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To the Graduate Council:

I am submitting herewith a thesis written by James G. Fair entitled "Effects of Epoxy-phenolic Enamel on DDT, DDD, and DDE During Blanching and Thermal Processing of 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.

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EFFECTS OF EPOXY-PHENOLIC ENAMEL ON DDT, DDD, AND DDE DURING BLANCHING AND THERMAL PROCESSING OF TURNIP GREENS

A Thesis

Presented to

the Graduate Council of

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In Partial Fulfillment

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by

James G. Fair

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ABSTRACT

The agricultural applications of pesticides have played an important role in increasing the yield of food products throughout the world. It is unfortunate that residues of pesticides are sometimes found on food products before and after processing. Attempts have been made to reduce the levels of various pesticides in food products, but they have been relatively unsuccessful. This study was designed to determine the effect of epoxy-phenolic enamel on levels of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and its metabolites in turnip greens during blanching and thermal processing.

Turnip greens were grown on three plots and were given periodic spray applications of DDT. The greens were harvested, washed, and blanched by steam for three minutes or in boiling water for three or five minutes. A steam-jacketed kettle was used for water blanching and was operated with and without an interior lining composed of epoxy-phenolic enamel coated tin plate. Greens blanched by steam or in water for three minutes were thermally processed subsequently at 121°C for 45 minutes. The greens were thermally processed in plain tin plate cans or epoxy-phenolic enamel lined cans.

Samples of greens were taken at the following times: prior to the first application of DDT, prior to washing, and immediately following each of the process treatments. The pesticides were extracted by macerating the plant tissue with acetonitrile followed by eluting the extract through a column of Florisil to remove other interfering

materials. The extract was analyzed quantitatively by gas liquid chromatography to determine levels of DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane], o,p'-DDT [1-trichloro-2-o-chlorophenyl-2-p-chlorophenylethane], and p,p'-DDT [1,trichloro-2,2-bis(p-chlorophenyl)ethane].

The data indicated that low concentrations of o,p'-DDT and p,p'-DDT were present in the turnip greens prior to the first application of the pesticide material. Residues remaining in or on the greens at the time of harvest were in excess of the established tolerance limits. Blanching methods and blanching times in water did not produce significant different levels of residue. Enamel lining in kettles or cans did not significantly reduce the levels of pesticides during blanching or thermal processing.

From the results of this study, it is evident that epoxy-phenolic enamel does not have an absorptive capacity for DDT or its metabolites, and is not an effective means of reducing residue levels.

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CHAPTER I

INTRODUCTION

The high quality, variety, and abundance of the American food supply is unequaled in the world today and is largely dependent upon the proper use of pesticides (67).* Without such usage, many important food crops could not be produced in this country, or at best, production would be limited and very costly to the consumer. This does not imply that other factors such as fertilization, mechanization, improved varieties, good cultural practices and things of this nature are not also important. All of these factors are essential to effective agricultural production; however, their effectiveness would be nullified greatly without the use of pesticides.

It has been determined that in agriculture no less than a third of the crop is lost because of pests and diseases, and if they were not systematically controlled, the loss would be even greater. Thus, according to the calculations of specialists, if the diseases and pests were not systematically controlled, then at best no more than 37 percent of a normal harvest of potatoes could be gathered, no more than 22 percent of cabbage, 10 percent of apples, nor 9 percent of peaches (59, 67).

It was at the end of World War II that many pesticides which were developed in wartime research became available to the agriculturist.

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

These and other new pest control chemicals which soon emerged from research laboratories quickly found wide acceptance and usage. Several types of pesticides were formulated, but the group of chlorinated hydrocarbons became one of the most important.

Chlorinated hydrocarbon pesticides have been used extensively since their introduction some 25 years ago. They have been applied directly to the soil and indirectly through spraying or dusting of crops. The chlorinated hydrocarbons and other chemicals have been used so extensively that they appear to be a permanent part of man's environment (51). Most of these compounds have persisted in soil from several to many years and have not been particularly effected by leaching action (57). Temperature, moisture, type of agricultural practices, soil, volatility of the compound, and possibly a number of other factors have controlled persistence of pesticides in the soil. As a result of persistence they have been translocated in water, plants, and animals throughout the food chain until they have reached man's food supply (51). Thus, man has come to accept the fact that small quantities of pesticide residues will be present in his food supply.

Barron (6) and Moss (66) stated that most data on pesticide residue content of food products has been based on samples taken from the grower or from the market in a fresh state. Within the last decade, however, there has been more emphasis placed on the processed product and the effects of processing on the residue content. Studies have been conducted also to investigate the intentional removal of organic pesticides by various techniques.

Carlin et al. (14) suggested that ferric ions from can walls may have catalyzed a decomposition of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane]. Later this theory was substantiated by Hemphill et al. (38) who stated that time and temperature alone did not account for the loss and degradation of DDT. They indicated that the loss might have been caused by the container. Hollowell (40) 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.'" The possibility that this phenomenon occurred was studied by Moss (66) who concluded that the can enamel did have an absorptive capacity for p,p'-DDT [1-trichloro-2,2-bis (p-chlorophenyl)ethane]. He further proposed that the enamel catalyzed the rearrangement of some of the p,p'-DDT to o,p'-DDT [1-trichloro-2-o-chlorophenyl-2-p-chlorophenylethane] after absorption.

The present study was conducted to examine the effects of epoxy-phenolic enamel on levels of pesticide residues in turnip greens during blanching and thermal processing. Of primary consideration were the effects of enamel lined steam kettles which were used for water blanching, and enamel lined cans which were used for thermal processing. Of secondary importance was the effect of normal processing upon residue levels in the turnip greens.

CHAPTER II

LITERATURE REVIEW

I. GENERAL PESTICIDE DEFINITION AND CLASSIFICATIONS

Of all methods used to control pests, the use of chemicals has been predominant, and such usage dates far back into history (29,56). However, the greatest development, the advent of the synthetic pesticide, is only about 30 years old (56). Up until 1944, the primary pesticide chemicals used were a few inorganic chemicals such as compounds of arsenic, lead, sulfur, and fluorine (18). Since then, a tremendous number of formulations has been developed and manufactured for use in agriculture, industry, and in health protection. In the United States more than 1,200 formulations have been manufactured which were based on DDT alone, and about 1,500 were based on other chlorinated hydrocarbons (59). In less than 20 years, the use of synthetic chemical pesticides in the United States has increased from a level of a few million pounds annually to nearly a billion pounds. Almost 60,000 pesticide formulations have been registered in the United States and each contained one or more of the 800 different pesticide compounds (73). The range and number of compounds now available as pesticides are of surprising magnitude and of diverse classifications.

It has been stated that the term "pesticide" was first used some 20 years ago as a collective noun or group classification to include all materials used to control, destroy, or mitigate pests (30). Those

compounds used mainly against insect pests were, as a group, called insecticides and further classified according to the main group of insects against which they were used. For example, aphicides were used against aphids and scalecides against scale insects. Also included in the major category of pesticides were acaricides, nematocides, fungicides, herbicides, molluscicides, algaecides, and rodenticides.

Insecticides were also categorized by their mode of action or the means by which they controlled the insects. Those insects which ate various parts of the plant were controlled by applying the pesticide to the plant parts. The material was consumed by the insect and acted as a stomach poison. The pesticide would destroy some insects that merely come into contact with it and would be classified as a contact poison. Many pesticides were classified on the basis of their chemical nature. Rudd (72) listed some of the major chemical categories as organic phosphate compounds, chlorinated hydrocarbon compounds, arsenical compounds, and carbamate compounds.

