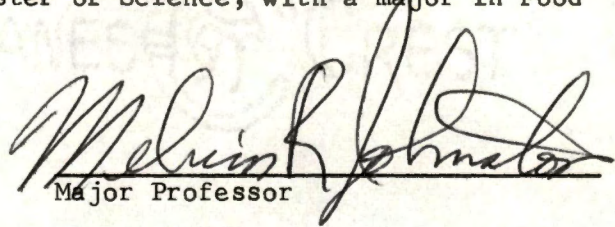


August 5, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Walter Robert Hollowell, entitled "Absorption and Thermal Decomposition of DDT and Heptachlor in Canned Turnip Greens and Roots (Brassica rapa).\" I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

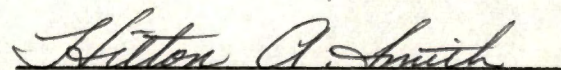

Major Professor

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Vice Chancellor for
Graduate Studies and Research

CRANESHOOT CREST

ABSORPTION AND THERMAL DECOMPOSITION OF DDT AND HEPTACHLOR IN CANNED
TURNIP GREENS AND ROOTS (BRASSICA RAPA)

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Walter Robert Hollowell
August 1968

CRANESHOOT CREST

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ABSTRACT

With the increased use of insecticides, much concern has arisen concerning the safety of treated food crops due to the possible retention of the insecticide in the processed product. Insecticidal residues, when present in food-plants, must be removed or decreased in concentration to levels established by the Food and Drug Administration before the food may be consumed.

This study was undertaken to determine whether insecticides are absorbed and stored by the plant; the effect of various methods of weathering, washing, and blanching on decreasing or removing an insecticide; and the effect of thermal processing on an insecticide should it not be removed by the above methods.

Turnips were grown on four plots: Control (no insecticide added); DDT-treated; heptachlor-treated; and DDT + heptachlor-treated. Each of the DDT-treated plots was sprayed with a foliar application of technical grade DDT and the heptachlor-treated plots had been treated with granular heptachlor in the previous planting. In Experiment One, samples of both the turnip greens and turnip roots were taken at final spraying, at harvest, after washing, and after blanching. In Experiment Two, turnip greens were fortified with technical DDT and thermally processed. Plant constituents were removed from any insecticide present by the extraction with acetonitrile followed by elution through a Floricil column to further remove interfering materials. The extraction product was then quantitatively

analyzed to determine which insecticides were present (heptachlor, heptachlor epoxide, DDE, TDE, and DDT). The results were analyzed statistically.

Under the conditions of the study, several conclusions were indicated. In Experiment One, washing and blanching of turnip greens effectively reduced residues of TDE and DDT, but had no significant effect on DDE, heptachlor, and heptachlor epoxide. None of the various processing treatments had a significant effect on root residues. In Experiment Two, thermal processing effected a significant rise in TDE, but had no effect on DDE or DDT. The heat process did not completely dechlorinate DDT to its metabolites, but did cause "feathering" of the can enamel in the DDT-fortified samples.

In general, the results of this study indicate that the processing treatments examined do not reduce the levels of insecticide residues in turnip greens sufficiently to meet Food and Drug Administration specifications for safe human consumption. In cases of high concentration it appears that some dilution procedure or multiple processing must be resorted to in order to meet Food and Drug Administration requirements.

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CHAPTER I

INTRODUCTION

The use of insecticides to control insects on food crops has resulted in an increased annual yield of food products. With the increased use of these insecticides, much concern has arisen concerning the safety of treated food crops due to the possible retention of the insecticide in the processed product. Thus, any insecticide used on food crops must be approved by the Food and Drug Administration (F. D. A.) which also establishes the length of time an insecticide must be allowed to dissipate before the crop is harvested.

In some cases, the insecticide does not entirely dissipate. This has resulted in the F. D. A. establishing "tolerance limits" for the persisting insecticides. The authority was invested in the F. D. A. by the Pure Food, Drug, and Cosmetic Act of 1938, and by the Miller Pesticide Residue Amendment in 1954 (77).^{*} In order to protect the consumer, the F. D. A. collects a "market basket" which includes all the food consumed by a teenage boy in a week and they check to see if the products are within the insecticide tolerance limits.

The organochlorine insecticides have been used to control agricultural insects since 1946, initially with the introduction of DDT and BHC, and subsequently with the introduction of the cyclodiene insecticides, such as aldrin, dieldrin, endrin, and heptachlor (77).

^{*}The numbers in parenthesis represent similarly numbered references in the Bibliography.

It has been demonstrated that some crops are capable of absorbing residues of insecticides from the soil. The degree of absorption is dependent upon the crop, the specific insecticide involved, its concentration in the soil, soil type, and climate (98). Crops absorbing the greatest amounts of residues includes roots (87, 89, 91), other vegetables (90, 91), and forage crops (70, 91, 96).

Until recently, the behavior of insecticide residues during the interval between harvest of the food crops and their arrival on the plate of the consumer has received little attention. For a significant amount of the fruits and vegetables consumed in the United States, commercial canning operations intervene between harvest and use. Many foods reach the plate of the consumer after processing and storing in tin cans. Since the effect of commercial canning operations on pesticide residues has not been thoroughly studied, a complete understanding of the fate of these compounds requires detailed information on their reactions throughout all parts of the food growing and processing operation.

The present study consisted of two parts: The first part was conducted to determine whether DDT, TDE, DDE, heptachlor, and heptachlor epoxide were translocated from the soil to the plant and what commercial methods of preparation and processing would remove or reduce these residues. The second part was undertaken to establish whether varying the thermal processing conditions would have an effect on a residue of DDT, TDE, or DDE.

CHAPTER II

LITERATURE REVIEW

I. GENERAL INSECTICIDE CLASSIFICATIONS AND CHARACTERISTICS

Although all insecticides are thought to be synthesized by man, cruciferous crops, such as turnips, produce natural toxins which affect some insects but do not harm man. One of these has been investigated by several workers (84, 92) and has been identified as 2-phenylethylisothiocyanate. This naturally occurring insecticide, which is present in both the turnip greens and root, is found primarily in the roots. Different varieties of turnips yield varying amounts of the insecticide with amounts ranging from 85 to 245 p.p.m. (124).

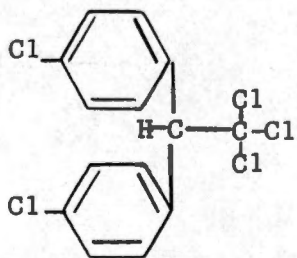
Synthetic insecticides can be divided into three basic groups: chlorinated hydrocarbons, organic phosphorous compounds, and organic sulfur compounds. The chlorinated hydrocarbon insecticides were the first type used on agricultural crops and still remain the predominant type. Two chlorinated hydrocarbons, DDT and heptachlor, are widely used. Usually, DDT is applied to the plant directly, while heptachlor is added to the soil. More DDT is produced annually than any other insecticide (130).

II. PHYSICAL AND CHEMICAL PROPERTIES OF DDT AND HEPTACHLOR

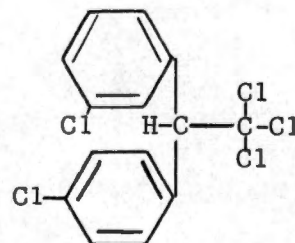
DDT is the derived name of dichloro-diphenyl-trichloroethane and is produced as a product of the Baeyer reaction between chloral and

chlorobenzene in the presence of concentrated sulfuric acid (52). Pure DDT (p,p' isomer) has a melting point between 108.5 and 109°C. and occurs as a long, tubular, biaxial, white compound (41). In the production of DDT, 66 to 77 per cent of that produced is p,p'-DDT and 8 to 21 per cent is o,p'-DDT (41). Insignificant amounts of p,p'-TDE, o,p'-TDE, p,p'-DDE and o,p'-DDE also are produced. (In this paper, TDE is used to represent DDD.)

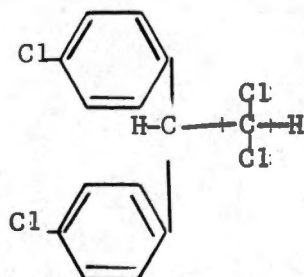
DDT is readily soluble in fat-soluble solvents (52) and long-chain alcohols (3), but is practically insoluble in water, dilute acids, and alkalies (24, 52). The active sites of DDT, its metabolites TDE (DDD) and DDE, heptachlor, and heptachlor epoxide are chlorine atoms. In the case of DDT, these chlorine groups comprise 50 per cent of the total molecular weight, but amount to only 18 per cent of the molecule in atom weight (12). The loss of chlorine atoms from the DDT molecule gives rise to TDE and DDE (66, 113).



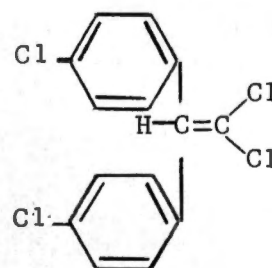
p,p'-DDT
1,1,1-trichloro-2,2-bis
(p-chlorophenyl) ethane



o,p'-DDT
1,1,1-trichloro-2(o-chlorophenyl)
-2-(p-chlorophenyl) ethane



p,p'-TDE
1,1-dichloro-2,2-bis
p-chlorophenyl ethane



p,p'-DDE
1,1-dichloro-2,2-bis(p-chlorophenyl)
ethylene

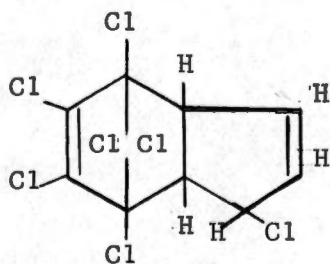
DDT is relatively stable, but decomposition results when it is heated to 100°C. (41). Dehydrochlorination causes detoxification and results either when DDT is in contact with iron and aluminum chlorides or when it is dissolved in organic solvents or in alkali and organic bases; in nature, enzymatic activity causes this dechlorination. (38, 41, 48, 128). Ultraviolet light (2, 41) and sunlight (41) have been shown to decompose DDT.

Technical DDT is formulated as a dust, a granular compound, a wettable powder, an emulsion, an aerosol, an insecticidal smoke, and for vaporizers (41).

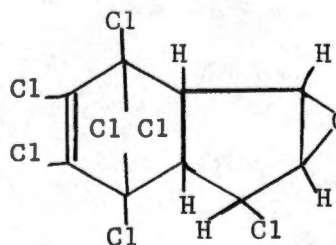
Harrell (63) reported that higher residues in corn were obtained with the granular application than with dust, while those of the spray were lowest. DDT is very toxic to mammals and is absorbed through broken skin. Both TDE and DDE, the metabolites of DDT, are less toxic than the parent compound with DDE being the least toxic (104). Klein (72), Ott (113), and Ritchey (115) have shown evidence to indicate that the conversion of DDT to its metabolites is a direct conversion rather than a

series of intermediate steps, and that DDE is not involved in the metabolic pathway of the conversion of DDT to TDE. DDT and its metabolites have been found in all types of food products, in food by-products, in clothing, and even in commercial cigarettes and cigarette smoke (22).

Heptachlor is the generic name of 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene. Pure heptachlor was a melting point of 95 to 96°C. and is a white crystalline solid (41). It is synthesized in the presence of benzoyl peroxide by the action of sulfuryl chloride on the condensation product of cyclopentadiene and hexachloropentadiene in carbon tetrachloride (12, 52). Heptachlor is readily soluble in paraffin hydrocarbons and aromatics such as benzene and xylene. The chlorine atoms which account for the toxicity of heptachlor amount to 40 per cent of the total molecular weight and only 25 per cent of the molecule in atom weight. The epoxidation of heptachlor results in a more toxic compound, heptachlor epoxide (8).



