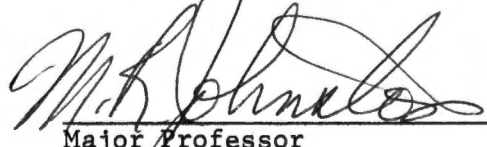


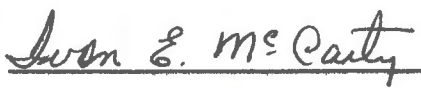
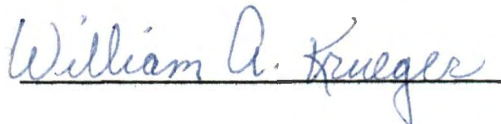
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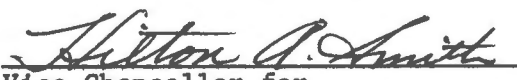
I am submitting herewith a thesis written by Bernard Phelps Moss entitled "Effects of Epoxy-Phenolic Can Linings on DDT in Thermally Processed Turnip Greens." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.


Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Chancellor for
Graduate Studies and Research

EFFECTS OF EPOXY-PHENOLIC CAN LININGS ON DDT IN
THERMALLY PROCESSED TURNIP GREENS

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
Bernard Phelps Moss

August 1970

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ABSTRACT

Insecticides are used on crops as a matter of course and are considered by most agricultural workers as essential for the provision of the nation's food at acceptable cost and quality. However, there is also a great deal of concern and research concerning the deleterious effects and the avoidance or removal of insecticide residues in food.

This study was undertaken to determine whether DDT is absorbed by epoxy-phenolic can lining during thermal processing, and whether residues in plain cans were affected differently from residues in cans lined with enamel.

Turnip greens were fortified with technical DDT and thermally processed. The insecticide was extracted from plant constituents with acetonitrile and then eluted through a Florosil column to remove other interfering materials. The extraction-elution product was then quantitatively analyzed to determine the presence of DDT or its metabolites DDD and DDE. The enamel was removed from the cans with an electrolytic solution and electric current and then treated the same as the plant material had been to extract insecticide residues.

Under the experimental conditions reported in this study, several results were indicated. The can enamel absorbed p,p'-DDT and apparently catalyzed the rearrangement of some of it to the o,p'-isomer after absorption. The can enamel did not significantly absorb DDD or DDE during thermal processing. Residues of o,p'-DDT in enamel of cans which had been processed at 121° C were significantly

higher than in the enamel of cans processed at 116° C. Cook times that achieve commercial sterilization values near Fo7 (normal process) are apparently optimum for the absorption-formation of o,p'-DDT.

Generally, it seems plausible that epoxy-phenolic plastics similar to the can linings observed in this experiment might be useful in adventitious removal of chlorinated pesticide residues. However, the reduction of residue in the food that it achieves, though significant, would probably not bring the level down to FDA tolerance if the canned product contained above-tolerance levels to begin with. Therefore, epoxy-phenolics in forms other than can lining should be studied as a means of reducing residues in food products. For example, partial cooking, prior to canning, in vats or heat exchangers lined with corrugated epoxy-phenolic resins might be more absorbent since a larger surface exposure of resin-to-food would result.

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CHAPTER I

INTRODUCTION

Most data available on pesticide residues in food are based on samples of food obtained from the grower or from the market in a fresh state. In recent years much research has been reported on the effects of commercial food processing on pesticide residues. Others have dealt with special techniques to reduce pesticide residues in foods.

The Food and Drug Administration (57)* has established tolerances of 1.0, 3.5, and 7.0 parts per million (ppm) for DDT and TDE (DDD), depending on the product.

Relatively few studies have dealt with the effect that container walls have on residues in the foods they contain.

Farrow and others (20) reported that residues of DDT in spinach converts in part to TDE (DDD) during thermal processing whether processed in plain tinplate, enameled tinplate, or glass containers. Later Hemphill and others (33) indicated that time and temperature alone did not explain the loss and degradation of DDT in green beans during thermal processing and that studies of the composition materials of the containers should be undertaken. It has been found that color of wax beans packed in enameled cans is significantly different from those packed in plain tinplate (67). Yellow color compounds are fat soluble as is DDT and most of its metabolites.

*The numbers in parentheses represent similarly numbered references in the bibliography.

Hollowell indicated that "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'" (35).

The present study was conducted especially to investigate the effect of epoxy-phenolic can linings on residue levels in thermally processed turnip greens and incidently to see if temperature differences and different cook times caused differing residue levels and/or characteristics.

CHAPTER II

LITERATURE REVIEW

I. GENERAL INSECTICIDE CLASSIFICATIONS

The term "pesticide" was coined about 1949. It is a collective noun to cover all materials used to control, destroy, or mitigate pests (25). Pesticides may be categorized in a number of ways but one common method is to group them according to the target species: Acaricides and Miticides--used against arachnids of the order Acarino to include the ticks and mites; Fungicides--used against rusts, mildews, and molds; Herbicides--used against higher order plant pests; insecticides--the largest classification since most pests are insects. Rodenticides--toxic to all orders of mammals but used against rats and other rodent pests as well as carnivores and sometimes birds (64). Thus, it is seen that insecticides are one of many classes of pesticides. Insecticides are subdivided further according to their general chemical composition into three basic groups: chlorinated hydrocarbons, organic phosphorous compounds, and carbamates (64). Many times insecticides are grouped according to their modes of action: contact poisons--when the poison is absorbed through the integument of the insect; stomach poisons--when it is necessary for the insect to ingest the poison for lethality; and systemic poison--wherein the insecticide is translocated through the protected plant, making it poisonous to the insect pest (50).

II. PHYSICAL AND CHEMICAL PROPERTIES OF DDT

DDT was first synthesized in 1874 but its insecticidal effectiveness was not discovered until 1939 by Paul Muller of the Geigy Company in Switzerland; it was patented in 1942. It is cheap, easy to make, stable, potent, and extremely persistent (58). According to Martin (50) by 1945, only six years after discovery of its insecticidal properties, there was a world production of 30 million pounds of DDT and the success of DDT gave rise to a search for related compounds with similar insecticidal properties. The compounds found as a result of this research are now grouped under the general name of chlorinated hydrocarbons and include such well-known formulations as Lindane, Aldrin, Rothane, Heptachlor, etc. (50).

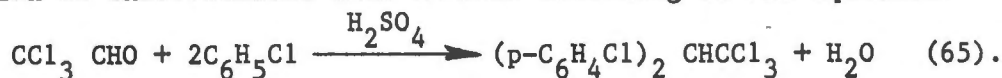
The name DDT is an abbreviation for dichlorodiphenyltrichloroethane. The designation is 2, 2 - bis (p-chlorophenyl) - 1, 1, 1 - trichloroethane in accordance with the IUPAC rules of nomenclature (58). It is also known by other names such as chlorophenothane, dicophane, pentachlorin, and Zerdane (24).

A number of isomers in addition to p, p'-DDT are possible and some such as o, p'-DDT; o, o'-DDT, etc., are present in the commercial product. These are less toxic to insects than the p, p'-isomer. The pure p, p'-isomer is a white crystalline solid, with a melting point of 108.5° C, and a vapor pressure of 1.9×10^{-7} mm. Hg at 20° C. It is nearly insoluble in water, soluble in most organic solvents, and moderately soluble in oils (24), (58).

The technical product may contain up to 30 percent of the o, p'-isomer. The specification universally used for DDT and approved

by the World Health Organization calls for a content of at least 70 percent of the p, p'-isomer (50).