II. PREPARATION AND PROPERTIES OF DDT

The chlorinated hydrocarbon now widely known as DDT was first synthesized and described in 1874 by Mr. Zeidler (29). The insecticidal properties, however, were not realized until more than 60 years later by Dr. Paul Muller. Dr. Muller was a research worker in the Basle laboratories of the Swiss Company of J. R. Geigy. He discovered the insecticidal property of DDT through research for mothproofing chemicals (29,84). Muller worked primarily with diphenyl sulfones and with the

substitution of various chemical groups in the molecule, DDT was resynthesized as the compound discovered earlier by Zeidler.

The original Baeyer Condensation which was used by Zeidler in his discovery of DDT has been the most widely used method for preparing the chemical (29,74). In this reaction, chloral and chlorobenzene react in the presence of sulfuric acid:

$$Cl_3CCHO + 2C_6H_5Cl \longrightarrow Cl_3CCH(C_6H_5Cl)_2 + H_2O$$

$$CCl_3CHO + {}_2C_6H_5Cl \longrightarrow (p\text{-}C_6H_4Cl)_2\ CHCCl_3 + H_2O.$$

The molar ratio of chloral to chlorobenzene varies, and the amount of sulfuric acid required is at least twice the total volume of chloral and chlorobenzene. After the mixture is heated to 60°C, it is cooled to room temperature and poured into an excess of water. The solid product separates and is filtered off for washing. DeOng (20) stated that three isomers have been identified from the Baeyer Condensation reaction: p,p'-DDT, o,p'-DDT, and o,o'-DDT [1-trichloro-2-2-bis(o-chlorophenyl) ethane].

DDT is the abbreviation given to dichlorodiphenyltrichloroethane by a member of the British Ministry of Supply. However, the exact chemical designation is 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (56,84). Technical DDT is a white amorphous powder and may contain up to 14 different chemical compounds (60). 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 and allows up to 30 percent of the o,p' isomer (56). Chemically pure p,p'-DDT consists of white needles

which melt at 109°C, has a density of 1.6, and a vapor pressure of 1.5 x 10^{-7} mm at 20°C (60). In alkaline solution, DDT is readily dehydrochlorinated to DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene]. This compound may then be oxidized to p,p'-dichlorobenzophenone catalyzed by ultraviolet radiation. The dehydrochlorination of DDT is also catalyzed by traces of iron, aluminum, and chromium salts. Highly purified DDT is stable to heat and does not decompose below 195°C. The technical grade of DDT, however, readily decomposes at 100°C. This property may be due possibly to impurities of iron (29,60).

DDT is the most permanent and durable of all commonly used contact insecticides due to its insolubility in water, its very low volatility, and its relative resistance to light and oxidation (60). DDT is stable to light in storage and remains active after long exposure to light, but there is accumulating evidence that light rays do have a marked effect on DDT when it is sprayed out in thin layers or films (84). Such reactions include both the radiation from ultraviolet lamps as well as direct sunlight (20). Both Chisholm et al. (16) and Lindquist et al. (48) performed studies that substantiated the decomposition of DDT by light. Lindquist indicated also that DDT was more rapidly and completely decomposed as a solution than as a dry solid. Also solutions made with volatile solvents evaporate more quickly and leave a more resistant residue.

DDE and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] are quite similar to the parent compound in many respects. In some instances they may be more resistant to decomposition, but show generally the same characteristics. Chemically, DDE undergoes the same reactions as DDT, but is more slowly dechlorinated. Both DDD and DDE are far less toxic

than DDT, but sometimes the metabolites are used to control certain insects such as leaf rollers which are not controlled readily by the parent compound (29).

The principle formulations in which DDT is used are 50 percent wettable powders, 25 percent emulsifiable concentrates, 5 percent dusts, and 10 percent aerosols (74). The particular formulation used depends upon the nature of both the product and the organism to be controlled.

III. PERSISTENCE OF CHLORINATED HYDROCARBON PESTICIDES

As previously mentioned, chlorinated hydrocarbon pesticides have been used so extensively that they appear to be a permanent part of man's environment. However, the length of time they will remain in the environment and the length of time before they find their way into the biological systems are not known (51). Cook (17) has defined "persistence" as related to the length of time that a pesticide residue resists removal under conditions that are not directly controllable by man. He also stated that persistence can be considered from at least two points of view: the length of time that the chemical has a protective capability and the length of time when there might be some hazard in consuming the product on which the chemical is applied.

Persistence is the result of many interacting factors of which volatility, solubility, stability, ease of hydrolysis, sensitivity, isomerization, and possibly others all play a part (17,56). Most chlorinated hydrocarbons have persisted in the soil for several years and have not been affected particularly by leaching action (57). Temperature,

moisture, type of agricultural practice, type of soil, soil microbial population, and other factors all control the persistence in soil (22,57).

The most common insecticide residues persisting in soils have been found to be chlorinated hydrocarbons of which DDT and dieldrin are the most widespread. Edwards (23) determined that on an average, DDT persisted longest with about 80 percent remaining after the first year as compared with 75 percent dieldrin, 60 percent lindane, 55 percent chlordane, 45 percent heptachlor, and 26 percent aldrin. After three years the residues had decreased to 50 percent, 40 percent, 15 percent, 10 percent, and 5 percent, respectively.

The type of soil seems to be one of the more important factors in determining the persistence of pesticide residues. This became obvious when it was observed that crops grown in soils treated heavily with chlorinated hydrocarbons were damaged more in light sandy soils than in heavy clays, mucks, and peats (23). In a study conducted by Harris and Sans (37), the influence of soil type on the degree of absorption was quite pronounced.

Lichtenstein et al. (47) pointed out that once the pesticide is in the soil, the degree of penetration and subsequent translocation are functions of the soil type as well as the properties of the chemical compound. Yaron et al. (88) determined that the nature of clay minerals did not effect the amount of aldrin absorbed; however, the mechanical composition and organic matter content of the soil did effect the amount of aldrin absorbed.

Baker and Applegate (5) investigated the effect of temperature and ultraviolet light on the persistence of methyl parathion and DDT.

Their results indicated that both factors accelerated the loss of pesticides, but temperature appeared to play a more important role in the loss of DDT than in the loss of methyl parathion.

IV. REMOVAL AND DECOMPOSITION OF PESTICIDE RESIDUES

Most of the research reported on removal of pesticide residues from foods have been from studies using regular commercial processing techniques or home processing procedures. The procedures were not intended to remove pesticides, however, the levels were in most cases lowered considerably (52).

It has been known for a long time that DDT is taken into the systems of dairy cattle after they have been sprayed with the pesticide or after they have consumed forage that has received applications of the chemical. Mann et al. (55) conducted an investigation to determine the effect of various processing procedures on the residue content of milk. They determined that the DDT content in the fat portion of milk and milk products was fairly constant and that the residue level was reduced very little.

Langlois et al. (46) conducted a study to determine the effects of processing and storage of dairy products on endrin, dieldrin, and heptachlor. They reported a loss of heptachlor and dieldrin during condensing and a loss of all pesticides studied during spray and drum drying. Butter and cheese contained less residue on a fat basis than raw milk because some of the residue separated into the skim milk and whey. Other products contained essentially the same amount of residue. In a similar study, Langlois et al. (44) determined the effects of processing

and storage on DDT and lindane. They reported that during drying of the milk into powder, there was some structural change in both pesticides. In general, however, all of the finished products other than dried milk contained essentially the same amount of residue as the raw milk when expressed on a fat basis.

