of FAO/WHO (1978):

Compound	ERL (mg/kg shell-free egg)
Total DDT	0.5
Lindane	0.1
Dieldrin + aldrin	0.1
Heptachlor	0.05

In addition, a Tolerance limit (T) of 0.2 mg/kg has been given for endrin. Total DDT was obtained as p,p'-DDT + o,p'-DDT + 1.11 (p,p'-DDD + p,p'-DDE), the factor 1.11 correcting for lower molecular weights (Skaare *et al.*, 1985).

Although the method could detect residues lower than 0.01 mg/kg the criterion of Cummings *et al.* (1966; 1967) was adopted by which values less than 0.01 mg/kg were regarded as unreliable.

6.2.6 Definition of terms

The two terms, Extraneous Residue Limit and Acceptable Daily Intake (ADI) referred to in this study are defined as follows:

Extraneous Residue Limit - Is the recommended maximum concentration of a pesticide residue in a food or food commodity that results from circumstances not designated to protect the food or food commodity against pest attack. It is expressed in mg of the residue per kg of the food or food commodity.

Acceptable daily intake - Is the daily intake (expressed as mg/kg body weight) which on the basis of all known facts about the chemical and its residues appear to be without appreciable risk during a life time.

Table 8 Number of eggs and feed samples collected from Embu and Meru.

Locality	Samples	
	Eggs	Feeds
Embu		
Runyenjes	95	8
Siakago	37	11
Gachoka	34	5
Embu Agricultural Institute	15	2
Meru		
Nithi	82	8
South Imenti	21	3
North Imenti	55	3
Mikinduri	28	2
Total	367	42

Fig. 12 Approximate areas (x) in Embu district where eggs and poultry feed were sampled.

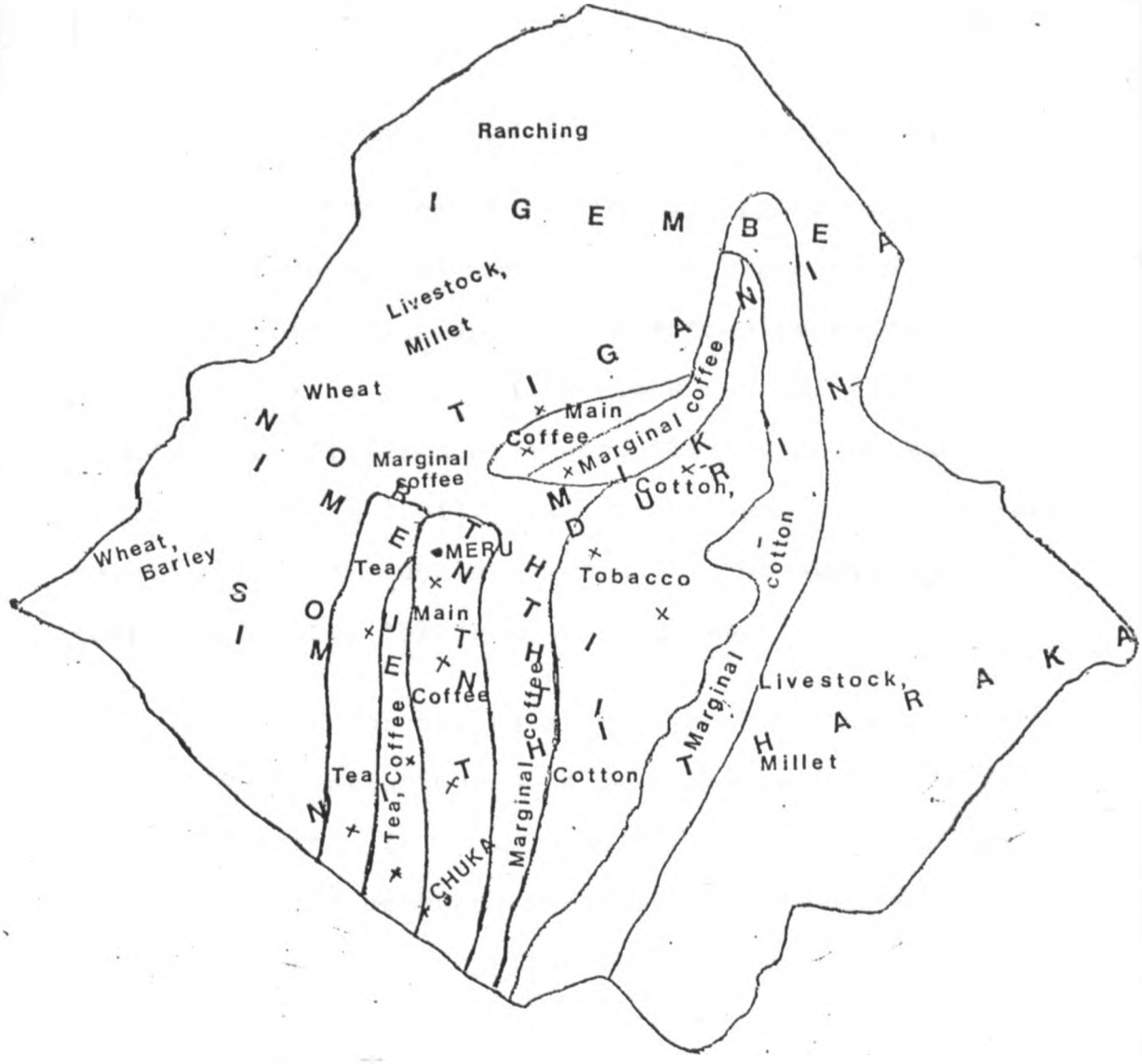


Fig. 13 Approximate areas (x) in Meru district where eggs and poultry feed were sampled.

6.3 RESULTS AND DISCUSSION

6.3.1 DDT group

DDT was found in all the 367 eggs analysed, and 27% of them had residues equal to or above the ERL (Table 9, p. 166).

DDT in eggs from enclosed versus free-range chickens

Total DDT - The mean total DDT in eggs from enclosed chickens (0.36 mg/kg) was below the ERL of 0.5 mg/kg, while the corresponding value for free range chickens (1.15 mg/kg) was more than 2 times the ERL (Table 9, p. 166). The difference between the two means was significant ($p < 0.01$). 42% of the eggs from free-range chickens had residue levels equal to or above the ERL, compared to only 15% from enclosed chickens.

The mean for the total DDT in the various localities showed large variations both for free-range and enclosed chickens' eggs. In most localities however, the eggs from free-range chickens had higher residue levels than those from enclosed chickens (Appendices 6, p.228 and 7, p.229).

The mean total DDT from Gachoka (Mwea) was 3.21 and 2.26 mg/kg in eggs from free-range and enclosed chickens respectively. These values are not much

different from the mean, 3.52 mg/kg, obtained by Kahunyo (1983), in 15 eggs from Embu, some of which were bought from a market in this area in 1981.

The occurrence of high DDT levels in eggs from free-range chickens agree with the findings of Hashemy-Tonkabony and Mosstofian (1979) in Iran. They suggested that the difference depended on the free-range chickens' collection of feeds in yards and farms extensively sprayed with pesticides.

The present study showed, that even within the same farms, eggs from free-range chickens had higher residue levels than those from enclosed chickens. For instance, in one farm the average residues for 10 eggs from enclosed versus 8 eggs from free-range chickens were 0.04 vs 0.28 mg p,p'-DDT/kg eggs and 0.05 vs 0.79 mg p,p'-DDE/kg eggs.

Ratio of p,p'-DDT to p,p'-DDE - Eggs from enclosed chickens contained, on average, about equal amounts of p,p'-DDT and p,p'-DDE, while eggs from free-range chickens, had twice as much p,p'-DDE as p,p'-DDT (Table 9, p.166). The difference between the means of the ratios was significant ($p < 0.01$). All eggs from Embu Agricultural Institute contained more p,p'-DDT than p,p'-DDE (Appendix 6, p.228).

Since the ratio of p,p'-DDT to p,p'-DDE was significantly lower in eggs from free-range chickens compared to eggs from enclosed chickens, this indicates that the free-range chickens had obtained the p,p'-DDT from a source after it had partly undergone an environmental transformation to p,p'-DDE. The enclosed chickens appeared to have had a more direct exposure. When a study is done in an area while DDT is in use, the ratio p,p'-DDT to p,p'-DDE is high (Albert *et al.*, 1980). Cecil *et al.* (1972) found that when chickens were continuously given DDT experimentally, the p,p'-DDT to p,p'-DDE ratio in eggs ranged between 25 and 75. In contrast they referred to eggs of wild birds, where DDE predominate as a result of environmental conversion from p,p'-DDT.

p,p'-DDD - This compound is another metabolite of p,p'-DDT, but also commercially available as an insecticide. Only low levels of it were detected (Table 10, p. 167). The mean value (0.01 mg/kg) for enclosed chickens' eggs was one third that for eggs from free-range chickens, although 51% compared to 40% of the eggs were positive for the residue. The highest p,p'-DDD concentration, 0.61 mg/kg, was found in an egg from a free-range bird.

o,p'-DDT - Low levels of o,p'-DDT were detected in 12% and 23% of eggs from enclosed and free-range chickens respectively (Table 10, p. 167). o,p'-DDT is an isomer of p,p'-DDT. Technical DDT may contain as much as 33% o,p'-DDT. The low levels reported may depend on the compound's very low tendency to accumulate in poultry tissues and eggs (Foster *et al.*, 1972). A similar observation was made by Bitman *et al.* (1971) with rats, when they found that not only o,p'-DDT but also other o,p'-analogues (o,p'-DDE and o,p'-DDD) were almost completely metabolised and excreted. Cecil *et al.* (1973) also reported that o,p'-DDT accumulates in tissues to levels equivalent to only 10% of those originally in the diet.

DDT in eggs from areas with differing agricultural activities

With the exception of eggs from Embu Agricultural Institute, the eggs were grouped according to the main cash crops of the various localities: rice, cotton-tobacco and coffee-tea growing areas.

Rice area

Total DDT - The highest mean concentration, 3 mg/kg,

occurred in this area where 79% of the samples had residues equal to or above the ERL (Table 11, p. 168). The standard deviation was large, about 3 mg/kg. The highest DDT-value in this study, 10.25 mg/kg, occurred in an egg from this area.

Ratio of p,p'-DDT to p,p'-DDE - On the average, the eggs contained about twice as much p,p'-DDE as p,p'-DDT (Table 11, p. 168).

Cotton-tobacco area

Total DDT - The mean DDT concentration was lower than in the rice area, and the difference significant ($p < 0.01$). Nevertheless, the mean exceeded the ERL about 3 times. 74% of the samples had residue levels equal to or above this limit. As in the rice area the standard deviation was large (Table 11, p. 168).

Ratio of p,p'-DDT to p,p'-DDE - The eggs contained about twice as much p,p'-DDE as p,p'-DDT (Table 11, p. 168).

Coffee-tea area

Total DDT - The mean total DDT was below the ERL.

Only 12% of the samples had values equal to or above this limit (Table 11, p. 168). The differences between means in eggs from this area and in those from the preceding two areas were significant ($p < 0.01$).

Ratio of p,p'-DDT to p,p'-DDE - The ratio (0.81) was about 60% higher than either of the two preceding ones and the differences were significant ($p < 0.01$).

p,p'-DDD and o,p'-DDT in eggs from the three areas

p,p'-DDD - The levels of p,p'-DDD were generally low in all the three areas, although the mean level in eggs from the rice area (0.09 $\pm$ 0.12 mg/kg) was about 5 times that in eggs from the cotton-tobacco area (0.02 $\pm$ 0.06 mg/kg), and about 9 times that from the coffee-tea area (0.01 $\pm$ 0.03 mg/kg). The highest recorded concentration, 0.61 mg/kg, was found in an egg from the rice area.

o,p'-DDT - The mean concentration of o,p'-DDT was about the same (0.01 mg/kg) in all the three areas.

Information from the questionnaires

A 5% DDT powder was used in some places, mainly against

maize stalk borers. A few farmers applied the powder around poultry houses to prevent entry of pests. Dicofol (Kelthane^{R}), a non-insecticidal DDT analogue, but an effective acaricide, was used in a few places. Some insects such as *Drosophila* and German cockroaches produce dicofol as a major metabolite of DDT (Matsumura, 1975). Cypermethrin, (Ambush c-y^{R}), a synthetic pyrethroid (Rundle and Forsyth, 1984) was widely used on cotton and cabbages. It appeared to be superseding the use of DDT on cotton. In the market, a 25% DDT emulsion, applicable against pests of cotton, cabbages, maize and beans was available.

In the coffee-tea-dairy areas farmers were increasingly using the more expensive but less persistent organophosphorus and carbamate insecticides, especially in the control of livestock pests. This may account for the low DDT levels in eggs from this area, and perhaps partly explain the lower levels in eggs from enclosed chickens since most of these eggs were from the coffee-tea area. Many of the answers with regard to pesticide usage, especially in the cotton-tobacco and in the rice areas, were found to be unreliable.

Residues in feeds

Total DDT - Feeds from enclosed poultry farms had a

mean total DDT content which was 4 times that of feeds from free-range chickens (Table 12, p.169).

Ratio of p,p'-DDT to p,p'-DDE - Since the p,p'-DDE values were mostly below 0.01 mg/kg, the ratios of p,p'-DDT to p,p'-DDE were calculated for only 38% of the samples. The ratios ranged from 2 to 50. p,p'-DDT is metabolised to p,p'-DDE and therefore p,p'-DDE's occurrence in higher concentrations in eggs than in feeds was expected.

p,p'-DDD - p,p'-DDD was detected in some feed samples, but the mean values was less than 0.01 mg/kg. Like p,p'-DDE it is a p,p'-DDT metabolite and this explains why it occurred in eggs more readily than in feeds.

o,p'-DDT - Although o,p'-DDT residues were low in feeds (Table 10, p.167), they occurred more frequently in feeds than in eggs. This was expected because of the rapid metabolism and excretion of o,p'-DDT.

Accumulation ratios - In feeding experiments, accumulation ratios (values of residues in eggs to those in

feeds), have been calculated. These values should be calculated when plateau concentrations of residues in eggs or fat are reached, otherwise too low ratios result (Kan and Tuinstra, 1976b). These workers have given their own ratio (1.3, for DDT) as well as ratios from the experiments of other researchers (0.3 to 1.6). To find out whether or not the residues found in feeds could account for the residues in eggs, an attempt was made to calculate the accumulation ratios for DDT in eggs from both types of farms. This was also done for a selected number of individual farms (Table 13, p. 170). It will be seen that the accumulation ratios for enclosed farms, with the exception of two, did not differ much from those obtained in feeding experiments.

Further, it is evident that in none of the farms with high DDT concentration in eggs, were the concentrations in the collected feed samples likely to be the major source of contamination. Caution should, however, be exercised in interpreting the Table because:

(a) Feeds were changed from time to time and were obtained from various manufacturers.

(b) Collection of eggs was done at varying intervals following the start of feeding of a given feed, and the plateau levels may not have been attained.

(c) Supplementary feeds such as cabbages were commonly fed to poultry, but were not analysed.

(d) Worms are capable of concentrating chemicals from the soil (Stickel *et al.*, 1965; Yadav *et al.*, 1981). Their intake by chickens may then result in contaminated eggs (Ladisch, 1970). This is particularly so for free-range chickens.

It would have been of interest if worms and cabbages had been analysed.

DDT has been used extensively in controlled feeding experiments. With low level feeding (Liska *et al.*, 1964; Stadelman *et al.*, 1965; Herrick *et al.*, 1969; Smith *et al.*, 1970a) the resulting concentrations in eggs agree fairly well with those reported for enclosed chickens in this study.

6.3.2 Cyclodiene group

Included in this group are dieldrin, aldrin, endrin, heptachlor and heptachlor epoxide.

Aldrin and dieldrin

Residues in eggs from enclosed versus free-range chickens

Aldrin was found in only 2 eggs (0.02 and 0.02 mg/kg). They also had the highest dieldrin concentration in this study (Appendix 13, p. 236, Eggs NE4 and NE6).

Dieldrin was the most frequently detected cyclodiene pesticide residue. All eggs from enclosed chickens had the residue (Table 14, p. 171). The mean value for these eggs was 0.16 mg/kg, 1.6 times the ERL of 0.1 mg/kg. Although 12% of the eggs from free-range chickens had no detectable amounts of dieldrin, the mean value for this group was as high as 0.61 mg/kg, about 6 times the ERL, due to a number of eggs with very high dieldrin contents. These eggs mainly came from Mikinduri and Gachoka areas (Appendices 13, p. 236 and 17, p. 240). Some eggs had values as high as 14 to 15 mg/kg, which are 140 to 150 times the ERL. Of the eggs from free-range chickens, 35% had values equal to or in excess of the ERL, about the same percentage (37) as for the other group. The difference between the means for eggs from enclosed and those from free-range birds was significant ($p < 0.01$).

Hashemy-Tonkabony and Mosstofian (1979) also found higher dieldrin residue levels in eggs from free-range than in those from enclosed chickens in Iran, as did Findlay and Hamilton (1968) in Britain.

Kahunyo (1983) found dieldrin levels in eggs to be low, not only in Embu where only 1 egg out of 15 had dieldrin (0.03 mg/kg), but in other districts as well.

In the present study, methodological experiments

showed that when Florisil, partially deactivated with 2% water (Ch. 4, p. 103) was used in the "old" method as employed by Kahunyo (1983), recoveries of dieldrin and endrin from the chromatographic column ranged from 0 to 42%. It has been recommended that each batch of Florisil should be standardized by verifying the pesticide elution pattern in the absence of sample because of its variability in adsorptive activity. Further the volume required to elute the 2 compounds is large compared to other chlorinated pesticides (Ch. 4, p. 103). These factors may possibly explain why Kahunyo's samples were mostly negative for dieldrin. Another possible explanation is that the use of dieldrin and/or aldrin has started recently.

Poor recovery of dieldrin from a Florisil column was recently reported by Al-Omar *et al.* (1985) (Ch. 4, p. 104). The poor recovery was suggested as a probable explanation when these workers found aldrin more frequently than dieldrin in lamb meat and beef.

Residues in eggs from areas with differing agricultural activities

Rice area - 76% of the samples were positive for dieldrin. Although only 35% of the samples had

residue levels equal to or above the ERL of 0.1 mg/kg, the mean dieldrin level was about 11 times this limit (Table 15, p. 172).

Coffee-tea area - Dieldrin was detected in 98% of the samples. The mean value was 3 times the ERL, and 37% of the samples, compared to 35% in the preceding area, had levels equalling or exceeding the limit (Table 15, p. 172). There was no significant difference between the means of the two areas.

Cotton-tobacco area - 89% of the samples were positive for dieldrin. The mean residue level exceeded the ERL, but was only half that obtained in the coffee-tea area. The residue levels in 50% of the samples equalled or exceeded the ERL (Table 15, p.172). The difference between this mean and that of eggs in the coffee-tea area was significant ($p < 0.05$). The difference was however, not significant when compared to the mean of the rice area.

Information from the questionnaires

Although dieldrin was detected in most of the eggs, not a single farmer reported using it. The accuracy

of these reports is questionable. The annual report for Eastern Province (1980) showed that 5,378 litres of a 8% dieldrin preparation had been used in Meru and Embu during the year. In the market a 18% dieldrin emulsion was also available.

A 2.5% preparation of aldrin was used in some areas during sowing. The annual report also showed that 437 kg of a 40% aldrin preparation was used in Embu. Since dieldrin is one of the several metabolic products of aldrin (Korte, 1972), aldrin is a potential source of dieldrin residues in living systems.

Residues in feeds

The mean total dieldrin (dieldrin + aldrin) for feeds from enclosed poultry farms was twice that for feeds from free-range farms (Table 16, p. 173). The mean aldrin level for feeds from enclosed farms was 0.01 mg/kg, while the corresponding value for feeds from free-range farms was less than 0.01 mg/kg. Waldron and Naber (1974b) found that dieldrin and other pesticides fortified in poultry feeds at levels of 0.01 and 0.05 mg/kg gave very low residues in eggs and tissues. Levels in feed, of 0.1 mg/kg gave 0.12 mg/kg, while 0.1 mg aldrin/kg feed gave 0.06 mg/kg in eggs after 32 weeks. Herrick *et al.* (1969) fed 0.114 mg aldrin/kg

feed for a week and found 0.01 mg dieldrin/kg eggs. It is therefore unlikely that the levels found in feeds in the present study could account for the residues in many of the corresponding eggs.