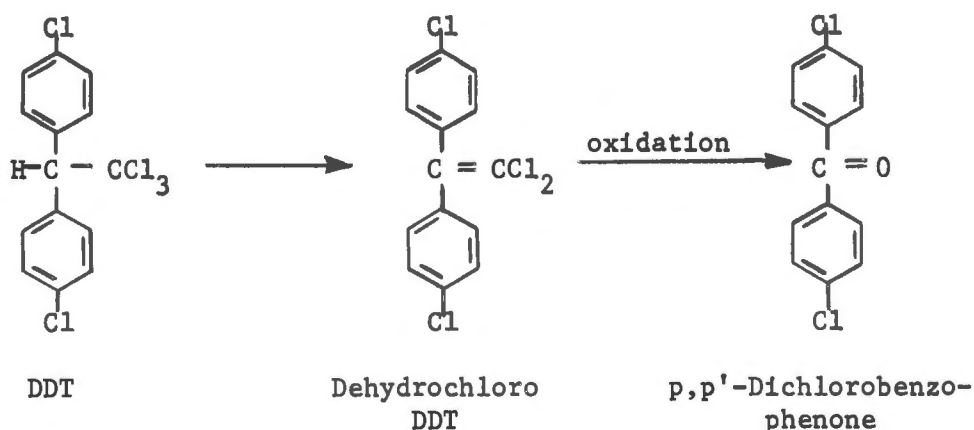
The basic reaction for the synthesis of DDT is the Bayer condensation of chlorobenzene with chloral according to the equation:



DDT is both a stomach poison and a contact poison; although without action as an ovicide and with little effect on pupal stages, its persistence ensures that the newly hatched larvae and emerging adults will receive a lethal dose. No satisfactory explanation of its lethal action is yet known but it causes violent tremors and muscular activity becomes uncoordinated (50).

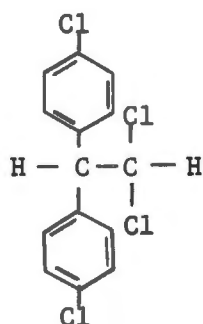
The persistence of DDT is due to its low volatility, low water solubility, and its chemical stability. However, when it is in solution it is readily decomposed by alkalies with loss of hydrochloric acid and its insecticidal activity. A similar dechlorination by an enzyme is thought to occur in those insects from which the continued use of DDT has resulted in the evolution of resistant strains (50), (23).

According to Fleck (23), in the presence of alkali, DDT readily loses HCl to form dehydrochloro DDT that in turn is quickly oxidized to p, p'-dichlorobenzophenone:

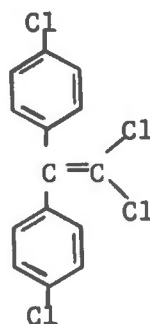


This same reaction takes place in the presence of catalysts. A tiny speck of anhydrous ferric chloride dropped into melted DDT will cause HCl to boil off and completely dehydrochlorinate the DDT in minutes. Even at ordinary temperatures other iron salts will cause the reaction to occur but at a slower rate (23).

The loss of chlorine atoms from the DDT molecule gives rise to DDD (TDE) and DDE (59).



p, p'-DDD



p, p'-DDE

Both of these metabolites of DDT are less toxic than the parent compound with DDE being the least toxic (54).

DDT decomposes to DDE and hydrochloric acid at temperatures above its melting point (37).

III. CHLORINATED HYDROCARBON RESIDUES

"Persistence" in connection with insecticides is considered as resistance to removal of an insecticide under conditions other than advertent removal by man, therefore, it refers to the length of time that a residue resists removal under conditions which may not be directly controllable by man. Persistence is affected by weather conditions--wind, rain, amount and intensity of sunlight, air temperature

and humidity (14). Chlorinated hydrocarbon insecticides are generally considered the persistent insecticides; the others are relatively short-lived. Plant characteristics also play a part--for example, 3 lbs of methoxychlor per acre on tomatoes resulted in 2 ppm at zero time but 2 lbs per acre on alfalfa resulted in 200 ppm of residue at zero time (14).

Formulations of the insecticide affect its persistence. Higher residues were obtained from sweet corn ear tips and silks when DDT granules were applied than when dust was applied, while those from spray applications were the lowest (31).

Klein et al. reported that both toxaphene and chlordane, two chlorinated hydrocarbon insecticides, "are rapidly lost on kale and persumably other vegetables by weathering." They reported that only trace amounts remained from an initial heavy application after 28 days (39).

There have been numerous investigations on soil retention of chlorinated hydrocarbons applied for crop protection and their translocation from the soil into subsequent crops grown on the same plot (44), (32), (46), (17), (19).

Eden et al. reported that DDT was not translocated from the soil into soybean plants (19). Lichtenstein reported that lindane was translocated from soil to the aerial parts of pea plants and that plants grown on plots containing aldrin and heptachlor contained only epoxides of these compounds within their leaves and stems, indicating that the parent compounds had been metabolized by the plant. No evidence of translocation of DDT was obtained (44). A similar study by Lichtenstein showed that apparently parathion, diazinon, aldrin, and lindane

were all translocated in pea plants but that the organophosphorous compounds were completely metabolized and detoxified while the latter two (chlorinated hydrocarbon) insecticides were not (46). Lichtenstein et al. reported that carrots absorb heptachlor and aldrin into the outer layers of the roots when grown in soils treated with those insecticides and that 96 to 99 percent of the residue could be removed by peeling the carrots (45). Duffy et al. stated that DDT and its metabolites could be absorbed by some plants from the soil (17).

Lamb et al. found DDT and DDT metabolite residues in potatoes grown in soil that had been treated over a five-year period with DDT (40). Harris et al. reported that trace amounts of DDT and DDE residues were absorbed by root crops grown in soils containing these residues (32).

As indicated by the literature cited above, DDT is not absorbed by plants from the soil to the extent that many other chlorinated hydrocarbons are. DDT residues and residues of its metabolites are, however, found in vegetable foods, probably from aerial application of that crop prior to harvest even though a time lapse is awaited between application and harvest for the purpose of natural removal of the pesticide (31), (41), (50).

Members of the University of Arizona Department of Entomology assessed the effects of the one year DDT moratorium in Arizona, begun in 1969. They found that alfalfa residues declined slightly, while beef fat residues remained the same as before the moratorium (68).

IV. INTENTIONAL REMOVAL OF PESTICIDES FROM FOOD PRODUCTS

Residues of DDT and its metabolites are widespread in the environment including man's food; a study conducted in England indicated a mean rate of ingestion of the DDT group there at .07 mg/person/day (51). Other studies have dealt with the effects normal food processing operations have on the removal of residues from food products (45), (33), (22), (21), (40), (41), (66).

Tressler's work in 1947 revealed that DDT was stable to heat in water and in acid buffer in Pyrex containers and only slightly decomposed in those media in ten cans, but it was decomposed to a greater degree when processed with various foods. He reported that his results indicated a destruction of from 20 to 27 percent of the DDT residues by thermal processing 25 minutes at 212° F. Lichtenstein and his coworkers reported that carrots had 96 to 99 percent of aldrin and heptachlor residues in the peel and thus the normal preparatory process of peeling the carrots significantly reduced the residues in the finished product (45). Hemphill et al. reported that washing, trimming, and cooking of green beans brought about a loss of p, p'-DDT, o, p'-DDT, and p, p'-DDE but an increase in p, p'-DDD, and o, p'-DDD. Apparently residues were within the vegetable tissue since washing reduced residues by only an average of 5.3 percent; trimming plus washing reduced residues by 9.2 percent; pressure cooking reduced residues by 62.9 percent; microwave cooking by 47.1 percent; and boiling by 47.1 percent (33).