Liska et al. (49) and Draper et al. (21) reported on the presence of chlorinated hydrocarbons in the eggs and tissue of chickens. Liska et al. (50) conducted a study to determine the effect of the method of cooking on residue content of chicken tissue. They reported that cooking the tissue of hens in water at 190°F to 200°F for three hours reduced residue content up to 90 percent. Ritchey et al. (70) conducted a similar experiment, but they used frying and baking procedures. They reported that both methods reduced the amount of residue present, but frying seemed to be more effective. The reduction of lindane and the breakdown of DDT was greater when the carcass was fried than when it was baked.

Carter et al. (15) compared five methods of cooking beef that contained residues of DDT. They reported that frying and pressure cooking removed small amounts of residue, but indicated from an overall standpoint that the residues were not materially decomposed nor lost.

Crosby (19) pointed out that a considerable volume of material exists in the files of industry and in the scientific literature on the effects of home and commercial processing of fruits and vegetables. As early as 1947, Tressler (80) reported that commercial canning at 100°C significantly reduced the DDT level in strained peaches, strained applesauce, green beans, and tomato juice. He also reported that part of the loss was caused by the use of cans rather than glass containers.

Farrow et al. (26) treated tomatoes with DDT, malathion, and carbaryl. They processed the tomatoes using both commercial and home preparative methods and reported that commercial canning and juicing operations removed virtually all DDT, malathion, and carbaryl residues. Home processing techniques removed all but traces of DDT and malathion, but higher residues of carbaryl still remained.

It was suspected that the occasional presence of DDD in canned products might result from the decomposition of DDT during processing and storage. Farrow et al. (25) conducted an experiment using infrared spectrophotometry, gas chromatography, and thin layer chromatography to identify the residues in canned spinach. They confirmed that p,p'-DDT did convert to p,p'-DDD during canning at 121°C.

In another study by Lamb et al. (43), the removal of DDT, parathion, and carbaryl by home and commercial processing of spinach was investigated. They reported that washing lowered the residue levels of all three pesticides, and that removal was increased by adding a detergent. Water blanching removed a large amount of the DDT, but steam blanching removed very little or no residue at all. After the spinach was heat processed and stored for five months, no DDT remained. Small amounts of DDD and DDE were found, but parathion and carbaryl residues remained unchanged during the storage period.

Carlin et al. (14) reported that after processing and 11 months storage, guthion residues in snap beans were very small, and DDT was eliminated completely. Elkins et al. (24) obtained similar results by processing green beans treated with DDT, malathion, and carbaryl. They reported that both home and commercial processing methods removed high

percentages of all three pesticides. They also reported that during thermal processing, p,p'-DDT was converted to p,p'-DDD.

As previously mentioned, most instances of residue removal are "side effects" of normal processing procedures. Liska and Stadelman (52) stated that special techniques to intentionally remove pesticide residues have not gained much support. However, they suggested that someday such techniques might become a necessity.

V. EFFECT OF CONTAINER ON RESIDUE CONTENT

In the very recent past, there has been an increasing amount of research carried out relating the effects of the container to the residue content of the enclosed food product. As early as 1947, Tressler (80) determined that DDT was stable to heat in water and to acid buffers in pyrex containers, but was slightly decomposed in these same media when processed in tin containers. DDT was decomposed to an even greater extent when processed with various food products. Ott and Gunther (68) observed that DDT decomposed to its metabolite, DDD, during the process of analyzing the sample on a gas chromatograph. They suspected that the reaction might have been a reductive dechlorination as a result of contact with the iron present in the stainless steel chromatographic column. The suspicion was substantiated by the work of Langlois *et al.* (45) and by Fleck and Haller (27,28) who indicated that DDT is easily decomposed by iron as well as by certain other metals.

Hernandez and Vosti (30) concluded that discoloration of asparagus did not occur if stannous ions were present. With the use of enameled cans for snap bean processing, it was observed that the beans packed in

enameled cans were not as bright as those packed in plain tin plate. This led to a study by Van Buren and Downing (31) who concluded that wax beans packed in enamel lined cans led to deterioration which increased with the length of storage. They further concluded that the darkening was increased by addition of iron, but was reversed by the addition of tin or stannous ions and the color was improved.

In a study to evaluate the effects of home preparation procedures on the chlorinated hydrocarbon residues in green beans, Hemphill et al. (38) observed that the degradation and losses of DDT could not be explained entirely on a time-temperature basis. They indicated that the composition of the container walls might have been a factor. This was consistent with the findings of Carlin et al. (14) who initiated a similar study on snap beans using heat processing and freezing procedures. They observed that after 11 months' storage, the DDT content was reduced by more than one-half in frozen beans and to zero in canned beans, as compared to the unwashed product. They also suggested that it was possible that the ferric ions present in the container walls had catalyzed the decomposition of DDT.

Farrow et al. (25) and Lamb et al. (43) observed the conversion of DDT to DDD during the thermal processing of spinach. This conversion was observed in spinach heated at temperatures in excess of 100°C in plain tinplate cans, enameled cans, and in glass test tubes. Hollowell (40) observed this conversion also, but to a lesser extent. In Hollowell's study, the cans containing DDT-fortified samples showed evidence of "feathering" of the can enamel as described by Tressler (80). The discoloration of the can occurred in varying degrees, but the cans containing unfortified samples showed no discoloration at all. Hollowell

indicated that this might have been due to the presence of DDT which could have extracted chlorophyll from the turnip greens and deposited the pigment along with the DDT on the can enamel. This phenomenon, if it actually occurs, could have explained the reduction in the amount of pesticide present in each sample after processing.

Moss (66) initiated a study of the phenomenon suggested by Hollowell (40). From this study he reported that the enamel did have the ability to absorb at least some of the DDT and to further catalyze its decomposition. However, the enamel did not absorb DDD nor DDE during the thermal processing.

VI. RESIDUE EXTRACTION

Generally speaking, the work of residue analysis and control covers a wide variety of sample types with each having a particular method that is most suitable. The residue analyst usually has two primary goals in mind: the identity of any residues present, and the quantity of residue present in the sample (1).

The first step in the analysis of pesticide residue is a separation of the pesticide from the plant or animal material by a process called extraction (10,78). There are various methods of extraction, but the one selected is governed by the type of pesticide and the product that is being analyzed. Wheeler and Frear (86) stated that methods for extracting pesticides from plant materials fall into three general categories: surface rinsing, exhaustive extraction, which is usually carried out in a Soxhlet apparatus, and maceration or blending with a solvent.

Surface rinsing is sometimes referred to as "stripping" or "tumbling" and usually involves placing the tissue to be analyzed in a container, adding the extraction solvent, and rotating or tumbling the material end over end for a period of time. The major advantage of this method is that the pesticide can be removed without including large amounts of the plant extractive (87,86). A serious limitation is that only the residue located on the surface of the product will be removed, and very little, if any, of the internal residue will be extracted (86).