Heptachlor
1,4,5,6,7,8,8-heptachloro-
3a,4,7,7a-tetrahydro-4,7-
endo-methanoindene



Heptachlor Epoxide
1,4,5,7,8,8a-heptachloro-2,3-
epoxy-2,3,3a,4,7,7a-hexahydro-4,
7-methanoindene

Heptachlor is stable at temperature from 150 to 160°C., to 5 per cent alkali or acid (117), and to light, moisture, and air (41). Formulation of heptachlor is as a 1.5 and 2.5 per cent dust, absorbed on clay particles, as an emulsion, and in oil solution (41). Heptachlor and heptachlor epoxide are very toxic to mammals, and are absorbed readily through the unbroken skin. Heptachlor has been used effectively for controlling many agricultural insects including flying, crawling, and burrowing types (8, 118). In soil, heptachlor epoxide has been shown to be more stable and toxic than heptachlor, although heptachlor is extremely stable (88). Heptachlor epoxide is less volatile and is not as readily absorbed by plants as heptachlor (70). Bruce (26) explained that the less polar compound, heptachlor, may be absorbed and translocated more readily than the more polar heptachlor epoxide.

III. CHLORINATED HYDROCARBON INSECTICIDE RESIDUE

TOLERANCE LEVELS

The Miller Pesticide Residue Amendment of 1954, authorized the establishment of tolerances for insecticide residues listed by crop categories by the Food and Drug Administration (F. D. A.). On the basis of many studies, these limits were established at the lowest level of safety, even though a larger amount would still be safe. The majority of the Food and Drug Administration tolerances have been in effect for a good many years. The United States was not the only country to establish tolerance levels for insecticides. The Netherlands has tolerance

levels which are similar to those in the United States; Germany established tolerances in January, 1968, which are a great deal lower than those the Food and Drug Administration established; the Common Market is in the process of developing standards similar to those of the United States (66).

As of February, 1968, the official tolerances as established by the Food and Drug Administration for fruit and vegetable foodstuffs are 7 p.p.m. for DDT and TDE and 0 p.p.m. for heptachlor and heptachlor epoxide. However, the tolerance for heptachlor and heptachlor epoxide may be raised to 0.1 p.p.m. in the case of cabbage and lettuce (66, 77).

IV. PHYSICAL AND CHEMICAL ASPECTS OF CHLORINATED HYDROCARBON INSECTICIDES IN THE SOIL

Lichtenstein (81, 82) listed the ten most important factors influencing the persistence of insecticides in soils as: soil moisture, soil temperature, the type of insecticide, cultivation, wind or air movement, mode of application of the insecticide, the type of insecticidal formulation, cover crops, the soil type, and soil microorganisms.

Adsorption of the insecticide is due to the attraction or repulsion between a solid surface and a vapor or solution. In soils, this attraction or repulsion is due to the interaction between the fields of force emanating from the surface of the soil molecules and the molecules or ions of the insecticide being used (10). Bailey (10) noted that a relationship appears to exist between the solubility of an insecticide in a

polar solution and its adsorption into the soil. Since pesticides have a low water solubility and a low vapor pressure, the majority of the chlorinated insecticides tend to persist in the soil either as the parent compound, as a metabolite, or as an oxidation product (129).

The moisture content of the soil system appears to have a great effect upon both the degree of adsorption and the bioactivity of insecticides present in both the aqueous and vapor phases. At low humidity and low temperature the insecticide may be highly adsorbed, while at high humidity and high temperature bioactivity will be enhanced to desorption of the insecticide (10, 11, 90). In contrast, Daniels (39) noted that greater quantities of heptachlor were detected in irrigated soils than in dryland soils when equal amounts of insecticide were used in both treatments, and Lichtenstein (80) observed that heptachlor persists longer than aldrin and aldrin dissipates more rapidly in wet soil than in dry soil.

Since adsorption is interrelated with both temperature and bioactivity, the bioactivity might be expected to be different at various temperatures. Temperature, through its effect on solubility, has an indirect influence on adsorption which is also related to the vapor pressure of the compound (10). The vapor pressure of organic compounds is temperature dependent; for example, when the temperature is increased, the vapor pressure will also increase.

The degree of acidity or alkalinity that a compound exhibits in aqueous solutions, as well as in the pH of the soil, also effects

adsorbability. It appears logical that the effect of pH on adsorption of a particular insecticide is manifest in determining the degrees of dissociation or association of the compound, that is, whether the compound is still a molecule or has dissociated into either cations or anions (10).

Since insecticides are generally applied to the soil in early spring when the soil temperature is generally very low, these conditions would favor adsorption. Then, in the summer months, as the soil temperature increases, the insecticide would be released (10).

Mattick (98) found that during the day immediately following application of an insecticide there is an initial rise in the residue which decreases over a period of weeks, until a minimum amount is present, while Barthel (15) noted a 50 per cent decrease of heptachlor six months after application. Although there is a loss of the insecticide from the soil, the amount that remains tends to accumulate over the years (44, 90, 109, 128) Murphy (109) stated that once an insecticide becomes "fixed" in the soil, there is little loss even in warm weather. Soon after the application of heptachlor to the soil, there is a rapid loss which in turn is followed by an extended period during which there is little loss of the insecticide. At this time metabolic products of the insecticide are found (15, 109, 110). Lichtenstein (90), Barthel (15), Weatherholtz (135), and King (70) found heptachlor to be quite persistent in the soils with the epoxidation to heptachlor epoxide quite slow and the total conversion to be about 15 to 20 per cent of the heptachlor applied.

The importance of the mode of cultivation in the loss of insecticide from the soil has been established by Lichtenstein (81) and Harris (64).

They found a higher residue in the uncultivated soils of orchards and alfalfa fields and a considerably lower residue in cultivated fields and vegetable crop lands.

Volatilization is still another means of insecticidal removal from the soil (15, 70), but cover crops such as alfalfa may tend to decrease the volatilization of insecticides (53). Volatilization may be enhanced by air currents which serve to keep the volatilized molecules in motion.

The ability of the soil to contain insecticide residues is dependent also upon the method of application of the insecticide and the insecticidal formulation. An insecticide which is forced into the soil by plowing or disking will be more permanent, and its residues will be more persistent than one which is applied by crop-dusting methods. Insecticides which are impregnated in clay or are applied in granular or dust form will be more readily available to the soil than those in an oil form. Those formulations which are emulsifiable will be the most easily adsorbed into the soil (85).

The soil type has a great bearing on the retention of insecticides. For example, a sandy soil will be much less prone to retain residues than a clay type soil (14, 64, 87, 137). Harris (64) demonstrated that the organic content of the soil also affects the sorption and inactivation of the insecticides. Hence a soil that contains a large amount of organic components, such as loam or muck, will not lose the insecticides as rapidly as a non-organic soil such as a sandy soil. Associated with the

soil type are the soil microbes. Since DDT is relatively stable in the soil, it will not be degraded unless certain common soil microorganisms are present (25). The main soil microorganism, Aerobacter aerogenes, reductively dechlorinates o,p'-DDT to o,p'-DDE by a metabolic pathway which appears to be specific to the DDT insecticides (12, 102).

V. CHLORINATED HYDROCARBON INSECTICIDE METABOLISM AND TRANSLOCATION IN PLANTS

Lichtenstein and Schultz (89) described mechanisms by which an insecticide applied to a soil might contaminate the aerial parts of plants grown in that soil. Those mechanisms are: rain or other physical factors resulting in the deposition of small amounts of treated soil on the foliage, volatilization of the insecticide from the soil and subsequent penetration through the leaf cuticle, and potential penetration of the compound into the roots and subsequent translocation into the aerial parts of the plants.

Lichtenstein (80) noted that the absolute amounts of residues recovered from crop tissues of plants grown on highly contaminated soils were higher than from those grown on slightly contaminated soils.

Insecticides may become more toxic when they are allowed to undergo oxidation, isomerization, transalkylation, hydrolysis, or ionization. In the latter, the ions formed may reattack the insecticide to yield highly toxic materials (33).

Several workers (76, 96) have presented evidence showing that the peroxidase in plants functions as an oxidase and activates molecular

oxygen toward aromatic substrates. In order for the peroxidase to function at its full capacity of insecticidal metabolism, the EDTA-Fe⁺² complex is necessary (76, 96). Lichtenstein (89) observed that epoxidation of heptachlor to heptachlor epoxide apparently took place within the plant tissue after heptachlor had been absorbed through the root system.

Hacskaylo and Ball (60) found that leaves that were deficient in certain minerals have a reduced metabolic rate and therefore, metabolize insecticides at a slower rate than leaves with adequately supplied minerals. The leaves that were deficient in Fe⁺² had the lowest oxygen analogue present. This decreased rate was probably associated with reduced cytochrome oxidase and the peroxidase activity (60).

It is not now known whether an insecticide enters the plant through a dissolved, gaseous, or solid contact phase. Penetration of insecticides may first be by stomatal and/or cuticular absorption. Cellular absorption may then follow. Both surfaces of the leaf function in the absorption of chemicals; usually the lower epidermis is more penetrable than the upper epidermis. Entry into the leaf is through the cuticle and is by diffusion, as well as by absorption, and not all areas of the leaf are equally permeable (51).

The persistence of the absorbed insecticide may be due to the waxy and fatty constituents of the plant. Foy (51) has indicated that the waxy and fatty constituents may form an important pool for holding fat-soluble compounds. Anderson (5) and Starr (125) found that the highest concentration of the residue found was located in the peel oil of oranges and in the oil of the peppermint.

Gannon (53) reported that at least two factors are necessary for translocation and metabolism of insecticides to occur; they are plant material and light. Harrett (68) has found that translocation of an insecticide will occur under unfavorable conditions, such as light starvation for sixty hours.

Smith and Dyer (123) observed that 2,2-Dechloropropionic acid is readily absorbed from the soil by the cotton plant and accumulates in the actively growing tissues. As the tissues mature, the residue is retranslocated to more actively growing areas. On the other hand, wheat stem has been reported to contain more pesticide than does the head (137).

Saha and McDonald (119) reported translocation of pesticides into the wheat grain, while the data of Roan and Srivastava (116) suggested that spray-applied emulsified insecticides penetrated to the interior of the grain.

There have been many reports of translocation of insecticides into food products. A few of these products are cucumbers (91), Alaskan pea plants (83), tomatoes (79), apples (37), and all varieties of root and leafy crops (65, 80, 90). In the case of the apple trees and tomato plants, although translocation did occur, no residue was found in the fruits (37, 79). Eden and Arthur (46) showed that there was no translocation of insecticides into the soybean.

Lichtenstein and co-workers (86) and Harris and Sans (65) found that certain root crops, notably carrots and potatoes, absorbed measurable amounts of insecticide from the soil, while other crops absorbed

none. Of all the crops which absorbed insecticides, carrots contained the highest concentration. Although heptachlor and other insecticides were translocated, no evidence of the translocation of DDT was observed in these root crop studies.

Although the physical properties of soil usually favor leaching, Taschenburg (128) concluded that DDT penetrates downward into the soil only by mechanical mixing, not by leaching. Lichtenstein and Schultz (86) observed that the vertical distribution of insecticidal residues within carrot tissue corresponded to the vertical distribution within the various soil layers. They also observed that insecticidal residues were less along the carrot rows than in areas where no carrots had grown (86).