Only 0.5% (2) of the 367 egg samples had aldrin. In contrast, although feed samples were few, 33% had aldrin.

Endrin

In eggs

Endrin was detected in some of the eggs with high dieldrin levels. It is an isomer of dieldrin. Chemically the two are very similar, but unlike dieldrin which is one of the most persistent chemicals known endrin can easily be degraded by heat and light (Matsumura, 1975). The detected levels were low, with only 3 eggs obtained from free-range chickens in the rice growing area, having levels above the Tolerance limit (T) of 0.2 mg/kg (Table 17, p. 174). The residue was detected in only 14 eggs, 13 of which came from free-range chickens.

Hashemy-Tonkabony and Mosstofian (1979) found endrin in eggs from both free-range and enclosed chickens with the mean for the former eggs being about 3 times that for the latter.

In poultry endrin build-up in fat is high during exposure, but residues fall rapidly after cessation of exposure (Brooks, 1974b).

Information from the questionnaires

None of the farmers reported use of endrin.

Residues in feeds

Not a single of the feed samples analysed had endrin.

Heptachlor and heptachlor epoxide

Eggs

Neither in the present study nor in that by Kahunyo (1983) were these compounds found. However, Kahunyo found heptachlor in some of the chicken fat samples.

Information from the questionnaires

None of the farmers interviewed reported use of heptachlor.

Residues in feeds

Traces of heptachlor epoxide appeared in 6 feed samples.

6.3.3. Hexachlorocyclohexane (HCH) group

This group consists mainly of α-, β- and γ-isomers. A HCH preparation that contains at least 99% of the γ-isomer is called lindane (Matsumura, 1975). The α-isomer occurred in quantifiable amounts in only 17 eggs (0.01 to 0.04 mg/kg) and the β-isomer in 32 eggs (0.01 to 0.11 mg/kg), while lindane occurred in 244 eggs (0.01 to 0.53 mg/kg).

Lindane residues in eggs from free-range and enclosed chickens

The mean levels were low in both groups, but the mean for eggs from free-range birds was three times that for the other group and the difference significant ($p < 0.05$). Eight per cent of the eggs from free-range and none from enclosed chickens had residue concentrations equal to or greater than the ERL of 0.1 mg/kg (Table 14, p. 171). The highest recorded value, 0.53 mg/kg, occurred in an egg from free-range chickens.

While there were large variations in residue levels of total DDT and dieldrin between and within localities and also between enclosed and free-range chickens, there was less variation with lindane (Appendices 9,p. 231 and 10, p. 232). In Siakago, there were

4 samples with quantifiable amounts of β-HCH, but not lindane. The total HCH in other eggs consisted mainly of lindane.

Lindane in eggs from areas of differing agricultural activities

Rice area - As with DDT and dieldrin, this area had the highest lindane levels. 21% of the eggs had a value equal to or greater than the ERL, but the mean concentration was below this limit. The residue was quantifiable in 38% of the samples (Table 15, p. 172).

Cotton-tobacco area - As with total DDT residues, this area was second to the preceding one in residue levels of lindane. Quantifiable amounts were found in 50% of the samples, while 9% had levels equal to or above the ERL (Table 15, p. 172). The difference between the means in eggs from this area and in those from the rice area was not significant.

Coffee-tea area - Lindane was quantifiable in 49% of the samples. The levels were below the ERL in all eggs (Table 15, p. 172). The difference between the means

of this and the rice area was significant ($p < 0.01$), as it was, when compared to that of the cotton-tobacco area ($p < 0.05$).

Information from the questionnaires

Lindane was reported to be used widely, especially on cabbages, as a 0.65% preparation. These cabbages were often fed to enclosed chickens as a supplement to commercial feeds and were also accessible to free-range birds.

Residues in feeds

Lindane occurred in 89% and 47% of the samples from enclosed and free-range poultry farms respectively (Table 18, p. 175).

While the mean lindane value in feeds from enclosed poultry farms was 5 times that of the eggs, the corresponding relationship for free-range farms was 0.7 (Tables 14, p. 171 and 18, p. 175). The observation that some feed samples had higher lindane levels than the corresponding eggs indicates that either the feeds had been introduced just prior to the sampling, or more likely, lindane underwent rapid elimination as has been

reported (Ware and Naber, 1961; Cummings *et al.*, 1966; Smith *et al.*, 1970a; Foster *et al.*, 1972; Waldron and Naber, 1974b).

Accumulation ratios - As with DDT, accumulation ratios were calculated (Table 19, p. 176). Kan and Tuinstra (1976b) have given their own ratio (0.13) and ratios from the work of others (0.2 to 0.5). It will be seen that for enclosed chickens' eggs, the ratios generally agree with the literature values. Accordingly, the feed was a likely source of the residue in these eggs, while this was less evident for eggs from free-range chickens.

In interpreting Table 19, the limitations enumerated during the discussion on DDT should be borne in mind.

Seven samples had quantifiable amounts of the α- isomer (Appendix 19, p. 242). The isomer may have occurred as a contaminant of the lindane used, or as a result of microbial conversion from lindane.

No feed sample had the more persistent β-isomer.

6.3.4 Polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB)

PCBs are chlorinated mixtures of environmentally stable

compounds, arising as pollutants from their industrial applications. They are therefore expectedly higher in samples from the industrialised than in those from the less industrialised countries.

HCB is a chlorinated anti-fungal agent, used as seed dressing. Since the early 1970's it has been recognized as an environmental contaminant comparable to the insecticide DDT and the industrial chemicals PCBs (Taylor and Keenan, 1970).

Although both PCBs and HCB could be detected by the method used in this study, they were not found. PCBs have been reported in eggs, for instance, in Canada (Mes *et al.*, 1974) and France (Richou-Bac and Venant, 1980). HCB has also been reported in eggs, for example, in Switzerland and U.S.A. (FAO/WHO/UNEP, 1982).

Several unidentified peaks, suspected to represent PCBs were observed in each of 4 eggs from a farm in Embu. Analysis of these eggs in Norway showed that the peaks were not PCBs, but their identity remained unknown (Skaare, 1984. Personal communication).

6.3.5 Probable effect of drought

Sampling was done between May and October 1984 when there was widespread drought in Kenya. This resulted

in free-range hens laying fewer eggs than under normal conditions. Enclosed chickens were generally better fed than free-range ones and most probably produced more eggs in a given period. Cecil *et al.* (1973) noted that of two pullet groups fed with a pesticide contaminated diet, one group, fed with a 1.5% calcium containing diet had a lower feed consumption than the other, whose diet contained 3.5% calcium. Further, they observed that the group with the lower feed consumption also produced significantly fewer eggs, suggesting less chances of residue excretion. It is therefore reasonable to assume that the more eggs produced in a given period, the smaller the fraction of the initial pesticide load in the hen is contained in each egg, all other factors that influence pesticide excretion being constant. The assumption, if valid, may account for part of the difference observed in residue levels between the eggs of enclosed and those of free-range chickens in this study.

6.3.6 Present results compared with findings in other countries

Pesticide residues in eggs

Total DDT - According to the available literature, both the mean and the maximum concentrations of total DDT are higher in the two Kenyan studies, than

reported in eggs from any other country (Table 20, p. 177).

Ratio of p,p'-DDT to p,p'-DDE - Both the present results and Kahunyo's data gave a low p,p'-DDT to p,p'-DDE ratio (Table 20, p. 177). The low ratios probably reflect a more rapid degradation of DDT in tropical environments compared to temperate ones (Ch. 2, p. 13). Perfect (1980) reported much lower DDT accumulation in tropical soils than expected on the basis of experience gained in temperate climates.

Dieldrin and aldrin - The dieldrin levels reported in the present study are much higher than those reported in other countries (Table 21, p. 178). Except for eggs from free-range chickens in Iran, the present mean values are the only ones that exceed the ERL of 0.1 mg/kg. The 2 compounds are readily available in the Kenyan market.

Endrin - The occurrence of this compound in eggs has only been reported from Iran and in the present study, with the present maximum value (0.78 mg/kg)

exceeding the highest Iranian value (0.13) by 6 times (Table 22, p. 179). The means are below the Tolerance limit of 0.2 mg/kg.

Heptachlor and heptachlor epoxide - None of these residues have been found in Kenyan eggs. In other countries low levels have been reported (Table 22, p. 179). The mean residue level in eggs from Spain is about 0.06 mg/kg, on a whole-egg basis and therefore approximately equal to the ERL of 0.05 mg/kg. The maximum value in eggs from Iran and Germany are above this limit.

Lindane - The levels reported in some countries are higher while in others they are lower than those of the present study (Table 23, p. 180). In this study only the maximum value for eggs from free-range birds exceeds the ERL, while in Iran both maximum values are above this limit. On a whole-egg basis, the mean lindane value in egg yolk reported by Ehrenstorfer and Guenther (1974) corresponds to a value of about 0.22 mg/kg, which is above the ERL of 0.1 mg/kg.

Pesticide residues in feeds

The available information on analyses of poultry feeds

from other countries is very limited. Some results from U.S.A., Britain, Turkey and Kenya are shown in Table 24, p. 181 .

DDT group - As in the present study, low levels of residues are reported. Feeds from Turkey have the highest mean total DDT reported, which is 3 times the value for Kenyan feeds.

Ratio of p,p'-DDT to p,p'-DDE - With only two exceptions, the p,p'-DDT to p,p'-DDE ratios given in Table 24 are greater than 1.

Dieldrin and aldrin - As in the present study, these two compounds are found in the feeds in other countries at low mean levels. Some maximum values, especially for aldrin, are, however, several times the present values.

Heptachlor and heptachlor epoxide - These two compounds are reported in feeds from U.S.A., at low levels. In the present study only traces of heptachlor epoxide appeared in 6 samples (Appendix 19, p. 242).

Lindane - Lindane was also found in feeds, at low levels. The mean in the present study is equal to that in feeds from Britain, higher than those for feeds from U.S.A. and lower than that for feeds from Turkey.

Since residues in feeds have been reported at comparable levels from all countries in contrast to the levels in eggs, this gives support to the earlier assumption that free-range birds were getting the residues from other sources, apart from the analysed feeds.

6.3.7 Present results related to the Acceptable Daily Intakes - toxicological implications

The ADIs are arrived at by competent people and are based on an overall evaluation of all the known facts from toxicological studies of chemicals and their residues. For instance, special risks to the young and the aged and such factors as interactions, are taken into account (Brooks, 1974b).

Total DDT - The ADI of DDT in man is 0.005 mg/kg body weight (FAO/WHO/UNEP, 1982). This has been related to the present findings (Table 25, p.182). Although this approach is somewhat arbitrary, it will be seen that, alone, the egg with the highest DDT concentration had

enough residue to cover the ADI for a 15 kg, about 3 year old child, for a whole week. It is of course likely that the child would also get the residue from other sources.

Dieldrin - The ADI of dieldrin is 0.0001 mg/kg body weight. The egg with the highest dieldrin level had a content which is 117 times the ADI for a 70 kg adult and 547 times that for a 15 kg child. Even the average dieldrin values for the eggs exceeded the ADI for both the adult and the child by 2.7 and 13 times respectively (Table 25, p. 182).

Lindane - The ADI of lindane is 0.01 mg/kg body weight. With the results of the present study, several eggs would have to be consumed by both the child and the adult for the ADI to be reached (Table 25, p. 182).

Toxicological implications

The residues that reach man through foods are more likely to result in chronic than in acute toxicity. It is, however, uncertain to what extent the levels reported here can cause chronic toxicity, as are the manifestations of such toxicity. It is therefore difficult to give the actual toxicolo-

gical implications of these results. Since the ADI is easier to exceed in the child than in the adult, the child is at a greater risk. Effects are dose-dependent and therefore should be manifest first in those people with high intake levels (McEwen and Stephenson, 1979). The child has the added disadvantage that the insecticides tend to accumulate in the CNS during its ontogeny.

Reproduction can potentially be affected and the effect can only be manifested in adulthood.

The effects will be influenced by other factors such as the length of exposure and the type of insecticide involved. Cyclodiene compounds are more toxic than DDT. Illnesses to plant workers in the early days during production of aldrin, dieldrin and endrin were common (McEwen and Stephenson, 1979) until sanitation was improved. LD_{50} values determined on the rat are the most widely used index of toxicity (McEwen and Stephenson, 1979). Assuming the validity of this index, the cyclodienes should be more toxic than DDT, to man.

Malnutrition may also influence the toxicity of residues. Animal experiments have shown that toxicity may increase, for example in conditions of protein deficiency (Berlin *et al.*, 1979).

The results from Embu and Meru indicate that

there is a need to introduce better practices in the use of the chlorinated hydrocarbon insecticides especially dieldrin/aldrin at least in some parts of Kenya. Many of the farmers interviewed had little knowledge of the chemicals they were using. With some background information on pesticides, the farmers would be able to select and use them more judiciously. Enlightening the people on this aspect is one of the objectives of the Pest Control Products Board and the Pesticide Chemicals Association of Kenya (Omamo, 1983; Wainaina, 1983).

Finally, periodic monitoring of pesticide chemicals in various food items is necessary to enable stringent legislative measures to be taken for restricting pesticide contamination in food.

Table 9 Total DDT residues and p,p'-DDT to p,p'-DDE ratios in eggs from enclosed and free-range chickens in Embu and Meru.

Poultry farms	No. of eggs			Total DDT (mg/kg eggs)	Ratio p,p'-DDT/p,p'-DDE	
	Total	Positive	≥ERL	*Mean* ± S.D. (Range)	*Mean* ± S.D. (Range)	% >1.00
Enclosed	211	211	32	*0.36* ± 0.54 (0.01 - 2.62)	*0.97* ± 0.47 (0 - 3.00)	34
Free-range	156	156	66	*1.15** ± 1.81 (0.02 - 10.25)	*0.53* ± 0.42 (0 - 2.03)	11

Positive : ≥ 0.01 mg DDT/kg eggs.

Extraneous Residue Limit (ERL) of total DDT : 0.5 mg/kg eggs.

* Mean ≥ERL.

Table 10 p,p'-DDD and o,p'-DDT (mg/kg sample) in eggs and feeds from enclosed and free-range poultry farms.

Poultry farms	No. of samples	p,p'-DDD		o,p'-DDT	
		Mean ± S.D. (Range)	No. positive	*Mean* ± S.D. (Range)	No. positive
		E G G S			
Enclosed	211	*0.01* ± 0.04 (<0.01 - 0.45)	107	*<0.01* (<0.01 - 0.41)	25
Free-range	156	*0.03* ± 0.07 (<0.01 - 0.61)	63	*0.01* ± 0.02 (<0.01 - 0.08)	36
		F E E D S			
Enclosed	27	*<0.01* (<0.01 - 0.04)	8	*0.02* ± 0.03 (<0.01 - 0.17)	20
Free-range	15	(-)	0	*0.01* ± 0.02 (<0.01 - 0.04)	5

Positive : ⩾ 0.01 mg p,p'-DDD or o,p'-DDT/kg sample
<0.01 : below the detection limit
(-) : no sample had quantifiable amounts

Table 11 Total DDT residues and p,p'-DDT to p,p'-DDE ratios in eggs from three areas in Embu and Meru of different agricultural activities.

Main agricultural activity	No. of eggs			Total DDT (mg/kg eggs	Ratio p,p'-DDT/p,p'-DDE	
	Total	Positive	≥ ERL	*Mean* ± S.D. (Range)	*Mean* ± S.D. (Range)	% > 1.00
Rice, marginal cotton	34	34	27	*2.93** ± 2.71 (0.21 - 10.25)	*0.64* ± 0.35 (0.22 - 1.65)	12
Cotton, tobacco	54	54	40	*1.39** ± 1.34 (0.04 - 6.07)	*0.51* ± 0.46 (0 - 1.95)	13
Coffee, tea	264	264	32	*0.34* ± 1.05 (0.01 - 2.40)	*0.81* ± 0.45 (0 - 2.33)	24

Positive : ≥ 0.01 mg DDT/kg eggs.

Extraneous Residue Limit (ERL) of total DDT: 0.5 mg/kg eggs.

*Mean ≥ERL

Table 12 p,p'-DDT and total DDT residues in poultry feeds from Embu and Meru.

Poultry farms	No. of samples		p,p'-DDT (mg/kg feed)		Total DDT (mg/kg feed)	
	Total	positive	*Mean* ± S.D.	Range	*Mean* ± S.D.	Range
Enclosed	27	24	*0.08* ± 0.10	(<0.01 - 0.50)	*0.12* ± 0.14	(<0.01 - 0.71)
Free-range	15	7	*0.02* ± 0.04	(<0.01 - 0.15)	*0.03* ± 0.06	(<0.01 - 0.20)
Enclosed and free-range	42	31	*0.06* ± 0.09	(<0.01 - 0.50)	*0.09* ± 0.12	(<0.01 - 0.71)

Positive : ≥ 0.01 mg DDT/kg feed

<0.01 : below the detection limit

Table 13 Accumulation ratios of DDT (ratio of the concentration in eggs to that in corresponding feed) in eggs from Embu and Meru, compared to accumulation ratios obtained in feeding experiments (Kan and Tuinstra, 1976b).

Sample number, type of farm	No. of eggs	Residues of p,p'-DDT + p,p'-DDE (mg/kg sample)		Accumulation ratios
		In eggs	In feed	
Accumulation ratios in feeding experiments				*0.3 - 1.6*
All enclosed (E) *farms*	211	0.15 + 0.17 = 0.32	0.08 + 0.01 = 0.09	*3.6*
All free-range (F) *farms*	156	0.34 + 0.71 = 1.05	0.02 +<0.01 = 0.02	>52
$AE_{1-20,}^{E}$	20	0.08 + 0.07 = 0.15	0.08 + 0.01 = 0.09	1.7
$BE_{1-23,}^{E}$	23	0.07 + 0.10 = 0.17	0.50 + 0.01 = 0.51	0.3
$FE_{1-4,}^{E}$	4	0.17 + 0.20 = 0.37	0.13 + 0.05 = 0.18	2.1
$ME_{1-10,}^{E}$	10	0.70 + 1.27 = 1.97	0.03 +<0.01 = 0.03	66
$RE_{1-10,}^{E}$	10	0.55 + 0.30 = 0.83	0.06 + 0.02 = 0.08	10
$*DE_{1-10,}^{E}$	10	0.04 + 0.05 = 0.09	0.13 + 0.01 = 0.14	0.6
$*DFr_{1-8,}^{F}$	8	0.28 + 0.79 = 1.07	<0.01	>107
$NE_{1-6,}^{F}$	5	1.81 + 1.49 = 3.30	0.08 + 0.01 = 0.09	37
$QE_{1-3,}^{F}$	3	0.05 + 0.48 = 0.53	0.02 +<0.01 = 0.02	27
$QE_{4-6,}^{F}$	3	0.05 + 0.24 = 0.29	0.01 +<0.01 = 0.01	29
$QE_{26-30,}^{F}$	5	0.11 + 0.66 = 0.77	<0.01	>77

* From the same farm.

Table 14 Dieldrin and lindane residues (mg/kg eggs) in eggs from enclosed and free-range chickens in Embu and Meru.

Poultry farms	No. of eggs	Dieldrin Mean ± S.D. (Range)	Dieldrin No. positive	Dieldrin No. ≥ERL	Lindane Mean ± S.D. (Range)	Lindane No. positive	Lindane No. ≥ERL
Enclosed	211	*0.16** ± 0.26 (0.01 - 1.44)	211	78	*0.01* ± 0.01 (<0.01 - 0.04)	173	0
Free-range	156	*0.61** ± 2.06 (<0.01 - 14.90)	138	55	*0.03* ± 0.09 (<0.01 - 0.53)	71	12

Positive : ≥ 0.01 mg dieldrin or lindane/kg eggs.

Extraneous Residue Limit (ERL) : 0.1 mg dieldrin or lindane/kg eggs.

* Mean ≥ ERL

<0.01 : below the detection limit.