Van Middelem et al. (82) combined tumbling with blending and determined that this procedure removed three times the amount of pesticide as tumbling alone. Klein et al. (42) reported that surface rinsing was inadequate for removing DDT from frozen leafy vegetables, and his conclusions were substantiated by Hardin and Sarten (36). Wilderman and Shuman (85) reported also that the method was not effective for analyzing firm fruits or hay.

Exhaustive extraction or Soxhlet extraction, is one of the methods routinely used for dry products. Usually the samples are finely ground by some type of mill and then extracted with a nonpolar solvent. The primary limitation for this method is that the sample must be dried. It may be dried by air or by heat, but air drying is extremely slow and heating may alter the composition of the pesticide residue (86).

Extraction by masceration usually consists of blending the sample with a solvent for a few minutes and then separating the liquid phase by decanting, centrifugation, or filtering (7,86). Thornburg (79) listed several commonly used solvents for extracting and pointed out some of the problems encountered with each. Hardin and Sarten (36) compared extraction

procedures using the tumbling method, tumbling plus blending, and blending first with isopropyl alcohol and then with hexane. They concluded that the double blending variation offered the most complete recovery of the pesticides studied.

Burke and Porter (13) compared the effectiveness of several blending procedures for removing field applied p,p'-DDD from kale. They reported that the acetonitrile extraction as employed by Mills, Onley, and Gaither (62) gave the most satisfactory results. They also reported that the method was effective for extraction of parathion and diazone.

Gunther and Blinn (35) pointed out that a "solvent bridge," blending first with a water miscible solvent and then a water immiscible solvent, must be employed in order to obtain efficient extraction from leafy vegetables. Jones and Riddick (41) considered acetonitrile and n-hexane as a solvent pair, but found that certain interfering substances were not removed.

Wheeler et al. (87) reported that blending was not completely quantitative when applied to plants containing only internal residues. He suggested that it might be due to incomplete penetration of the solvents. Klein et al. (42) compared the three methods of extraction, Soxhlet, tumbling, and blending, and reported that blending with isopropyl alcohol followed by blending in benzene gave the most satisfactory results.

VII. RESIDUE CLEANUP

After a pesticide residue has been extracted from the sample material, many compounds other than the residue are present in the extract.

Often these include lipids, waxes, pigments, and other nonpolar compounds (3,10). Therefore, a cleanup procedure is needed which will adequately remove the interfering compounds without removing or destroying the residue content.

There are primarily two factors which determine the method and degree of cleanup required for a particular sample. These factors include the nature of the sample material and the ultimate method of analysis (1). Certain vegetables of low fat content yield an extract that contains few interfering compounds, depending upon the method of extraction. On the other hand, waxy plant tissues or fatty animal tissues contain much more co-extracted material and require a much more stringent method of cleanup. Analysis by gas liquid chromatography requires a very clean sample while thin layer chromatography is able to retain a portion of contaminating material at the base line and does not require the same degree of purity as gas chromatography (1).

Thornburg (78) and Cook (18) pointed out that as more pesticides have been developed, more sophisticated and specific methods of analysis have also been developed. Many of the newer procedures have resulted in more thorough means of extraction, and therefore, have necessitated the development of more thorough methods of cleanup. Ideally the material co-extracted with the residue should be completely eliminated, but this is very difficult to achieve. It is especially difficult to purify those extracts which contain large amounts of fats, oils, or colored material. Burke (12) pointed out that interfering materials, when concentrated and injected into a gas chromatograph, will almost always cause erroneous results. Such effects as pesticide decomposition,

poor resolution, low recoveries, or extraneous responses often result. Burke and Giuffrida (11) reported that unclean samples result in a number of peaks not due to pesticide content and make the recording difficult to interpret.

Column chromatography has been one of the most widely used and readily adapted methods of cleanup employed in residue analysis. There are a large variety of different absorbant materials used in columns: combinations of carbon-Celite, magnesium oxide-Celite, potassium hydroxide-Celite, sulfuric acid-Celite, and charcoal (2,4,31,61,65,75). For chlorinated hydrocarbon pesticides, the most widely used absorbant has been Florisil (58).

After the residue is absorbed onto the column, then it must be eluted with the proper solvents. The Mills, Onley, and Gaither procedure which is suitable for 21 different chlorinated hydrocarbons, utilizes two eluting solvents. After the residues are eluted from the column, the elutant is ready to be analyzed by gas chromatography or other such methods.

VIII. GAS LIQUID CHROMATOGRAPHY OF PESTICIDE RESIDUES

Before 1960, gas liquid chromatography was not successfully utilized in determining pesticide residue content. This was chiefly due to the nonspecificity of the available detection systems (12). When such detection devices were employed, very complex chromatograms resulted which were difficult to interpret. With the introduction of specific detectors, however, gas chromatography became a very useful method of pesticide determination.

Bevenue (8) described gas chromatography as a method of separation of vaporized components by distribution between a fixed stationary phase and a moving inert gas phase. He also added that in gas liquid chromatography, the stationary phase is a nonvolatile liquid distributed on a solid support. The basic gas chromatographic apparatus consists of a carrier gas supply, sample injection port, column, and some type of detector and recording device (12). Nitrogen, argon, helium, or hydrogen are commonly used as carrier gases. The stationary phase is prepared by impregnation of an inert solid with a nonvolatile organic liquid. This results in a free flowing powder which is packed into a column (10). Several studies have been conducted to determine the best column packing materials (11,12,76,77). Bonelli et al. (9) suggested that the best general purpose column material for pesticides is a nonpolar silicone.

Burke (12) refers to the column as the "heart" of a gas chromatographic system. This is where the actual component separation occurs and is also the area where absorption and degradation may occur. Columns have been made of aluminum, stainless steel, copper, and other metals, as well as glass. Several authors have reported that metal columns cause a decomposition of DDT and suggested that only glass columns be used (8,11,45,64,68,69).

When the sample is injected into the hot column through the sample injection port, it immediately vaporizes. The carrier gas forces the vapor to flow through the column material, however, the vapor components do not travel at the same rate. Their rate of flow depends on the difference in partition coefficients between the liquid stationary phase and the

mobile gas phase. Thus, they emerge from the column into the detector at different times and are detected accordingly. Usually, the response of the detector is fed into a strip chart recorder and each component is registered on the chart as a peak. The distance of the peak from the solvent front is called the retention time and is characteristic for each pesticide.

Several detectors are available for detecting and measuring pesticide residues. However, the electron capture detector is by far the most sensitive (10,54). It is capable of measuring some pesticides in amounts as low as ten picograms. It is extremely responsive to chlorinated hydrocarbons and relatively insensitive to nonpolar organic compounds. This reduces background "noise" and makes it possible to detect peaks from pesticides even when impurities are in the sample (10). Burke (12) indicated that the greatest disadvantage of the electron capture detector is its lack of complete specificity for chlorinated compounds.

The basic principle of the electron capture detector is the affinity of chemical compounds for free electrons (10). The electron affinity cell consists of an anode, a cathode, and a radioactive source of Beta particles. Suitable sources of Beta particles are tritium, strontium, promethium, or nickel. When only the carrier gas is in the cell, the Beta particles produce positive ions and low energy electrons. By applying a potential to the electrodes, the electrons will migrate to the anode and produce a current. When a substance capable of absorbing electrons enters the cell, part of the electrons will be removed in the

form of negative molecular ions and the current will be decreased. It is this decrease in current which is registered on the strip chart recorder (32, 53, 83).