VI. PHYSICAL REMOVAL AND DECOMPOSITION OF CHLORINATED HYDROCARBON RESIDUES FROM FOOD PRODUCTS

"Residue," as described by Gunther and Blinn (57) and as cited by Barron (12), is that portion of an insecticide that remains after run-off, evaporation, or sloughing. This residue can be either an internal residue, one that penetrates through the cuticle, or an external residue which remains on the outside of the cuticle (12). The residue can also be absorbed or adsorbed by the root system.

Cook (35), as cited by Barron (12), has shown that when insecticides are applied at equal rates to different crops, the initial residue after application varies directly with the surface area per unit weight of the product. For example, crops of a leafy nature, such as spinach, cabbage,

turnip greens, and alfalfa, would have a much higher residue than a crop such as apples or oranges when equal amounts of an insecticide are applied.

Hardee and co-workers (61) and Klein and Link (74) cited growth and weathering as the main factors which contribute to the decrease of insecticide residues on any plant. Gunther, et al. (58) and Linscott (93) have found that the attrition effect of as little as 0.13 inch of rain will cause a significant loss of an insecticide.

It is evident that suspension residues, which consist partly of solid material adhering to the surface, are easier to wash off than emulsion residues, which are firmly bound to the cuticle of the fruit and dissolved in it (57). Anderson, et al. (5) found that 30 per cent of an insecticidal residue is removed by standard washing procedures and that the remaining residue is entirely within the peel.

Several authors have found that boiling an insecticide-contaminated foodstuff, such as potatoes in water, results in a rapid loss of insecticide (84, 86, 112). The boiling water being in contact with the potato is the determining factor since Ordas, et al. (112) proved that potatoes boiled in water lost all the pesticide contained in the peel. However, when similar potatoes were baked, there was a considerable, but not complete loss of residue. Ritchey, Young, and Essary (115) noted that chicken carcasses containing residues of DDT, TDE, and DDE cooked by either frying or baking had an increase in TDE and a decrease in both DDT and DDE. This change indicates that during the cooking process DDT and DDE were converted to TDE.

In root crops the insecticidal residue, when present, is located in the peel; therefore, peeling will reduce the residue very drastically (86). Work by Lichtenstein, Myrdal, and Schultz (86) indicated that the peel (epidermal layer) of carrots contained all the insecticidal residues and that peeling reduced the residue level by 90 per cent. When potatoes, which contained a residue, were peeled and then analyzed, the amount of residue was decreased by 75 per cent (80). The same has been shown to be true for other root crops.

Hemphill, et al. (67) noted a decrease in residues when green beans were cooked in a pressure cooker after they had been washed and trimmed. They observed degradation of p,p'-DDT to p,p'-TDE also when the green beans were boiled, but this breakdown was more pronounced when the beans were pressure cooked. Bills and Sloan (19) reported that pasteurization has little effect on DDT in milk; this can be explained that in order to dechlorinate the DDT, a longer process time than that used in pasteurization is required.

VII. THERMAL PROCESSING AND ITS EFFECT ON CHLORINATED HYDROCARBONS

Generally, after a food product has been washed, boiled, and/or peeled it is placed in a tin can, thermally processed, and stored. Several workers (32, 36, 50, 131) have suggested that ferric ions and aluminum salts from the can catalyze the decomposition of DDT. Farrow (48) substantiated this belief by observing the conversion of DDT to TDE

in spinach processed in plain and enameled tinplate and noted that a smaller amount was converted in glass containers.

To render canned foods commercially sterile, the heat treatment or process is designed to destroy spoilage organisms (namely spores of Clostridium botulinum) at the slowest heating part within the container. Depending upon the container and the rate of heat penetration, portions of food adjacent to the outer surfaces of the cans are "over processed". Since there is considerable variation in the rate of heat penetration from one type of food product to another, in any study of the effect of processing on insecticide residues, it is necessary to investigate a range of heat treatments that will include several variations from the "normal" process.

Heat transfer occurs in canned foods by conduction, convection, or by a combination of the two (4). The sterilization value of a process is defined as F_0 and is the equivalent value of the process in terms of minutes at 121°C. (250°F.) when no time is required in heating to 121°C. (250°F.) or cooling to sublethal temperatures (4).

There are three methods of calculating the time-temperature relationship for thermal processing of foods to obtain the F_0 value of the process. One of these methods is the graphical method originated by Bigelow and is referred to as the "general method" (1). It consists of the graphing of heating and cooling data and then converting these values to an F_0 value and other thermal processing values. The second method is the formula method devised by Ball. It consists of recording the

heat penetration data on semi-logarithmic paper and then using a formula to obtain the values essential to thermal processing. The third and final method is the nomogram method of Olsen, Stevens, and Roberts. It is a graphical solution of the equations given in the Ball (formula) method (4). For each increase of 10°C. (18°F.) in temperature beyond the optimum conditions for growth of Clostridium botulinum, a tenfold increase in the destruction of the organism occurs. Such an increase in temperature results in a doubling of the rate of chemical reactions within the can (42). This increased chemical activity may cause the breakdown of any insecticide which may be present within the can. Tressler (131) reported that commercial canning at 100°C. (212°F.) significantly reduced the DDT level in several food products, part of the effect being due to the use of metal cans rather than glass containers.

Farrow, Elkins, and Cook (48) investigated the conversion of DDT to its metabolites during thermal processing of DDT-fortified liquefied spinach. They found that after processing at 121°C., for the normal thermal processing time, 52 per cent of the DDT was converted to TDE, DDE, or other decomposition products which did not register on the electron-capture detector. At four times the normal process time at 121°C., 78 per cent of the DDT was decomposed to TDE. Carlin, Hibbs, and Dalm (32) reported that a 50-per cent decrease in insecticide occurs when snap beans were frozen and a 100-per cent reduction when they were home-canned, with no insecticide residue being found in the brine. Other workers have found that insecticide residues in green beans were reduced 5.3 per cent by

washing, and only slightly by trimming (67). Hemphill, et al. (67) concluded that the usual home washing and/or trimming are relatively ineffective in reducing insecticide residues.

Barron (12) reported that washing turnip greens reduced residues of DDT, TDE, and heptachlor; blanching reduced the level of heptachlor; thermal processing reduced the concentration of DDT residues; and storage of the canned product at room temperature caused an increase in TDE. He noted further that washing of turnip roots reduced heptachlor, and that peeling reduced DDT, TDE, DDE, heptachlor, and heptachlor epoxide residues.

VIII. EXTRACTION OF CHLORINATED HYDROCARBON INSECTICIDES FROM FOOD PRODUCTS

The removal of insecticide residues from foodstuffs is referred to as the "cleanup method". This method can be divided into two parts: the gross cleanup and the separation of interferences. Gross cleanup is accomplished by removing the insecticide residues from the plant or animal material. The separation of interferences is the removal of other materials which are extracted with the residue and must be physically or chemically removed before qualitative or quantitative analysis can be made.

In order to achieve reproducible results in insecticide residue work, it is essential that the plant constituents be separated from the residues. There are many suggested methods for removing chlorinated hydrocarbon residues from food material, some being excellent and others

being quite unsatisfactory. Although there are many extraction methods (30, 62, 106, 137, 138), the methods officially recognized by the Food and Drug Administration are described in detail in the Pesticide Analytical Manual (13, 45).

Early investigators used a tumbling (stripping) method in which the product was "tumbled" in the presence of a solvent, the solvent removing any insecticide present (132, 138). Other extraction methods include a Soxhlet extraction, vacuum sublimation, and blending of the plant material in a solvent by means of an Osterizer or Waring Blendor. Although the Soxhlet method is an excellent one, it is very time-consuming. Vacuum sublimation uses low pressure (2 to 5 mm Hg) and moderate temperatures (30 to 60°C.) to sublime or distill common insecticides which are sufficiently volatile. This separation will occur only when the plant extractives accompanying the residue have appreciably higher vapor pressures than the insecticides (99). The blending method is the most commonly used method since it takes a relatively short time and gives results comparable to those of the other two methods (30, 71, 137).

Various solvents have been used with the blending method in an attempt to isolate the solvent system. Hardin and Sarten (62) tried tumbling and blending with hexane and with isopropyl alcohol and hexane. Although the recoveries were suitable (85 to 95 per cent), emulsions were easily formed and were difficult to remove. Klein, et al. (73) reported that emulsions formed with a hexane-isopropyl alcohol combination could be prevented by the addition of sodium sulfate. Other solvents

such as ethyl acetate (Watts-Storherr method) and methylene chloride (Benson-Finocchiaro) have been tried in extractions, but the recoverability of the insecticides was lower than those with acetonitrile.

The method of Mills, Onley, and Gaither (106) was the first blending method to use acetonitrile as the extraction solvent. The use of acetonitrile eliminated several time-consuming and cumbersome steps of earlier methods (12). This method involves blending of the plant tissue in acetonitrile. The residue is then transferred to ether and finally eluted through a Florisil column. Burke and Porter (31) found that when dry or partially dehydrated samples are used, acetonitrile containing 35 per cent water effectively removes any residue present. They noted also that a decrease in sample moisture content causes a proportional decrease in recoverability of the insecticides. The methods of Klein, et al. (75), Samuel (120), and Moats and Kotula (107) are very similar to the method of Mills, et al. (106) but in some cases are specific to high-fat products.

Mumma, et al. (108) used an exhaustive extraction method which utilizes a Soxhlet extraction on the filter cake obtained from the blending method. This method is used successfully with a partially dehydrated product. Burke and Porter (30) reported that the method of Mills et al. (106) with an acetonitrile extraction and sample-size adjustment factor gave essentially the same p,p'-DDT residue recovery as did the exhaustive procedure.

Once the insecticide has been removed from the plant tissue by acetonitrile and transferred to ether, the extract is eluted by column

chromatography to absorb interfering substances such as chlorophylls, xanthophylls, and carotenoids. The column absorbent most commonly used is Florisil, although cellulose (136), activated charcoal (9), magnesium oxide-celite mixtures (106), and polyethylene-coated alumina (69) also are used. Florisil is a synthetic magnesium silicate widely used in analytical methods for pesticide residues. It is activated at 623°C. and held at 155°C. until used.

Since all oily material causes irreversible damage to a gas-liquid chromatography column, all the oily substances must be removed before gas-liquid chromatography is attempted (27). McKinley, et al. (101) and Anglin, et al. (6) used cooling to -70°C. to precipitate interfering waxes, pigments, and fats from high-fat samples. Saponification of fatty and waxy materials can be used rather than column chromatography when large amounts of the fatty or waxy materials are present (101). The main disadvantage of this method is that certain insecticides, such as DDT, decompose due to their instability in the presence of strong alkali. However, this method is acceptable for removal of fats from samples containing heptachlor. Barron (12) cited Davidow (40) as using a hexane slurry of 30 g. celite, 9 ml. concentrated sulfuric acid, and 9 ml. fuming sulfuric acid to render 5 g. fat. Although this method does render the fat, its severity limits the number of insecticides that can be recovered.

IX. GAS-LIQUID CHROMATOGRAPHY OF CHLORINATED HYDROCARBON INSECTICIDES

The insecticide residue, once it has been removed from interfering material by cleanup, must be quantified. Some methods for measuring the types and amounts of insecticide are: bioassay, channel-layer chromatography, column chromatography, colorimetric determination, complete combustion, coulometric determination, fluorometric determination, gas chromatography, infrared spectroscopy, mass spectroscopy, paper chromatography, thin-layer chromatography, total organic chlorine, and other methods (47, 49, 54, 59, 77, 80, 97, 121).

Gas chromatography, or more correctly gas-liquid chromatography, is a method in which components of a gaseous mixture are separated by elution through a column containing a stationary non-volatile liquid phase on an inert support and a mobile vapor phase in a gas chromatograph (122).