In a series of reports Lamb and Farrow reported on the effects of home and commercial canning processes on the residues of different

insecticides in different food products (40), (41), (21), (22). In potatoes containing low concentrations of o, p'-DDT; p, p'-DDT; and p, p'-DDE at harvest it was found that commercial washing operations removed about 20 percent of the total DDT residue from potatoes and lye peeling plus washing removed about 94 percent. Thermal processing decreased the residue level another 5 percent (40). Minimum and maximum commercial washing removed 17 and 48 percent of the DDT residues, respectively, from spinach that had been treated with DDT. Use of a detergent in the water increased the percent removal. After commercial water blanching, 38 to 60 percent of the DDT was removed--steam blanching removed little or no residue. After commercial canning and storage for five months, no DDT remained but small amounts of DDD and DDE were found (41). Tomatoes grown on separate plots were treated with DDT, malathion, and carbaryl and harvested the same day, then prepared for serving by commercial canning, home canning, and kitchen procedures and residue determinations were made at appropriate points. Commercial canning and juicing operations removed virtually all DDT, malathion, and carbaryl residues. In all instances, removal of residue was 99 percent or better (21). In an experiment to evaluate the effect of normal commercial washing and canning procedures on residues in a number of products, it was found that washing plus blanching plus canning removed from: tomatoes--99 percent, green beans--83 percent, spinach--91 percent, potatoes--96 percent (22). A pilot study of DDT and its derivatives in apples as affected by preparation procedures showed that washing removed only 10 to 15 percent of the DDT present. Peeling removed an additional 30 to 40

percent from the apples, indicating that wax in the peel must absorb the DDT residues (49).

The above literature indicates that normal preserving processes tend to remove significant percentages of residues from food crops but nevertheless, residues of insecticides are found in foods of both plant and animal origin to an extent that is a concern to scientists of all disciplines, legislators, and leaders in the chemical and food industries. The reason for this concern is not high residues of DDT but the fact that DDT accumulates in the fat and is not readily biodegradable (58), (51), (12), (16), (63), (18), (72), (34), (62), (26).

There have been numerous studies on procedures that go beyond normal food processing operations for the purpose of further reducing insecticide residues (48). However, Liska and Stadelman reported "special processing techniques intentionally to remove all chemical residues have not gained much support. In many cases, industrial concerns do not want to get involved because of the cost and the possibility of adverse publicity. So long as an adequate supply of raw agricultural food is available we can afford this luxury. Someday, as with removal of radioactive materials, it may be necessary to develop techniques to improve adventitious removal or to get complete removal of chemical residues from processed foods" (49).

One reasonably effective method of decreasing DDT residues in milk and meat is to feed activated charcoal to dairy or meat animals along with or after feeding of feeds that contain residues (48), (3). Some workers have tried special washing solutions such as dilute aqueous sodium silicate on pears and apples but others suggest that

peeling is the only effective means of residue removal (15). Others have used oven heat and vapors of common solvents (water, iso-PrOH, benzene, and pentane) to remove residues from alfalfa hay, with relative success (4). It has been found that chicken carcasses contaminated with chlorinated hydrocarbons can have 40 to 50 percent of residues removed by simmering in water at 190° F to 200° F for three hours and 95 percent of residues can be removed when heat treatment of 250° F is applied under pressure for one to three hours. Heptachlor is the exception to the above findings; it was found the most tightly held of the insecticides studied (47).

Microbiological studies have indicated that Aerobacter aerogenes converts DDT to DDD (61), (53), rumen microorganisms in vitro convert DDT to DDD (27), and the aerobic cheese microorganisms Geotrichum candidum (a yeast) and the bacterium Brevibacterium linens degrade DDE and DDT to almost complete disappearance. It is suggested that the pesticide degrading activity of these surface organisms might diffuse into the cheese much as cheese ripening enzymes do (43).

V. EFFECT OF CONTAINER ON PESTICIDE

RESIDUES IN FOOD PRODUCTS

The observation of a possible container effect was reported by Carlin et al. (11). In their study, container effect was mentioned in reference to the metal of the can in which beans were processed. They suggested that ferric ions from the can may have catalyzed decomposition of DDT. Previously, Ott and Gunther (59) suggested that

reductive dechlorination or hydrogenolysis might occur due to contact of DDT with the ferrous ions associated with a stainless steel chromatographic column.

As previously noted, Lamb et al. reported that after commercial canning and five months' storage, no DDT remained, but small amounts of TDE (DDD) and DDE were found in the spinach under study (41). This could plausibly indicate a slow catalytic effect of the container.

Farrow et al. found that in commercially canned spinach, which had been fortified to 10 ppm with p, p'-DDT prior to thermal processing, from 10 to 50 percent of the DDT was converted, depending on the cook time, to TDE during canning at 250° F. DDE did not increase as a result of processing. The conversion was observed in spinach processed in plain tinplate, enameled tinplate, and glass containers (20).

Tressler found that DDT was stable to heat in water and in acid buffer in Pyrex containers but slightly decomposed in these media in tin cans (66).

VI. EXTRACTION AND CLEANUP OF CHLORINATED HYDROCARBON RESIDUES FOR GLC

Before pesticides can be analyzed by chromatographic methods, they must be extracted from the environments in which they occur and isolated from interfering materials. This process is called extraction and cleanup (10). The methods used will vary depending upon the chemical nature of the pesticide and the physical and chemical nature of the material that the residue is in or on (10). Burchfield et al. gave complete instructions on procedures for extracting the various pesti-

cides from water, vegetable foods, meat, and milk (10). The methods officially recognized by the Food and Drug Administration are described in detail in the Pesticide Analytical Manual (5).

Extraction

The methods employed to extract pesticides from plant materials fall into three general categories: surface rinsing, exhaustive extraction (usually in a Soxhlet apparatus), and maceration or blending with solvent (70).

Surface rinsing. This method usually entails the placing of the product to be analyzed in a container, adding the extraction solvent, and rotating or tumbling the mixture end-over-end for a given period of time. Hand agitation of the container, rolling on the floor and/or allowing the product to stand in the solvent for 18 to 24 hours are variations of this method. The advantages of this method are that it is very simple and the extracts are relatively clean (13), (52), (71). This technique has the limitation that it can only be applied to surface residues which will dissolve readily in the extracting solvent and thus does not extract pesticides that may be in the inner tissues rather than on the surface (38).

Exhaustive or Soxhlet extraction. This technique is routinely used with dry samples. Samples are finely subdivided by grinding and then extracted with a suitable solvent. This method has several limitations. For most efficient extraction the sample must be dried. Drying may be accomplished with or without the use of heat; the latter may

be slow, and the former may alter the physical condition of the sample. Drying may cause loss of pesticides which are appreciably water soluble or co-distillable with water (70).

Macerating or blending with solvents. This procedure differs from the surface-rinse method in that the plant material is comminuted to fine particles, usually in the presence of the extraction solvent. This homogenizes solid and solvent together and theoretically provides for the quantitative removal of internal as well as external pesticide residues. The usual procedure is to macerate or blend plant material with the solvent for a few minutes in a high speed blender such as the Waring Blender, allow the solvent to separate from the solid material, and decant the liquid phase. Sometimes centrifugation is used to separate the two phases (70). Originally, samples were blended in the presence of a single, non-polar solvent. Unsatisfactory recoveries of known quantities of added pesticides have demonstrated the inefficiency of the single solvent. It was subsequently found that blending of plant tissues in the presence of a polar-non-polar solvent combination (such as acetone-hexane) produced high recoveries (38).

The extraction of chlorinated hydrocarbon pesticides from lean and fatty animal tissues can be accomplished by grinding the tissues with sand and a dehydrating agent such as sodium sulfate followed by extraction with hexane (10).

Cleanup

The second step of preparing a sample for residue analysis, cleanup, is necessary to remove those interfering substances that

were extracted with the residue from the original food sample. A commonly used cleanup step involves partitioning the sample between two immiscible solvents. This is important in removing pigments, waxes, and fats. The hexane-dimethylformamide system and the petroleum ether-acetonitrile system are examples of solvent systems used (36).

A final cleanup step, if necessary, may be accomplished by filtering the extract obtained from the partitioning step through a column composed of a suitable absorbant material, such as Florosil, Attaclay, Florosil-Celite, magnesium oxide, magnesium oxide-Celite, or aluminum oxide (36).