The principal means of identifying a component eluted from the chromatographic column is the retention time of the peak recorded on the strip chart (8). Variations in retention time may occur as samples are injected over a period of time. This requires that the instrument be checked very frequently with standards if possible.

The area of the peak is directly related to the concentration of the represented compound. However, the peak may be adversely effected by temperature, gas-flow fluctuations, sample volume, purity of sample, a dirty detector, a dirty column, or other factors of this nature (8). Gaul (33) described five methods for quantifying chromatographic peaks: peak height, product of peak height x width of the peak at half height, area as a product of retention time and peak height, triangulation, and disc integration. He reported that in the electron capture detection of aldrin, heptachlor epoxide, and dieldrin, there was no significant difference in the five methods. However, Bevenue (8) suggested that peak area is usually more reliable than peak height.

Bevenue (8) also suggested that the preferred and most reliable procedure for the quantitative analysis of a given pesticide is by injecting a series of pesticides in increasing concentrations. The peak areas are then calculated and a standard reference curve is constructed. Within the limits of gas the chromatograph, the relation of the concentration to peak area will be a straight line function.

CHAPTER III

MATERIALS AND METHODS

I. EXPERIMENTAL DESIGN

On August 9, 1971, three plots or replications of trunip greens were hand planted at The University of Tennessee Plant Science Farm, Knoxville. The study was conducted with Purple Top White Globe Turnips (Brassica rapa) c.v. Each plot was 32 ft. by 40 ft. and consisted of eight rows, 40 ft. long. Initially there was a lack of moisture and spray irrigation was employed. The greens within each plot were relatively uniform in size; however, the greens were not uniform in size among plots.

On September 7, 1971, a 100-gram sample was selected randomly from each of the three plots. Each sample was washed in approximately five gallons of water (26°C) for one minute and allowed to drain for three minutes on a screen. The samples were chopped with a hand knife on a cutting board and thoroughly mixed, and three 25-gram portions from each sample were weighed and placed into 12 oz. screw cap jars. The jars were placed in a freezer and stored at -27°C until they were removed for extraction of the pesticides. These nine samples represented the pesticide level of the turnip greens prior to the first application of DDT.

After the first set of samples had been removed from the field on September 7, 1971, DDT was applied to the plant surfaces at the rate of 1.5 pounds per acre in 2.0 gallons of water per plot. A 50 percent

wettable powder formulation was used and applications were made with a hand sprayer. The spraying procedure was repeated on September 14, September 24, and October 1, 1971.

On October 4, 1971, a 100-gram sample was selected randomly from each of the three plots. Each sample was washed for 2.5 minutes in five gallons of water (35°C). and 2.5 minutes in five gallons of water (20°C). After draining on a screen for three minutes, the greens were chopped and thoroughly mixed, and three 25-gram portions from each sample were weighed and placed into 12 oz. screw cap jars. These nine samples represented the residue level of the greens prior to blanching or thermal processing. The jars were stored in a freezer at -27°C until they were removed for extraction of the pesticide.

On October 5, 1971, greens from plot I were hand harvested by cutting two to three inches above the ground. They were placed in baskets and transferred to the laboratory where an initial 28-pound lot was weighed out. The lot was divided into four, seven-pound sublots to facilitate washing and handling. Each sublot was washed for 2.5 minutes in 18 gallons of water (35°C), and for 2.5 minutes in 18 gallons of water (20°C). The greens were continuously agitated by hand during the washing and the containers were thoroughly scrubbed between each washing. Each sublot was chopped into approximately one- to two-inch lengths and mixed thoroughly.

Sublot number one was divided into two, 3.25-pound portions. One portion was placed on a blanching tray and steam blanched at atmospheric pressure for three minutes. After being blanched, the greens were sprayed with water until cooled and allowed to drain for three minutes on a screen.

A 100-gram sample was selected from the blanched greens, and from this sample, three 25-gram portions were weighed and placed into 12 oz. screw cap jars to be stored at -27°C.

From the same tray of blanched greens, three 330-gram portions were selected and placed in three number 303 x 407 plain tin plate cans to be thermally processed. All cans were received from Bush Brothers and Company of Dandridge, Tennessee. The other 3.25-pound portion was treated similarly, except the three 330-gram portions were placed in enamel lined cans.

Sublot number two was divided into two 3.25-pound portions. For treatment of the greens, two gallons of water were placed in a three gallon capacity steam-jacketed kettle and brought to boiling. One 3.25-pound portion of greens was immersed in the boiling water and blanched for three minutes. The greens were stirred continuously during the process with a large wooden spoon. The temperature was maintained at approximately 100°C during the blanching. The greens were poured onto a blanching tray, sprayed with water until cooled, and allowed to drain three minutes. Samples were taken as in the first 3.25-pound portion mentioned of sublot number one. The water blanching procedure was repeated on the other 3.25-pound portion of sublot number two, and samples were selected as in sublot number one.

Sublot number three was divided into two 3.25-pound portions. A section of enameled tin plate, also received from Bush Brothers and Company, was cut so as to simulate an interior lining of a steam-jacketed kettle. After the liner was placed inside the kettle with the enamel

facing toward the inside, two gallons of water were added and heated to boiling. One 3.25-pound portion of greens was immersed in the water and blanched for three minutes. The greens were stirred continuously during the process with a large wooden spoon. The greens were poured onto a blanching tray, sprayed with water until cooled, and allowed to drain three minutes. Samples were taken as in the first 3.25-pound portion mentioned of sublot number one.

The enamel liner was removed from the kettle and the kettle was thoroughly scrubbed. A new liner was placed in the kettle and the procedure was repeated for another 3.25-pound portion of greens from sublot number three. Samples were taken as in the second portion of sublot number one.

Sublot number four was divided into two 3.25-pound portions. Two gallons of water were placed into a steam-jacketed kettle and heated to boiling. One portion of greens was submerged in the water and blanched for five minutes. The greens were stirred continuously during the process with a large wooden spoon. The greens were poured onto a blanching tray, sprayed with water until cooled, and allowed to drain three minutes. A 100-gram sample was selected from which three 25-gram portions were weighed into 12 oz. screw cap jars for storage at -27°C. The other portion of sublot number four was treated in a similar manner except that an enamel liner was placed inside the kettle. A 100-gram sample was selected and subsequent three 25-gram portions were weighed into 12 oz. screw cap jars for storage at -27°C.

After all cans were filled, they were prepared for thermal processing. Two grams of salt and 100 ml of boiling water were added to each can.

The cans were sealed immediately and placed in a retort to be processed at 121°C for 45 minutes. Moss (66) observed that this process induced the maximum amount of p,p'-DDT sorption by the enamel. After processing, the cans of greens were cooled by allowing cold water to flow over them for 20 minutes.

Soon after cooling, the contents of each can were placed into a quart size Mason jar and blended on an Osterizer blender for 60 seconds. A 25-gram sample was selected from each blending and placed into a 12 oz. screw cap jar for storage at -27°C.

Greens from plots II and III were harvested similarly to greens from plot I, and were processed on October 6 and October 7, respectively. All jars containing samples were labeled and stored at -27°C until removed for extraction of pesticides.

II. EXTRACTION AND CLEANUP OF SAMPLES

The Mills, Onley, and Gaither procedure (62) for extraction and cleanup of nonfatty vegetables was used to prepare the samples for analysis by gas liquid chromatography. The samples were removed from the screw cap jars in groups of three at a time, and all 117 samples were cleaned up prior to initiating the final step of analysis. Extreme care was taken in the cleanup phase to remove as much as possible of the co-extracted material that would interfere with the proper functioning of the electron capture detector.