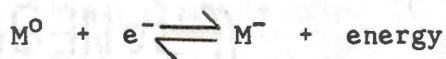
The column which contains the inert support phase can be made of stainless steel, glass, or aluminum. Some authors feel that borosilicate pyrex glass is superior to any other column since evidence has been shown by Langlois, et al. (78), Minyard and Jackson (103), and Ott and Gunther (113) that there is a breakdown of some insecticides when they come in contact with hot metal columns. The optimum column size is 0.125 inch i.d. and 3 to 6 feet in length.

The support material used in the column is generally diatomaceous earth in various mesh sizes. The advantage of diatomaceous earth is that

it is quite inert and has a high ratio of surface area to volume (28).

Goodwin, et al. (56) found that diatomaceous earth decomposed the insecticide, and that the active sites which caused this decomposition could be removed by mixing small amounts of polar stationary phase with the non-polar stationary phase. Burke (28) found that when the support material adsorbed the sample, washing the diatomaceous earth with acid or alkali would correct the problem. For the most complete separation of chlorinated hydrocarbon insecticides, Beynon and Elgar (18) suggested silicone-base greases. Unfortunately, these greases will not separate p,p'-TDE from o,p'-DDT. At this stage in column technology, no one column has been found which will completely separate all the compounds presented to it. For insecticides, the best general purpose columns are the non-polar silicones (21).

Electron-capture is the result of the affinity of functional groups such as halogens, to attract free, low energy electrons (133). The electron-capture detector was devised by Lovelock and Lipsky in 1960. Their theory was that in an ionized gas the negative charge carriers may be either free electrons or negative molecular ions. An important process leading to the formation of negative ions is the reversible reaction in which neutral molecules (M^0) capture free electrons (e^-).



The energy liberated by the reaction or required to dissociate the negative ion is called the electron affinity of the molecule (95).

A beta radiation source, such as tritium foil or nickel, is used as a source of free electrons.

The electron-capture detector is more sensitive than the flame ionization or thermal conductivity conductors, and is used almost exclusively with chlorinated hydrocarbon insecticides (103, 127). The electron-capture detector, unlike others, measures the loss of signal rather than a positively generated electrical current.

As the nitrogen carrier gas flows through the detector, a tritium source ionizes the nitrogen molecules and "slow" electrons are formed. These "slow" electrons migrate to the anode under fixed potential; 90 volts is used for routine pesticide analysis. Collected, these "slow" electrons produce a steady current. Then, if a sample containing electron-absorbing molecules is introduced, this current will be reduced. The loss of current is a measure of the amount and electron affinity of the compound, and is usually amplified by an electrometer and recorded on a strip-chart recorder as a peak. The electron-capture detector gives a very linear response over a dynamic range of approximately 1,000. The sensitivity is adequate for measuring most pesticides in the low parts-per-billion range (43).

Gaul (55) has described five methods of quantifying gas chromatographic peaks. These methods are not significantly different and are: measuring peak height, multiplying peak height by peak width at half height, determining peak area by triangulation, attaching a mechanical or electrical integrator to the recorder, and by cutting and weighing peaks.

CHAPTER III

MATERIALS AND METHODS

I. EXPERIMENTAL DESIGN

Experiment One: Translocation From Previous Crops

On August 31, 1966, four plots, 60 ft. by 12 ft., were prepared for planting at Mound Range No. 5, University of Tennessee, Knoxville Farms. Immediately prior to planting, all plots were disked to a depth of 5 in. During the previous planting, on March 12, 1966, technical heptachlor was applied to plots No. 2 and No. 4 at the rate of 10 lb./acre.

On September 1, 1966, all plots were fertilized with 6-12-12 fertilizer at the rate of 500 lb./acre. The plots were culti-packed. Purple Top globe (Brassica rapa) seed, which had been obtained from a local seed company was broadcast over the plots at the rate of 3 lb./acre.

On September 23, October 3, and October 13 technical DDT (78 per cent p,p'-isomer) was applied to plots No. 3 and No. 4 at the rate of 1.5 lbs./acre. A cart-mounted sprayer with nozzles mounted 1.5 ft. apart on a 6 ft. boom was used to apply the DDT.

The plots were partially harvested on October 19. Prior to sampling, the weight of the leaves from each plot was adjusted to 16-lb. Each 16-lb. lot was divided into 8 lb. sub-lots to facilitate handling during the washing

process. Each sub-lot was washed by submersion and agitation for 2.5 min. in 18.5 gal. warm (35°C.) water, followed by a similar wash for 2.5 min. in cool water (18°C.). The sinks were thoroughly cleaned between washings. The two sub-lots from each lot were recombined and thoroughly mixed.

The leaves were spread on blanching trays, and blanched for 3 min. in an atmospheric blancher. Ten-g. random samples were taken in three replications as follows: after the final DDT application; at harvest, after adjustment of lot weight; after washing and recombining the sub-lots; and after blanching. The leaves were sampled by randomly selecting about 100 g. of leaves, chopping them with a knife, mixing, and selecting a 10-g. sample. The leaf samples were placed in 12-oz. screw cap jars and placed in a -10°C. air blast freezer. They later were removed to a 0°C. still air freezer and held there until analyzed.

The turnip roots were allowed to mature until November 10, at which time they were harvested by hand. Lots from each plot were adjusted to 16 lbs. prior to sampling. Each lot was washed statically with cold water for one min., then for 3 min. in a rotary pea washer (Food Machinery Corporation).

The washed turnips were peeled in a rotary abrasive peeler at the rate of 7.5 lbs. for 5 min., then were washed statically with cold water to remove any remaining peel. The turnips were cut in half and then were quartered.

Ten-g. random samples in three replications were taken as follows: at harvest, after adjustment of lot weight; after washing; and after peeling and second washing.

Experiment Two: Thermal Dechlorination of DDT

In February, 1968, six bushels of turnip greens were purchased from a local distributor. Each bushel was divided into three 8-lb. lots and washed by submersion and agitation (to simulate actual in-plant washing procedures) first in 18.5 gal. warm (35°C.) water for 5 min., and then for 5 min. in cool (18°C.) water. The sinks were thoroughly cleaned between washings to insure against cross-contamination.

The leaves were spread on blanching trays, and allowed to drain for 5 min. They were then blanched for 5 min. in an atmospheric steam blancher and packed into 303 x 406 (No. 303) cans. In order that the insecticide would be approximately in the center of the can, each can was first packed half-full. Then 1 ml. of 2,500 p.p.m. emulsified technical DDT (61 p,p'-isomer) was added and the can was filled to 11.7 oz., which gave a final concentration of 5.65 p.p.m. Next, each can was filled to within 0.25 in. of the top with 2.0-per cent NaCl brine (88°C. $\pm$ 3°), sealed, and placed immediately into a steam-jacketed kettle filled with water at 60° $\pm$ 5°C.

The cans were sterilized at 116 and 121°C. in a retort equipped with an automatic controlling and a recording instrument for indicating process times and temperatures. The F_0 (commercial sterilization) values used were $F_0 = 4$ (under normal process), $F_0 = 7$ (normal process), and $F_0 = 10$ (over normal process) with B_B values (process time) varying from 39 min. to 85 min. (See Table I).

TABLE I
PROCESS CONDITIONS USED FOR PROCESSING TURNIP
GREENS-FORTIFIED WITH TECHNICAL DDT

Process Temperature	Sterilization Values		
	$F_0 = 4$	$F_0 = 7$	$F_0 = 10$
116°C.	59 ¹	72	85
121°C.	39	45	50

¹Process time in minutes at each set of conditions.



The process-time values were calculated using the Broken Heating Curve Nomogram method of Olson, et al. (111) and the following values (16):

j (lag factor) = 1.79

f_h (first heating curve slope) = 28.1 min.

f_2 (second slope of the heating curve) = 42.1 min.

X'_{bh} (number of min. from beginning of process to the point of break in the heating curve) = 3.4

Retort Temperature = 116 and 121°C.

Initial Temperature = 60°C.

F_0 (sterilization value) = 4, 7, and 10

B_B (calculated process time in min.) = 39, 45, 50, 59, 72, and 85.

The treatments consisted of processing three cans at each set of conditions (i.e., $F_0 = 4$, Retort Temperature = 116°C.; $F_0 = 4$, Retort Temperature = 121°C.; etc.). In order to determine reproducibility, each treatment was repeated four times. Immediately following heat processing, each sample was cooled to 39°C. by immersion into water at 18°C. for 30 min. The cans were stored at room temperature for two days.

Each can was opened and its contents were quantitatively transferred into a quart Mason jar and liquified for two min. at high speed. Ten-g. samples of the homogenate were randomly taken, placed in 12-oz. screw-cap jars, and stored in a still-air freezer (0°C.) until analyzed.

II. SAMPLE CLEANUP PRIOR TO GAS CHROMATOGRAPHY

A complete cleanup of the turnip greens is essential, since the wide variety of chemical entities found in the plant will rapidly foul the electron-capture detector, and thereby lower its sensitivity.

The Mills-Onley-Gaither (106) method of cleanup for chlorinated pesticide residues in nonfatty foods was used to prepare all samples for gas chromatography.

Reagents

Acetonitrile--Analytical Reagent (AR) grade. It was purified by glass distilling one gal. acetonitrile in the presence of 1 ml. of 85-per cent phosphoric acid and 30 g. phosphorous pentoxide at 81 to 82°C. (care was taken to not allow the temperature to exceed 82°C.). The first 100-ml. fraction and the last 150-ml. fraction of each gal. distilled was discarded.

Petroleum ether--AR grade. It was glass redistilled at 30 to 60°C., with the first 100-ml. fraction and the last 150-ml. fraction of each gal. distilled discarded.

Diethyl ether--AR grade, anhydrous. It was purified by washing twice with an amount of distilled water equal to twice the volume of the ether. The ether was washed with 50 to 100 ml. saturated NaCl solution with all aqueous layers discarded. The washed ether was stored in a F flask in the presence of excess anhydrous sodium sulfate. It was shaken for several minutes before use to remove any water which might be present.

Elution solvent--6 per cent diethyl ether in redistilled petroleum ether.

Florisil--80 to 100 mesh, pesticide grade. Activated at 623°C. by manufacturer and stored at 175°C. for at least 72 hours prior to use. (Floridan Company, Tallahassee, Fla.).

Celite 545--(Johns-Manville Company).

Sodium Sulfate--AR grade, anhydrous.

n-Hexane--AR grade. Washed in an equal volume of concentrated sulfuric acid and in an equal volume of fuming sulfuric acid, and glass redistilled at 68°C. with the first 50-ml. and last 100-ml. fraction of each distillation discarded.

Apparatus

Graduated cylinders--100 ml. and 250 ml. with 24/40 F glass stoppers.

Separatory funnels--500 ml. and 1000 ml. with Teflon stopcocks.

Kuderna-Danish concentration apparatus (Konte's Glass Company, Vineland, New Jersey)--500 ml. size complete with 3-ball Snyder column and Mills-type collection tube.

Vacuum filtration apparatus--3.5 in. Büchner funnel fitted with a No. 8 rubber stopper; 35/42 to 24/40 F reducing adapter; and 24/40 F vacuum take-off adapter (Konte's Glass Company).

Chromatographic columns (Konte's Glass Company)--300 mm. x 23 mm. i.d. with Teflon stopcock.

Flint glass bottles (Sargent)--15 ml. with bakelite screw cap containing a conical polyethylene liner.

Erlenmeyer flasks--500 ml. and 1000 ml. with 24/40 F glass stoppers.