Following absorption of the sample onto a column, selective elution of the residues from the column is accomplished by passing various solvent elutants, such as ethyl ether in petroleum ether or ethyl ether in hexane, through the column. The elutant is usually concentrated to a definite volume and may be directly injected into a gas chromatograph, or it may be evaporated to dryness and the residue redissolved in another solvent for gas chromatograph, paper, or thin-layer chromatographic analysis. Colorimetric procedures may be used at this point. Thin-layer chromatography has also been used as a cleanup step (36).

VII. GAS-LIQUID CHROMATOGRAPHY OF CHLORINATED HYDROCARBON INSECTICIDES

After the pesticide has been extracted from the sample and interfering compounds removed, the residue is ready for analysis. The techniques available for residue analysis include spectrophotometry;

gas, paper, and thin-layer chromatography; halide analyses; isotope methods; biochemical and biological methods (73).

Gas chromatography is a method of separation of vaporized components which are distributed between a fixed stationary phase and a moving inert gas phase (8).

The stationary phase is prepared by impregnating an inert solid with a nonvolatile organic liquid (8). A free-flowing powder is formed, which is then packed into a tube (called a column) made of glass (10), synthetic polymer, copper, aluminum, or stainless steel (8). Many writers have indicated glass is the best tubing to use because there is evidence that hot metal columns cause the breakdown of some insecticides (59), (6).

The basic gas chromatographic apparatus consists of the units shown in the diagram below.

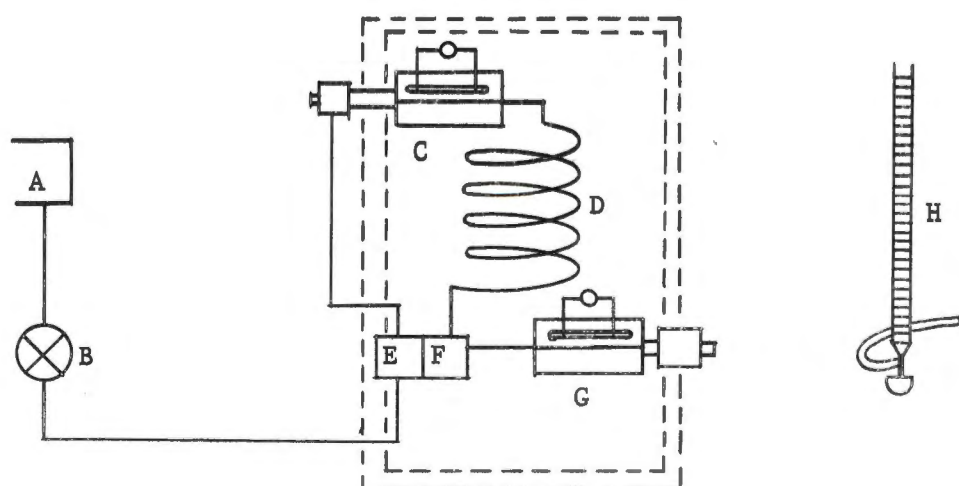


Figure 1. Gas chromatograph apparatus.

A. Carrier gas, B. Pressure regulator, C. Heated inlet, D. Heated column, E. Reference filaments, F. Detector filaments, G. Heated collector, H. Bubble flow meter.

The sample is introduced into the heated inlet C; carrier gas A passing through pressure regulator B will carry the sample through chromatograph column D; chromatographed components from D will be sensed by detector F and the response will be picked up by a recording potentiometer. Fractional collection of effluent gases may be effected at G for further analysis by infrared, ultraviolet, or mass spectrometry. Burette H may be filled with soap solution, attached to gas outlet valve G, and the rate of gas flow may be determined by timing the rise of the soap bubbles in the burette (8), (1).

It should be pointed out that separation occurs in the column of the gas chromatograph apparatus. A solution containing a mixture of pesticides is injected into the hot column and the pesticides will vaporize and be carried through the column by the moving carrier gas. The pesticides travel through the column at different rates because of their different partition coefficients between the liquid stationary phase and the mobile gas phase. Consequently, they emerge from the far end of the column at different times, and pass through the detector which electronically detects their concentrations. The response of the detector is fed into a strip chart recorder so that the presence of each compound is registered as a measurable peak as it emerges from the chromatograph. The horizontal distance of the peak from the origin is called the retention time. It indicates the time the compound is retained by the apparatus and is characteristic for each pesticide. The area under the peak is proportional to the amount of compound which has passed through the detector. Thus through the use of gas chromatography, it is possible to obtain

both qualitative and quantitative measure of the pesticides which are present in a sample (8), (69).

Several types of detectors are successful in the detection and measurement of pesticide residues but the most sensitive is the electron capture detector (10). The basic part of the electron capture detector is the electron affinity cell shown below.

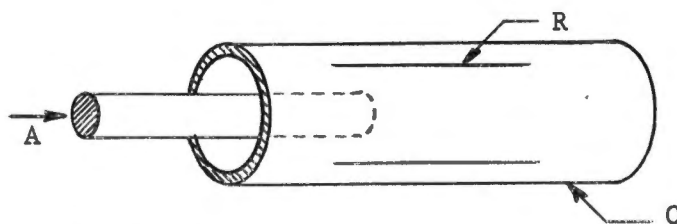


Figure 2. Electron affinity cell.

A is the anode and the gas entrance, C is the cathode and R is the radioactive beta source. Suitable sources are tritium, strontium 90, promethium 147, or nickel 63. With only pure gas in the system, a potential applied to the electrodes will cause electrons to flow from the beta source R to the anode A and thus establish a flow of current. The applied potential determines the number of electrons collected at the anode from the beta source. When a substance which can absorb electrons enters the cell at A, part of the electrons will be removed in the form of negative molecular ions. This decrease in the number of electrons from the source to the anode causes a corresponding decrease in the current. The decrease in current is amplified and displayed on the strip chart recorder (28), (60).

After obtaining a strip chart from the recorder displaying peaks caused by the injected samples and standards, the concentrations of the unknown samples may be calculated from the peak areas of the

standards. Gaul (29) describes five methods for quantifying gas chromatographic peaks and concludes that they are not significantly different. These methods 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

On December 16, 1969, seventy-two pounds of chopped frozen turnip greens (two cartons, each containing 12 three-pound boxes) were purchased from a local distributor for use in this experiment and stored at 6 to 10° F.

The greens were thawed when needed by placing a three-pound box of greens in a microwave oven for 2 to 4 minutes. This thawed the mass enough that it could be broken up and placed into number 303 cans.

The 48 number 303 cans used in this experiment were provided by Bush Brothers, Incorporated from the Heekin Can Company manufacturing plant adjacent to their cannery at Dandridge, Tennessee. Twenty-four of the cans used were plain tin plate and the other 24 were lined with J E enamel, an epoxy-phenolic coating (30).

The cans were filled approximately half full with turnip greens, one ml of 2500 µg/ml DDT was added, then greens added to a total weight of 330 grams, and finally 95 ml of a 2 percent NaCl brine was added before the can was steam evacuated and sealed, giving a total weight of 426 grams.

After sealing, the cans were thermally processed, four cans at a time, in an upright steam retort equipped with automatic temperature/pressure control and a recording instrument for indicating process times and temperatures. The Fo (commercial sterilization) values used

ranged from $F_o = 2.4$ to $F_o = 9.4$ with B_D values (process time) varying from 39 minutes to 85 minutes as shown in Table 1. The recommended process is $F_o = 7$. F_o is the equivalent value of the process in terms of minutes at 250°F , when no time is required in heating to 250°F or cooling to sublethal temperatures (2).