Reagents

The following reagents were used in the extraction and cleanup procedure:

Acetonitrile--Analytical Reagent grade from Fisher Scientific Company. It was purified by glass-distilling one gallon of the reagent in the presence of one ml of 85 percent phosphoric acid and 30 grams of phosphorous pentoxide. The temperature of distillation was maintained between 81°C and 82°C with the first and last 100 ml fractions of distillate being discarded.

Celite 545--from Fisher Scientific Company.

Petroleum Ether--Analytical Reagent grade from Fisher Scientific Company. This was purified by glass-distilling at 30°C to 60°C. The first and last 100 ml fractions of distillate were discarded.

Sodium Chloride--certified A. C. S. from Fisher Scientific Company.

Sodium Sulfate--Analytical Reagent grade, anhydrous, from Fisher Scientific Company.

Florisil--80 to 100 mesh, pesticide grade from Fisher Scientific Company. This was activated at 623°C by the manufacturer prior to delivery, and was stored at 155°C to 160°C at least 72 hours prior to use.

Ethyl Ether--Analytical Reagent grade from Fisher Scientific Company. This was purified by washing twice with an amount of distilled water equal to twice the volume of the ether. It was washed a third time using 50 to 100 ml of saturated sodium chloride solution, and all aqueous layers were discarded. The washed ether was stored in a 2000 ml flask over an excess of anhydrous sodium sulfate, and was shaken for several minutes prior to use to remove any water that might have been present.

n-Hexane--pesticide quality from Matheson, Coleman, and Bell Chemical Company. This reagent was especially purified by the manufacturer

for use in the determination of pesticide residues by gas liquid chromatography with an electron capture detector. It was also certified to contain no more than ten parts per trillion of compounds that would interfere with detection of chlorinated hydrocarbon pesticides.

Apparatus

The following apparatus were used in the extraction and cleanup procedure:

Erlenmeyer Flasks--2000 ml with 24/40 ℨ glass stoppers.

Osterizer Blender

Mason Jars--pint size with Osterizer blending heads.

Vacuum Filtration Apparatus--set up for filtering three individual samples simultaneously. This consisted of 3.5 inch Buchner funnels, number 8 rubber stoppers, 35/42 to 24/40 ℨ reducing adapters and 24/40 ℨ vacuum take-off adapters (Kontes Glass Company), number 2 Whatman filter paper, and rubber tubing for connecting to the vacuum source.

Graduated Laboratory Cylinders--100 ml and 250 ml with 24/40 ℨ glass stoppers.

Separatory Funnels--500 ml and 1000 ml with teflon stopcocks and glass stoppers.

Chromatographic Columns--from Kontes Glass Company. These were 300 mm x 23 mm i.d. with teflon stopcocks.

Glass Beads

Quartz-Glass Wool

Kuderna-Danish Concentration Apparatus--500 ml from Kontes Glass Company. These consisted of the flasks, three-ball Snyder Columns, and 10 ml concentrator tubes.

Flint Glass Bottles--15 ml from Sargent Company. These had bakelite screw caps with conical polyethylene liners.

Burgess Vibro-tool

Pipettes--10 ml size

Steam Distillation Apparatus--for sample concentration with Kuderna-Danish concentration apparatus.

Reagent Distillation Apparatus--for glass-distilling solvents. This included an electric heating element, glass-distillation tube, and a 3000 ml round-bottomed flask.

Ovens--for evaporating samples to dryness and activating the Florisil. One was a forced air oven and adjusted to maintain a temperature of 40°C. The other oven maintained a temperature of 155°C to 160°C.

Procedure

The sample was removed from the freezer and allowed to thaw approximately 15 minutes at room temperature. It was transferred from the storage jar into a pint Mason jar. A volume of acetonitrile equal to 150 ml was used to rinse any adhering material from the sides of the storage jar into the Mason jar. Ten grams of Celite 545 were added to the sample, an Osterizer blending head affixed to the blender jar, and the sample was blended. The samples that had been thermally processed and previously blended for 60 seconds were blended 30 seconds with the acetonitrile and Celite. The samples that had not been previously blended were blended for 60 seconds with the acetonitrile and Celite.

A vacuum filtration apparatus was set up so that it was possible to filter three individual samples simultaneously into three 250-ml graduated laboratory cylinders. After the samples had been blended for the appropriate length of time, the blending jar, Osterizer head, and filter cake were rinsed in 50 ml of acetonitrile which was allowed to flow into the 250-ml graduated laboratory cylinder. The volume of the acetonitrile filtrate was noted and recorded.

The acetonitrile filtrate was transferred to a 1000-ml separatory funnel. One hundred ml of petroleum ether were measured into the graduated laboratory cylinder used to collect the filtrate. The volume was noted and recorded, and this rinse was added to the separatory funnel containing the filtrate. The material was shaken for 60 seconds to force the pesticide residue from the acetonitrile into the petroleum ether phase. About 10 ml of saturated sodium chloride solution and 600 ml of distilled water were added to the separatory funnel and it was shaken for another 60 seconds. The solution was allowed to separate and the aqueous layer of acetonitrile was discarded. The petroleum ether extract was rinsed twice with 200 ml of distilled water to remove any remaining acetonitrile from the petroleum ether phase. This was accomplished by swirling the petroleum ether extract and water gently to prevent the formation of an emulsion. After each washing, the aqueous layer was discarded.

The petroleum ether was transferred to a 100 ml glass stoppered graduated laboratory cylinder and the volume was noted and recorded. One teaspoon of anhydrous sodium sulfate was added to the cylinder and it

was inverted several times to remove any remaining water. After the sodium sulfate was added, the extract was not permitted to stand for more than one hour to avoid decomposition of the residue (63).

The chromatographic column was prepared by plugging the bottom opening with quartz-glass wool and adding about 40 grams of reactivated Florisil topped with one teaspoon of anhydrous sodium sulfate to the column. The column was packed by vibrating it about 60 seconds with a vibro-gun. A Kuderna-Danish concentrator flask with attached concentrator tube was placed under the column to receive the elutant. A glass bead was placed in the tube to facilitate the evaporation.

The column was prewetted with 60 ml of petroleum ether. Just as the last of the 60 ml sank into the sodium sulfate and Florisil, the petroleum ether extract was poured onto the column material and allowed to percolate through into the collection apparatus. After the sample sank into the column material, 200 ml of the solvent, 6 percent ethyl ether in petroleum ether, were added to the column. The flow rate of the eluting solvent was regulated to about 5 ml to 10 ml per minute.

After the elutant had moved through the column and was collected in the Kuderna-Danish flask and concentrator tube, a three-ball Snyder column was attached and the apparatus was placed on a steam bath. The solution was evaporated to approximately 10 ml, with care taken not to evaporate it to dryness. The concentrator tube was removed and placed in a forced air oven where it was evaporated to dryness at approximately 40°C. The residue in the concentrator tube was dissolved in exactly 10 ml of n-hexane and transferred to a 15 ml flint glass bottle. The sample

bottle was tightly capped, labeled, and stored at 4°C until removed for analysis by gas liquid chromatography.