Procedure

The fractionation scheme of chlorinated hydrocarbon insecticides from food products is presented in Figure I.

The sample was quantitatively removed from the storage jar into a pint Mason jar. Any adhering material was rinsed into the Mason jar with 150 ml. acetonitrile. Ten-g. of Celite was added to the sample, and an Osterizer blending head was attached to the jar.

Although Klein (72) reported that there is no significant difference between extraction results when different lengths of blending times are used, the leaf samples were blended 90 sec. and the root samples 120 sec. in Experiment One, and the homogenate was blended 60 sec. in Experiment Two.

A vacuum filtration apparatus was attached to a 250-ml. graduated cylinder, and the slurry was filtered by vacuum. The filter cake and Mason jar were rinsed with 50 ml. acetonitrile. The volume of the acetonitrile filtrate was observed and recorded.

The acetonitrile filtrate was transferred to a 1000-ml. separatory funnel. One hundred ml. of petroleum ether was measured into the graduated cylinder used for the acetonitrile filtrate and the volume recorded. About ten to twenty-five ml. of saturated NaCl solution was added to prevent the formation of emulsions; the separatory funnel was then filled with 600 ml. distilled water and shaken well for two minutes to force the residue from the acetonitrile phase into the petroleum ether phase. The solution was allowed to separate and the aqueous layer of

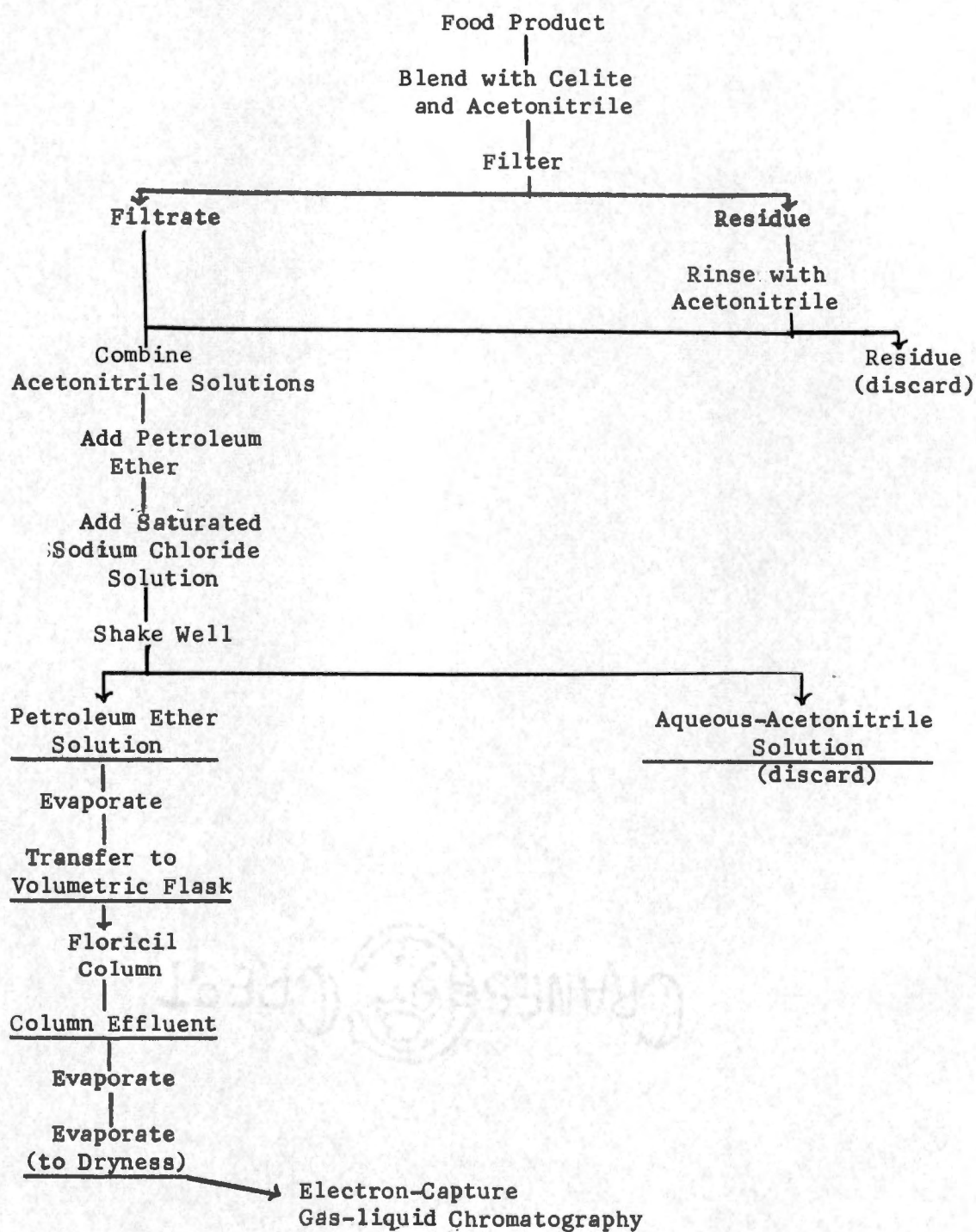


Figure I. Pesticide Fractionation Scheme.

layer of acetonitrile-water was discarded. The petroleum ether was rinsed twice with 200 ml. distilled water with gentle swirling (to prevent the formation of emulsions) to remove any acetonitrile remaining in the ether; with the washings discarded. The petroleum ether was transferred to a 100-ml. glass-stoppered graduated cylinder and the volume recorded. Ten-g. (0.5 in. layer) anhydrous sodium sulfate was added to the graduated cylinder and shaken well to remove any water.

The solvent was then transferred to a Kuderna-Danish concentration flask, and the sodium sulfate was washed twice with 15 ml. petroleum ether and added to the solvent. The solvent was evaporated on a steam bath to about 5 ml. The concentration extract, after cooling to room temperature, was transferred to a 10-ml. volumetric flask. The extracts were not made to volume at this time.

The chromatographic column was prepared by plugging the bottom opening with a small plug of quartz-glass wool. Forty g. of reactivated Florisil was added to the column and was topped with 10 g. (0.5 in. layer) anhydrous sodium sulfate. The column was packed by means of vibration for two min. and prewetted with 60 ml. redistilled petroleum ether. With the prewet being discarded. A Kuderna-Danish concentration flask and collection tube were placed as receiver.

The concentrated extract was made to 10 ml. Two ml. of the extract, in the case of turnip greens (Experiments One and Two), and 4 ml. of the extract, in the case of the turnip roots (Experiment One), was placed on the prewetted Florisil column (to simplify dilution of

the sample for gas chromatography). The sample and 200 ml. of the elutant were allowed to percolate through the column at a rate of 5 to 10 ml./min. A 3-ball Snyder column was attached, and the apparatus was placed on a steam bath. Care was taken not to evaporate it to dryness at this time, since insecticides are decomposed by the resulting elevated temperatures when allowed to go to dryness (29, 34, 109). The collection tube was removed after the apparatus had cooled, the solvent was evaporated to dryness in a forced-air oven at 36°C. The residue in the collection tube was dissolved in redistilled n-hexane to a volume of 10 ml. and transferred to a 15-ml. flint glass bottle and stored at 4°C. until chromatographed.

The sample weight represented by elutants after extraction, was calculated from the following formula:

$$S_r = S_o \times \frac{F}{T} \times \frac{P}{R}$$

where

S_r = actual sample weight (after elution)

S_o = sample weight in g.

F = volume of acetonitrile filtrate

T = total volume (ml. water contained in sample + ml. acetonitrile + ml. acetonitrile rinse - 5 ml. correction for shrinkage)

P = volume petroleum ether extract

R = volume petroleum ether added.

III. GAS CHROMATOGRAPHIC ANALYSIS OF SAMPLES

A Micro Tek Model DDS 162 gas chromatograph equipped with a d.c. 150 μ c. tritium foil electron-capture detector and a 60 x 0.125 in. i.d. spiral aluminum tube. This tube was packed with a 5-per cent Dow DC-11 silicone grease on 60 to 80 mesh, acid washed, silanized, Chromosorb W (w/w) and was used throughout the experimental work. Prepurified nitrogen (Matheson Company, Morrow, Ga.) was used as the carrier gas with a flow rate of approximately 70 ml./min. and an inlet pressure of 40 p.s.i. Optimum reproducibility and linearity were obtained at a column temperature of 175°C., the detector was maintained at 195°C., and the inlet temperature was 185°C. The detector attenuation (input x output) was set at $10^2 \times 8$, which is quite sensitive ($10^2 \times 8$ is twice as sensitive as $10^2 \times 16$; $10^2 \times 16$ is twice as sensitive as $10^2 \times 32$; etc.). However, the "noise" response was only 1 per cent.

Analytical crystalline standards (98.8 and 97.9 per cent pure) of heptachlor and heptachlor epoxide were obtained from the Velsicol Chemical Corporation (Chicago, Ill.). They were made into working standard solutions, ranging from 0.05 to 10.0 p.p.m.

Prepared analytical standard solutions of p,p'-DDT, p,p'-TDE, and DDE (100 p.p.m.--w/v) were obtained from Analabs, Incorporated (Hamden, Conn.). These solutions were combined and diluted to a working range of 0.25 to 10.0 p.p.m.

All samples in Experiment One were analyzed for heptachlor, heptachlor epoxide, DDE, TDE, DDT, and the samples in Experiment Two were

analyzed for DDE, TDE, and DDT. Since aluminum columns elute off o,p'-DDT and p,p'-TDE at the same time as a single peak (27), this peak was treated as TDE and reported as such (23). The recorder response and retention times of the five standards are presented in Figure II.

A Hamilton 10 μ l. syringe was used to inject the sample into the chromatograph. Injections ranging from 0.8 μ l to 3.4 μ l were used, depending upon the concentration of the sample. The leaf samples in Experiment One, and all the samples in Experiment Two required dilution from 0.2 to 0.1 of the original sample, with redistilled n-hexane in order to bring the residue concentration within a readable range on the recorder. Occasionally, samples had to be diluted and chromatographed several times before they were within the readable range of the recorder.

Each day, prior to operation, a "profile" of the chromatograph was made. This was made by setting the voltage of the machine, to the dynamic range at which optimum results are obtained. Linearity was determined by injecting varying amounts of standard into the machine and plotting the peak area vs. concentration.