Table 15 Dieldrin and lindane residues (mg/kg eggs) in eggs from three areas in Embu and Meru of different agricultural activities.

Main agricultural activity	No. of eggs	Dieldrin			Lindane		
		Mean ± S.D.	No. positive	No. ≥ERL	*Mean* ± S.D.	No. positive	No. ≥ERL
Rice, marginal cotton	34	*1.12** ± 3.52 (<0.01 - 14.90)	26	12	*0.08* ± 0.14 (<0.01 - 0.53)	25	7
Coffee, tea	264	*0.31** ± 0.98 (<0.01 - 9.74)	260	98	*0.01* ± 0.01 (<0.01 - 0.07)	130	0
Cotton, tobacco	54	*0.15** ± 0.18 (<0.01 - 0.69)	48	27	*0.04* ± 0.11 (<0.01 - 0.47)	27	5

Positive : ≥ 0.01 mg dieldrin or lindane/kg eggs.

Extraneous Residue Limit (ERL) : 0.1 mg dieldrin or lindane/kg eggs.

* Mean ≥ ERL

<0.01 : below the detection limit

Table 16 Dieldrin and total dieldrin (aldrin + dieldrin) residues in poultry feeds from Embu and Meru.

Poultry farms	No. of samples Total	positive	Dieldrin (mg/kg feed) *Mean* ± S.D.	Range	Total dieldrin (mg/kg feed) *Mean* ± S.D.	Range
Enclosed	27	20	*0.01* ± 0.01	(<0.01–0.06)	*0.02* ± 0.02	(<0.01–0.07)
Free-range	15	3	*<0.01* ± 0.01	(<0.01–0.03)	*0.01* ± 0.01	(<0.01–0.05)
Enclosed and free-range	42	23	*0.01* ± 0.01	(<0.01–0.06)	*0.01* ± 0.02	(<0.01–0.07)

Positive : ⩾ 0.01 mg dieldrin/kg feed

<0.01 : below the detection limit

Table 17 Endrin residues (mg/kg eggs) in eggs from free-range and enclosed chickens in Embu and Meru.

Locality Main agric, products	FREE - RANGE				ENCLOSED			
	No. of eggs			*Mean* ± S.D.	No. of eggs			*Mean* ± S.D.
	Total	No. positive	No. ≥T	(Range)	Total	No. positive	No. ≥T	(Range)
Gachoka Rice, marginal cotton	24	5	3	*0.07* ± 0.19 (<0.01 - 0.78)	10	-	-	-
Mikinduri Coffee	16	5	0	*0.01* ± 0.02 (<0.01 - 0.06)	12	-	-	-
Nithi Coffee, tea	19	2	0	*<0.01* (<0.01 - 0.08)	63	-	-	-
N/Imenti Coffee, tea, cotton	32	1	0	*<0.01* (<0.01 - 0.01)	23	-	-	-
Runyenjes Coffe, tea,	32	-	-	-	63	1	-	*<0.01* (<0.01 - 0.0
Siakago Cotton, tobacco	27	-	-	-	10	-	-	-
S/Imenti Coffee, tea	6	-	-	-	10	-	-	-
Embu Agric. Inst. Coffee	-	-	-	-	10	-	-	-

Positive : ≠0.01 mg endrin/kg eggs

<0.01 : below the detection limit

\- : no residues

Tolerance limit (T) : 0.2 mg endrin/kg eggs

Table 18 Lindane residues in poultry feeds from Embu and Meru.

Poultry farms	No. of samples		Lindane (mg/kg feed)
	Total	Positive	*Mean* ± S.D. (Range)
Enclosed	27	24	*0.05* ± 0.09 (<0.01 - 0.39)
Free-range	15	7	*0.02* ± 0.03 (<0.01 - 0.09)
Both	42	31	*0.04* ± 0.08 (<0.01 - 0.39)

Positive : ⩾ 0.01 mg lindane/kg feed

<0.01 : below the detection limit

Table 19 Accumulation ratios of lindane (ratio of the concentration in eggs to that in corresponding feed) in eggs from Embu and Meru, compared to accumulation ratios obtained in feeding experiments (Kan and Tuinstra, 1976b).

Sample number, type of farm	No. of eggs	Residues mg/kg sample		Accumulation ratio
		In eggs	In feed	
Accumulation ratios in feeding experiments				*0.13 - 0.5*
All enclosed (E) farms	211	0.01	0.05	*0.2*
All free-range (F) farms	156	0.03	0.02	*1.5*
$AE_{1-20,}^{E}$	20	0.01	0.04	0.3
$BE_{1-23,}^{E}$	23	0.02	0.25	0.1
$FE_{1-4,}^{E}$	4	0.04	0.04	1.0
$ME_{1-10,}^{E}$	10	0.03	0.08	0.4
$RE_{1-10,}^{E}$	10	0.02	0.01	2.0
$*DE_{1-10,}^{E}$	10	0.02	0.14	0.1
$*DFr_{1-8,}^{F}$	8	0.02	<0.01	>2
$NE_{1-6,}^{F}$	5	0.12	<0.01	>12
$QE_{1-3,}^{F}$	3	<0.01	0.01	<1
$QE_{4-6,}^{F}$	3	<0.01	0.01	<1
$QE_{26-30,}^{F}$	5	0.01	0.06	0.2

* From the same farm

Table 20 Total DDT residues (mg/kg) in eggs from various countries.

Country	Sample description	Total DDT* Mean	Max.	p,p'-DDT / p,p'-DDE	Reference
GREAT BRITAIN	96 samples of 12 eggs each from enclosed chickens	0.037**		1.00	Findlay and Hamilton (1968)
	67 samples of 12 eggs each from free-range chickens	0.038**		0.88	
	all 163 samples	<0.038**		1.00	
NORWAY	116 eggs	0.01**	0.45		Bjerk (1970)
U.S.A.	Egg yolk, No. not given	0.072	0.110	-	Foster *et al.* (1972)
ITALY		0.027		-	Riva *et al.* (1973) : cited by Campanini *et al.* (1980).
GERMANY	egg white	0.011		3.50	Ehrenstorfer and Guenther (1974)
	egg yolk	0.095		0.60	
CANADA	100 samples of 12 eggs each	0.012	0.314	0.71	Mes *et al.* (1974)
GERMANY 1973-76	970 samples of 30 eggs each	0.0075		0.60	Dorn and Knoppler (1977)
IRAN	143 samples of 5 eggs each from commercial breeds	0.17		1.61	Hashemy-Tonkabony and Mosstofian (1979)
	78 samples of 5 eggs each from free-range chickens	0.50		1.06	
	all 221 samples	0.25		1.61	
SPAIN	50 yolks	0.79		7	Serrano *et al.* (1979)
FRANCE		<0.01			Richou-Bac and Venant (1980)
KENYA	145 eggs	0.64	9.40	0.49	Kahunyo (1983)
KENYA	211 eggs from enclosed chickens	0.36	2.62	0.97	Present study
	156 eggs from free-range chickens	1.15	10.25	0.53	
	all 367 eggs.	0.70	10.25	0.78	

* Total DDT has been calculated as shown in Ch. 6, p. 132

** Estimates from the information given in the paper.

Table 21 Dieldrin/aldrin residues (mg/kg) in eggs from various countries.

Country	Sample description	Dieldrin/aldrin Mean	Max.	Reference
GREAT BRITAIN	96 samples of 12 eggs each from enclosed chickens	<0.01*	0.01	Findlay and Hamilton (1968)
	67 samples of 12 eggs each from free-range chickens	0.014*	0.06	
	All 163 samples	<0.014*	0.06	
ITALY	40 eggs	0.0034		Riva *et al.* (1973): cited by Campanini *et al.* (1980).
CANADA	100 samples. Each sample = 12 eggs	0.001	0.006	Mes *et al.* (1974).
GERMANY	egg white egg yolk	0.011 0.09		Ehrenstorfer and Guenther (1974).
GERMANY	970 samples of 30 eggs each	0.0005	0.10	Dorn and Knoppler (1977).
IRAN	143 samples of 5 eggs each from commercial breeds	0.026	0.185	Hashemy-Tonkabony and Mosstofian (1979).
	78 samples of 5 eggs each from free-range chickens.	0.168	0.31	
	All 221 samples	0.031	0.31	
SPAIN	50 yolks	0.271		Serrano *et al.* (1979).
FRANCE		<0.005		Richou-Bac and Venant (1980).
KENYA	145 whole eggs	<0.01	0.07	Kahunyo (1983).
KENYA	211 eggs from enclosed chickens	0.16	1.44	Present study
	156 eggs from free-range chickens	0.61	14.90	
	All 367 eggs	0.35	14.90	

* Estimates based on the information given in the paper.

Table 22 Endrin and heptachlor/heptachlor epoxide residues (mg/kg) in eggs from various countries.

Country	Sample description	Endrin		Heptachlor/heptachlor epoxide		Reference
		Mean	Max.	Mean	Max.	
ITALY	96 eggs			0.023* 0.045**		Troncone *et al.* (1970): cited by Campanini *et al.* (1980).
CANADA	100 samples of 12 eggs each			0.002* 0.001**	0.010 0.003	Mes *et al.* (1974).
GERMANY	egg white			0.01* 0.002**		Ehrenstorfer and Guenther (1974).
	egg yolk			0.06* 0.016**		
GERMANY	970 samples of 30 eggs each			0.0004**	0.10	Dorn and Knoppler (1977)
IRAN	143 samples of 5 eggs each from enclosed chickens	0.007	0.013	0.019***	0.223	Hashemy-Tonkabony and Mosstofian (1979).
	78 samples of 5 eggs each from free-range chickens	0.021	0.130	0.032***	0.092	
	all the 221 samples	0.017	0.130	0.023***	0.223	
SPAIN	50 yolks			0.18*		Serrano *et al.* (1979).
FRANCE				<0.005		Richou-Bac and Venant (1980).
KENYA	145 eggs					Kahunyo (1983).
KENYA	211 egss from enclosed chickens	<0.01	0.04			Present study
	156 eggs from free-range chickens	<0.01	0.78			
	all 367 eggs	<0.01	0.78			

* Heptachlor

** Heptachlor epoxide

*** Both

Table 23 Lindane residues (mg/kg) in eggs from various countries.

Country	Sample description	Lindane Mean	Lindane Max.	Reference
GREAT BRITAIN	96 samples of 12 eggs each from enclosed chickens	0.018*	0.03	Findlay and Hamilton (1968).
	67 samples of 12 eggs each from free-range chickens	0.018*	0.04	
	All 163 samples	0.018*	0.04	
U.S.A.	egg yolk	<0.001		Foster *et al.* (1972).
ITALY	40 eggs	0.0032		Riva *et al.* (1973): cited by Campanini *et al.* (1980).
CANADA	100 samples of 12 eggs each	0.003	0.01	Mes *et al.* (1974).
GERMANY	egg white	0.031		Ehrenstorfer and Guenther (1974).
	egg yolk	0.65		
GERMANY	970 samples of 30 eggs each	0.0038	0.10	Dorn and Knoppler (1977).
IRAN	143 samples of 5 eggs each from enclosed chickens	0.058	0.259	Hashemy-Tonkabony and Mosstofian (1979).
	78 samples of 5 eggs each from free-range chickens	0.039	0.97	
	All 211 samples	0.052	0.97	
SPAIN	50 yolks	0.18		Serrano *et al.* (1979).
FRANCE		<0.005	0.01	Richou-Bac and Venant (1980).
KENYA	145 eggs	<0.01	0.04	Kahunyo (1983)
KENYA	211 eggs from enclosed chickens	0.01	0.04	Present study
	156 eggs from free-range chickens	0.03	0.53	
	All 367 eggs	0.02	0.53	

* Estimates based on the information given in the paper.

Table 24 Organochlorine pesticide residues (mg/kg) in poultry feeds from various countries.

Country	Sample type and number	Total DDT Max. (mean)	p,p'-DDT / p,p'-DDE	Dieldrin Max. (mean)	Aldrin Max. (mean)	Lindane Max. (mean)	Heptachlor Max. (mean)	Heptachlor epoxide Max. (mean)	Reference
GREAT BRITAIN		(0.01)*	>1	0.01 (<0.01)		0.12 (0.04)			Findlay and Hamilton (1968).
U.S.A.	Control ration in an experiment	(0.044)	0.39						Foster *et al.* (1972).
U.S.A.	Soy bean meal 234	(0.006)	6.8	0.028 (0.0013)	0.278 (0.006)	0.013 (0.0004)	0.024 (0.0012)	0.007 (0.0002)	Waldron and Naber (1974a).
U.S.A.	Corn mean 203	(0.009)	19.5	0.203 (0.0025)	1.909 (0.0156)	0.033 (0.0007)	0.029 (0.0014)	0.036 (0.006)	Waldron and Naber (1974a).
U.S.A.	Fats 232	(0.151)	0.12	0.389 (0.0192)	8.395 (0.0393)	0.172 (0.0042)	0.734 (0.0113)	0.062 (0.0024)	Waldron and Naber (1974a).
U.S.A.	Alfa alfa meal 226	(0.0792)	2.39	0.183 (0.0072)	0.322 (0.008)	0.109 (0.0018)	0.130 (0.0055)	0.138 (0.0042)	Waldron and Naber (1974a).
TURKEY	Non-commercial and commercial feeds, 43**	(0.281)		(0.020)	(0.132)	(0.218)			Akman *et al.* (1976).
KENYA	Non-commercial and commercial feeds, 42	0.71 (0.09)	12	0.06 (0.01)	0.06 (0.01)	0.39 (0.04)			Present study.

* Estimates based on the information given in the paper.

** Poultry and cattle feed

Table 25 Mean and maximum residues of total DDT, dieldrin and lindane in 211 eggs from enclosed and 156 eggs from free-range chickens in Meru and Embu, related to the Extraneous Residue Limits (ERLs) for eggs and the Acceptable Daily Intakes (ADIs) of the compounds by man.

	Residue (mg/kg)	ERL (mg/kg)	ADI (mg/kg b.w.t.)	Residue in a 55 g egg	Days the ADIs are covered by the 55 g egg for a: 70 kg man	15 kg child
DDT						
Enclosed chickens						
Mean	0.36	0.5	0.005	0.02	0.06	0.3
Max.	2.62			0.144	0.4	2
Free-range						
Mean	1.15	0.5	0.005	0.063	0.15	1
Max.	10.25			0.564	1.5	7.5
Enclosed and free-range						
Mean	0.70	0.5	0.005	0.039	0.1	0.52
Max.	10.25			0.564	1.5	7.5
DIELDRIN						
Enclosed						
Mean	0.16	0.1	0.0001	0.009	1.3	6
Max.	1.44			0.079	11	53
Free-range						
Mean	0.61	0.1	0.0001	0.034	5	23
Max.	14.90			0.820	117	547
Enclosed and free-range						
Mean	0.35	0.1	0.0001	0.019	2.7	13
Max.	14.90			0.820	117	547
LINDANE						
Enclosed						
Mean	0.01	0.1	0.01	0.0006	0.001	0.004
Max.	0.04			0.002	0.003	0.015
Free-range						
Mean	0.03	0.1	0.01	0.002	0.003	0.013
Max.	0.53			0.03	0.042	0.195
Enclosed and free-range						
Mean	0.02	0.1	0.01	0.001	0.001	0.007
Max.	0.53			0.03	0.042	0.195

CHAPTER SEVEN

GENERAL CONCLUSIONS

1. Tests on the gas liquid chromatographic technique showed that all the 13 chlorinated hydrocarbon insecticides of interest in this study could be adequately identified and quantitated.

2. A comparison of an extraction and clean-up procedure so far used in our laboratory with an alternative newer one demonstrated that at a "low" spiking level the recoveries of all 13 compounds from spiked eggs were significantly better with the "new" method. Both methods yielded more complete recoveries at a "high" spiking. The "new" method still gave the best results, although the differences in recoveries were significant in its favour for fewer compounds than at the "low" spiking.

3. The methodological studies showed that some compounds such as aldrin were lost at the liquid-liquid partitioning step of the "old" method. Their recoveries could be improved by changing the dimethylformamide to hexane ratio at the partitioning step. Others like dieldrin were retained in the Florisil column. The recoveries could, however, be improved by increasing the amount of the deactivating water added to Florisil or the

volume of the eluting solvent mixture.

4. Comparison of the overall cost of chemicals revealed a cost of Ksh. 36 per sample with the "new" method against Ksh. 258 with the "old" method. The "new" method was also less time-consuming.

5. Satisfactory clean-up was obtained with both methods. However, pigment removal by base clean-up of the "new" method was incomplete. This could possibly cause faster detector contamination and column deterioration.

6. In the 367 eggs collected, 10 residues were detected in various combinations and in the following order of frequency. p,p'-DDE (in 100% of the eggs), p,p'-DDT (98%), dieldrin (95%), lindane (66%), p,p'-DDD (46%), o,p'-DDT (17%), β-HCH (9%), α-HCH (4.6%), endrin (4%) and aldrin (0.5%).

7. 27% of the eggs had residues at or above the ERL of 0.5 mg total DDT/kg eggs. The mean concentration, 0.70 mg/kg (Range 0.01 - 10.25), of the 367 eggs exceeded the ERL. p,p'-DDT and/or its major metabolite p,p'-DDE accounted for most of the total DDT. p,p'-DDD and o,p'-DDT accounted for the rest.

8. The mean dieldrin concentration, 0.35 mg/kg eggs

(Range 0.01 - 14.90), exceeded the ERL value of 0.1 mg/kg, 3.5 times. 36% of the eggs had residues at or above the ERL. At least part of the dieldrin in the eggs is likely to have originated from metabolic conversion of aldrin.

9. Only 3.3% of the eggs had lindane residues at or above the ERL of 0.1 mg/kg eggs. The mean concentration was 0.02 mg/kg (Range 0.01 - 0.53). Despite the reported extensive use of lindane, the concentrations of the residue were low. This probably depends on the fast elimination rate of the compound from the body.

10. Of the three areas with different agricultural activities, the rice, cotton-tobacco and coffee-tea areas, the rice area had the highest mean and maximum concentrations of all the compounds detected. Except for dieldrin, the cotton-tobacco area ranked second and the coffee-tea area the last. It was difficult, however, to obtain reliable information on the pesticide usage, especially from farmers in the rice and cotton-tobacco areas. In the coffee-tea areas, there seemed to be more frequent use of the more expensive but less persistent organophosphate and carbamate pesticides. This observation offers a possible explanation for the relatively low DDT content in most eggs from these areas.

11. The 156 eggs from free-range chickens had significantly higher residue concentrations of total DDT, dieldrin and lindane, compared to the 211 eggs collected from enclosed chicken farms. This probably depends on the access of the former chickens to farm yards treated with pesticides in the control of crop pests.

12. In contrast to the eggs, feed samples from farms with enclosed chickens had higher mean residue levels than feed samples from farms with free-range chickens. With a few exceptions, only the levels of DDT and lindane in feed from farms with enclosed chickens were found likely to account for the egg levels. Other potential sources not analysed such as earth-worms, which are known to concentrate chemicals from the soil and supplementary feeds like cabbages, might have been responsible for the residues not accounted for.

13. The mean ratio of p,p'-DDT to p,p'-DDE, 0.97, in eggs from enclosed chickens was significantly higher than the ratio, 0.53, in the eggs from free-range chickens. This indicates that the enclosed chickens had a more direct exposure to p,p'-DDT, while free-range chickens obtained more of the compound after environmental conversion to p,p'-DDE.

14. While the results with respect to DDT and lindane agreed well with those of an earlier study in Kenya, the high dieldrin levels sharply contrasted the previous findings. As indicated by the methodological studies, this could be due to dieldrin's retention in the Florisil column of the "old" method. Another possible explanation is that the use of dieldrin and/or aldrin has started recently.

15. According to the literature available, the total DDT and dieldrin levels in eggs in the present study are higher than those reported from any other country. The levels in the analysed feeds are, however, comparable to the feed levels reported from other countries.

16. When the present findings are related to the Acceptable Daily Intakes (ADIs) of a 70 kg adult man and a 15 kg child, the DDT content of each egg was, on the average, one tenth of the ADI for the adult and one half of the ADI for the child. The amount of DDT in the egg with the highest concentration was 1.5 times and 7.5 times the ADIs for the adult and the child.