TABLE 1
PROCESS CONDITIONS FOR PROCESSING TURNIP GREENS
FORTIFIED WITH TECHNICAL DDT

RT	Process Time in Minutes	Time Equivalent to Minutes at 250°F
116°C (240°F)	59*	3.6
	72	6.4
	85	9.4
121°C (250°F)	39	2.4
	45	4.3
	50	6.8

*Process time in minutes at each set of conditions.

The F_o values were calculated using the Formula Method of Ball (2), and the following values(7):

j (lag factor) = 1.79

f_h (first heating curve slope) = 28.1 min.

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

$x'bh$ (number of minutes from beginning of process to the point of break in the heating curve) = 34

Retort temperature = 116 and 121° C (240 and 250° F)

Initial temperature = 46° C (115° F)

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

Corresponding F_o (sterilization value) = 2.4, 4.3, 6.8, 3.6, 6.4, 9.4

The treatment consisted of processing four cans at each set of conditions (i.e., F_o = 3.6, Retort temperature = 116° C; F_o = 2.4, Retort temperature = 121° C; etc.) Two of the cans had enameled interiors and two were plain tin plate. Immediately after heat processing, the cans were cooled by placing them in cold running water (18° C) for 20 minutes. This treatment was carried out until all sets of conditions shown in Table 1 were accomplished, four cans to each set of conditions, for a total of 24 cans. The entire procedure was then repeated giving a final total of 48 cans.

As Table 1 shows, there were six different actual F_o values, but for convenience of comparison and statistical analysis on the computer, the lowest F_o value for 116° C and 121° C were both labeled F_o 4, the median F_o values as F_o 7, and the highest F_o values as F_o 10. Thus, the first cans that were cooked at retort temperature 116° C for 59 minutes were labeled F_o 4 116 E_1 , F_o 4 116 E_2 , F_o 4 116 P_1 , F_o 4 116 P_2 . The letter E indicates an enameled can, the letter P indicates a plain can. The subscripts 1 and 2 indicate the observation. These four cans made up the first replication. Following this example, the experimental design is outlined in Table 2.

The cans were stored at room temperature. The P_1 , P_2 , E_1 , and E_2 cans with F_o values of 4, 7, and 10 were processed at 116° C on

TABLE 2
LABELS ON CANS

RT	Conditions		Replication I		Replication II	
	B _B	Fo	Observation 1	Observation 2	Observation 1	Observation 2
116° C	59	3.6	Fo 4 116 E ₁	Fo 4 116 E ₂	Fo 4 116 E ₃	Fo 4 116 E ₄
			Fo 4 116 P ₁	Fo 4 116 P ₂	Fo 4 116 P ₃	Fo 4 116 P ₄
	72	6.4	Fo 7 116 E ₁	Fo 7 116 E ₂	Fo 7 116 E ₃	Fo 7 116 E ₄
			Fo 7 116 P ₁	Fo 7 116 P ₂	Fo 7 116 P ₃	Fo 7 116 P ₄
121° C	85	9.4	Fo10 116 E ₁	Fo10 116 E ₂	Fo10 116 E ₃	Fo10 116 E ₄
			Fo10 116 P ₁	Fo10 116 P ₂	Fo10 116 P ₃	Fo10 116 P ₄
	39	2.4	Fo 4 121 E ₁	Fo 4 121 E ₂	Fo 4 121 E ₃	Fo 4 121 E ₄
			Fo 4 121 P ₁	Fo 4 121 P ₂	Fo 4 121 P ₃	Fo 4 121 P ₄
121° C	45	4.3	Fo 7 121 E ₁	Fo 7 121 E ₂	Fo 7 121 E ₃	Fo 7 121 E ₄
			Fo 7 121 P ₁	Fo 7 121 P ₂	Fo 7 121 P ₃	Fo 7 121 P ₄
	50	6.8	Fo10 121 E ₁	Fo10 121 E ₂	Fo10 121 E ₃	Fo10 121 E ₄
			Fo10 121 P ₁	Fo10 121 P ₂	Fo10 121 P ₃	Fo10 121 P ₄

December 22, 1969 and opened on January 2, 1970--a span of 11 days. The Fo 10 116 E₃, E₄, and P₃ and P₄ cans were also processed and opened on the same respective dates. The P₃, P₄, E₃ and E₄ cans for Fo 4 and Fo 7 values at 116° C were processed and opened, respectively, on December 23, 1969, and January 2, 1970. All cans processed at 121° C were processed on December 24, 1969, and opened on January 2, 1970.

On January 2, 1970 all 48 cans were opened, their contents quantitatively transferred to a quart Ball jar and blended on an Oster blender for two minutes. In each case, 100 ml of distilled water were added to the sample to aid blending. Approximately 200 grams of each blended sample were transferred to a labeled jar and stored at 6 to 10° F until time for further analytic procedures.

II. SAMPLE CLEANUP PRIOR TO GAS CHROMATOGRAPHY

The Mills, Onley, Gaither Procedure (55) of cleanup for chlorinated pesticide residues in nonfatty foods was used to prepare all samples for gas chromatography.

Reagents

Methyl cyanide (CH₃CN) Analytical Reagent (AR) grade. Purified by glass distilling one gallon CH₃CN in the presence of 1 ml of 85 percent phosphoric acid and 30 g. phosphorous pentoxide at 81 to 82° C. The first and last 100 ml fractions of each gallon distilled were discarded.

Celite 545--(Johns-Manville Company).

Diethyl ether--AR grade, anhydrous. Purified by washing twice with twice its volume of distilled water. The ether was then washed with 50 to 100 ml saturated NaCl solution with all aqueous layers discarded. The washed ether was stored in a $\frac{T}{S}$ flask in the presence of excess anhydrous Na_2SO_4 .

Elution solvent--6 percent diethyl ether in redistilled petroleum ether.

Florosil--80 to 100 mesh, pesticide grade. Activated at 623°C by manufacturer and stored at 155 to 160°C for at least 72 hours prior to use. (Floridan Company, Tallahassee, Florida)

Hexane--pesticide quality, Matheson, Coleman, and Bell Chemical Company, Norwood, Ohio. Guaranteed to not contain more than 10 ppt of compounds that interfere with gas chromatography of chlorinated pesticides.

Petroleum ether--AR grade. Redistilled at 30 to 60°C with the first and last 50 ml fractions of each gallon distilled discarded.

Sodium sulfate--AR grade, anhydrous.

Graduated cylinders--100 ml and 250 ml with $24/40 \frac{T}{S}$ glass stoppers.

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

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

Vacuum filtration apparatus--3.5 inch Buchner funnel fitted with No. 8 rubber stopper; $35/42$ to $24/40 \frac{T}{S}$ reducing adapter; and $24/40 \frac{T}{S}$ vacuum take-off adapter (Kontes Glass Company).

Chromatographic columns (Kontes 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 $\frac{T}{S}$ glass stoppers.

Procedure

Fifty grams of the homogenized sample were transferred from the storage jar into a blender jar; three teaspoons of Celite and 150 ml of CH_3CN were added, the blender jar was capped with an Osterizer blending head and the sample was blended for 60 seconds.

A vacuum filtration apparatus was attached to a 250 ml graduated cylinder, and the blended sample filtered by vacuum. The blender jar and filter cake were rinsed with 50 ml of CH_3CN . The volume of the methyl cyanide filtrate was observed and recorded.

The methyl cyanide filtrate was transferred to a 1000 ml separatory funnel. One hundred ml of petroleum ether were measured into the graduated cylinder used for the methyl cyanide filtrate and the volume recorded. This ether was then poured into the separatory funnel, followed by about 10 ml of saturated NaCl solution and 600 ml distilled water. The separatory funnel was shaken for one to two minutes to force the pesticide residue from the methyl cyanide phase into the petroleum ether phase. The solution was allowed to separate and the aqueous layer of methyl cyanide was discarded.