Verification of the location of each residue was accomplished by chromatographing a sample, chromatographing the standard ('spike') solution, then chromatographing a 'spiked' sample (equal in dilution and volume to both the standard and the sample). If a residue was present, the peak area of the 'spiked' sample would be equal to the area of the standard used for 'spiking' plus the area of the residue present in the sample. There was no observation of interfering materials causing peak

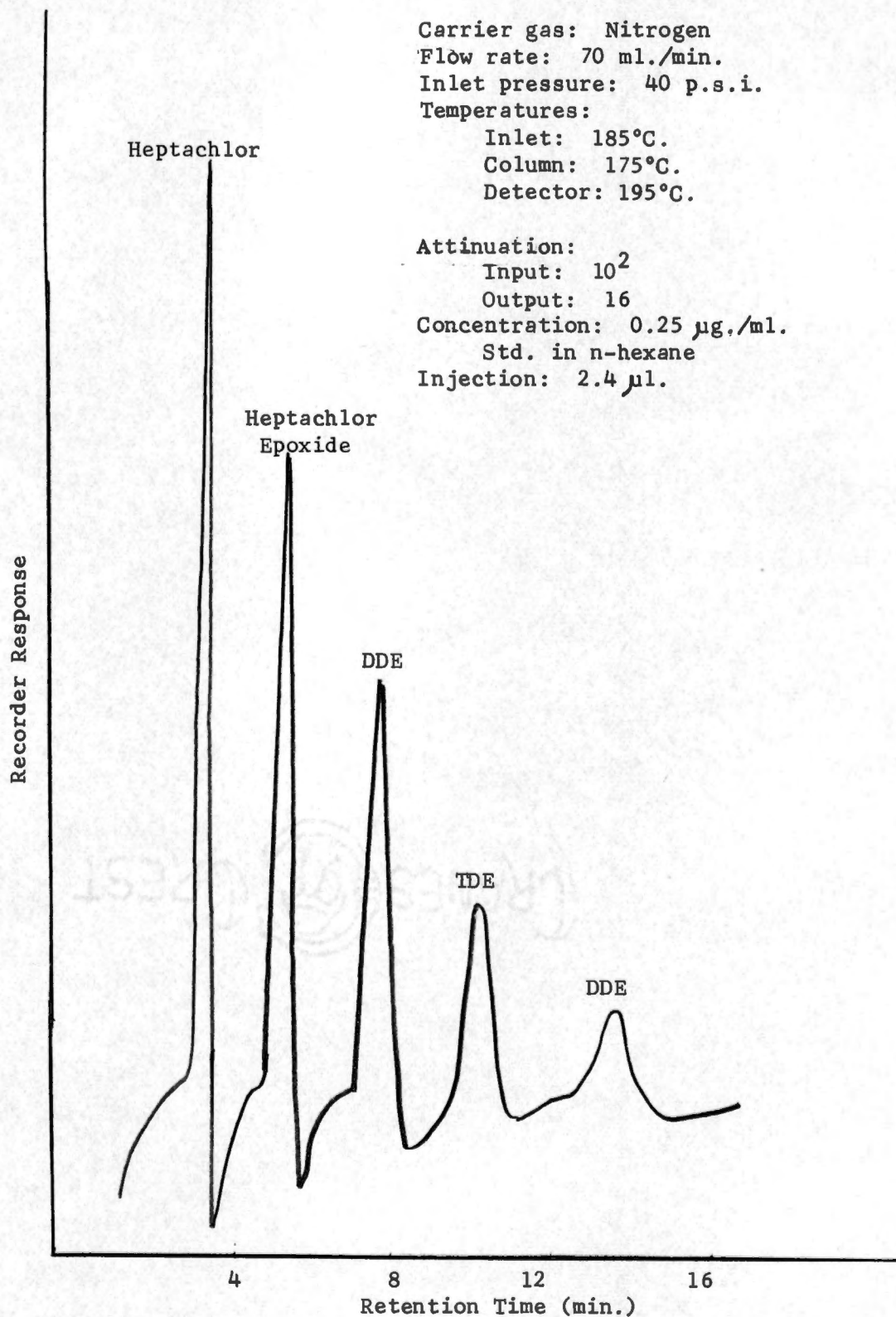


Figure II. Chromatogram of Five Chlorinated Hydrocarbon Insecticides.

shifting of the retention time. The chromatographic peaks was quantified by calculating the product of peak height and width of peak at half-height (55) and were compared to the areas of standard solutions injected at the beginning of each day and at 4-hour intervals throughout the day. The actual residue present was calculated in p.p.m. on the IBM Model 7040 digital computer (The University of Tennessee Computing Center) by the HOLWEL subroutine, using the following fomula:

$$\text{p.p.m.} = \frac{\left[\left(\frac{A_s}{I_s} \right) \times \left(\frac{A_u}{I_u} \right) \times D \times V \right]}{G}$$

where

A_s = area obtained from standard solution

I_s = amount of standard solution injected

C_s = concentration of standard solution (in $\mu\text{g./ml.}$)

A_u = area obtained from sample

I_u = amount of sample injected

C_u = concentration of sample

D = dilution factor

V = volume of sample after cleanup

G = sample weight (after extraction),

As a check procedure, a study was made to determine the percentage of pesticides recoverable using the cleanup procedure previously described. In this test, 10 g. of commercially processed turnip greens was fortified with 100 p.p.m., 0.1 p.p.m, and 0.0 p.p.m. of each of the insecticides

studied, and then analyzed. The recovery percentage of each insecticide may be found in Table II.

IV. STATISTICAL ANALYSIS

The experimental data from which all statistical analyses were computed are found in Tables XX through XXII in the Appendix.

The data were adjusted to give a composite DDT residue and a composite heptachlor residue. The composite heptachlor residues were determined by the following formula:

Composite heptachlor = heptachlor + 0.975 heptachlor epoxide, where 0.975 is the ratio of the molecular weight of the heptachlor to the molecular weight of the heptachlor epoxide. The composite DDT residue was determined by the following formula:

Composite DDT = DDT + 1.079 TDE + 1.111 DDE, where 1.079 and 1.111 are the ratios of the molecular weight of DDT to the molecular weights of TDE and DDE respectively. The composite residue calculation was carried out by a WATFOR program written for the IBM Model 7040 digital computer (The University of Tennessee Computing Center).

The ANOVAR subroutine (20) on the IBM Model 7040 digital computer (The University of Tennessee Computing Center) was used to perform the analysis of variance calculations. Duncan's multiple range test subroutine (114) on the IBM 360 Model 40 digital computer (The University of Tennessee Computing Center) was used to determine the significance of mean differences in both experiments.

TABLE II
PERCENTAGE CLEANUP RECOVERY OF INSECTICIDE RESIDUES OF DDT,
TDE, DDE, HEPTACHLOR, AND HEPTACHLOR EPOXIDE
IN TURNIP GREENS

Replicate ¹	Initial Concen- tration (p.p.m.)	Insecticide Recovery ²				
		DDT	TDE	DDE	Heptachlor	Heptachlor Epoxide
A	100	97.2	99.0	94.7	92.0	87.0
B	100	95.0	97.6	96.0	89.5	86.5
A	0.1	93.4	89.9	88.0	80.1	79.8
B	0.1	90.2	91.0	87.5	77.3	80.3
A	0	----	----	----	----	----
B	0	----	----	----	----	----

¹Each replicate is the average of two samples.

²In percentage of original concentration.

The BMDQ2D subroutine on the IBM Model 7040 computer (The University of Tennessee Computing Center) was used for the correlation between residue concentrations and composite residue concentrations.

CHAPTER IV

RESULTS AND DISCUSSION

I. DDT AND DDT METABOLITE RESIDUES IN TURNIP GREENS

The analyses of variance of DDT and DDT metabolite residues in turnip greens are shown in Table III. Results of Duncan's multiple range test are given in Tables IV and V. This test was made only when the F test in the analysis of variance indicated significance at the 0.01 level of probability. Duncan's test was applied only in those cases in which the F test indicated significance of differences at either the 0.05 or 0.01 level of probability of chance occurrence. The correlations of DDE, TDE, and DDT with one another and with the composite DDT are presented in Table VI.

Analyses of variance (Table III) indicate that there were significant ($P < 0.01$) differences between treatments with respect to amounts of DDE residues, TDE residues, and DDT residues. Process differences were significant ($P < 0.01$) with respect to amounts of TDE residues and DDT residues.

There were no significant process differences with respect to amounts of DDE residues. Neither was there any significance demonstrated with respect to replicate differences or with respect to interaction of the main effects in the analysis.

TABLE III
ANALYSES OF VARIANCE OF DDT AND DDT METABOLITE
RESIDUES IN TURNIP GREENS

Source	d. f.	DDE		TDE		DDT	
		M. S.	F	M. S.	F	M. S.	F
Treat- ment	3	4.2543	7.887**	9.1704	18.700**	200.4059	11.194**
Process	3	1.1141	2.065	3.7902	7.729**	103.0115	5.754**
Repli- cates	2	0.7515	1.393	0.0988	0.201	0.7650	0.043
TxPxR	18	0.4690	0.869	0.3353	0.684	3.4258	0.191
Error	21	0.5394		0.4904		17.9033	
Total	47						

**Throughout this presentation, significance levels will be denoted by asterisks, one asterisk for the 0.05 level and two asterisks for the 0.01 level. Although significance at the 0.1 level was tested, in no case was significance at this level detected.

TABLE IV
RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
INSECTICIDAL TREATMENT ON DDT AND DDT METABOLITE
RESIDUES IN TURNIP GREENS¹

Treatment	Means ²		
	DDE	TDE	DDT
Control	0.025 a	0.053 a	0.132 a
DDT	1.242 b	1.640 b	8.326 b
Heptachlor	0.122 a	0.335 a	0.370 a
DDT + Heptachlor	0.908 ab	1.745 b	5.909 b

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of twelve observations.

TABLE V
RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF PROCESS ON
DDT AND DDT METABOLITE RESIDUES IN TURNIP GREENS¹

Process	Means ²		
	DDE	TDE	DDT
Final Spray	0.851 a	1.777 b	7.944 b
Harvest	0.824 a	0.701 a	3.197 ab
Wash	0.298 a	0.549 a	2.150 a
Blanch	0.323 a	0.747 a	1.455 a

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of twelve observations.

TABLE VI
CORRELATION MATRIX OF DDT AND DDT METABOLITES
IN TURNIP GREENS

	DDT	TDE	DDE
Composite DDT	0.9851	0.9262	0.6621
DDE	0.5664	0.5613	
TDE	0.8727		

The mean residues of DDE, TDE, and DDT from each treatment are shown in Table IV, page 47). In no case were there any significant differences between the control- and the heptachlor-treated plots or the DDT- and DDT + heptachlor-treated plots. The DDE residues of the DDT + heptachlor-treated plots showed no significant treatment differences. The lack of a significant difference between the control- and the heptachlor-treated plots can be attributed to the possibility of heptachlor having been present in the soil as a residual from previous plantings. No significant differences between the DDT- and the DDT + heptachlor plots would be expected because of similarity of treatment.

The mean residues of DDT and DDT metabolites at each process level are shown in Table V, page 48. In all cases, washing and blanching significantly ($P < 0.01$) reduced the residues in turnip greens. TDE and DDT residues were significantly ($P < 0.01$) reduced by weathering between the final insecticidal spray treatment and harvest.

The correlation matrix (Table VI) shows that the DDT, TDE, and DDE were mainly converted from the composite DDT directly and that there was little stepwise dechlorination from DDT to TDE and TDE to DDE. This substantiates the observations of Klein, et al. (72), Ott (113), and Ritchey, et al. (115).

II. HEPTACHLOR AND HEPTACHLOR EPOXIDE RESIDUES IN TURNIP GREENS

Results of analyses of variance of amounts of heptachlor and heptachlor epoxide in turnip greens are contained in Table VII, while the

TABLE VII
ANALYSES OF VARIANCE OF HEPTACHLOR AND HEPTACHLOR
EPOXIDE RESIDUES IN TURNIP GREENS

Source	d. f.	Heptachlor		Heptachlor Epoxide	
		M. S.	F	M. S.	F
Treatments	3	0.0001	0.457	0.0006	4.866**
Process	3	0.0002	0.694	0.0000	0.183
Replicates	2	0.0001	0.520	0.0000	0.295
TxPxR	18	0.0002	0.775	0.0001	1.012
Error	21	0.0003		0.0001	
Total	47				

Duncan's multiple range test for treatments is found in Table VIII, and the correlation of composite heptachlor, heptachlor, and heptachlor epoxide is found in Table IX.