    More alarming results were obtained with regard to dieldrin. On the average, the dieldrin content of each egg was 3 times the ADI for the adult

and 13 times that of the child. The amount of dieldrin in the egg with the highest concentration was 117 times the ADI for the adult and 547 times the ADI for the child.

17. Accordingly, the present findings indicate that there is a need to ensure improved practices in the use of some chlorinated hydrocarbon pesticides, especially aldrin/dieldrin, at least in parts of Kenya. The answer to the question of whether or not these compounds should be banned is less obvious. Over the years considerable experience has been gained with a compound such as DDT, and some consider DDT to still be one of the safest and least hazardous insecticides for general use.

REFERENCES

ABBOTT, U.K.; CRAIG, R.M. AND KEITH, J.O. (1964). Effects of malathion spray on embryonated chicken eggs exposed under field conditions at Tuodumne Meadow Yosemite National Park. *Poultry Sci., 43,* 1297.

AHMAD, N. (1979). Clean-up of biological samples for determining p,p'-DDT and its metabolites. *J. Assoc. Off. Anal. Chem., 62,* 1150-1154.

AKMAN, M.S; SANLI, Y. AND CEYLAN, S. (1976). Estimation of organochlorine insecticide residues in samples of animal feed. *Ankara Universitesi Veteriner Falkultesi Dergisi, 23,* 103-114.

ALBERT, L.; MENDEZ, F.; CEBRIAN, M.E. AND PORTALES, A. (1980). Organochlorine pesticide residues in human adipose tissue in Mexican cities. *Arch. Environ. Health, 35,* 262-269.

AL-OMAR, M.; AL-BASSOMY, M.; AL-OGAILY, N. AND SHEBL, D.A. (1985). Residue levels of organochlorine insecticides in lamb and beef from Baghdad. *Bull. Environ. Contam. Toxicol., 34,* 509-512.

ANDERSON, J.M. AND PETERSON, M.R. (1969). DDT: Sublethal effects on brook trout nervous system. *Science, 164,* 440-441.

ANNUAL REPORT FOR EASTERN PROVINCE (1980). p. 43. Ministry of Agriculture, Kenya.

AVRAHAMI, M. AND GERNERT, I.L. (1972). Hexachlorobenzene antagonism to dieldrin storage in adipose tissue of female rats. *N.Z.J. Agric. Res., 15,* 783-787.

AVRAHAMI, M. AND STEELE, R.T. (1972). Hexachlorobenzene: II Residues in laying pullets fed hexachlorobenzene in their diet and the effects on egg production, egg hatchability and on chickens. *N.Z.J. Agric. Res., 15,* 482-488.

BARNES, J.M. (1976). Hazards to people. In *Pesticides and Human Welfare.* Gunn, D.L. and Stevens, J.G.R. (eds.). p. 188, Oxford Univ. Press, Oxford.

BERLIN, A.; WOLFF, A.H. AND HASEGAWA, Y. (1979). *The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants.* P. 22 . Martinus Nijhoff Publishers, London.

BEROZA, M. AND BOWMAN, M.C. (1965). Extraction of insecticides for clean-up and identification. *J. Assoc. Off. Agric. Chem., 48,* 358-370.

BITMAN, J. AND CECIL, H.C. (1970). Oestrogenic activity of DDT analogues and polychlorinated biphenyls. *J. Agric. Food. Chem., 18,* 1108-1112.

BITMAN, J.; CECIL, H.C.; HARRIS, S.J. AND FRIES, G.F. (1969). DDT induces a decrease in egg shell calcium. *Nature, 224,* 44-46.

BITMAN, J.; CECIL, H.C.; HARRIS, S.J. AND FRIES, G.F. (1971). Comparison of DDT effect on pentobarbital metabolism in rats and quail. *J. Agric. Food Chem., 19,* 333-338.

BJERK, J.E. (1970). Residues of organochlorine insecticides in hens' eggs produced in Norway, 1970. *Nord. Vet. Med., 23,* 288 - 292.

BJERK, J.E. AND SUNDBY, R. (1970). Residues of organochlorine insecticides and PCBs in terrestrial and aquatic organisms. Norwegian part of OECD programme 1967/68. *Norsk Vet. Tidsskr., 82,* 241-246.

BROOKS, G.T. (1974a). *Chlorinated Insecticides. Vol. I: Technology and Application.* 249 pp. CRC Press, Cleveland.

BROOKS, G.T. (1974b). *Chlorinated Insecticides. Vol. II : Biological and Environmental Aspects.* pp. 74, 122-124 and 147, CRC Press, Cleveland.

BUNYAN, P.J.; PAGE, J.M.J. AND TAYLOR, A. (1966). *In vitro* metabolism of p,p'-DDT in pigeon liver. *Nature, 210* 1048 - 1049.

BURCHFIELD, H.P. AND WHEELER, R.J. (1966). Analysis by microcoulometric gas chromatography. *J. Assoc. Off. Anal. Chem., 49,* 651 - 661.

CAIN, B.W. (1981). Nationalwide residues of organochlorine compounds in wings of adult mallards and black ducks, 1979 - 1980. *Pest. Monit. J., 15,* 128 - 134.

CARSON, R.L. (1962). *Silent Spring.* 5th reprint, 1969. 304 pp. Hamish and Hamilton Ltd., London.

CASARETT, L.J.; FRYER, G.C.; YAUGER, W.L.JR. AND KLEMMER, H.W. (1968). Organochlorine pesticide residues in human tissue - Hawaii. *Arch. Environ. Health., 17,* 306 - 311.

CECIL, H.C.; BITMAN, J.; FRIES, G.F.; HARRIS, S.J. AND LILLIE, R.J. (1973). Changes in eggshell quality and pesticide content of laying hens or pullets fed DDT in high or low calcium diets. *Poultry Sci., 52,* 648 - 653.

CECIL, H.C.; FRIES, G.F.; BITMAN, J.; HARRIS, S.J.; LILLIE, R.J. AND DENTON, C.A. (1972). Dietary p,p'-DDT, o,p'-DDT or p,p'-DDE and changes in eggshell characteristics and pesticide accumulation in egg contents and body fat of caged white leghorns. *Poultry Sci., 51,* 130 - 139.

CLARKE, M.L.; HARVEY, D.G. AND HUMPHREYS, D.J. (1981). *Veterinary Toxicology.* 2nd ed., p. 9. Bailliere Tindall, London.

CODEX ALIMENTARIUS COMMISSION OF FAO/WHO (1978). *Guide to Codex Maximum Limits for Pesticide Residues.* Pp. 67, 87, 96 and 105.

COPPLESTONE, J.F. (1977). A global view of pesticide safety. In *Pesticide Management and Insecticide Resistance.* Watson, D.L. and Brown, A.W.A. (eds.) pp. 153 - 154. Acad. Press Inc., New York.

COULSON, D.M.; CAVANAGH, L.A.; DE VRIES, J.R. AND WALTHER, B. (1960). Microcoulometric gas chromatography of pesticides. *J. Agric. Food Chem.*, *8*, 399 - 402.

CREMLYN, R. (1980). *Pesticides: Preparation and Mode of Action.* 240 pp. John Wiley and Sons, Ltd., London.

CROSBY, D.G. AND MOILAMEN, K.W. (1977). Vapour phase photodecomposition of DDT. *Chemosphere, 6,* 167 - 172.

CUMMINGS, J.G.; EIDELMAN, M.; TURNER, V.; REED, D.; ZEE, K.T. AND COOK, R.E. (1967). Residues in poultry tissues from low level feeding of five chlorinated hydrocarbon insecticides to hens. *J. Assoc. Off. Anal. Chem., 50,* 418 - 425.

CUMMINGS, J.G.; ZEE, K.T.; TURNER, V.; QUINN, F. AND COOK, R.E. (1966). Residues in eggs from low level feeding of five chlorinated hydrocarbon insecticides to hens. *J. Assoc. Off. Anal. Chem., 49,* 354 - 364.

DE FAUBERT MAUNDER, M.J.; EGAN, H.; GODLY, E.W.; HAMMOND, E.W.; ROBURN, J. AND THOMSON, J. (1964). Clean-up of animal fats and dairy products for the analysis of chlorinated pesticide residues. *Analyst, 89,* 168 - 174.

DE VOS, R.H.; BOWMAN, J. AND ENGEL, A.B. (1972). Residues of organochlorine pesticides in broilers fed feed fortified with known levels of these compounds. *Pest. Sci., 3,* 421 - 432.

DICKES, G.J. AND NICHOLAS, P.V. (1976). *Gas Chromatography in Food Analysis.* P. 57. Butterworth and Co. Ltd., London.

DOMENJOZ, R. (1944). Cited by SPINDLER, M. (1983). DDT: Health aspects in relation to man and risk/benefit assessment based thereupon. *Res. Rev., 90,* 1 - 34.

DONALDSON, W.E.; SHEETS, T.J. AND JACKSON, M.D. (1968). Starvation effects of DDT residues in chicken tissues. *Poultry Sci., 47,* 237 - 243.

DORN VON, P. AND KNOPPLER, H.O. (1977). Analysation of eggs and poultry meat for chlorinated hydrocarbons, arsenic and for residues of lead,

cadmium and mercury. *Berl. Munch. Tierarztl. Wschr., 90,* 137 - 140 (Engl. abstr.).

DRIVER, D.; BREWER, R.N. AND COTTIER, G.J. (1976). Pesticide residues in eggs and chicks from laying hens fed low levels of several chlorinated hydrocarbon pesticides. *Poultry Sci., 55,* 1544 - 1549.

DUGGAN, R.E. AND WEATHERWAX, J.R. (1967). Dietary intake of pesticide chemicals. *Science, 157,* 1006 - 1010.

ECOBICHON, D.J. AND SASCHENBRECKER, P.W. (1969). The re-distribution of stored DDT in cockerels under the influence of food deprivation. *Toxicol. Appl. Pharmacol., 15,* 420 - 432.

EDWARDS, C.A. (1973). *Environmental Pollution by Pesticides.* Edwards, C.A. (ed.), p. 4. Plenum Press, London.

EHRENSTORFER, S. AND GUENTHER, H.O. (1974). Pesticide residues in chicken eggs and powdered eggs. *Deut. Lebensm. Rundsch., 70,* 105 - 109. (Engl. Abstr.).

EL ZORGANI, G.A. (1976). Persistence of organochlorine insecticides in the Gezira soil under cotton. *Bull. Environ. Contam. Toxicol., 15,* 378 - 382.

EL ZORGANI, G.A. (1980). Residues of organochlorine pesticides in fishes in Sudan. *J. Environ. Sci. Health, B15,* 1091 - 1098.

EPA (1980). *Analysis of Pesticide Residues in Human and Environmental Samples.* Watts, R.R. (ed.). Sections 2, 3 and 4. Health Effects Research Laboratory, Environmental Toxicology Division, Research Triangle Park, North Carolina.

EPA (1985). Status of cancelled pesticides. *Vet. Hum. Toxicol., 27,* 387.

FAO/WHO (1982). *Pesticide Residues in Food* - 1981. Evaluations 1981, p. 548. Food and Agricultural Organization of the United Nations, Rome.

FAO/WHO/UNEP (1982). *Summary and Assessment of Data Received from the FAO/WHO Collaborating Centres for Food Contamination Monitoring.* p. 31 and pp. 61 - 63. National Food Administration, Uppsala.

FEIL, V.J.; LAMOUREX, C.H.; STYRVOKY, E.; ZAYLSKIE, R.G.; THACKER, E.J. AND HOLMAN, G.M. (1973). Metabolism of o,p'-DDT in rats. *J. Agric. Food. Chem., 21,* 1072 - 1078.

FEIL, V.J.; LAMOUREUX, C.H. AND ZAYLSKIE, R.G. (1975). Metabolism of o,p'-DDT in chickens. *J. Agric. Food Chem.*, *23*, 382 - 388.

FERRY, D.G.; McQUEEN, E.G. AND OWEN, D. (1973). Failure of ethanol and phenobarbitone to reduce organochlorine pesticide residues in sheep. *N.Z. J. Agric. Res.*, *16*, 105 - 110.

FIMREITE, N.; BREVIK, E.M. AND TORP, R. (1982). Mercury and organochlorines in eggs from a Norwegian Gannet colony. *Bull. Environ. Contam. Toxicol.*, *28*, 58 - 60.

FINDLAY, E. AND HAMILTON, G.A. (1968). Pesticide residues in foodstuffs in Great Britain : VIII - Organochlorine pesticide residues in eggs and poultry. *J. Sci. Food Agric.*, *19*, 609 - 611.

FLEMING, W.J. AND CROMARTIE, E. (1981). DDT residues in young wood ducks (*Aix sponsa*) near a former DDT manufacturing plant. *Pest. Monit. J.*, *14*, 115 - 118.

FOSTER, T.S. (1974). Physiological and biological effects of pesticide residues in poultry. *Res. Rev.*, *51*, 69 - 121.

FOSTER, T.S.; MORLEY, H.V.; YASTHA, R.P.; GREENHALGH, R. AND HUNT, J.R. (1972). Residues in eggs and tissues of hens fed a ration containing low levels of pesticides with and without charcoal. *J. Econ. Entomol., 65,* 982 - 988.

FRANK, L.G.; JACKSON, R.M.; COOPER, J.E. AND FRENCH, M.C. (1977). A survey of chlorinated hydrocarbon residues in Kenyan birds of prey. *East Afr. Wildl. J., 15,* 295 - 304.

FREUND, J.E. (1973). *Modern Elementary Statistics.* 4th ed., p. 280. Prentice-Hall Inc., Englewood Cliffs.

GOODWIN, E.S.; GOULDEN, R. AND REYNOLDS, J.G. (1961). Rapid identification and determination of residues of chlorinated pesticides in crops by gas liquid chromatography. *Analyst, 86,* 697 - 709.

GREICHUS, Y.A.; GREICHUS, A.; AMMAN, B.D. AND HOPCRAFT, J. (1978). Insecticides, polychlorinated biphenyls and metals in African lake ecosystems. III Lake Nakuru, Kenya. *Bull. Environ. Contam. Toxicol., 19,* 454 - 461.

GUTHRIE, F.E. AND DONALDSON, W.E. (1970). Distribution of DDT and dieldrin in the avian embryo. *Toxicol. Appl. Pharmacol., 16,* 475 - 481.

HALL, E.T. (1971). Variations of Florisil activity. Method to increase retentive properties and improve recovery and elution patterns of insecticides. *J. Assoc. Off. Anal. Chem., 54,* 1349 - 1357.

HANSEN, D.J. (1969). Avoidance of pesticides by untrained sheepshead minnows. *Trans. Amer. Fish. Soc., 98,* 426 - 429.

HASHEMY-TONKABONY, S.E. AND MOSSTOFIAN, B. (1979). Chlorinated pesticide residues in chicken egg. *Poultry Sci., 58,* 1432 - 1434.

HATCH, R.C. (1982). Poisons causing nervous stimulation or depression. In *Veterinary Pharmacology and Therapeutics.* Booth, N.H. and McDonald, L.E. (eds.), 5th ed., p. 1001. Iowa State Univ., Press, Ames.

HAYES, W.J. JR. (1982). *Pesticides Studied in Man.* Pp. 172 - 283. Williams and Wilkins, London.

HEATH, R.G.; SPANN, J.W. AND KREITZER, J.F. (1969). Marked DDE impairement of mallard reproduction in controlled studies. *Nature, 224,* 47 - 48.

HENNY,C.J.; OLSON, R.A. AND MECKER, D.L. (1977). Residues in common flicker and mountain blue bird eggs one year after a DDT application. *Bull. Environ. Contam. Toxicol., 18,* 115 - 122.

HERRICK, G.M.; FRY, J.L.; FONG, W.G. AND GOLDEN, D.C. (1969). Pesticide residues in eggs resulting from the dusting and short-term feeding of low levels of chlorinated hydrocarbon insecticides to hens. *J. Agric. Food Chem., 17,* 291 - 295.

HICKEY, J.J. AND ANDERSON, D.W. (1968). Chlorinated hydrocarbons and eggshell changes in raptorial and fish eating birds. *Science, 162,* 271 - 273.

HILL, D.S. (1983). *Agricultural Insect Pests of the Tropics and their Control.* 2nd ed., pp. 50 - 58. Cambridge Univ. Press, Cambridge.

HOFFMAN, D.J. AND EASTIN, W.C. JR. (1982). Effects of lindane, paraquat, toxaphene and 2,4,5-Trichlorophenoxyacetic acid on mallard embryo development. *Arch. Environ. Contam. Toxicol., 11,* 79 - 86.

HOLDEN, A.V. AND MARSDEN, K. (1969). Single stage clean-up of animal tissue extracts for organochlorine residue analysis. *J. Chromat., 44,* 481 - 492.

HUANG, C.C. (1973). Effect on growth but not on chromosomes of the mammalian cells after treatment with three organophosphorus insecticides. *Proc. Soc. Exp. Biol. Med., 142,* 36 - 40.

INGEBRIGTSEN, K.; BREVIK, E.M. AND NAFSTAD, I. (1983). Distribution and elimination of HCB after a single oral exposure in Japanese quail.*(Cortunix cortunix japonica). J. Toxicol. Environ. Health, 8,* 845 - 856.

INGEBRIGTSEN, K.; SKAARE, J.U. AND TEIGEN, S.W. (1984). Organochlorine residues in two Norwegian puffin *(Fratercula arctica)* colonies. *J. Toxicol. Environ. Health, 14,* 813 - 828.

INTERIOR DEPARTMENT, OFFICE OF ENDANGERED SPECIES, FISH AND WILDLIFE SERVICE (1986). Dicofol stays in California if Rohm and Haas pays to help falcon recovery. *Pest. and Toxic Chem. News, 14,* 39 - 40.

JAETZOLD, R. AND SCHIMDT, H. (1983). *Farm Management Handbook of Kenya, Vol. II. Natural Conditions and Farm Management Information, Part C, East Kenya (Eastern and Coast Provinces).* pp. 21 - 137. Ministry of Agriculture, Kenya and the German Agricultural team.

JAMES, A.T. AND MARTIN, A.J.P. (1952). Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem. J., 50,* 679 - 690.

JEFFERIES, D.J. AMD WALKER, C.H. (1966). Uptake of p,p'-DDT and its post-mortem breakdown in avian liver. *Nature, 212,* 533.

JOHNSON, L.D.; WALTZ, R.H.; USSARY, J.P. AND KAISER, F.E. (1976). Automated gel permeation chromatographic clean-up of plant extracts for pesticide residue determination. *J. Assoc. Off. Anal. Chem., 59,* 174 - 187.

JONES, F.J.S. AND SUMMERS, D.D.B. (1968). Relations between DDT in diets of laying birds and viability of their eggs. *Nature, 217,* 1162 - 1163.

JUSZKIEWICZ, T. (1983). The veterinary problems of pesticide monitoring and analysis. *Vet. Res. Commun., 7,* 113 - 118.

KAHUNYO, J.K.M. (1983). *Organochlorine Pesticide Residues in Chicken Fat and Eggs.* M.Sc. Thesis, Univ. of Nairobi. 122 pp.

KALLMAN, J.B. AND ANDREWS, A.K. (1963). Reductive dechlorination of DDT to DDD by yeast. *Science, 141,* 1050 - 1051.

KAN, C.A. (1978). Accumulation of organochlorine pesticides in poultry. A review. *J. Agric. Food Chem., 26,* 1051 - 1055.

KAN, C.A. AND JONKER-DEN ROOYEN, J.C. (1978). Accumulation and depletion of some organochlorine pesticides in high producing laying hens. *J. Agric. Food Chem., 26,* 935 - 940.

KAN, C.A.; JONKER-DEN ROOYEN, J.C.; TUINSTRA, L.G.M.TH.; ROOS, A.H. AND TRAAG, W. (1978). Possible influence of sex and embryonic content on accumulation of some organochlorine pesticides in broilers. *J. Agric. Food Chem., 26,* 618 - 621.

KAN, C.A. AND TUINSTRA, L.G.M.TH. (1976a). Effect on performance of a low level mixture of organochlorine insecticides in broiler breeder feed. *J. Agric. Food Chem.*, *24*, 772 - 775.