III. GAS LIQUID CHROMATOGRAPHIC ANALYSIS OF SAMPLES

A Micro Tek Model MT-220 gas chromatograph equipped with a Nickel-63 high temperature electron capture detector was used to analyze the pesticide residue concentrations in all samples. The column utilized was glass, U-shaped, and 6 ft. x 4 mm i.d. It was packed with 3 percent OV17 (phenol substituted silicone) on 100 to 200 Gas Chrom Q. Purified nitrogen was used as the carrier gas with a flow rate of approximately 50 ml/min. (column flow rate) and an inlet pressure of 40 p.s.i. The column temperature was maintained at 235°C, the detector at 300°C, and the inlet temperature at 230°C. The detector attenuation (input x output) was set at 10 x 64 for all samples except those taken prior to the first application of pesticide material. The residue concentration of those samples was lower and the sensitivity of the instrument was increased to an attenuation of 10 x 32.

Prepared analytical standard solutions of p,p'-DDT, o,p'-DDT, DDD, and DDE were obtained from Analabs, Incorporated (Hamden, Conn.). The standards were in concentrations of 100 ppm (parts per million) and were combined and diluted to working standard solutions of 5.0, 1.0, 0.1, 0.01, and 0.001 ppm concentrations.

A Hamilton 10 µl syringe was used to inject the samples into the gas chromatograph. Injections of 1.0 µl were used for all standards and all samples except those collected the day preceding harvest. This group of samples was more concentrated and injections of 0.4 µl were used. The

samples collected prior to the first application of pesticide material were taken directly from the 15 ml flint glass bottles and injected into the chromatograph. All other samples were diluted to 0.1 of the original concentration of the 10 ml of concentrate.

The individual standards were injected into the chromatograph to determine their relative retention times. The combined standards injected resolved in the order: DDE, DDD, o,p'-DDT, and p,p'-DDT. The sample residues were identified by injecting and comparing retention times with the standard retention times.

The residue concentrations were quantified by calculating the product of peak height and width of peak at half height and comparing with the products of the standard peaks. The results represented the ppm of pesticide residue in the 10 ml concentrated solutions.

IV. STATISTICAL METHODS

The experimental data from which all statistical analyses were calculated are found in Table A-1 in the Appendix. The data were analyzed by the analysis of variance method and the analyses were carried out in two parts: the effects of blanching with steam or with water in lined or unlined kettles, and the effects of blanching followed by thermal processing.

The analysis of the effects of blanching was a 5 x 3 x 3 factorial design (treatment levels, replications, and observations). The analysis of the effects of blanching followed by thermal processing was a 6 x 3 x 3 factorial design (treatment levels, replications, and observations).

Significant differences between means were determined by Duncan's Multiple Range Test (71). The 0.10 level of probability was applied for the analyses of variance and for the mean pesticide residue values.

CHAPTER IV

EXPERIMENTAL RESULTS

Samples of greens selected prior to the first application of DDT contained residues of o,p'-DDT and p,p'-DDT as presented in Table A-1 in the Appendix. The presence of DDT in the untreated greens indicate that the isomers of the pesticide may have persisted in the soil from previous applications and were translocated from the soil into the greens. Samples of greens selected after they were sprayed with DDT but prior to blanching, contained residues of DDE, DDD, o,p'-DDT, and p,p'-DDT as presented in Table A-1 in the Appendix.

I. GREENS SUBJECTED TO BLANCHING

Results of the analyses of variance for DDT and DDT metabolite residues which remained after blanching are shown in Table 1. The analyses indicated that there were no significant differences between blanching with steam or water, between water blanching in unlined kettles and lined kettles, or between water blanching three minutes or five minutes. Interaction between kettle lining and time was significant for DDE, but not for the other isomers.

Replications were significantly different for all isomers. The interaction between replications and treatments was significant for DDD, o,p'-DDT, and p,p'-DDT.

The mean parts per million of pesticide residues present in the greens after they were blanched are presented in Table 2. The data

TABLE 1

ANALYSIS OF VARIANCE OF PESTICIDE RESIDUES IN TURNIP GREENS SUBJECTED TO BLANCHING

Source	d.f.	DDE M. S.	DDD M. S.	o,p'-DDT M. S.	p,p'-DDT M. S.
Total	44				
Treatments	4	0.2063	0.1546	2.1150	8.4386
Steam vs. Water	1	0.0132	0.0385	1.3284	17.7266
Unlined vs. Lined Kettle	1	0.0035	0.1434	1.1453	11.0945
3 min. vs. 5 min.	1	0.1340	0.1531	1.1001	1.4693
Lining x Time	1	0.6746[a]	0.2820	4.8863	3.4639
Replications	2	3.8193[a]	0.6217[a]	2.4470[a]	5.7672[a]
Reps x Treatments[b]	8	0.1712	0.1631[a]	5.4783[a]	6.1991[a]
Observations/Reps, Treatments[c]	30	0.1810	0.0298	0.9317	1.2524

[a]F-ratios are significant at the 0.10 level of probability.

[b]Replications x treatments mean square is used as the error term for treatment effects.

[c]Observations/replications, treatments mean square is used as the error term for replications and for replications x treatments.

TABLE 2

PARTS PER MILLION OF PESTICIDE RESIDUES IN TURNIP GREENS SUBJECTED TO BLANCHING[1]

Process	DDE	DDD	o,p'-DDT	p,p'-DDT
Blanching Method				
Steam[2]	2.278[a]	0.762[d]	6.691[g]	11.101[j]
Water[3]	2.235[a]	0.689[d]	6.261[g]	9.532[j]
Kettle Lining				
Unlined[4]	2.226[b]	0.752[e]	6.083[h]	8.977[k]
Lined[4]	2.245[b]	0.626[e]	6.440[h]	10.087[k]
Time				
3[4]	2.174[c]	0.623[f]	6.086[i]	9.330[m]
5[4]	2.296[c]	0.754[f]	6.436[i]	9.734[m]

[1]Pairs of means followed by the same letter are not significantly different at the 0.10 level of probability.

[2]Each value is the mean of 9 observations.

[3]Each value is the mean of 36 observations.

[4]Each value is the mean of 18 observations.

indicated that there were no significant differences between blanching with steam or water, between water blanching in an unlined kettle or a lined kettle, or between water blanching three minutes or five minutes.

The results of interaction between kettle lining and time are shown in Table 3. The interaction was significant for DDE, but was not significant for DDD, o,p'-DDT, or p,p'-DDT.

II. GREENS SUBJECTED TO BLANCHING AND THERMAL PROCESSING

Results of the analyses of variance for DDT and DDT metabolite residues which remained after blanching was followed by thermal processing are shown in Table 4. The analyses indicated that methods of processing had a significant effect on DDE, o,p'-DDT, and p,p'-DDT. Greens which were steam blanched and subsequently thermally processed in different types of cans showed no significant difference in the remaining levels of residues. Greens which were water blanched three minutes in an unlined kettle and subsequently thermally processed in different types of cans resulted in significantly different levels of DDE, but the remaining residues of DDD, o,p'-DDT, and p,p'-DDT were not significantly different. The level of residues which remained in greens that were blanched three minutes in a lined kettle and thermally processed in different types of cans were not significant for any of the isomers.

The method of blanching had a significant effect on the residues of o,p'-DDT and p,p'-DDT when blanching was followed by thermal processing. When greens were water blanched three minutes in an unlined or lined kettle and subsequently thermally processed, the amount of residues of DDE and o,p'-DDT were significantly different.