The petroleum ether extract was rinsed twice with 200 ml distilled water by gently swirling (to prevent the formation of

emulsions) to remove any CH_3CN remaining in the ether. The washings were discarded. The petroleum ether was transferred to a 100 ml glass-stoppered graduated cylinder and the volume recorded. One teaspoon (about 0.5 inch layer) anhydrous Na_2SO_4 was added to the graduated cylinder to remove water. The ether-salt mixture was never permitted to stand for more than one hour to avoid loss of pesticide residues (56).

The chromatographic column was prepared by plugging the bottom opening with a pinch of quartz-glass wool. About forty g of reactivated Florosil was added to the column and topped with a teaspoon of anhydrous Na_2SO_4 . The column was packed by means of vibration for two minutes and pre-wetted with 60 ml of redistilled petroleum ether. A Kuderna-Danish concentration flask and collection tube were placed at the bottom of the column as a receiver and the pre-wetting petroleum ether was allowed to drip into it. Just as the pre-wet ether sank into the top of the Florosil column, the petroleum ether extract of the sample was poured onto the column and allowed to percolate through into the Kuderna-Danish collection flask and tube. After the meniscus of the sample had moved into the column, 200 ml of the 6 percent diethyl ether in petroleum ether elution solvent were added. Sample and elutant traveled through the column at a rate of 5 to 10 ml/minute. After all solutions had passed through the column and were collected in the Kuderna-Danish collection flask, a 3-ball Snyder column was attached and the apparatus placed on a steam bath where the entire collection was concentrated to a volume of 3 to 10 ml. The collection tube was then removed and placed in a forced air oven at 36°C and evaporated to dryness. The residue in the collection tube (incidentally,

not visible) was dissolved in 10 ml of pesticide quality hexane and transferred to a 15 ml flint glass bottle, tightly capped, labeled, and stored at 4° C until time for gas chromatographic analysis.

III. CLEANUP OF CAN ENAMEL PRIOR TO GAS CHROMATOGRAPHY

After the turnip greens had been removed from all 48 cans, the enamel was removed from the 24 enameled cans by the following procedure:

Both ends of the can were removed with a can opener. The can was placed in a 1000 ml beaker of concentrated NaHCO_3 solution and the negative lead of a 6-volt battery charger was clipped onto the can; the positive lead of the charger was clipped to a carbon rod inserted through, but not touching the can into the NaHCO_3 solution. The charger was plugged up and a vigorous evolution of gas was visible from the solution. After about one hour the enamel loosened from the can and could be easily pulled off and placed into a labeled jar. After the enamel was removed from the can body, the same solution was used to remove the enamel from the two ends of the can. After removing the enamel, the solution was evaporated to about 500 ml on a Kjeldahl heater. The solution was then filtered on a Buchner funnel by vacuum and the filtrate transferred to a labeled separatory funnel. The enamel was blended with Celite and methyl cyanide by the same procedure described for the greens. The blended enamel plus CH_3CN was filtered through the same Buchner funnel and filter paper used to filter the solution. A final 50 ml of the CH_3CN were used to rinse the solution beaker and the blender jar before being poured into the Buchner funnel.

This final filtrate was added to the separatory funnel with the electrolytic solution and from this point onward the enamel sample was cleaned up for gas chromatography following the same procedure described for greens.

Thus, a total of 72 samples were prepared for gas chromatographic analysis; 24 extracted from the greens of the plain cans, 24 from the greens of the enameled cans, and 24 from the enamel of the enameled cans.

IV. GAS CHROMATOGRAPHIC ANALYSIS OF SAMPLES

A Micro Tek Model MT-220 gas chromatograph equipped with a Nickel-63 high temperature electron-capture detector and a 6 ft. x 4 mm i.d. U-shaped column was used to analyze the residues in all samples. The column was packed with SE 30 on gas chrom Q and was used throughout the experimental work. Purified nitrogen was used as the carrier gas with a flow rate of approximately 80 cc/minute and an inlet pressure of 40 p.s.i. Column temperature was maintained at 180 to 195° C, the detector at 350° C, and the inlet temperature at 230° C. The detector attenuation (input x output) was set at 10 x 32. About half the samples were run a second time and the attenuation was then set at 10 x 16.

Prepared analytical standard solutions of p, p'-DDT, p, p'-DDD, and DDE (100 ppm-w/v) obtained from Analabs, Inc., Hamden, Conn. were used to form the standard solutions by combining and diluting them with pesticide quality hexane to concentrations of 0.1 ppm, 0.5 ppm, 1.0 ppm, 5.0 ppm, and 10.0 ppm.

A Hamilton 10 μ l syringe was used to inject the sample into the chromatograph. Injections of 1.0 μ l were used the first time through; injections of 3.0 μ l were used on the samples and standards that were repeated.

Relative retention times of DDT, DDD, and DDE were found by injecting standard solutions of these compounds into the chromatograph and then measuring the distance from the first line of the solvent peak to the apex of the solution peak in mm. The combined standard solution peaks occurred in the order DDE, DDD, and DDT.

The residue of a sample was identified by measuring the retention time and comparing this to the retention times of the known standards. All samples showed residues of all three compounds in the DDT "family."*

The residues were quantified by calculating the product of peak height and width of peak at half-height (29) and comparing them with the products obtained from the peaks of the standard solutions. These comparisons were used to calculate the ppm of pesticide residues in the 10 ml concentrated solutions of the samples.

In order to form a basis for statistical analysis the values in ppm for the 10 ml concentrated solutions were converted to milligrams of residue in the greens of each can and milligrams of residue

* A standard of o, p'-DDT was not used, but when an unknown peak from GLC consistently showed up in the enamel the relative retention time was found to be that given in the literature for o, p'-DDT. This compound was quantified by calculating peak area and comparing with the appropriate standard areas (10).

that had adhered to the enamel of the enameled cans. The following calculations were used:

A. $\text{ppm} \times 10 = \text{total } \mu\text{g in 10 ml of concentrated solution.}$

(Or 50 g. of greens)

B. $\text{Total } \mu\text{g} \times .001 = \text{total mg in 10 ml of concentrated solution.}$

C. For total mg/can in the greens:

$$\text{Total mg in 10 ml conc. soln} \times \frac{426 + 100}{50}$$

Where 50 is the weight in grams of homogenized greens in the original sample, 426 is the total weight of the can contents, and 100 is the volume of distilled water added in the first blending.

D. To obtain total mg/can in the enamel formulas A and B

above are sufficient since the sample of enamel represents 100 percent of the enamel of the can.

V. STATISTICAL ANALYSIS

All statistical analyses were computed from the experimental data found in Tables A-1 and A-2 in the Appendix.

The data were adjusted to give a composite DDT residue by the following formula:

Composite DDT = $\text{DDT} + 1.079 \text{ DDD} + 1.111 \text{ DDE}$, where 1.079 and 1.111 are the ratios of the molecular weight of DDT to the molecular weight of DDD and DDE, respectively.

The experimental data given in the tables are parts per million values. In order to convert these data to total milligrams they were multiplied by .1052. This procedure was based on the following reasoning: one millileter of 2500 ppm DDT solution contains 2.5

milligrams of DDT. Therefore, 2.5 milligrams of DDT were added to each can prior to thermal processing. Each can then contained 330 grams of greens, 95 grams of brine, and one gram of the DDT solution for a total content weight of 426 grams. To aid blending 100 ml of distilled water were added. Only 50 grams of blended contents from each can were analyzed, so the ppm found in this aliquot was multiplied by .01 to convert ppm to the mg of residue in the 50 grams; this figure was then multiplied by $\frac{526}{50}$, or 10.52, to compute the mg of residue in the entire contents of the can. Obviously, these two steps can be consolidated into one by multiplying the ppm in the 50 gram samples by .1052, and this was programmed as an additional step into the ANOVAR subroutine (9) on the IBM model 7040 digital computer at the University of Tennessee Computing Center to perform the analysis of variance calculations.