With respect to heptachlor residues in turnip greens (Table VII) there were no significant differences between treatments, processes, replicates, nor was interaction significant (treatment-process-replication). Treatments were significantly ($P < 0.01$) different in heptachlor epoxide residues, while there was no significance with respect to process difference, replicate difference, or interaction.

The effect of insecticide treatments are shown in Table VIII. There were no significant treatment-plot differences with respect to heptachlor residue samples. There were significant ($P < 0.01$) differences between the DDT-treated plots and the control-, the heptachlor-, and the DDT-treated plots indicating that no DDT residue was present. The lack of significant differences between the control-treated plot and the other plots can be assumed to be the result of residual heptachlor from previous crops.

The correlation matrix (Table IX) shows that the heptachlor and the heptachlor epoxide were derived from the composite heptachlor, while the conversion of heptachlor to heptachlor epoxide was not as readily apparent.

TABLE VIII

RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
INSECTICIDAL TREATMENT ON HEPTACHLOR AND HEPTACHLOR
EPOXIDE ON TURNIP GREENS¹

Treatment	Means ²	
	Heptachlor	Heptachlor Epoxide
Control	0.007 a	0.017 b
DDT	0.000 a	0.000 a
Heptachlor	0.002 a	0.009 ab
DDT + Heptachlor	0.006 a	0.007 ab

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of twelve observations.

TABLE IX
CORRELATION MATRIX OF HEPTACHLOR AND HEPTACHLOR
EPOXIDE IN TURNIP GREENS

	Heptachlor	Heptachlor Epoxide
Composite Heptachlor	0.8989	0.8150
Heptachlor Epoxide	0.4787	

III. DDT AND DDT METABOLITE RESIDUES IN TURNIP ROOTS

The results of the experiments concerning DDT on turnip roots are found in Tables X through XII. In the analyses of variance (Table X), significant ($P < 0.01$) differences were observed between treatments with respect to amounts of DDE residues and significant ($P < 0.05$) differences were observed between treatments with respect to amounts of DDT residues. There were no significant differences between treatments in TDE residues, nor was there significance with respect to treatment-process-replicate interaction in any of the residues.

Table XI contains the results of the insecticide treatments and shows significant differences between the control- and heptachlor-treated plots and the DDT- and DDT + heptachlor-treated plots, which may be due to improper sampling, drift of the insecticidal spray, or both. There were no significant differences between treatments in the TDE residues or between the control-, DDT-, and heptachlor-treated plots in the DDT residues. There were significant ($P < 0.05$) differences in DDT residues in the case of DDT + heptachlor-treated plots, and since there was no significance also from the DDT-treated plot, improper sampling, insecticidal drift, or both could explain this unexpected result.

The correlations of DDT and DDT metabolites in turnip roots (Table XII) indicate that the conversion of DDT to its metabolites is a direct-path conversion and not a step-wise one. It shows also that the persistence of DDT in root crops is less than in leaf crops.

TABLE X
ANALYSES OF VARIANCE OF DDT AND DDT METABOLITE
RESIDUES IN TURNIP ROOTS

Source	d. f.	DDE		TDE		DDT	
		M. S.	F	M. S.	F	M. S.	F
Treat- ments	3	0.0009	7.737**	0.0015	1.382	0.0107	4.065*
Process	2	0.0001	0.916	0.0012	1.056	0.0002	0.079
Repli- cates	2	0.0001	0.697	0.0002	0.207	0.0011	0.406
TxPxR	12	0.0002	1.692	0.0002	0.192	0.0013	0.504
Error	16	0.0001		0.0011		0.0026	
Total	35						

TABLE XI
RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
INSECTICIDAL TREATMENT ON DDT AND DDT METABOLITE
RESIDUES IN TURNIP ROOTS

Treatment	Means ¹		
	DDE ²	TDE ²	DDT ³
Control	0.019 b	0.000 a	0.016 a
DDT	0.000 a	0.000 a	0.022 a
Heptachlor	0.019 b	0.019 a	0.027 a
DDT + Heptachlor	0.003 a	0.025 a	0.090 b

¹Each mean is expressed as p.p.m. and is the average of nine observations.

²Means followed by the same letter are not significantly different at the 0.01 level of probability.

³Means followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE XII
CORRELATION MATRIX OF DDT AND DDT METABOLITES
IN TURNIP ROOTS

	DDT	TDE	DDE
Composite DDT	0.7884	0.8377	0.2939
DDE	-0.0969	0.2916	
TDE	0.3666		

IV. HEPTACHLOR AND HEPTACHLOR EPOXIDE RESIDUES IN TURNIP ROOTS

The analyses of variance of heptachlor and heptachlor epoxide residues in turnip roots is presented in Table XIII. The Duncan's multiple range test, Table XIV, tests significance between treatments. Table XV contains the correlations of the heptachlor and heptachlor epoxide with each other and with the composite heptachlor.

The analysis of variance tests indicate no significant difference between treatments, processes, or replicates, and no significance of treatment-process-replicate interaction with respect to amount of heptachlor residue. Treatments were significantly ($P < 0.01$) different from each other in amount of heptachlor epoxide residue, but there were no significant differences between processes, replicates, and no significant interaction.

The Duncan's multiple range test shows that there is no significant difference between treatments even though there is statistical significance ($P < 0.01$) in the analysis of variance (Table XIII). Occasional inconsistencies between the analysis of variance and the range test do arise, though very rarely. In such cases the F test can be relied upon, but one cannot go beyond stating that significant differences did occur. No statement can be made as to which differences were significant.

The correlation matrix, Table XV, shows that heptachlor and heptachlor epoxide are derived mainly from the composite heptachlor and that the conversion of heptachlor epoxide from heptachlor is not readily apparent.

TABLE XIII
ANALYSES OF VARIANCE OF HEPTACHLOR AND HEPTACHLOR
EPOXIDE RESIDUES IN TURNIP ROOTS

Source	d. f.	Heptachlor		Heptachlor Epoxide	
		M. S.	F	M. S.	F
Treatments	3	0.0000 ¹	1.046	0.0000	5.818**
Process	2	0.0000	0.326	0.0000	0.560
Replicates	2	0.0000	0.579	0.0000	0.399
TxPxR	12	0.0000	0.520	0.0000	0.940
Error	16	0.0000		0.0000	
Total	35				

¹The zero mean squares shown are simply a result of truncation of calculated results. The computer output shows only four digits to the right of the decimal point. The non-zero F values indicate that the actual mean square obtained by the computer were non-zero.

TABLE XIV

RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
INSECTICIDAL TREATMENT ON HEPTACHLOR AND HEPTACHLOR
EPOXIDE RESIDUES IN TURNIP ROOTS¹

Treatment	Means ²	
	Heptachlor	Heptachlor Epoxide ²
Control	0.001 a	0.009 a
DDT	0.004 a	0.008 a
Heptachlor	0.003 a	0.004 a
DDT + Heptachlor	0.004 a	0.009 a

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of nine observations.

TABLE XV
CORRELATION MATRIX OF HEPTACHLOR AND HEPTACHLOR
EPOXIDE IN TURNIP ROOTS

	Heptachlor	Heptachlor Epoxide
Composite Heptachlor	0.7111	0.7319
Heptachlor Epoxide	0.0413	

V. EFFECT OF THERMAL PROCESSING OF DDT-FORTIFIED
CANNED TURNIP GREENS

The results of thermal processed DDT-fortified canned turnip greens are found in Tables XVI through XIX.

The analyses of variance shows no significant difference between treatments, processes, replicates, and no significance of treatment-process-replication interaction with respect to DDE residues. There were significant ($P < 0.01$) differences between treatments and processes but no significance with respect to replicate differences or interaction. There were no significant treatment, process, or replication differences and no significant interaction with respect to DDT residues.

Duncan's multiple range test showed no significant differences in the experiment when it was reproduced four times in the residues of DDE and DDT. A significant ($P < 0.01$) difference was observed in TDE residues indicating a lowered reproducibility with this metabolite. Table XVIII shows that the six processes had no significant effect on the DDE residues or DDT residues. All processes had no significant effect on the TDE residues, except the above normal process at 116°C . which caused a significant ($P < 0.01$) increase in the metabolite. This is consistent with the findings of Farrow, et al. (48).

The correlation matrix, Table XIX, shows that DDT and DDE were mainly converted from the composite DDT directly with TDE converted to a lesser extent. While the observation of the conversion of DDT to TDE is consistent with the findings of Farrow, et al. (48), the conversion

TABLE XVI
ANALYSES OF VARIANCE OF DDT AND DDT METABOLITE RESIDUES
IN THERMALLY PROCESSED TURNIP GREENS

Source	d. f.	DDE		TDE		DDT	
		M. S.	F	M. S.	F	M. S.	F
Treat- ments	3	0.0485	1.986	1.1542	4.770**	9.7653	2.188
Process	5	0.0170	0.695	1.4203	5.870	3.9703	0.890
Repli- cates	2	0.0109	0.477	0.6580	2.720	0.9322	0.209
TxPxR	30	0.0078	0.320	0.1749	0.723	0.8023	0.180
Error	31	0.0244		0.2419		4.4630	
Total	71						

TABLE XVII
RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
TREATMENT ON DDT-TREATED TURNIP GREENS¹

Treatment ³	Means ²		
	DDE	TDE	DDT
1	0.159 a	0.696 a	0.725 a
2	0.264 a	0.993 ab	2.359 a
3	0.212 a	0.967 ab	0.944 a
4	0.270 a	1.314 b	1.073 a

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of eighteen observations.

³Each number denotes three observations of each of the six processes as described in Table XVIII.

TABLE XVIII
RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR THE EFFECT OF
THERMAL PROCESSING ON DDT-TREATED TURNIP GREENS¹

Process ³	Means ²		
	DDE	TDE	DDT
F ₀ 4, 116°C.	0.189 a	0.930 a	1.073 a
F ₀ 4, 121°C.	0.180 a	0.535 a	0.782 a
F ₀ 7, 116°C.	0.219 a	1.033 a	1.582 a
F ₀ 7, 121°C.	0.258 a	0.886 a	0.909 a
F ₀ 10, 116°C.	0.274 a	1.597 b	1.983 a
F ₀ 10, 121°C.	0.239 a	0.975 a	0.619 a

¹Within each column, means followed by the same letter are not significantly different at the 0.01 level of probability.

²Each mean is expressed as p.p.m. and is the average of twelve observations. As pointed out on page 68, the imperfect recovery after processing may be attributed to "feathering".

³F₀4 is below process, F₀7 is normal process, and F₀10 is above normal process.

TABLE XIX

CORRELATION MATRIX OF DDT AND DDT METABOLITES IN
THERMALLY PROCESSED TURNIP GREENS

	DDT	TDE	DDE
Composite DDT	0.8948	0.6723	0.9317
DDE	0.7418	0.7655	
TDE	0.2716		

of TDE to DDE and the low correlation of DDT being converted to TDE is not.

The cans containing the DDT-fortified samples showed evidence of "feathering" of the can enamel (J-enamel) as described by Tressler (131), with varying degrees of discoloration, while the control cans (not fortified with DDT) showed none. This phenomenon could be due to the presence of DDT. DDT, being a non-polar compound, might be able to extract chlorophyll from the greens and deposit the pigment on the can enamel. The reduction in the amount of insecticide present in each sample after processing may be accounted for by the deposition of part of the insecticide on the enamel of the can, i.e. "feathering".

A white precipitate was observed in the fill brine in both the control and the DDT-fortified samples. This probably was calcium oxalate which was precipitated from the greens during the thermal processing operation.

The presence of p,p' -DDT was observed in all cases; hence, the complete dechlorination of DDT as reported by Farrow, et al. (48) did not occur in the present study.