KAN, C.A. AND TUINSTRA, L.G.M.TH. (1976b). Accumulation and excretion of certain organochlorine insecticides in broiler breeder hens. *J. Agric. Food Chem.*, *24*, 775 - 778.

KANJA, L. (1986). A study of levels and sources of organochlorine pesticide residues in mothers' milk in Kenya. Personal communication.

KAPHALIA, B.S.; HUSAIN, M.M.; SETH, T.D.; KUMAR, A. AND KRISHNA MURTI, C.R. (1981). Organochlorine pesticide residues in some Indian wild birds. *Pest. Monit. J.*, *15*, 9 - 13.

KAPHALIA, B.S. AND SETH, T.D. (1981). DDT and BHC residues in some body tissues of goats, buffalo and chickens, Lucknow-India. *Pest. Monit. J.*, *15*, 103 - 106.

KLEIN, A.K.; LAUG, E.P.; DATTA, P.R.; WATTS, J.O. AND CHEN, J.T. (1964). Metabolites: reductive dechlorination of DDT to DDD and isomeric transformation of o,p'-DDT to p,p'-DDT *in vivo*. *J. Assoc. Off. Agric. Chem.*, *47*, 1129 - 1145.

KOEMAN, J.H.; OUDENJANS, R.C. AND HUSMAN, E.A. (1967). Danger of chlorinated hydrocarbon insecticides in birds' eggs. *Nature, 215,* 1094 - 1096.

KOEMAN, J.H. AND PENNINGS, J.H. (1970). An orientational survey on the side effects and environmental distribution of insecticides used in tse-tse control in Africa. *Bull. Environ. Contam. Toxicol., 5,* 164 - 170.

KOEMAN, J.H.; PENNINGS, J.H.; DE GOEIJ, J.J.M.; TSIOE, P.S.; OLINDO, P.M. AND HOPCRAFT, J. (1972). A preliminary survey of the possible contamination of Lake Nakuru in Kenya with some metals and chlorinated hydrocarbon pesticides. *J. Appl. Ecol., 9,* 411 - 416.

KORTE, F. (1972). Chemistry and metabolism of terminal residues of organochlorine compounds-state of art. In *Fate of Pesticides in Environment : Proceedings of the Second International IUPAC Congress of Pesticide Chemistry Vol. VI.* Tahori, A.S. (ed.). pp. 217, 219 and 221. Gordon and Breach Science Publishers, London..

KROGER, M. (1968). Effects of various physical treatments on certain organochlorine hydrocarbon insecticides found in milk fat. *J. Dairy Sci, 51,* 196 - 198.

LADISCH, H. (1970). The contamination of chicken eggs with organochlorine compounds. *Z. Gesamte Hyg., 17,* 599 - 602.

LANGLOIS, B.E.; STEMP, A.R. AND LISKA, B.J. (1964). Analysis of animal food products for chlorinated insecticide residues. I Column clean-up of samples for electron capture gas chromatographic analysis. *J. Milk Food Tech., 27,* 202 - 204.

LILLARD, D.A. AND NOLES, R.K. (1973). Effect of force molting and induced hyperthyroidism on the depletion of DDT residues from the laying hen. *Poultry Sci., 52,* 222 - 228.

LINCER, J.L.; ZALKIND, D.; BROWN, L.H. AND HOPCRAFT, J. (1981). Organochlorine residues in Kenya Rift Valley lakes. *J. Appl. Ecol., 18,* 157 - 172.

LISKA, B.J.; LANGLOIS, B.E.; MOSTERT, G.C. AND STADELMAN, W.J. (1964). Residues in eggs and tissues of chickens on rations containing low levels of DDT. *Poultry Sci., 43,* 982 - 984.

LOVELOCK, J.E. AND LIPSKY, S.R. (1960). Electron affinity spectroscopy- a new method for identification of functional groups in chemical compounds separated by gas chromatography. *J. Am. Chem. Soc., 82,* 431 - 433.

LUDKE, J.L. (1974). Interaction of dieldrin and DDE residues in Japanese quail (*Cortunix coturnix japonica*). *Bull. Environ. Contam. Toxicol.*, *11*, 297 - 302.

MAITHO, T.E. (1978). *Pesticide Residues in Animal Products in Kenya : Organochlorine and Organophosphorus Insecticides in Milk and Body Fat.* M.Sc. Thesis, Univ. of Nairobi. 134 pp.

MATSUMURA, F. (1975). *Toxicology of Insecticides.* pp. 55, 57, 60, 61, 107, 194, 305, 363 and 364. Plenum Press, London.

McBLAIN, W.A.; LEWIN, V. AND WOLFE, F.H. (1974). Limited accumulation of DDT in eggs, fat and livers of Japanese quail. *Poultry Sci.*, *53*, 84 - 88.

McCASKEY, T.A.; STEMP, A.R.; LISKA, B.J. AND STADELMAN, W.J. (1968). Residues in egg yolks and raw and cooked tissues from laying hens administered selected chlorinated hydrocarbon insecticides. *Poultry Sci.*, *47*, 564 - 569.

McCULLY, K.A. AND McKINLEY, W.P. (1964). Determination of chlorinated pesticide residues in fat by electron capture gas chromatography. *J. Assoc. Off. Agric. Chem.*, *47*, 652 - 659.

McCULLY, K.A.; VILLENEUVE, D.C. AND McKINLEY, W.P. (1966). Metabolism and storage of DDT residues in beef cattle. *J. Assoc. Off. Anal. Chem.*, *49*, 966 - 973.

McEWEN, F.L. AND STEPHENSON, G.R. (1979). *The Use and Significance of Pesticides in the Environment.* pp. 4, 14, 309 - 332 and 420 - 452. John Wiley and Sons, New York.

McLEOD, H.A. (1973). *Analytical Methods for Pesticide Residues in Foods.* Revised 1st ed., Sections 3, 6 and 12. Health Protection Branch, Ottawa.

MENZER, R.E. (1970). Effect of chlorinated hydrocarbons in the diet on the toxicity of several organophosphorus insecticides. *Toxicol. Appl. Pharmacol.*, *16*, 446 - 452.

MES, J.; COFFIN, D.E. AND CAMPBELL, D. (1974). Polychlorinated biphenyl and organochlorine pesticide residues in Canadian chicken eggs. *Pest. Monit. J.*, *8*, 8 - 11.

METCALF, R.L. (1973). A century of DDT. *J. Agric. Food Chem.*, *21*, 511 - 519.

MILLS, P.A. (1959). Detection and semiquantitative estimation of chlorinated organic pesticide residues in food by paper chromatography. *J. Assoc. Agric. Chem., 42,* 734 - 740.

MORGAN, D.P. AND ROAN, C.C. (1969). Renal function in persons occupationally exposed to pesticides. *Arch. Environ. Health, 19,* 633 - 636.

MORGAN, K.J.; ZABIK, M.E. AND FUNK, K. (1972). Lindane, Dieldrin and DDT residues in raw and cooked chicken and chicken broth. *Poultry Sci., 51,* 470 - 475.

MUINAMIA, C.K. (1976) "Chlorinated and phosphorus containing pesticide residues in foodstuffs and feedstuffs in Kenya". 1st East African Conference on Entomology and Pest Control, Nairobi.

MULLER, H.D. AND LOCKMAN, D.C. (1972). Fecundity and progeny growth following subacute insecticide ingestion by the mallard. *Poultry Sci., 51,* 239 - 241.

MURPHY, D.A. AND KORSCHGEN, L.J. (1970). Reproduction, growth and tissue residues of deer fed dieldrin. *J. Wildl. Mgmt., 34,* 887 - 903.

NABER, E.C. (1977). The impact of contamination by organochlorine insecticides on poultry nutrition and feeding. *Fed. Proc., 36,* 1880 - 1887.

NABER, E.C. AND WARE, G.W. (1961). Effect of BHC or lindane ingestion on its occurrence in eggs and body tissues. *Poultry Sci., 40,* 1435.

NIETHAMMER, K.R.; BASKETT, T.S. AND WHITE, D.H. (1984). Organochlorine residues in three heron species as related to diet and age. *Bull. Environ. Contam. Toxicol., 33,* 491 - 498.

O'BRIEN, R.D. (1967). *Insecticides: Action and Metabolism.* pp. 125, 133 and 291. Acad. Press, London.

OLIELO, T.K. (1985). Acceptable limits of organochlorine pesticide residues. Kenya Bureau of Standards. Personal communication.

OMAMO, O. (1983). "Pests and diseases lead to big losses". 25th Anniv. Cel. Pest. Chem. Assoc. Kenya, Nairobi. Nov. 1983.

ONLEY, J.H. AND MILLS, P.A. (1962). Detection of chlorinated pesticide residues in eggs. *J. Assoc. Off. Agric. Chem., 45,* 983 - 987.

OSADCHUK, M.; ROMACH, M. AND McCULLY, K.A. (1971). Methods in residue analysis. *International Union of Pure and Applied Chemistry. Congress of Pesticide Chemistry*, Israel, Part 4, pp. 357 - 383.

PANEL ON DETERMINATION OF ORGANOCHLORINE PESTICIDES IN FOODSTUFFS OF ANIMAL ORIGIN (1979). Determination of residues of organochlorine pesticides in animal fats and eggs. *Analyst, 104*, 425 - 433.

PEAKALL, D.B. (1969). Effect of DDT on calcium uptake and vitamin D metabolism in birds. *Nature, 224*, 1219 - 1220.

PERFECT, J. (1980). The environmental impact of DDT in a tropical agro-ecosystem. *Ambio, 9*, 16-21.

PINTO, J.D.; CAMIEN, M.N. AND DUNN, M.S. (1965). Metabolic fate of p,p'-DDT in rats. *J. Biol. Chem., 240*, 2148 - 2153.

RADELEFF, R.D. (1970). *Veterinary Toxicology.* 2nd ed. pp. 235 - 236 and 238 - 240. Lea and Febiger, Philadelphia.

RATCLIFFE, D.A. (1967). Decrease in eggshell weight in certain birds of prey. *Nature, 215,* 208 - 210.

RATCLIFFE, D.A. (1970). Changes attributable to pesticides in egg breakage frequency and egg shell thickness in some British birds. *J. Appl. Ecol., 7,* 67 - 107.

REEVES, R.G.; WOODHAM, D.W.; GANYARD, M.C. AND BOND, C.A. (1977). Preliminary monitoring of agricultural pesticides in a co-operative tobacco pest management project in North Carolina 1971 - first year study. *Pest. Monit. J., 11,* 99 - 106.

REPORT OF A CONSULTATION ON ORGANOCHLORINE COMPOUNDS (1980). WHO/UNEP *Pilot Project on Assessment of Human Exposure to Pollutants Through Biological Monitoring.* P. 4.

RICHOU-BAC, L. AND VENANT, A. (1980). Contamination des denrees d'origine animale par les residus de composes organochlores persistants. Pesticides et diphenyles polychlores. *Revue tech. vet. l'aliment, 19,* 13 - 19.

RITCHEY, S.J.; YOUNG, R.W. AND ESSARY, E.O. (1972). Effects of heating and cooking method on chlorinated hydrocarbon residues in chicken tissues. *J. Agric. Food Chem., 20,* 291 - 293.

RIVA, M.; CARISANO, A. AND DAGHETA, A. (1973). Cited by CAMPANINI, G.; MAGGI, E. AND ARTIOLI, D. (1980). Present situation of organochlorine pesticide residues in food of animal origin in Italy. *Wld. Rev. Nutr. Diet, 35,* 129 - 171.

RUNDLE, J.C. AND FORSYTH, B.A. (1984). The treatment and eradication of sheep lice and ked with cyhalothrin - a new synthetic pyrethroid. *Aust. Vet. J., 61,* 396 - 399.

SAUTER, E.A. AND STEELE, E.E. (1972). The effect of low level pesticide feeding on the fertility and hatchability of chicken eggs. *Poultry Sci., 51,* 71 - 76.

SAWYER, A.D. (1966). Study of extraction procedures for chlorinated organic pesticides in fresh eggs. *J. Assoc. Off. Anal. Chem., 49,* 643 - 647.

SCOTT, M.L.; ZIMMERMAN, J.R.; MARINSKY, S.; MULLENHOFF, P.A.; RUMSEY, G.L. AND RICE, R.W. (1975). Effect of PCBs, DDT and mercury compounds upon egg production, hatchability and shell quality in chickens and Japanese quail. *Poultry Sci., 54,* 350 - 367.

SCOTT, T.G.; WILLIS, Y.L. AND ELLIS, J.A. (1959). Some effects of a field application of dieldrin on wildlife. *J. Wildl. Mgmt., 23,* 409 - 427.

SERICANO, J.L. AND PUCCI, A.E. (1984). A simplified confirmatory technique for organochlorine residues. *Bull. Environ. Contam. Toxicol., 33,* 138 - 143.

SERRANO, C.J.M.; GARCIA, R.A.; SANTIAGO, L.D. AND INFANTE, M.F. (1979). Residues of organochlorine pesticides in egg yolk 2. Eggs with broken shells. *Archivos de Zootecnia, 28,* 95 - 101.

SHERMA, J. (1979). *Manual of Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Samples.* Pp. 1 - 2, 34, 88, 114 and 124. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park.

SIMPSON, C. (1970). *Gas Chromatography.* p. 9. Koganpage, London.

SKAARE, J.U. (1983, 1984). Personal communication.

SKAARE, J.U.; STENERSEN, J.; KVESETH, N. AND POLDER, A. (1985). Time trends of organochlorine chemical residues in seven sedentary marine fish species from a Norwegian Fjord during the period 1972 - 1982. *Arch. Environ. Contam. Toxicol.*, *14*, 33 - 41.

SMART, N.A.; HILL, A.R.C. AND ROUGHAN, P.A. (1974). Chlorinated hydrocarbon pesticide residues in foodstuffs: comparison of methods. *J. Assoc. Off. Anal. Chem.*, *57*, 153 - 164.

SMITH, S.I.; WEBER, C.W. AND REID, B.L. (1970a). Dietary pesticides and contamination of yolks and abdominal fat of laying hens. *Poultry Sci.*, *49*, 233 - 237.

SMITH, S.I.; WEBER, C.W. AND REID, B.L. (1970b). The effect of injection of chlorinated hydrocarbon pesticides on hatchability of eggs. *Toxicol. Appl. Pharmacol.*, *16*, 179 - 185.

SNEDECOR, G.W. AND COCHRAN, W.G. (1967). *Statistical Methods*. 6th ed. pp. 103 and 115. Iowa State Univ. Press, Ames.

SOULSBY, E.J.L. (1978). *Helminths Arthropods and Protozoa of Domesticated Animals.* 6th ed. Pp. 376 - 522. Bailliere Tindall and Cassell Ltd., London.

SPINDLER, M. (1983). DDT : Health aspects in relation to man and risk/benefit assessment based thereupon. *Res. Rev., 90,* 1 - 34.

STADELMAN, W.J.; LISKA, B.J.; LANGLOIS, B.E.; MOSTERT, G.C. AND STEMP, A.R. (1965). Persistence of chlorinated hydrocarbon insecticide residues in chicken tissues and eggs. *Poultry Sci., 44,* 435 - 437.

STEMP, A.R.; LISKA, B.J.; LANGLOIS, B.E. AND STADELMAN, W.J. (1964). Analysis of egg yolk and poultry tissues for chlorinated insecticide residues. *Poultry Sci., 43,* 273 - 275.

STEMP, A.R.; LISKA, B.J.; WESLEY, R.L. AND STADELMAN, W.J. (1965). Effect of high temperature cooking on chlorinated insecticide residues in chicken tissues. *Poultry Sci., 44,* 1417.

STICKEL, W.H.; HAYNE, D.W. AND STICKEL, L.F. (1965). Effects of heptachlor contaminated earthworms on woodcocks. *J. Wildl. Mgmt., 29,* 132 - 146.

STREET, J.C. (1964). DDT antagonism to dieldrin storage in adipose tissue of rats. *Science, 146,* 1580 - 1581.

STREET, J.C. AND BLAU, A.D. (1966). Insecticide interactions affecting residue accumulation in animal tissues. *Toxicol. Appl. Pharmacol., 8,* 497 - 504.

STREET, J.C.; CHADWICK, R.W.; WANG, W. AND PHILIPS, R.L. (1966). Insecticide interactions affecting residue storage in animal tissues. *J. Agric. Food Chem., 14,* 545 - 549.

SUNDSTRÖM, G.; JANSSON, B. AND JENSON, S. (1975). Structure of phenolic metabolites of p,p'-DDE in rat, wild seal and guillemot. *Nature, 255,* 627 - 628.

SWEDISH NATIONAL FOOD ADMINISTRATION (1983). *Assessment of Human Exposure to Selected Organochlorine Compounds Through Biological Monitoring.* Slorach, S.A. and Vaz, R. (eds.) p. 17 and pp. 27-28. Uppsala.

TANNOCK, J.; HOWELLS, W.N. AND PHELPS, R.J. (1983). Chlorinated hydrocarbon pesticide residues in eggs of some birds in Zimbabwe. *Environ. Pollut. Ser. B, 5,* 147 - 155.

TAYLOR, A.; REA, R.E. AND KIRBY, D.R. (1964). An improved procedure for the extraction of organochlorine pesticides from animal tissue. *Analyst, 89*, 497 - 498.

TAYLOR, I.S. AND KEENAN, F.P. (1970). Studies on the analysis of hexachlorobenzene residues in foodstuffs. *J. Assoc. Off. Anal. Chem., 53*, 1293 - 1295.

THOMPSON, R.P.H.; STATHERS, G.M.; PILCHER, C.W.T.; McLEAN, A.E.M.; ROBINSON, J. AND WILLIAMS, R. (1969). Treatment of unconjugated jaundice with dicophane. *The Lancet, 2*, 4 - 6.

TODOROKI, T.; IMAI, K.; MATSUMOTO, K. AND KANO, S. (1983). Initial deactivation of Florisil adsorbent for column chromatographic separation of lipids. *Analyst, 108*, 1267 - 1269.

TRONCONE, A.; STANCO, F. AND DAMIANO, S. (1970). Cited by CAMPANINI, G.; MAGGI, E. AND ARTIOLI, D. (1980). Present situation of organochlorine pesticide residues in food of animal origin in Italy. *Wld. Rev. Nutr. Diet, 35*, 129 - 171.

TSCHIRLEY, F.H. (1979). The role of pesticides in increasing agricultural production. In *Pesticides : Contemporary Roles in Agriculture, Health and Environment*. Sheets, T.J. and Pimentel, D. (eds.) Pp. 3 - 20 Humana Press, Clifton.

WAGSTAFF, D.J.; McDOWELL, J.R. AND PAULIN, H.J. (1981). Egg binding in broiler chickens for heptachlor. *Poultry Sci., 60,* 1578 - 1580.

WAIBEL, G.P.; SPEERS, G.M. AND WAIBEL, P.E. (1972). Effects of DDT and charcoal on performance of white leghorns. *Poultry Sci., 51,* 1963 - 1967.

WAINAINA, J. (1983). "Educating people about the use of pesticides". 25th Anniv. Cel. Pest. Chem. Assoc. Kenya, Nairobi. Nov. 1983.

WALDRON, A.C. AND NABER, E.C. (1974a). Importance of feed as unavoidable source of pesticide contamination in poultry meat and eggs. 1 Residues in feedstuffs. *Poultry Sci., 53,* 1359 - 1371.

WALDRON, A.C. AND NABER, E.C. (1974b). Importance of feed as an unavoidable source of pesticide contamination in poultry meat and eggs : 2. Residues in eggs and tissues. *Poultry Sci., 53,* 1428 - 1435.

WARE, G.W. AND NABER, E.C. (1961). Lindane in eggs and chicken tissues. *J. Econ. Entomol., 54,* 675 - 677.

WASSERMANN, M.; ROGOFF, M.G.; TOMATIS, L.; DAY, N.E.; WASSERMANN, D.; DJAVAHERIAN, M. AND GUTTEL, C. (1972). Storage of organochlorine insecticides in the adipose tissue of people in Kenya. *Ann. Soc. belge Med. trop., 52,* 509 - 514.

WELLS, C.E. (1967). Validation study of a method for pesticide residues in foods and animal feeds. *J. Assoc. Off. Anal. Chem., 50,* 1205 - 1215.