To avoid incorrect results, the ppm figures from the analysis of the enamel were divided by 10.52 and the resultant figures punched into data cards so that when the computer multiplied these input data by .1052, it was in effect multiplying the enamel ppm by .01. By this process the total in the enamel of each can was calculated and expressed as milligrams.

The Duncan's Multiple Range Test (for comparing means of o, p'-DDT resulting from cook times) was carried out by calculator in accordance with the instructions of LeClerc (42).

CHAPTER IV

RESULTS AND DISCUSSION

Statistical results concerning the DDT residues in the thermally processed DDT-fortified turnip greens are shown in Tables 3 through 11.

TABLE 3
DUNCAN'S MULTIPLE RANGE TEST FOR o, p'-DDT

Cook Time	Longest (Fo 10)	Shortest (Fo 4)	Median (Fo 7)
Mean Mg Residue	.008	<u>.018</u>	<u>.020</u>

The analysis of variance appears in Tables 4 and 5. Table 4 shows the analysis of variance of residues from greens. Table 5 shows the analysis of variance after the residues from the enamel were added to the residues from the greens of the same can.

The greens of the enameled cans contained significantly less p, p'-DDT residues than the greens of the plain cans. Composite DDT, DDD, and DDE were not significantly different in the greens due to can type. No o, p'-DDT residues were found in the greens of either type of can. Different cooking temperatures effected greater differences than any other factor. All residues were higher in greens processed at 121° C than in those processed at 116° C. Different

TABLE 4
ANALYSIS OF VARIANCE OF INSECTICIDE RESIDUES IN GREENS
OF PLAIN CANS AND GREENS OF ENAMELED CANS

Source	df	Composite- DDT MS	p,p'-DDT MS	o,p'-DDT MS	DDE MS	DDD MS
Temp	1	1.3293*	.1298*	0	.0512	.2517**
Time	2	.1371	.0255	0	.0028	.0317
Can	1	.1946	.1496*	0	.0014	.0078
Te x ti	2	.4330	.0464	0	.0211	.0783
Te x Can	1	.3672	.0669	0	.0281	.0223
Reps	1	.0628	.0088	0	.0060	.0043
Error	<u>35</u>	.1843	.0264	0	.0123	.0267
Total	47					

*Significant at 0.05 level.

**Significant at 0.01 level.

TABLE 5

ANALYSIS OF VARIANCE OF INSECTICIDE RESIDUES IN GREENS OF PLAIN CAN
AND GREENS AND ENAMEL OF ENAMELED CAN

Source	df	Composite- DDT MS	p,p'-DDT MS	o,p'-DDT MS	DDE MS	DDD MS
Temp	1	.9564*	.0717	.0020**	.0454	.2204**
Time	2	.2132	.0467	.0007*	.0031	.0371
Can	1	.0003	.0369	.0111**	.0027	.0000
Te x Ti	2	.4722	.0608	.0002	.0210	.0843
Te x Can	1	.6099	.1233	.0020**	.0327	.0330
Reps	1	.0933	.0143	.0000	.0059	.0065
Error	<u>35</u>	.1862	.0265	.0002	.0126	.0263
Total	47					

* Significant at 0.05 level.

** Significant at 0.01 level.

TABLE 6
MEAN TOTAL MG. RESIDUE--GREENS ONLY

Residue Source	Comp	p,p'-DDT	o,p'-DDT	DDE	DDD
Greens of Plain Can	1.695 ^a	0.344 ^d	0.00 ^f	0.299 ^h	0.944 ⁿ
Greens of Enameled Can	1.568 ^a	0.233 ^c	0.00 ^f	0.310 ^h	0.918 ⁿ

Figures followed by the same letter are not significantly different.

TABLE 7
MEAN TOTAL MG. RESIDUE PER CAN INCLUDING ENAMEL

Residue Source	Comp	p,p'-DDT	o,p'-DDT	DDE	DDD
Greens of Plain Can	1.695 ^a	0.344 ^d	0.000 ^f	0.299 ^h	0.944 ⁿ
Greens + Enamel of Enamel Can	1.690 ^a	0.289 ^d	0.030 ^g	0.314 ^h	0.944 ⁿ

Figures followed by the same letter are not significantly different.

TABLE 8
MEAN TOTAL MG. RESIDUE PER CAN, ENAMEL ONLY

Residue Source	Comp	p,p'-DDT	o,p'-DDT	DDE	DDD
Enamel	0.122	0.056	0.030	0.004	0.026

TABLE 9

MEAN TOTAL MG. RESIDUE RESULTANT OF COOK
TEMPERATURE--GREENS ONLY RESIDUE

Residue Source Greens Only	Comp.	p,p'-DDT	o,p'-DDT	DDE	DDD
116° C	1.465 ^a	0.236 ^c	0.00 ^e	0.272 ^f	0.859 ^g
121° C	1.798 ^b	0.340 ^d	0.00 ^e	0.337 ^f	1.003 ^h

(Figures superscripted by the same letter are not significantly different.)

TABLE 10

MEAN TOTAL MG. RESIDUE RESULTANT OF COOK
TEMPERATURE--INCLUDING ENAMEL RESIDUE

Residue Source Greens and Greens + Enamel	Comp.	p,p'-DDT	o,p'-DDT	DDE	DDD
116° C	1.551 ⁱ	0.278 ^k	0.022 ^m	0.276 ^p	0.876 ^r
121° C	1.834 ^j	0.355 ^k	0.009 ⁿ	0.337 ^p	1.012 ^s

(Figures superscripted by the same letter are not significantly different.)

TABLE 11

MEAN TOTAL MG. RESIDUE RESULTANT OF COOK TEMPERATURE--ENAMEL ONLY

Residue Source Enamel Only	Comp	p,p'-DDT	o,p'-DDT	DDE	DDD
116° C	0.076 ^t	0.042 ^v	0.022 ^x	0.004 ^z	0.017 ^a
121° C	0.036 ^u	0.015 ^w	0.009 ^y	0.000 ^z	0.009 ^b

(Figures superscripted by the same letter are not significantly different.)

commercial sterilization (F_0) values caused no significant differences in the residues of the greens.

When the data of the residues in enamel were pooled with the residues in the greens of enameled cans, two important changes occurred (Table 5, page 36). There was no longer a significant difference between can types with regard to p, p'-DDT residues and, secondly there was a highly significant difference ($p > 0.01$) between can types in o, p'-DDT. The second change occurred because o, p'-DDT was found only in the can enamel--not in the greens of either can type.

Since o, p'-DDT was not found in the greens of either can type, and the p, p'-DDT was less in the enameled can than in the plain can, it may be concluded that p, p'-DDT is partially absorbed by the enamel and some of that absorbed is rearranged to the o, p'-isomer. Another possible explanation for the o, p'-isomer in the enamel is that the o, p'-isomer may have been present in the greens of both cans but was completely destroyed by thermal processing in the plain cans and partially absorbed by the enamel in the enameled cans.

All residues were higher in the enamels processed at 116°C than in those processed at 121°C (Table 11, page 38). Since o, p'-DDT was found only in the enamel, the statistics describing it in Table 5, page 36, tell essentially how the factors of time and temperature affected it in the enamel. Tables 5 and 11 show that the o, p'-DDT residues were significantly less when the cook temperature was 121°C . Table 3, page 34, and Table 5 show that the longest cook times resulted in significantly less of the o, p'-isomer than resulted from median and shortest cook times. Table 3 further indicates that the median cook times may be optimum for occurrence of the o, p'-isomer residue.

In review, this discussion is an attempt to answer some salient questions about the experiment. First, did the can enamel make a difference in the residues? Yes. Greens from the enameled cans contained significantly less p, p'-DDT (Tables 4 and 6, pages 35 and 37). This is further indicated by the fact that there was no longer a significant difference between can types when the residues in the enamels were added to the residues in greens of the enameled cans (Table 5, page 36). Second, did the other factors, cook time and cook temperature, make a difference in the residues in greens? Yes. Only temperature caused different residue levels, cook time had no effect (Table 4 and Table 9, page 38) on residue levels in the greens.