CHAPTER V

SUMMARY

The first experiment was conducted to determine the effect of insecticidal translocation from residues of previous crops, and the effect, if any, of commercial processing techniques on the removal of this residue.

Under the conditions of the study reported in this paper, it was found that:

1. The washing of turnip greens reduced residues of TDE and DDT, but had no significant effect on DDE, heptachlor, or heptachlor epoxide residues.
2. The steam blanching of turnip greens reduced residues of TDE and DDT, but had no significant effect on DDE, heptachlor, or heptachlor epoxide.
3. Commercial processing of turnip roots showed no significant difference between processes.

The second experiment was conducted to determine whether varying the thermal processing conditions would have an effect on a residue of DDT, TDE, or DDE.

Under the conditions of the study reported in this paper, it was found that:

1. Thermal processing effected a significant rise in TDE metabolites, but had no significant effect on DDE or DDT.

2. Thermal processing does not completely dechlorinate DDT to its metabolites.

3. Thermal processing causes "feathering" of the can enamel in the presence of DDT.

In general, the results of this study indicate that the processing treatments examined do not reduce the levels of insecticide residues in turnip greens sufficiently to meet Food and Drug Administration specifications for safe human consumption. In cases of high concentration it appears that some dilution procedure or multiple processing must be resorted to in order to meet Food and Drug Administration requirements.

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APPENDIX



TABLE XX

RESIDUES OF HEPTACHLOR, HEPTACHLOR EPOXIDE, DDT, AND DDT
METABOLITES IN TURNIP GREENS

Source ²	Rep.	Insecticide ¹				
		Heptachlor	Heptachlor Epoxide	DDE	TDE	DDT
C-1	1	.000	.000	.160	.444	1.186
C-1	2	.000	.033	.000	.000	.000
C-1	3	.000	.014	.000	.000	.000
C-2	1	.000	.018	.047	.000	.000
C-2	2	.001	.010	.042	.000	.283
C-2	3	.000	.025	.000	.000	.000
C-3	1	.079	.038	.000	.000	.115
C-3	2	.001	.009	.000	.000	.000
C-3	3	.000	.004	.046	.189	.000
C-4	1	.000	.013	.000	.000	.000
C-4	2	.000	.014	.000	.000	.000
C-4	3	.003	.023	.000	.000	.000
D-1	1	.000	.000	1.682	3.290	21.013
D-1	2	.000	.000	1.594	3.090	17.871
D-1	3	.000	.000	1.785	3.453	20.519
D-2	1	.000	.000	1.172	2.053	11.845
D-2	2	.000	.000	5.336	.555	3.884
D-2	3	.000	.000	.532	1.092	3.983
D-3	1	.000	.000	.512	.945	3.313
D-3	2	.000	.000	.578	.897	4.150
D-3	3	.000	.000	.598	1.192	5.431
D-4	1	.000	.000	.406	1.086	3.369
D-4	2	.000	.000	.276	.947	2.016
D-4	3	.000	.000	.428	1.085	2.520
DH-1	1	.000	.000	.914	1.860	6.559
DH-1	2	.000	.053	1.495	2.833	12.207
DH-1	3	.000	.000	1.235	2.546	11.540
DH-2	1	.000	.000	.837	1.493	5.279
DH-2	2	.000	.000	1.511	2.374	10.165
DH-2	3	.070	.029	.411	.846	2.931
DH-3	1	.000	.000	.752	1.171	4.714
DH-3	2	.000	.000	.478	.902	3.664
DH-3	3	.000	.000	.610	1.292	4.412

TABLE XX (CONTINUED)

Source ²	Rep.	Insecticide ¹				
		Heptachlor	Heptachlor Epoxide	DDE	TDE	DDT
DH-4	1	.000	.000	.668	1.746	3.401
DH-4	2	.000	.000	.651	1.458	2.681
DH-4	3	.000	.000	1.340	2.425	3.357
H-1	1	.000	.006	.036	.000	.146
H-1	2	.000	.000	1.230	3.448	4.076
H-1	3	.000	.000	.087	.358	.216
H-2	1	.001	.009	.000	.000	.000
H-2	2	.002	.013	.000	.000	.000
H-2	3	.014	.011	.000	.000	.000
H-3	1	.002	.012	.000	.000	.000
H-3	2	.002	.012	.000	.000	.000
H-3	3	.001	.009	.000	.000	.000
H-4	1	.002	.010	.045	.000	.000
H-4	2	.000	.009	.000	.000	.000
H-4	3	.004	.014	.068	.214	.000

¹All values are expressed in p.p.m.

²Letter indicates test plot from which samples were taken: C, control; D, DDT; DH, DDT + heptachlor; H, heptachlor. Number indicates process level from which samples were taken: 1, final insecticidal spray; 2, harvest; 3, wash; 4, blanch.

TABLE XXI

RESIDUES OF HEPTACHLOR, HEPTACHLOR EPOXIDE, DDT, AND
DDT METABOLITES IN TURNIP ROOTS

Source ²	Rep.	Insecticide ¹				
		Heptachlor	Heptachlor Epoxide	DDE	TDE	DDT
C-1	1	.000	.006	.000	.000	.000
C-1	2	.001	.008	.027	.000	.000
C-1	3	.003	.012	.036	.000	.141
C-2	1	.003	.012	.036	.000	.000
C-2	2	.001	.008	.028	.000	.000
C-2	3	.000	.008	.000	.000	.000
C-3	1	.001	.011	.000	.000	.000
C-3	2	.004	.014	.031	.000	.000
C-3	3	.000	.003	.010	.000	.000
D-1	1	.002	.007	.000	.000	.000
D-1	2	.002	.007	.000	.000	.000
D-1	3	.005	.007	.000	.000	.000
D-2	1	.004	.010	.000	.000	.000
D-2	2	.004	.009	.000	.000	.000
D-2	3	.004	.008	.000	.000	.000
D-3	1	.004	.011	.000	.000	.000
D-3	2	.004	.006	.000	.000	.112
D-3	3	.004	.006	.000	.000	.090
DH-1	1	.002	.010	.000	.000	.078
DH-1	2	.002	.012	.000	.000	.057
DH-1	3	.001	.008	.000	.000	.080
DH-2	1	.003	.009	.000	.000	.085
DH-2	2	.004	.012	.000	.000	.083
DH-2	3	.003	.011	.000	.000	.125
DH-3	1	.002	.004	.000	.072	.113
DH-3	2	.016	.010	.000	.083	.084
DH-3	3	.004	.005	.027	.073	.106
H-1	1	.010	.005	.029	.089	.128
H-1	2	.008	.000	.044	.084	.000
H-1	3	.004	.003	.026	.000	.000

TABLE XXI (CONTINUED)

Source ²	Rep.	Insecticide ¹				
		Heptachlor	Heptachlor Epoxide	DDE	TDE	DDT
H-2	1	.001	.003	.000	.000	.117
H-2	2	.001	.003	.000	.000	.000
H-2	3	.002	.006	.029	.000	.000
H-3	1	.002	.006	.022	.000	.000
H-3	2	.001	.006	.000	.000	.000
H-3	3	.001	.007	.020	.000	.000

¹All values are expressed in p.p.m.

²Letter indicates test plot from which samples were taken: C, control; D, DDT; DH, DDT + Heptachlor; H, Heptachlor. Number indicates process level from which samples were taken: 1, harvest; 2, wash; 3, peel.

TABLE XXII

DDT AND DDT METABOLITE RESIDUES RESULTING FROM DECOMPOSITION
DUE TO THERMAL PROCESSING

Source ²	Treatment ³	Rep.	Insecticide ¹		
			DDE	TDE	DDT
I	1	1	.184	.884	.062
I	1	2	.111	.453	.027
I	1	3	.176	.556	.044
II	1	1	.067	.213	.296
II	1	2	.069	.396	.012
II	1	3	.067	.197	.343
III	1	1	.237	.946	1.780
III	1	2	.292	.824	1.811
III	1	3	.495	2.153	4.896
IV	1	1	.189	1.160	.047
IV	1	2	.205	1.228	.041
IV	1	3	.172	2.152	.031
I	2	1	.130	.350	1.016
I	2	2	.145	.400	1.227
I	2	3	.137	.378	.664
II	2	1	.037	.141	.138
II	2	2	.040	.125	.116
II	2	3	.074	.224	.408
III	2	1	.136	.393	.415
III	2	2	.107	.397	.718
III	2	3	.305	1.042	2.698
IV	2	1	.321	1.078	4.102
IV	2	2	.384	.939	3.320
IV	2	3	.343	.951	4.163
I	3	1	.198	.995	.061
I	3	2	.166	.944	.250
I	3	3	.169	.971	.540
II	3	1	.089	.454	.633
II	3	2	.061	.219	.253
II	3	3	.308	1.378	4.076

TABLE XXII (CONTINUED)

Source ²	Treatment ³	Rep.	Insecticide ¹		
			DDE	TDE	DDT
III	3	1	.312	1.222	2.018
III	3	2	.131	.824	.024
III	3	3	.279	.830	2.207
IV	3	1	.287	1.592	.314
IV	3	2	.456	1.924	.498
IV	3	3	.177	1.047	.038
I	4	1	.361	1.027	3.541
I	4	2	.214	.701	2.913
I	4	3	.129	.394	1.556
II	4	1	.413	1.042	3.591
II	4	2	.505	1.414	4.870
II	4	3	.463	1.309	4.400
III	4	1	.110	.675	.018
III	4	2	.145	.820	.012
III	4	3	.115	.753	.015
IV	4	1	.171	.473	1.113
IV	4	2	.168	.521	1.104
IV	4	3	.304	1.503	.669
I	5	1	.095	.614	.113
I	5	2	.152	1.005	.221
I	5	3	.339	2.025	.610
II	5	1	.348	2.435	.498
II	5	2	.410	1.411	4.870
II	5	3	.386	2.364	.614
III	5	1	.315	1.215	.208
III	5	2	.230	1.361	.120
III	5	3	.179	1.292	.060
IV	5	1	.281	1.746	.053
IV	5	2	.270	1.734	.028
IV	5	3	.284	1.959	.033
I	6	1	.061	.332	.010
I	6	2	.053	.179	.181
I	6	3	.048	.313	.015

TABLE XXII (CONTINUED)

Source ²	Treatment ³	Rep.	Insecticide ¹		
			DDE	TDE	DDT
II	6	1	.431	1.353	5.313
II	6	2	.488	1.426	5.282
II	6	3	.503	1.782	6.750
III	6	1	.145	.985	.000
III	6	2	.142	.831	.000
III	6	3	.140	.847	.000
IV	6	1	.087	.339	.533
IV	6	2	.357	1.322	2.730
IV	6	3	.410	1.990	.503

¹All values are expressed in p.p.m.

²Roman numeral indicates run replicate: I, first run; II, second run; III, third run; IV, fourth run.

³Number indicates process conditions: 1, F₀⁴, 116°C.; 2, F₀⁴, 121°C.; 3, F₀⁷, 116°C.; 4, F₀⁷, 121°C.; 5, F₀¹⁰, 116°C.; 6, F₀¹⁰, 121°C.

VITA

The author was born February 28, 1944, in Brigham City, Utah. He was graduated from George Washington High School, Denver, Colorado, in 1962. He attended Colorado State University, Fort Collins, Colorado, from 1962 to 1966 and received a Bachelor of Science degree in Animal Nutrition. In September, 1966, he entered The University of Tennessee Graduate School, and was appointed Assistant in Food Technology the following June. Since September, 1966, he has been working to complete the requirements for the degree, Master of Science.

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