WESLEY, R.L.; STEMP, A.R.; HARRINGTON, R.B.; LISKA, B.J.; ADAMS, R.L. AND STADELMAN, W.J. (1969). Further studies on depletion of DDT residues from laying hens. *Poultry Sci., 48,* 1269 - 1275.

WESLEY, R.L.; STEMP, A.R.; LISKA, B.J. AND STADELMAN, W.J. (1966). Depletion of DDT from commercial layers. *Poultry Sci., 45,* 321 - 324.

WHITEHEAD, C.C. (1971). The effects of pesticides on production in poultry. *Vet. Rec., 88,* 114 - 117.

WHO (1971). Cited by SPINDLER, M. (1983). DDT : Health aspects in relation to man and risk/ benefit assessment based thereupon. *Res. Rev.*, *90*, 1 - 34.

WHO (1979). Cited by SPINDLER, M. (1983). DDT : Health aspects in relation to man and risk/ benefit assessment based thereupon. *Res. Rev.*, *90*, 1 - 34.

WOOD, N.F. (1969). Extraction and clean-up of organochlorine pesticide residues by column chromatography. *Analyst*, *94*, 399 - 405.

WRIGHT, F.C.; RINER, J.C. AND YOUNGER, R.L. (1972). Residues in chickens given DDT. *J. Agric. Food Chem.*, *20*, 17 - 19.

YADAV, D.V.; MITTAL, P.P.K.; AGARWAL, H.C. AND KRISHNA PILLAI, M.K. (1981). Organochlorine insecticide residues in soil and earthworms in the Delhi area India, August to October, 1974. *Pest Monit. J.*, *15*, 80 - 85.

Appendix 1 Mean per cent recoveries (six replicates) ± S.D. of pesticides with the "new" and "old" methods from eggs spiked at the "low" level. The dimethylformamide to hexane ratio of the "old" method was 1:4.

Compound	ppm* added	"NEW METHOD" per cent recovery	S.D.	"OLD METHOD" per cent recovery	S.D.
p,p'-DDE	0.100	96.6	5.0	45.4	11.9
p,p'-DDT	0.260	103.2	6.8	69.9	11.1
p,p'-DDD	0.190	109.1	4.2	72.7	4.6
o,p'-DDT	0.225	95.8	3.8	58.0	7.0
o,p'-DDD	0.200	96.6	5.0	76.0	5.1
Lindane	0.025	117.3	7.0	72.8	7.2
α-HCH	0.025	76.0	3.6	50.4	9.2
β-HCH	0.100	77.2	2.3	68.2	2.8
Aldrin	0.050	77.7	4.1	28.0	3.7
Dieldrin	0.120	84.6	6.2	60.0	7.6
Endrin	0.200	70.8	5.2	59.5	6.2
Heptachlor	0.025	74.0	3.3	48.0	4.9
Heptachlor epoxide	0.080	82.1	7.8	68.3	4.7

* in µg of pesticide/g of egg.

Appendix 2 Mean per cent recoveries (six replicates) ± S.D. of pesticides with the "new" and "old" methods from eggs spiked at the "high" level. The dimethylformamide to hexane ratio of the "old" method was 1:4.

Compound	ppm* added	"NEW" METHOD per cent recovery	S.D.	"OLD" METHOD per cent recovery	S.D.
p,p'-DDE	0.200	95.2	5.1	64.8	14.7
p,p'-DDT	0.520	103.3	9.2	94.2	7.3
p,p'-DDD	0.380	104.1	6.9	106.5	7.4
o,p'-DDT	0.450	96.2	5.4	82.3	8.9
o,p'-DDD	0.400	92.1	3.3	102.0	7.5
Lindane	0.050	87.3	4.7	87.0	9.1
α-HCH	0.050	95.3	3.5	85.3	6.5
β-HCH	0.200	90.8	4.3	101.4	7.9
Aldrin	0.100	83.7	5.4	35.3	7.8
Dieldrin	0.240	90.0	7.6	72.8	9.5
Endrin	0.400	92.9	7.1	71.0	8.1
Heptachlor	0.050	82.3	6.5	59.0	9.9
Heptachlor epoxide	0.160	84.5	5.5	88.3	6.6

* in µg of pesticide/g of egg.

Appendix 3 Mean per cent recoveries (six replicates) ± S.D. of pesticides with the "old" method from eggs spiked at the "low" level. The dimethylformamide to hexane ratio was 1:2.

Compound	ppm* added	per cent recovery	S.D.
p,p'-DDE	0.100	75.6	4.8
p,p'-DDT	0.260	78.0	6.0
p,p'-DDD	0.190	84.0	4.4
o,p'-DDT	0.225	80.0	4.5
o,p'-DDD	0.200	82.8	4.8
Lindane	0.025	62.0	7.9
α-HCH	0.025	74.7	4.1
β-HCH	0.100	98.5	6.3
Aldrin	0.050	57.3	5.0
Dieldrin	0.120	72.1	5.5
Endrin	0.200	77.0	3.3
Heptachlor	0.025	74.7	4.1
Heptachlor epoxide	0.080	86.7	5.4

* in µg of pesticide/g of egg.

Appendix 4

UNIVERSITY OF NAIROBI

Department of Public Health, Pharmacology and Toxicology
Chairman
Tel. 592211

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

Faculty of Veterinary Medicine
P.O. Box 29053
Telegrams:
UNIVET Kabete

QUESTIONNAIRE TO BE FILLED IN DURING COLLECTION OF EGGS AND POULTRY FEED SAMPLES

1. Sample No. ..... 2. Name of farm (if any) .....
   ..... 3. Owner of farm .....
4. Division ..... 5. Location .....
6. Date ..... 7. No. of birds in the farm .....
8. Other animals and their numbers .....
   .....
9. Free-range/enclosed poultry keeping? .....
10. System (e.g. deep-litter) .....
11. Feed used for poultry .....
    .....
12. Source of the feed .....
13. Crops grown on the farm .....
    .....
14. Common crops and animal pests in the area .....
    .....
    .....
15. Chemicals used for pest control recently/in the past .....
    .....
16. Average length of time for keeping layers .....
17. Any other relevant information .....
    .....

Appendix 5 Residue concentrations (mg/kg eggs) of some compounds found in nine eggs analysed in Norway (in parentheses) and in our laboratory.

Sample No.	p,p'-DDE	p,p'-DDT	Lindane	Dieldrin
NE_2	0.65	1.07	0.05	0.83
	(0.73)	(1.07)	(0.03)	(0.67)
NE_6	1.95	2.00	0.09	14.47
	(1.53)	(1.89)	(0.08)	(13.48)
BE_{21}	0.11	0.19	0.02	1.44
	(0.09)	(0.18)	(0.01)	(0.27)
BE_{23}	0.97	1.23	0.04	5.85
	(1.00)	(1.40	(0.03)	(11.16)
KE_1	2.24	1.47	0.10	0.05
	(1.78)	(1.43)	(0.06)	(-)
LE_5	3.33	1.47	-	-
	(3.77)	(1.35)	(t)	(-)
FE_1	0.17	0.15	0.05	0.05
	(0.15)	(0.11)	(0.03)	(0.06)
FE_2	0.15	0.13	0.03	0.05
	(0.15)	(0.11)	(0.03)	(0.08)
FE_3	0.12	0.11	0.03	0.07
	(0.11)	(0.10)	(0.04)	(0.06)

t = Trace amounts

\- = Not detected

Appendix 6 Total DDT residues and ratios of p,p'-DDT to p,p'-DDE in eggs from enclosed chickens in various localities of Embu and Meru.

Locality Main agric. products	No. of eggs	Sum DDT (mg/kg eggs) *Mean* ± S.D. (Range)	% egg ≥PRL	Ratio p,p'-DDT/p,p'-DDE *Mean* ± S.D. (Range)	% > 1
Gachoka (Mwea) Rice, marginal cot.	10	*2.26** ± 0.77 (1.39 - 4.16)	100	*0.56* ± 0.20 (0.31 - 0.96)	0
Siakago Cot., tob.	10	*0.50** ± 0.28 (0.33 - 1.26)		*1.20* ± 0.43 (0.67 - 1.95)	50
Nithi Coffee, tea	63	*0.38* ± 0.45 (0.03 - 2.10)	25	*1.02* ± 0.45 (0.36 - 2.24)	37
Embu Agric. Institute Coffee	15	*0.35* ± 0.11 (0.22 - 0.65)	7	*1.73* ± 0.43 (1.13 - 3.00)	100
Runyenjes Coffee, tea	63	*0.20* ± 0.20 (0.08 - 1.61)	3	*0.87* ± 0.29 (0.17 - 1.73)	25
Mikinduri Coffee	12	*0.19* ± 0.08 (0.10 - 0.35)	0	*1.08* ± 0.61 (0.36 - 2.33)	42
N/Imenti Cof., tea, cot.	23	*0.15* ± 0.05 (0.06 - 0.28)	0	*0.90* ± 0.40 (0.40 - 1.88)	35
S/Imenti Coffee, tea	15	*0.08* ± 0.03 (0.03 - 0.11)	0	*0.52* ± 0.23 (0 - 0.80)	0

Positive : ≥ 0.01 mg DDT/kg eggs
Extraneous Residue Limit (ERL) of total DDT : 0.5 mg/kg eggs
*mean ≥ ERL
Cot. = cotton
Cof. = coffee
tob. = tobacco

Appendix 7 Total DDT residues and ratios of p,p'-DDT to p,p'-DDE in eggs from free-range chickens in various localities of Embu and Meru.

Locality	No. of eggs			Total DDT (mg/kg eggs)	Ratio p,p'-DDT/p,p'-DDE	
Main agric. products	Total	Positive	≥ ERL	*Mean* ± S.D. (Range)	*Mean* ± S.D. (Range)	% > 1.00
Gachoka (Mwea) Rice	24	24	17	*3.21** ± 3.14 (0.21 - 10.25)	*0.54* ± 0.40 (0.22 - 1.65)	17
N/Imenti Cof., tea, cot.	32	32	17	*1.43** ± 1.73 (0.01 - 6.07)	*0.52* ± 0.42 (0 - 1.68)	13
Siakago Cot., tob.	27	27	20	*0.96** ± 0.71 (0.04 - 2.48)	*0.29* ± 0.22 (0 - 0.74)	0
Runyenjes Coffee, tea	32	32	11	*0.76** ± 0.75 (0.09 - 2.40)	*0.52* ± 0.39 (0.11 - 2.03)	9
Nithi Coffee, tea	19	19	2	*0.18* ± 0.18 (0.03 - 0.72)	*0.88* ± 0.55 (0.17 - 2.00)	32
Mikinduri Coffee	16	16	0	*0.10* ± 0.06 (0.02 - 0.29)	*0.65* ± 0.33 (0 - 1.25)	6
S/Imenti Coffee, tea	6	6	0	*0.06* ± 0.03 (0.02 - 0.11)	*0.30* ± 0.37 (0 - 1.00)	0

Positive : ≥0.01 mg DDT/kg eggs

Extraneous Residue Limit (ERL) of total DDT : 0.5 mg/kg eggs

*Mean ≥ERL

cot. = cotton

cof. = coffee

tob. = tobacco

Appendix 8 Dieldrin residues (mg/kg eggs) in eggs from free-range and enclosed chickens in various localities of Embu and Meru.

Locality	Free-range				Enclosed			
Main agric. products	Dieldrin				Dieldrin			
	No. of eggs	*Mean* ± S.D. (Range)	No. positive	No. ≥ERL	No. of eggs	*Mean* ± S.D. (Range)	No. positive	No. ≥ERL
Mikinduri	16	*1.84** ± 3.07	15	6	12	*0.06* ± 0.02	12	0
Coffee		(<0.01 - 9.74)				(0.03 - 0.09)		
Gachoka	24	*1.55** ± 4.14	18	9	10	*0.09* ± 0.03	10	3
Rice, marginal cot.		(<0.01 - 14.90)				(0.06 - 0.14)		
Nithi	19	*0.51** ± 1.09	18	5	63	*0.06* ± 0.06	63	11
Coffee, tea		(<0.01 - 3.99)				(0.01 - 0.39)		
Runyenjes	32	*0.32** ± 1.02	32	11	63	*0.34** ± 0.36	63	47
Coffee, tea		(<0.01 - 5.85				(0.01 - 1.44)		
N/Imenti	32	*0.18** ± 0.22	29	15	23	*0.11** ± 0.09	23	11
Cof., tea, cot.		(<0.01 - 0.75)				(0.01 - 0.37)		
Siakago	27	*0.08* ± 0.07	23	10	10	*0.09* ± 0.03	10	3
Cot., tob.		(<0.01 - 0.26)				(0.05 - 0.16)		
S/Imenti	6	*0.02* ± 0.02	5	0	15	*0.22** ± 0.40	15	0
Coffee, tea		(<0.01 - 0.05)				(0.02 - 1.29)		
Embu Agric. Inst.					15	*0.04* ± 0.01	15	0
Coffee						(0.03 - 0.05)		

Positive : ≥0.01 mg dieldrin/kg eggs

Extraneous Residue Limit (ERL) : 0.1 mg dieldrin/kg eggs

* Mean ≥ERL

<0.01 : below the detection limit

cot. = cotton

cof. = coffee

tob. = tobacco

Appendix 9 Lindane and total HCH residues (mg/kg eggs) in eggs from enclosed chickens in various localitites of Embu and Meru.

Locality	No. of eggs	Lindane			Total HCH	
Main agric. produce		*Mean* ± S.D. (Range)	No. positive	No. >ERL	*Mean* ± S.D. (Range)	No. positive
Gachoka	10	*0.03* ± 0.01	10	0	*0.03* ± 0.01	10
Rice, marginal cot.		(0.03 - 0.05)			(0.03 - 0.05)	
Embu Agric.Inst.	15	*0.02* ± 0.01	15	0	*0.02* ± 0.01	15
Coffee		(0.01 - 0.03)			(0.01 - 0.03)	
Siakago	10	*0.01* ± 0.01	8	0	*0.01* ± 0.01	8
Cot. tob.		(<0.01 - 0.02)			(<0.01 - 0.02)	
S/Imenti	15	*0.01* ± 0.01	9	0	*0.01* ± 0.01	9
Coffee, tea		(<0.01 - 0.01)			(<0.01 - 0.01)	
Nithi	63	*0.01* ± 0.01	42	0	*0.01* ± 0.01	42
Coffee, tea		(<0.01 - 0.02)			(<0.01 - 0.02)	
Runyenjes	63	*0.01* ± 0.01	62	0	*0.02* ± 0.03	62
Coffee, tea		(0.01 - 0.04)			(<0.01 - 0.21)	
N/Imenti	23	*0.01* ± 0.005	16	0	*0.01* ± 0.005	
Cof., tea, cot.		(<0.01 - 0.01)			(<0.01 - 0.01)	
Mikinduri	12	*0.01* ± 0.004	9	0	*0.01* ± 0.004	9
Coffee		(<0.01 - 0.02)			(<0.01 - 0.01)	

Positive : ≥0.01 mg lindane or total HCH/kg eggs

Extraneous Residue Limit (ERL) : 0.1 mg lindane/kg eggs

<0.01 : below the detection limit

cot. = cotton

cof. = coffee

tob. = tobacco

Appendix 10 Lindane and total HCH residues (mg/kg eggs) in eggs from free-range chickens in various localities of Embu and Meru.

Locality Main agric. produce	No. of eggs	Lindane			Total HCH	
		Mean ± S.D. (Range)	No. positive	No. ≥ERL	*Mean* ± S.D. (Range)	No. positive
Gachoka (Mwea)	24	*0.10** ± 0.16	15	7	*0.12** ± 0.17	15
Rice, marginal Cot.		(<0.01 - 0.53)			(<0.01 - 0.54)	
E/Imenti	32	*0.06* ± 0.14	15	5	*0.06* ± 0.14	15
Cof., tea, cot.		(<0.01 - 0.47)			(<0.01 - 0.47)	
Mikinduri	16	*0.01* ± 0.01	8	0	*0.01* ± 0.01	8
Coffee		(<0.01 - 0.01)			(<0.01 - 0.01)	
Runyenjes	32	*0.01* ± 0.01	23	0	*0.02* ± 0.02	24
Coffee, tea		(<0.01 - 0.04)			(<0.01 - 0.09)	
Nithi	19	*<0.01*	3	0	*<0.01*	3
Coffee, tea		(<0.01 - 0.03)			(<0.01 ± 0.03)	
Siakago	27	*<0.01*	7	0	*0.02* ± 0.03	11
Cot., tob.		(0.01 - 0.05)			(<0.01 - 0.14)	
S/Imenti	6	*<0.01*	0	0	*<0.01*	0
Coffee, tea		(-)			(-)	

Positive : ≥0.01 mg lindane or total HCH/kg eggs

Extraneous Residue Limit (ERL) : 0.1 mg lindane/kg eggs

*Mean ≥ERL

<0.01 : below detection limit

(-) : all values were below the detection limit

cot. = cotton

cof. = coffee

tob. = tobacco

Appendix II Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Runyenjes division