CHAPTER V

SUMMARY

This experiment was conducted to determine the effect of epoxy-phenolic can lining on DDT residues in canned turnip greens during thermal processing.

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

1. The can enamel absorbed p, p'-DDT during thermal processing, making this residue significantly less ($p > 0.05$) in the greens of enameled cans than in greens of plain cans cooked at the same time and temperature.
2. The p, p'-DDT was apparently partially converted to o, p'-DDT after absorption to the can enamel.
3. Can enamel did not significantly absorb DDE or DDD during thermal processing.
4. Median cook times (near those recommended by the National Cannery Association) were optimum for residues of o, p'-DDT to occur in the enamel.
5. A cooking temperature of 121°C achieved significantly higher residues of p, p'-DDT in the can enamel than a cook temperature of 116°C .
6. All insecticide residues except DDE were significantly lower in greens cooked at 116°C than in those cooked at 121°C , regardless of the type of can the greens were contained in. This is the inverse of what was expected.

To afford a better explanation of the events associated with the epoxy-phenolic can lining, it would be necessary to have more complete knowledge of the chemistry of epoxy-phenolic can lining than is currently available in the literature. DDT is known to have a high affinity for plant and animal fats, perhaps there are some chemical similarities between the can enamel studied and those fat compounds. A study of effects of "cook-in" plastic bags on DDT might be worthwhile since the most toxic parent compound of DDT is absorbed by the can lining studied. Partial cooking, prior to canning, in vats or heat exchangers lined with corrugated epoxy-phenolic resins might be more absorbent than can linings since the resin-to-food surface ratio would be larger.

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APPENDIX

APPENDIX

TABLE A-1

PPM RESIDUES IN 10-ML SOLUTIONS CONTAINING THE
RESIDUES FROM 50 GRAMS OF GREENS

Can Label	Composite DDT	p,p'-DDT	o,p'-DDT	DDE	DDD
Fo 4 116 E1	14.530	2,480	0.000	3.070	8.010
E2	13.510	2.280	0.000	2.660	7.670
E3	19.260	4.830	0.000	3.250	10.030
E4	14.530	2.480	0.000	3.070	8.010
Fo 7 116 E1	15.230	2.070	0.000	3.120	8.980
E2	13.560	1.610	0.000	2.600	8.400
E3	12.440	1.630	0.000	2.600	7.340
E4	18.540	2.820	0.000	3.890	10.560
Fo10 116 E1	11.900	1.320	0.000	2.570	7.160
E2	15.520	1.700	0.000	3.540	9.160
E3	12.330	1.140	0.000	2.770	7.520
E4	8.450	.500	0.000	1.230	6.100
Fo 4 116 P1	16.250	3.500	0.000	2.710	9.030
P2	15.830	3.530	0.000	2.710	8.610
P3	17.740	3.310	0.000	3.610	9.660
P4	16.540	3.280	0.000	3.010	9.190
Fo 7 116 P1	17.080	3.270	0.000	2.920	9.790
P2	5.970	.350	0.000	.400	4.800
P3	7.010	.600	0.000	.620	5.300
P4	9.650	1.300	0.000	1.200	6.500

TABLE A-1 (continued)

Can Label	Composite DDT	p,p'-DDT	o,p'-DDT	DDE	DDD
Fo10 116 P1	8.540	.700	0.000	.650	6.600
P2	17.130	3.810	0.000	2.920	9.340
P3	15.070	2.280	0.000	3.100	8.660
P4	17.560	3.150	0.000	3.790	9.450
Fo 4 121 E1	17.240	2.980	0.000	3.220	9.900
E2	14.510	2.860	0.000	2.740	7.980
E3	19.130	3.230	0.000	3.360	11.280
E4	18.180	3.020	0.000	3.700	10.240
Fo 7 121 E1	20.310	3.820	0.000	3.890	11.280
E2	22.420	5.060	0.000	4.670	11.280
E3	9.890	.600	0.000	2.100	6.450
E4	10.730	.400	0.000	.900	8.650
Fo10 121 E1	13.830	1.100	0.000	3.200	8.500
E2	10.520	.750	0.000	1.410	7.600
E3	14.300	1.780	0.000	3.360	8.140
E4	16.750	2.610	0.000	3.750	9.240
Fo 4 121 P1	18.640	4.300	0.000	3.580	9.600
P2	8.830	1.350	0.000	1.100	5.800
P3	11.460	1.350	0.000	1.820	7.500
P4	23.860	5.700	0.000	4.920	11.760
Fo 7 121 P1	23.850	5.310	0.000	4.920	12.120
P2	19.230	3.810	0.000	3.510	10.680
P3	25.500	5.310	0.000	5.060	13.500

TABLE A-1 (continued)

Can Label	Composite DDT	p,p'-DDT	o,p'-DDT	DDE	DDD
P4	20.250	4.640	0.000	3.390	10.980
Fo10 121 P1	17.070	3.600	0.000	2.950	9.450
P2	12.380	1.150	0.000	1.850	8.500
P3	17.130	3.860	0.000	3.790	8.400
P4	24.090	9.080	0.000	3.720	10.080

TABLE A-2

PPM RESIDUES IN 10-ML SOLUTIONS CONTAINING THE
RESIDUES FROM THE CAN ENAMEL

Can Label	Composite DDT	p,p'-DDT	o,p'-DDT	DDE	DDD
Fo 4 116 E1	2.297	1.060	.635	.117	.437
E2	2.292	1.060	.635	.117	.437
E3	2.476	1.454	.366	.076	.531
E4	2.476	1.454	.366	.076	.531
Fo 7 116 E1	1.647	.615	.368	.080	.514
E2	.974	.560	.300	.056	.048
E3	1.839	.908	.463	.115	.313
E4	2.402	.887	1.013	.130	.332
Fo10 116 E1	1.648	.607	.327	.094	.563
E2	.323	.171	.086	.010	.053
E3	1.097	.510	.285	.065	.214
E4	.352	.174	.086	.010	.076
Fo 4 121 E1	.664	.380	.171	.010	.095
E2	.386	.233	.081	.010	.057
E3	.890	.257	.228	.010	.366
E4	.948	.532	.257	.015	.133
Fo 7 121 E1	.236	.138	.066	.010	.019
E2	.915	.352	.399	.010	.143
E3	1.613	.595	.352	.010	.608
E4	.462	.257	.071	.010	.114

TABLE A-2 (continued)

Can Label	Composite DDT	p,p'-DDT	o,p'-DDT	DDE	DDD
Fo10 121 E1	.472	.257	.124	.010	.076
E2	.303	.138	.114	.101	.038
E3	.305	.157	.090	.010	.043
E4	1.005	.066	.057	.771	.024

VITA

Bernard Phelps Moss, son of William F. and Elizabeth Moss, was born May 18, 1940 near Petersburg, Illinois. He received his elementary education in small rural schools in central Illinois and south central Kentucky and graduated from Hiseville High School, Hiseville, Kentucky in 1958. He attended Berea College, Berea, Kentucky from 1958 to 1962 where he received the B. A. degree in Biology, June, 1962.

From September, 1962 to August, 1963 he was employed by the health department at Ashland, Kentucky as a sanitarian. His military service was with the U. S. Air Force from August, 1963 to December, 1967, as a munitions officer in Thailand and various bases in the western United States.

He entered the University of Tennessee in January, 1968, as an adult special student and was admitted to the graduate school in June, 1968. From August, 1968 to August, 1970 he was employed as an Assistant-in-Food Technology while studying to complete the requirements for the Master of Science degree.

He married the former Betty Jo Girdler on June 1, 1961, and they have one daughter, Sylvia Karen, age 5.