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
AE1a*	0.08	0.09	0.02	t	0.20	1.13	0.01	-	-	*0.15*	-
AE1b	0.07	0.08	0.02	t	0.20	1.14	0.01	-	t	*0.15*	-
AE2a	0.09	0.11	0.03	t	0.24	1.22	0.01	-	t	*0.23*	-
AE2b	0.09	0.10	0.02	t	0.23	1.11	0.01	-	t	*0.22*	-
AE3a	0.07	0.07	0.02	t	0.17	1.00	0.01	-	-	*0.12*	-
AE3b	0.05	0.05	0.01	t	0.13	1.00	0.01	-	-	*0.13*	-
AE4a	0.07	0.08	0.02	t	0.18	1.14	0.01	-	-	*0.21*	-
AE4b	0.07	0.08	0.02	t	0.18	1.14	0.01	-	-	*0.20*	-
AE5a	0.08	0.09	0.02	t	0.20	1.13	0.01	-	t	*0.15*	-
AE5b	0.08	0.10	0.02	t	0.21	1.25	0.01	-	-	*0.15*	-
AE6a	0.08	0.09	0.02	t	0.20	1.13	0.01	-	-	*0.15*	-
AE6b	0.09	0.10	0.02	t	0.22	1.11	0.01	-	-	*0.15*	-
AE7a	0.08	0.08	0.02	t	0.19	1.00	0.01	-	-	*0.14*	-
AE7b	0.08	0.08	0.02	t	0.19	1.00	0.01	-	-	*0.13*	-
AE8a	0.07	0.09	0.02	t	0.19	1.29	0.01	-	-	*0.29*	-
AE8b	0.07	0.09	0.02	t	0.19	1.29	0.01	-	-	*0.29*	-
AE9a	0.08	0.09	0.02	t	0.20	1.13	0.01	-	t	*0.46*	-
AE9b	0.08	0.09	0.02	t	0.20	1.13	0.01	-	t	*0.45*	-
AE10a	0.05	0.07	0.02	t	0.15	1.40	0.01	-	t	*0.11*	-
AE10b	0.05	0.07	0.02	t	0.15	1.40	0.01	-	-	*0.12*	-
AE11a	0.06	0.09	0.02	t	0.18	1.50	0.01	-	-	0.09	-
AE11b	0.06	0.08	0.02	t	0.17	1.33	0.01	-	-	*0.10*	-
AE12a	0.08	0.07	0.02	t	0.18	0.88	0.01	-	-	*0.19*	-
AE12b	0.08	0.07	0.02	t	0.18	0.88	0.01	-	-	*0.20*	-
AE13	0.06	0.08	0.02	t	0.17	1.33	0.01	-	-	0.07	
AE14	0.06	0.06	0.02	t	0.15	1.00	0.01	-	-	0.09	-
AE15	0.04	0.04	0.01	t	0.09	1.00	0.01	-	-	0.09	-
AE16	0.05	0.06	0.01	-	0.13	1.20	0.01	-	-	*0.10*	-
AE17	0.06	0.07	0.01	t	0.15	1.17	0.01	-	-	*0.13*	-
AE18	0.07	0.07	0.02	t	0.17	1.00	0.01	-	-	*0.20*	-
AE19	0.06	0.07	0.02	t	0.16	1.17	0.01	-	-	*0.14*	-
AE20	0.07	0.07	0.02	t	0.17	1.00	0.01	-	-	*0.11*	-
BE1	0.08	0.05	-	-	0.14	0.63	0.02	-	-	*0.50*	-
BE2	0.09	0.06	t	t	0.16	0.67	0.02	-	-	*1.34*	-
BE3	0.13	0.07	0.01	t	0.22	0.54	0.02	-	-	*0.84*	-
BE4	0.08	0.06	0.01	t	0.16	0.75	0.02	-	-	*0.61*	-
BE5	0.09	0.06	0.02	t	0.18	0.67	0.01	-	-	*0.49*	-
BE6	0.12	0.08	0.02	t	0.23	0.67	0.02	-	-	*1.20*	-
BE7	0.08	0.03	t	t	0.12	0.38	0.04	t	-	*0.69*	-
BE8	0.07	0.04	0.01	t	0.13	0.57	0.01	-	-	*0.72*	-
BE9	0.10	0.05	t	t	0.16	0.50	0.02	-	-	*0.80*	-
BE10	0.12	0.07	0.02	t	0.22	0.58	0.02	-	-	*0.89*	-
BE11	0.06	0.05	t	-	0.12	0.83	0.01	-	-	*0.37*	-
BE12	0.09	0.06	0.01	-	0.17	0.67	0.01	-	-	*0.78*	-
BE13	0.11	0.08	0.02	t	0.22	0.73	0.01	-	-	*1.28*	-
BE14	0.09	0.05	0.01	t	0.16	0.56	0.01	-	-	*0.62*	-
BE15	0.07	0.04	0.01	t	0.13	0.57	0.02	-	-	*0.50*	-
BE16	0.08	0.06	0.01	t	0.16	0.75	0.01	-	-	*0.64*	-
BE17	0.10	0.06	0.01	t	0.18	0.60	0.01	-	-	*0.78*	-
BE18	0.11	0.06	0.02	t	0.20	0.55	0.01	-	-	*0.68*	-
BE19	0.09	0.06	0.02	t	0.18	0.67	0.01	-	-	*0.55*	-
BE20	0.09	0.05	0.01	t	0.16	0.56	0.01	-	-	*0.55*	-
BE21	0.11	0.19	0.01	t	0.31	1.73	0.02	-	t	*1.44*	0.04
BE22	0.12	0.15	t	t	0.28	1.25	0.01	-	-	*0.21*	-
BE23	*0.97*	*1.23*	0.05	0.03	*2.40*	1.27	0.04	0.04	0.01	*5.85*	-
BE24	0.11	0.15	t	t	0.27	1.36	0.01	-	-	*0.21*	-
CE1	0.08	0.05	0.01	0.01	0.16	0.63	0.01	t	t	*0.10*	-
CE2	0.06	0.04	0.01	t	0.12	0.67	0.01	t	-	*0.25*	-
CE3	0.04	0.03	0.01	t	0.08	0.75	0.01	-	-	*0.21*	-
CE4	0.04	0.03	0.01	t	0.08	0.75	0.01	-	-	*0.12*	-
CE5	0.07	0.04	0.01	0.02	0.15	0.57	0.02	t	t	*0.22*	-
CE6	0.06	0.03	0.01	d	0.11	0.50	0.01	-	t	*0.22*	-
DE1a	0.06	0.03	0.01	-	0.11	0.50	0.02	-	-	0.03	-
DE1b	0.05	0.03	0.01	-	0.10	0.60	0.01	-	-	0.02	-
DE2	0.04	0.04	0.01	t	0.09	1.00	0.01	-	-	0.02	-
DE3	0.04	0.03	0.01	-	0.08	0.75	0.01	-	-	0.02	-
DE4	0.06	0.03	0.01	-	0.11	0.50	0.02	-	-	0.02	-
DE5	0.05	0.05	0.01	t	0.12	1.00	0.02	-	-	0.02	-
DE6	0.06	0.04	-	-	0.11	0.67	0.02	-	-	0.02	-
DE7	0.05	0.04	0.01	-	0.11	0.80	0.02	-	-	0.01	-
DE8	0.05	0.04	0.01	-	0.11	0.80	0.01	-	-	0.02	-
DE9	0.06	0.05	0.01	t	0.13	0.83	0.02	-	-	0.03	-
DE10	0.05	0.05	0.01	-	0.12	1.00	0.02	-	-	0.02	-

t = trace, peak, but < 0.01 mg/kg

- = no peak

italics = values ≥ ERL : DDT = 0.5 mg/kg; lindane and dieldrin = 0.1 mg/kg

*Samples AE1 - AE12 analysed in parallel

Appendix 11 (cont.)

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
DFE1	*1.34*	0.19	0.05	t	*1.74*	0.14	0.02	-	-	0.08	-
DFE2	*1.41*	0.21	0.05	t	*1.84*	0.15	0.02	-	-	0.07	-
DFE3	*1.79*	0.20	0.04	t	*2.23*	0.11	0.01	t	-	*0.10*	-
DFE4	0.23	0.19	t	0.01	0.46	0.11	0.03	-	-	0.04	-
DFE5	0.23	0.21	t	0.02	0.49	0.91	0.03	-	-	0.04	-
DFE6	0.23	0.25	t	0.01	*0.52*	1.09	0.02	-	-	0.03	-
DFE7	0.29	*0.59*	0.02	0.04	*0.97*	2.03	0.02	-	-	0.03	-
DFE8	*0.76*	0.38	t	0.02	*1.24*	0.50	0.02	-	-	0.08	-
EE1	0.19	0.04	t	-	0.25	0.21	-	-	-	*0.41*	-
EE2	0.27	0.05	0.01	-	0.36	0.19	-	-	-	*0.48*	-
EE3	0.24	0.04	t	t	0.31	0.17	t	t	-	*0.45*	-
EE4	0.27	0.06	0.02	t	0.38	0.22	t	0.01	t	*0.47*	-
FE1	0.17	0.15	0.14	0.18	*0.67*	0.88	0.03	t	0.05	0.05	-
FE2	0.15	0.13	0.05	0.04	0.39	0.87	0.03	t	0.02	0.05	-
FE3	0.12	0.11	0.09	0.07	0.41	0.92	0.03	-	0.03	0.07	-
FE4	0.36	0.30	0.45	0.41	*1.61*	0.83	0.07	-	0.14	*0.18*	-
FEr	0.23	0.11	0.02	-	0.39	0.48	0.01	-	-	*0.15*	-
GE1	0.27	0.03	0.05	-	0.39	0.11	-	-	-	0.06	-
GE2	0.30	0.16	0.15	-	*0.66*	0.53	t	-	-	0.08	-
HE1	0.15	0.06	0.01	-	0.25	0.40	0.01	-	-	0.04	-
HE2	0.14	0.07	0.02	t	0.25	0.50	0.01	-	-	0.06	-
HE3	0.13	0.08	0.01	-	0.23	0.62	0.01	-	-	0.05	-
HE4	0.13	0.08	0.01	-	0.23	0.62	0.01	-	-	0.05	-
HE5	0.12	0.06	0.01	-	0.20	0.50	0.01	-	-	0.04	-
HE6	0.16	0.07	0.01	-	0.26	0.44	0.01	-	-	0.05	-
HE7	0.13	0.08	0.01	-	0.23	0.62	0.01	-	-	0.06	-
IE1	0.05	0.03	t	-	0.09	0.60	t	-	-	0.01	-
IE2	0.05	0.04	t	-	0.10	0.80	t	-	-	0.01	-
IE3	0.12	0.04	0.01	-	0.18	0.33	0.01	-	t	0.01	-
IE4	0.15	0.05	0.01	-	0.23	0.33	0.01	-	t	0.01	-
IE5	0.05	0.03	t	-	0.09	0.60	0.01	-	t	0.01	-
PE1	*1.01*	0.44	0.03	0.02	*1.61*	0.44	0.01	0.01	0.01	*0.31*	-
PE2	*1.29*	*0.74*	0.04	0.05	*2.26*	0.57	0.01	0.02	0.01	*0.27*	-
PE3	*0.98*	0.45	0.03	0.03	*1.60*	0.46	t	0.01	0.01	*0.28*	-
PE4	*1.11*	*0.53*	0.03	0.03	*1.82*	0.48	0.01	0.01	t	*0.39*	-

Appendix 12 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Siakago division

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
QE1	0.47	0.05	t	t	*0.57*	0.11	t	t	-	0.03	-
QE2	0.49	0.05	t	t	*0.59*	0.10	t	t	-	0.03	-
QE3	0.47	0.05	-	-	*0.57*	0.11	t	t	-	0.02	-
QE4	0.22	0.03	-	-	0.27	0.14	t	t	-	*0.18*	-
QE5	0.29	0.09	t	t	0.41	0.31	t	-	-	*0.18*	-
QE6	0.21	0.02	-	-	0.26	0.14	t	-	-	*0.26*	-
QE7	*1.03*	0.31	t	0.01	*1.46*	0.30	t	t	t	0.08	-
QE8	*1.17*	*0.83*	0.02	0.05	*2.20*	0.71	t	t	t	0.09	-
QE9	*1.32*	0.29	0.01	0.01	*1.78*	0.22	t	t	t	0.09	-
QE10	*1.35*	*0.91*	0.02	0.05	*2.48*	0.67	t	t	t	0.08	-
QE11	*1.23*	*0.83*	0.02	0.05	*2.27*	0.67	t	t	t	0.09	-
QE12	*1.06*	*0.66*	0.02	0.04	*1.90*	0.62	t	t	-	*0.11*	-
QE13	0.32	0.27	t	t	*0.63*	0.84	0.02	t	-	*0.16*	-
QE14	0.25	0.25	t	t	*0.53*	1.00	0.01	t	-	0.09	-
QE15	0.18	0.19	t	t	0.39	1.06	0.01	t	-	0.03	-
QE16	0.17	0.17	t	t	0.36	1.00	0.01	t	-	0.07	-
QE17	0.15	0.28	t	t	0.45	1.87	0.02	t	-	0.09	-
QE18	0.16	0.15	t	t	0.33	0.94	0.01	t	-	0.05	-
QE19	0.14	0.18	t	t	0.34	1.29	0.01	t	-	0.07	-
QE20	0.14	0.19	t	t	0.35	1.36	0.01	t	-	0.07	-
QE21	*0.74*	0.25	t	t	*1.07*	0.34	t	t	0.02	-	-
QE22	0.47	0.17	t	t	*0.69*	0.36	t	t	0.01	-	-
QE23	*0.50*	0.17	t	t	*0.73*	0.34	t	t	0.01	-	-
QE24	0.39	0.76	0.04	0.03	*1.26*	1.95	t	t	-	*0.11*	-
QE25	0.21	0.14	t	t	0.37	0.67	t	t	-	*0.12*	-
QE26	*0.61*	0.10	t	t	*0.78*	0.16	0.01	t	0.01	*0.16*	t
QE27	*0.81*	0.11	t	t	*1.01*	0.14	0.01	t	0.02	*0.16*	-
QE28	*0.66*	0.11	t	t	*0.84*	0.17	0.01	t	0.02	*0.11*	-
QE29	0.45	0.08	-	-	*0.58*	0.18	t	t	0.01	*0.12*	-
QE30	*0.75*	0.13	t	t	*0.96*	0.17	0.01	t	0.02	*0.15*	-
QE31	*0.84*	*0.62*	0.04	0.04	*1.61*	0.74	0.05	0.02	0.07	*0.11*	-
QE32	*0.75*	0.32	0.02	0.01	*1.18*	0.43	0.02	0.01	0.04	0.06	-
QE33	*0.87*	0.34	0.01	t	*1.32*	0.39	0.02	0.01	0.04	0.07	-
QE34	0.07	t	-	-	0.08	0.00	t	-	-	0.02	-
QE35	0.08	0.01	-	-	0.10	0.13	t	-	-	0.02	-
QE36	0.08	0.01	-	-	0.10	0.13	t	-	-	0.02	-
QE37	0.04	t	-	-	0.04	0.00	t	-	-	-	-

Appendix 13 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Gusaka (Mwea) division.

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin	Aldrin
JE1a	0.13	0.06	0.02	t	0.22	0.46	t	-	-	*0.38*	-	-
JE1b	0.13	0.04	0.02	t	0.20	0.31	t	-	-	*0.37*	-	-
JE2a	0.13	0.05	0.01	t	0.20	0.38	t	-	-	*0.26*		
JE2b	0.12	0.05	0.01	t	0.19	0.42	t	-	-	*0.29*	-	-
JE3	*0.97*	0.26	0.04	t	*1.38*	0.27	0.02	-	-	-	-	-
JE4	*1.96*	0.29	0.05	t	*1.52*	0.27	t	-	-	-	-	-
JE5	*1.20*	0.33	0.04	-	*1.71*	0.28	t	-	-	-	-	-
KE1	*2.24*	*1.47*	0.07	0.08	*4.11*	0.66	*0.10*	0.03	0.03	0.05	-	-
KE2	*1.89*	*1.34*	0.08	0.07	*3.60*	0.71	0.08	0.02	0.03	0.06	-	-
KE3	*2.46*	*1.26*	0.08	0.08	*4.16*	0.51	0.09	0.02	0.03	0.06	-	-
LE1	*6.63*	*1.53*	0.16	t	*9.07*	0.23	-	-	-	-	-	-
LE2	*6.72*	*1.45*	0.14	-	*9.06*	0.22	-	-	-	-	-	-
LE3	*7.38*	*1.87*	0.17	t	*10.25*	0.25	-	-	-	-	-	-
LE4	*3.00*	*1.38*	0.19	t	*4.92*	0.46	-	-	-	-	-	-
LE5	*3.33*	*1.47*	0.22	t	*5.41*	0.44	-	-	-	-	-	-
ME1	*1.14*	*1.10*	0.05	t	*2.42*	0.96	0.04	-	-	0.07	-	-
ME2	*0.90*	0.46	t	t	*1.46*	0.51	0.03	-	-	0.08	-	-
ME3	*1.14*	*0.76*	0.04	0.02	*2.09*	0.67	0.03	-	-	*0.14*	-	-
ME4	*1.24*	*0.76*	0.08	t	*2.21*	0.60	0.03	-	-	0.08	-	-
ME5	*1.63*	*1.22*	0.10	0.02	*4.16*	0.75	0.05	-	-	*0.13*	-	-
ME6	*1.31*	*0.56*	t	t	*2.01*	0.43	0.04	-	-	*0.10*	-	-
ME7	*0.86*	0.40	0.03	t	*1.39*	0.47	0.02	-	-	0.06	-	-
ME8	*1.56*	*0.83*	0.05	t	*2.68*	0.53	0.03	-	-	0.07	-	-
ME9	*1.45*	0.47	0.03	t	*2.15*	0.32	0.03	-	-	0.07	-	-
ME10	*1.43*	0.44	0.03	t	*2.06*	0.31	0.04	-	-	0.08	-	-
NE1	*2.84*	*3.91*	0.61	t	*7.74*	1.38	*0.23*	0.01	0.11	*4.24*	0.78	-
NE2	*0.65*	*1.07*	0.16	t	*1.97*	1.65	0.05	0.01	0.04	*0.83*	0.03	-
NE4	*1.51*	*1.94*	0.32	t	*3.97*	1.28	*0.11*	t	0.06	*14.90*	0.46	0.02
NE5	*0.50*	0.13	t	t	*0.69*	0.26	*0.14*	t	t	*1.34*	0.09	-
NE6	*1.85*	*2.00*	0.14	0.02	*1.34*	1.03	0.09	0.01	0.06	*14.47*	0.32	0.02
NE7	0.32	0.12	-	t	0.48	0.38	0.02	t	0.01	*0.13*	-	-
NE8	0.25	0.12	t	t	0.40	0.48	*0.53*	t	0.01	0.04	-	-
NE9	0.22	0.11	t	t	0.34	0.50	*0.49*	t	0.01	0.04	-	-
NE10	0.46	0.16	t	t	0.67	0.35	0.06	t	0.01	*0.13*	-	-
NE11	0.27	0.12	t	t	0.42	0.44	0.03	-	0.01	0.08	-	-
NE12	0.24	0.12	t	t	0.39	0.50	*0.43*	t	0.01	0.05	-	-

Appendix 14 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Embu Agricultural Institute.

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
OE1	0.13	0.17	0.02	-	0.34	1.31	0.02	-	-	0.04	-
OE2	0.09	0.17	0.03	t	0.30	1.89	0.01	-	-	0.03	-
OE3	0.10	0.15	0.04	t	0.31	3.00	0.01	t	-	0.05	-
OE4	0.08	0.16	0.03	t	0.28	2.00	0.02	-	-	0.04	-
OE5	0.07	0.13	0.03	t	0.24	1.86	0.02	t	-	0.03	-
OE6	0.08	0.12	0.03	t	0.24	1.50	0.02	t	-	0.03	-
OE7	0.12	0.19	0.06	t	0.39	1.58	0.02	t	-	0.05	-
OE8	0.13	0.22	0.06	t	0.43	1.69	0.02	t	-	0.03	-
OE9	0.08	0.09	0.04	t	0.22	1.13	0.01	t	-	0.03	-
OE10	0.14	0.22	0.06	t	0.44	1.57	0.03	t	-	0.04	-
OE11	0.13	0.21	0.05	t	0.41	1.62	0.02	t	-	0.05	-
OE12	0.11	0.17	0.05	t	0.35	1.55	0.01	t	-	0.04	-
OE13	0.19	0.39	0.04	t	0.65	2.05	0.03	t	-	0.03	-
OE14	0.12	0.17	0.04	t	0.35	1.42	0.01	t	-	0.05	-
OE15	0.10	0.18	0.04	t	0.34	1.80	0.02	t	-	0.04	-

Appendix 15 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Nithi division

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
RE1	0.34	*0.64*	0.01	0.01	*1.04*	1.88	0.02	t	t	0.07	-
RE2	0.34	*0.56*	0.02	0.01	*0.97*	1.65	0.02	t	t	0.07	-
RE3	0.25	*0.51*	0.02	0.01	*0.82*	2.04	0.01	-	t	0.08	-
RE4	0.26	0.43	0.02	0.01	*0.75*	1.65	0.01	-	t	0.08	-
RE5	0.34	*0.57*	0.03	0.01	*0.99*	1.68	0.02	t	t	0.09	-
RE6	0.25	*0.56*	0.02	0.01	*0.87*	2.24	0.02	t	t	0.08	-
RE7	0.29	*0.51*	0.02	0.01	*0.86*	1.76	0.01	t	t	0.07	-
RE8	0.28	*0.56*	0.03	0.01	*0.91*	2.00	0.02	t	t	*0.11*	-
RE9	0.28	*0.58*	0.02	0.01	*0.92*	2.07	0.03	t	t	*0.10*	-
RE10	0.39	0.55	0.03	0.01	*1.02*	1.41	0.01	t	t	*0.10*	-
RE11	0.13	0.06	-	-	0.20	0.46	0.03	t	t	0.05	-
RE12	0.11	0.08	t	t	0.20	0.73	0.03	t	-	0.05	-
RE13	0.14	0.05	t	t	0.21	0.36	0.03	-	-	0.04	-
RE14	0.10	0.07	t	t	0.18	0.70	0.03	-	-	0.03	-
RE15	0.11	0.04	-	-	0.16	0.36	0.03	t	-	0.05	-
RE16	0.10	0.06	t	t	0.17	0.60	0.03	-	-	0.05	-
RE17	*1.37*	0.41	t	t	*2.10*	0.30	0.02	-	t	*0.14*	-
RE18	*0.81*	0.34	0.02	-	*1.26*	0.42	0.02	-	-	*0.11*	-
RE19	0.36	0.44	0.01	0.01	*0.86*	1.22	0.01	-	-	*0.39*	-
RE20	0.47	*0.50*	0.04	0.01	*1.07*	1.06	0.02	t	-	0.09	-
RE21	*0.75*	*0.60*	0.04	0.01	*1.48*	0.80	0.02	t	-	*0.13*	-
RE22	*0.63*	*0.57*	t	0.02	*1.29*	0.90	0.02	t	-	0.07	-
RE23	0.08	0.07	t	-	0.16	0.88	0.01	t	-	0.06	-
RE24	0.16	0.10	t	-	0.28	0.63	0.01	-	-	*0.15*	-
RE25	0.08	0.07	t	-	0.16	0.88	0.01	-	t	0.07	-
RE26	0.08	0.09	0.01	0.01	0.20	1.13	0.02	-	-	0.08	-
RE27	0.10	0.09	t	t	0.20	0.90	0.01	-	-	0.08	-
RE28	0.07	0.08	t	t	0.16	1.14	0.01	t	-	0.05	-
RE29	0.15	0.07	-	-	0.24	0.47	0.01	t	-	0.09	-
RE30	0.10	0.08	t	t	0.19	0.80	0.01	t	-	0.08	-
RE31	0.08	0.09	-	t	0.18	1.13	0.01	t	-	0.06	-
RE32	0.06	0.05	-	-	0.12	0.83	0.01	-	-	0.06	-
RE33	0.17	0.02	-	-	0.21	0.12	t	-	-	0.07	-
RE34	0.32	0.35	t	0.01	*0.72*	1.09	t	-	-	0.07	-
RE35	0.23	0.26	t	t	*0.52*	1.13	t	t	t	0.06	-
RE36	0.16	0.03	-	-	0.21	0.19	t	-	-	0.06	-
RE37	0.18	0.03	-	-	0.23	0.17	-	-	-	0.06	-
RE38	0.16	0.03	-	-	0.21	0.19	-	-	-	0.05	-
RE39	0.05	0.04	-	-	0.10	0.80	-	-	-	-	-
RE40	0.04	0.02	-	-	0.06	0.50	t	-	-	*0.34*	-
RE41	0.23	0.20	t	t	0.46	0.87	0.02	t	-	*0.13*	-
RE42	0.10	0.11	t	t	0.22	1.10	0.01	t	-	*0.12*	-
RE43	0.04	0.04	-	-	0.08	1.00	t	-	-	0.03	-
RE44	0.03	0.03	-	-	0.06	1.00	t	-	-	0.03	-
RE45	0.05	0.04	-	t	0.10	0.80	t	-	-	0.07	-
RE46	0.04	0.02	-	-	0.06	0.50	t	-	-	0.04	-
RE47	0.02	0.01	-	-	0.03	0.50	t	-	-	0.06	-
RE48	0.02	0.02	-	-	0.04	1.00	t	-	-	0.04	-
RE49	0.03	0.04	-	-	0.07	1.33	t	-	-	0.02	-
RE50	0.08	0.05	-	-	0.14	0.63	t	-	-	0.03	-
RE51	0.03	0.03	-	-	0.06	1.00	t	-	-	0.03	-
RE52	0.05	0.06	t	t	0.12	1.20	t	t	-	0.02	-
RE53	0.05	0.05	0.01	t	0.11	1.00	0.01	-	-	0.03	-
RE54	0.02	0.03	t	t	0.05	1.50	t	-	-	0.02	-
RE55	0.06	0.04	-	-	0.11	0.67	t	-	-	0.02	-
RE56	0.03	0.03	-	-	0.06	1.00	-	t	-	0.01	-
RE57	0.08	0.04	t	t	0.13	0.50	t	t	-	0.03	-
RE58	0.07	0.06	-	-	0.14	0.86	0.01	t	-	0.04	-
RE59	0.03	0.03	-	-	0.06	1.00	t	t	-	0.02	-
RE60	0.04	0.05	t	t	0.09	1.25	t	-	-	0.02	-
RE61	0.03	0.04	-	-	0.07	1.33	t	-	-	0.02	-
RE62	0.07	0.06	t	t	0.14	0.86	0.01	t	-	0.03	-
RE64	0.09	0.06	t	t	0.16	0.67	0.01	t	-	0.03	-
RE65	0.05	0.03	t	t	0.09	0.60	t	t	-	0.02	-
RE66	0.03	0.04	t	-	0.07	1.33	t	-	-	0.02	-
RE67	0.03	0.03	-	-	0.06	1.00	t	t	-	0.02	-
RE68	0.07	0.06	-	-	0.14	0.86	0.01	t	-	0.04	-
RE69	0.08	0.16	0.01	t	0.26	2.00	0.01	t	-	*2.54*	t
RE70	0.12	0.14	0.01	0.01	0.28	1.17	t	t	-	*1.96*	0.01
RE71	0.08	0.06	-	t	0.15	0.75	t	t	-	*3.99*	0.05
RE72	0.06	0.06	-	t	0.13	1.00	0.03	t	-	0.01	-
RE73	0.05	0.04	t	t	0.10	0.80	0.01	-	-	0.03	-
RE74	0.05	0.04	t	t	0.10	0.80	0.01	-	-	0.17	-
RE75	0.08	0.09	t	t	0.18	1.13	0.01	-	t	0.04	-
RE76	0.07	0.06	t	t	0.14	0.86	0.01	t	-	0.02	-
RE77	0.05	0.04	t	t	0.10	0.80	0.01	-	-	0.01	-
RE80	0.02	0.03	-	-	0.05	1.50	t	t	-	0.05	-
RE81	0.03	0.03	t	-	0.06	1.00	t	t	-	0.03	-
RE82	0.01	0.02	-	t	0.03	2.00	t	t	-	0.02	-
RE83	0.04	0.03	t	-	0.07	0.75	t	t	-	0.05	-
RE84	0.03	0.03	-	-	0.06	1.00	t	t	-	0.06	-
RE85	0.02	0.01	-	-	0.03	0.50	t	-	-	0.17	-

Appendix 16 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from North Imenti division

Sample Number	p,p-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
SE1	0.09	0.04	0.01	t	0.15	0.44	0.01	t	-	0.07	-
SE2	0.06	0.04	0.01	t	0.12	0.67	0.01	-	-	0.04	-
SE3	0.05	0.02	0.01	t	0.09	0.40	t	-	-	*0.11*	-
SE4	0.06	0.03	0.01	t	0.11	0.50	0.01	-	-	0.05	-
SE5	0.06	0.04	-	t	0.11	0.67	0.01	-	-	*0.10*	-
SE6	0.06	0.04	0.01	t	0.12	0.67	0.01	t	-	0.03	-
SE7	0.07	0.04	0.01	t	0.13	0.57	0.01	t	-	*0.17*	-
SE8	0.07	0.05	0.01	t	0.14	0.71	0.01	t	-	*0.14*	-
SE9	0.06	0.04	0.01	t	0.12	0.67	0.01	t	-	*0.15*	-
SE10	0.05	0.05	-	t	0.11	1.00	0.01	t	-	0.06	-
SE11	0.07	0.03	0.01	t	0.12	0.43	0.01	-	-	*0.15*	-
SE12	0.06	0.04	0.01	t	0.12	0.67	0.01	-	-	0.04	-
SE13	*2.85*	*1.11*	0.23	0.04	*4.57*	0.39	*0.39*	-	-	*0.39*	-
SE14	*2.58*	*1.64*	0.34	0.03	*4.91*	0.64	*0.30*	-	-	*0.49*	-
SE15	*2.55*	*0.78*	0.02	0.02	*3.65*	0.31	*0.45*	-	-	*0.34*	-
SE16	*0.52*	*0.56*	0.01	0.03	*1.18*	1.08	0.01	-	-	-	-
SE17	*2.59*	*1.04*	0.04	0.03	*3.98*	0.40	*0.47*	-	-	*0.36*	-
SE18	*2.72*	*1.13*	0.03	0.03	*4.21*	0.42	*0.18*	-	-	*0.26*	-
SE19	*4.50*	*0.85*	0.18	0.03	*6.07*	0.19	0.03	-	-	*0.22*	-
SE20	*0.61*	0.11	-	-	*0.79*	0.18	0.01	-	-	0.04	-
SE21	*1.33*	*0.50*	t	0.02	*2.00*	0.38	t	-	-	*0.69*	t
SE22	*1.40*	*0.50*	0.02	0.02	*2.09*	0.36	t	-	t	*0.59*	-
SE23	*1.34*	0.41	t	t	*1.90*	0.31	t	-	-	*0.75*	t
SE24	*1.41*	0.47	0.01	0.02	*2.07*	0.33	t	-	t	*0.61*	t
SE25	*0.98*	0.19	t	t	*1.28*	0.19	0.01	-	-	-	-
SE26	*0.67*	0.09	t	t	*0.83*	0.13	0.01	t	-	0.01	-
SE27	*0.58*	0.20	0.01	t	*0.85*	0.34	0.03	-	-	0.04	-
SE28	*0.74*	0.34	0.02	0.01	*1.19*	0.46	0.02	-	t	*0.13*	-
SE29	*0.98*	*1.55*	0.05	0.02	*2.65*	1.68	0.02	-	t	*0.11*	-
SE67	0.06	0.07	t	t	0.14	1.17	t	-	-	0.05	-
SE68	0.09	0.12	t	t	0.22	1.33	0.01	-	-	0.05	-
SE69	0.09	0.10	t	t	0.20	1.11	0.01	-	-	0.06	-
SE70	0.09	0.12	t	t	0.22	1.33	0.01	-	-	0.06	-
SE71	0.03	0.02	t	-	0.05	0.67	t	-	-	0.05	-
SE72	0.06	0.05	t	-	0.12	0.83	-	-	-	0.08	-
SE73	0.03	0.02	-	-	0.05	0.67	t	-	-	0.09	-
SE74	0.03	0.03	-	-	0.06	1.00	-	-	-	0.06	-
SE75	0.03	0.03	-	-	0.06	1.00	t	t	-	0.08	-
SE76	0.03	0.02	-	-	0.05	0.67	-	-	-	0.02	-
SE77	0.09	0.10	-	t	0.20	1.11	0.01	t	-	0.03	-
SE78	0.09	0.14	t	t	0.24	1.56	0.01	t	t	0.03	-
SE79	0.08	0.06	-	-	0.15	0.75	t	t	-	0.02	-
SE80	0.03	0.03	-	-	0.06	1.00	t	t	-	0.01	-
SE81	0.16	0.10	-	-	0.28	0.63	-	-	-	*0.37*	-
SE82	0.07	0.11	t	t	0.19	1.57	0.01	t	-	*0.26*	-
SE83	0.05	0.07	t	0.01	0.13	1.40	t	-	-	*0.12*	-
SE84	0.08	0.15	0.01	t	0.24	1.88	0.01	-	-	*0.16*	-
SE85	0.06	0.07	t	t	0.14	1.17	t	-	-	*0.21*	-
SE86	0.16	0.02	-	-	0.20	0.13	-	-	-	*0.10*	-
SE87	0.17	0.05	t	-	0.24	0.29	t	-	-	*0.13*	-
SE88	0.18	0.06	t	-	0.26	0.33	t	t	-	*0.15*	0.01
SE105	0.04	0.02	-	-	0.06	0.50	-	-	-	0.03	-
SE106	0.01	t	-	-	0.01	0.00	-	-	-	t	-
SE107	0.02	t	-	-	0.02	0.00	-	-	-	0.01	-
SE108	0.02	t	-	-	0.02	0.00	t	t	-	0.02	-

Appendix 17 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from Mikinduri division.

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	β-HCH	Dieldrin	Endrin
SE30	0.05	0.05	-	-	0.11	1.00	0.01	-	-	0.01	-
SE32	0.05	0.04	-	-	0.10	0.80	0.01	-	-	0.01	-
SE33	0.04	0.03	-	-	0.07	0.75	0.01	-	-	t	-
SE34	0.04	0.04	t	-	0.08	1.00	0.01	t	-	0.02	-
SE37	0.04	0.02	-	-	0.06	0.50	t	-	-	0.02	-
SE38	0.03	0.03	-	-	0.06	1.00	t	-	-	0.02	-
SE39	0.04	0.03	-	-	0.07	0.75	t	-	-	0.03	-
SE40	0.07	0.02	t	-	0.10	0.29	0.01	0.01	t	*6.61*	0.03
SE41	0.10	0.08	t	t	0.19	0.80	0.01	0,02	t	*5.33*	0.06
SE42	0.04	0.05	-	-	0.09	1.25	t	t	-	*4.53*	0.02
SE43	0.02	t	-	-	0.02	0.00	t	t	-	*2.89*	0.01
SE44	0.04	0.02	-	-	0.06	0.50	t	t	-	*9.74*	0.03
SE45	0.14	0.05	t	t	0.21	0.36	0.01	-	-	0.08	-
SE46	0.13	0.09	t	t	0.23	0.69	0.01	-	-	0.09	-
SE48	0.17	0.14	t	t	0.33	0.82	t	-	t	0.03	-
SE49	0.18	0.15	t	t	0.35	0.83	t	t	t	0.04	-
SE50	0.06	0.04	t	t	0.10	0.80	0.01	t	-	0.07	-
SE51	0.06	0.04	t	-	0.11	0.67	0.01	t	-	0.07	-
SE52	0.12	0.05	t	t	0.18	0.42	0.01	t	-	0.07	-
SE53	0.08	0.14	t	t	0.23	1.75	0.01	t	-	0.04	-
SE54	0.05	0.09	t	t	0.15	1.80	0.01	-	-	0.04	-
SE55	0.05	0.06	t	t	0.12	1.20	t	t	-	0.03	-
SE56	0.06	0.08	0.01	t	0.16	1.33	0.01	t	-	0.05	-
SE57	0.03	0.07	-	-	0.10	2.33	0.01	t	-	0.07	-
SE58	0.08	0.02	-	t	0.11	0.25	0.01	t	-	0.06	-
SE59	0.04	0.02	-	-	0.06	0.50	t	t	-	0.04	-
SE60	0.04	0.02	t	-	0.06	0.5o	t	-	-	0.05	-
SE109	0.18	0.08	0.01	t	0.29	0.44	0.01	-	-	*0.11*	-

Appendix 18 Detailed data on organochlorine pesticide residues in eggs (mg/kg) from South Imenti division.

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	ε-HCH	Dieldrin	Endrin
SE 61	0.04	0.01	-	-	0.05	0.25	t	-	-	0.05	-
SE62	0.03	0.01	-	-	0.04	0.33	t	-	-	0.02	-
SE63	0.05	0.01	-	-	0.07	0.20	t	-	-	0.01	-
SE64	0.01	0.01	-	-	0.02	1.00	t	-	-	0.01	-
SE65	0.06	t	-	-	0.07	0.00	-	-	-	t	-
SE66	0.10	t	-	-	0.11	0.00	t	-	-	0.01	-
SE89	0.02	0.01	-	-	0.03	0.50	t	t	-	*0.96*	t
SE90	0.03	t	t	t	0.03	0.00	t	t	-	*1.29*	-
SE91	0.06	0.02	-	t	0.09	0.33	t	t	-	*0.62*	-
SE92	0.06	0.02	-	-	0.10	0.33	t	t	-	0.02	-
SE93	0.06	0.02	-	-	0.09	0.33	t	-	-	0.02	-
SE94	0.04	0.02	-	-	0.06	0.50	t	t	-	0.04	-
SE95	0.04	0.02	-	-	0.03	0.50	0.01	t	-	0.03	-
SE97	0.05	0.03	-	-	0.10	0.60	0.01	t	-	0.06	-
SE98	0.06	0.02	-	-	0.09	0.33	0.01	-	-	0.08	-
SE99	0.05	0.03	-	-	0.10	0.60	0.01	-	-	0.09	-
SE100	0.04	0.03	t	t	0.07	0.75	0.01	-	-	0.02	-
SE101	0.05	0.04	t	t	0.10	0.80	0.01	-	t	0.03	-
SE102	0.04	0.03	t	-	0.07	0.75	0.01	-	-	0.02	-
SE103	0.05	0.04	t	t	0.10	0.80	0.01	t	t	0.02	-
SE110	0.06	0.04	-	-	0.11	0.67	0.01	t	-	0.02	-

Appendix 19 Detailed data on organochlorine pesticide residues in poultry feed (mg/kg) from Kitale and Meru

Sample Number	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	Total DDT	p,p'-DDT / p,p'-DDE	Lindane	α-HCH	δ-HCH	Dieldrin	Aldrin	Type of feed
					ENCLOSED FARMS							
AF1	t	0.01	t	0.01	0.02	-	0.01	t	-	0.01	0.01	Growers mash
AF2	0.01	0.08	0.01	0.02	0.12	8	0.04	-	-	0.02	0.01	Layers mash
BF	0.01	0.50	0.03	0.17	0.71	50	0.25	t	-	0.01	0.02	Layers mash
CF	-	-	-	-	-	-	0.06	t	-	0.01	-	Layers mash
DF1	-	0.02	-	t	0.02	-	0.01	t	-	0.01	-	Layers mash
**DF2	0.01	0.18	0.01	0.05	0.25	18	0.02	-	-	0.01	-	Growers mash
DF3	0.01	0.13	0.01	0.03	0.18	13	0.39	-	-	0.01	-	Chick mash
FF	0.05	0.13	0.04	0.04	0.26	2.6	0.04	t	-	0.01	t	Layers mash
QF6	t	0.06	-	0.01	0.07	-	0.01	t	-	-	-	Layers mash
QF7	t	0.12	-	0.03	0.15	-	0.03	t	-	-	t	Growers mash
OF1	t	0.09	-	0.02	0.11	-	0.08	0.01	-	0.01	-	Layers mash
**OF2	0.01	0.13	0.01	0.04	0.19	13	0.21	-	-	0.01	0.03	Growers mash
MF	-	0.03	-	0.01	0.04	-	0.08	t	-	0.02	t	Layers mash
RF2	0.01	0.11	0.01	0.03	0.15	11	0.01	t	-	0.02	0.01	Chick mash
RF3	-	0.01	-	-	0.01	-	t	-	-	0.06	-	Millet
**RF4	0.01	0.07	t	0.02	0.10	7	0.01	t	-	0.01	0.06	Layers mash
RF5	-	0.02	-	-	0.02	-	0.01	-	-	0.02	-	Layers mash
RF6	0.01	0.08	t	0.02	0.11	8	0.02	t	-	0.01	0.01	Layers mash
**RF7	t	0.07	0.01	0.02	0.10	-	0.02	t	-	t	-	Layers mash
RF1'	0.02	0.06	-	0.02	0.10	3	0.01	-	-	t	-	Layers mash
SF1	0.01	0.09	t	0.03	0.12	9	0.01	t	-	t	-	Layers mash
SF3	0.01	0.05	t	0.01	0.07	5	0.01	t	-	0.01	0.01	Layers mash
SF4	-	-	-	-	-	-	t	-	-	-	-	Maize
**SF5	0.01	0.07	t	0.02	0.10	7	0.01	t	-	t	0.01	Growers mash
**SF6	0.01	0.09	t	0.02	0.12	9	0.01	t	-	t	0.01	Layers mash
SF7	0.01	0.03	-	0.01	0.05	3	0.03	0.01	-	0.01	0.01	Layers mash
SF8	t	-	-	-	-	-	-	-	-	-	-	Rice
					FREE-RANGE FARMS							
DF4	-	t	-	-	t	-	-	-	-	-	-	Maize
NF1	0.01	0.08	-	0.05	0.14	8	-	-	-	0.03	0.02	Maize
NF2	-	-	-	-	-	-	t	-	-	-	-	Rice
QF1	t	t	-	-	t	-	0.01	-	-	-	-	Maize
QF2	-	0.02	-	-	0.02	-	0.09	0.03	-	-	-	Sorghum
QF3	t	-	t	-	t	-	0.01	-	-	-	-	Millet
QF4	-	0.01	-	0.01	0.02	-	-	-	-	t	-	Sunflower
QF5	-	t	-	t	t	-	0.06	0.02		0.01	0.01	Maize
QF8	t	0.06	-	0.02	0.08	-	0.03	0.01	-	t	-	Millet
QF9	-	t	-	-	t	-	0.02	0.01	-	-	-	Maize
QF10	-	t	-	t	t	-	-	-	-	-	-	Millet
KF	t	0.02	-	t	0.02	-	t	t	-	-	t	Rice
RF8	-	0.03	-	0.01	0.04	-	t	-	-	-	-	Rice-Maize
SF4	0.01	0.15	t	0.04	0.20	- 15	0.01	t	-	0.01	0.02	Millet
QF11	-	t	-	-	t	-	0.06	0.02	-	t	-	Maize

** samples with traces of heptachlor epoxide.