TABLE 3

EFFECT OF KETTLE LINING x TIME INTERACTION ON PESTICIDE RESIDUES IN TURNIP GREENS SUBJECTED TO BLANCHING[1]

Time	DDE		DDD		o,p'-DDT		p,p'-DDT	
(min.)	Unlined	Lined	Unlined	Lined	Unlined	Lined	Unlined	Lined
3	2.028[a]	2.321[b]	0.598[g]	0.649[g]	5.540[m]	6.633[m]	8.464[s]	10.195[s]
5	2.423[b]	2.169[ab]	0.905[g]	0.602[g]	6.626[m]	6.246[m]	9.489[s]	9.979[s]

[1]All values are expressed as parts per million and are the mean of 9 observations. Means for each isomer followed by the same letter are not significantly different at the 0.10 level of probability.

TABLE 4

ANALYSIS OF VARIANCE OF PESTICIDE RESIDUES IN TURNIP GREENS SUBJECTED TO BLANCHING AND THERMAL PROCESSING

Source	d.f.	DDE M. S.	DDD M. S.	o,p'-DDT M. S.	p,p'-DDT M. S.
Total	53				
Treatments	5	0.5072[a]	0.1555	7.2156[a]	1.1006[a]
Steam, Plain vs. Enamel Cans	1	0.0162	0.0045	0.1574	0.1426
Unlined Kettle, Plain vs. Enamel Cans	1	0.6366[a]	0.2840	3.2956	0.7337
Lined Kettle, Plain vs. Enamel Cans	1	0.3934	0.1139	0.2319	0.0024
Steam vs. Water Blanching	1	0.2439	0.1408	17.2025[a]	3.8262[a]
Unlined vs. Lined Kettles	1	1.2462[a]	0.2341	15.1905[a]	0.7980
Replications	2	3.1568[a]	2.6688[a]	3.1222[a]	1.0088[a]
Reps x Treatments[b]	10	0.1946[a]	0.4026[a]	2.0601[a]	0.3562
Observations/Reps, Treatments[c]	36	0.0703	0.1657	0.9309	0.3780

[a]F-ratios are significant at the 0.10 level of probability.

[b]Replications x treatment mean square is used as the error term for treatment effects.

[c]Observations/replications, treatments mean square is used as the error term for replications and for replications x treatments.

Replications were significant for all isomers of DDT. The interaction between replications and treatments was significant for DDE, DDD, and o,p'-DDT.

The mean parts per million of pesticide residues present in the greens after they were blanched and thermally processed are presented in Table 5. The data indicate that thermal processing in different types of cans did not result in significantly different levels of residues in greens that had been steam blanched. Thermal processing in different types of cans produced a significant difference in the DDE residue level of greens that had been water blanched three minutes in an unlined kettle. Thermal processing in different types of cans did not result in significantly different levels of residues in greens that had been water blanched three minutes in a lined kettle.

When blanching was followed by thermal processing, the method of blanching had a significant effect on the amount of o,p'-DDT and p,p'-DDT remaining in the greens. The kettle lining resulted in significantly different residue levels of DDE and o,p'-DDT when blanching was followed by thermal processing.

III. RESIDUE LEVELS PRESENT IN GREENS BEFORE AND AFTER PROCESSING

Table 6 presents the mean levels of residues present in greens before and after processing. Both treatments, washing and blanching and thermal processing, decreased levels of all isomers studied except DDD, which was increased slightly by thermal processing. The data from Table 6 were not analyzed statistically, but were included to show the

TABLE 5

PARTS PER MILLION OF PESTICIDE RESIDUES IN TURNIP GREENS SUBJECTED TO BLANCHING AND THERMAL PROCESSING[1]

Process	DDE	DDD	o,p'-DDT	p,p'-DDT
Steam Blanched				
Plain Can[2]	1.474[a]	1.278[h]	6.241[m]	1.614[t]
Enamel Can[2]	1.414[a]	1.310[h]	6.054[m]	1.792[t]
Water Blanched In Unlined Kettle				
Plain Can[2]	1.330[b]	1.141[i]	6.027[n]	1.489[u]
Enamel Can[2]	1.676[c]	1.392[i]	5.172[n]	1.086[u]
Water Blanched In Lined Kettle				
Plain Can[2]	0.968[d]	1.185[j]	4.187[o]	0.978[v]
Enamel Can[2]	1.264[d]	1.025[j]	4.414[o]	1.001[v]
Blanching Method				
Steam[3]	1.444[e]	1.294[k]	6.147[p]	1.703[w]
Water[4]	1.302[e]	1.186[k]	4.950[q]	1.139[x]
Kettle Lining				
Unlined[3]	1.488[f]	1.266[l]	5.600[r]	1.278[y]
Lined[3]	1.116[g]	1.105[l]	4.300[s]	0.990[y]

[1]Pairs of means within columns followed by the same letter are not significantly different at the 0.10 level of probability.

[2]Each value is the mean of 9 observations.

[3]Each value is the mean of 18 observations.

[4]Each value is the mean of 36 observations.

TABLE 6

RESIDUE LEVELS PRESENT IN TURNIP GREENS BEFORE AND AFTER PROCESSING[a]

	DDE	DDD	o,p'-DDT	p,p'-DDT
Before Processing[b]	4.374	1.013	15.615	25.237
After Washing and Blanching[c]	2.244	0.703	6.347	9.846
After Thermal Processing[d]	1.349	1.222	5.349	1.327

[a]Values are expressed in parts per million.

[b]Each value is the mean of 9 observations.

[c]Each value is the mean of 45 observations.

[d]Each value is the mean of 54 observations.

initial levels of pesticide residues present and the overall effects of washing, blanching, and thermal processing on the levels of residues in the greens.

CHAPTER V

DISCUSSION

Edwards (23) stated that DDT is one of the most widespread pesticides found persisting in soil. The experimental data in Table A-1 in the Appendix indicate that o,p'-DDT and p,p'-DDT were present in the turnip greens prior to the first application of pesticide material. These isomers may have persisted in the soil from previous applications, however, past records of The University of Tennessee Plant and Soil Science Department indicate that DDT had not been applied to the soil during the last 10 years. It is possible that the pesticide material may have been transported into the soil or greens through the irrigation water or some other source.

The data indicate that at the time of harvest, residue levels of DDT well in excess of the tolerance level were observed in the turnip greens. Such high concentrations can be attributed to at least two factors: applications of pesticide material were made more frequently than is recommended and greens were harvested before the recommended waiting period after the final application of pesticide material.

Greens blanched by steam or by water showed no significant difference in the residue levels of any of the isomers studied. In every case, however, residue concentrations were slightly lower in the greens that had been water blanched, although not significantly less. Lamb *et al.* (43) reported that water blanching removed large amounts of DDT, but steam blanching removed only very small amounts of the residue. Greens

that were water blanched in an unlined or an enamel lined kettle showed no significant difference in the remaining levels of pesticide residues. The blanching time, three or five minutes, did not result in significantly different levels of residue.

The interaction between blanching time and kettle lining was significant only for the residue level of DDE. The greens which were water blanched five minutes in an unlined kettle had the highest concentration of DDE. The greens which were water blanched three minutes in an unlined kettle had the lowest remaining concentration of DDE, but the level of residue was not significantly less than the other levels.

Replications, or plots, were significantly different for all isomers studied. As mentioned earlier, greens within plots or replications were relatively uniform in size. However, the greens among plots were not uniform in size. Samples, although selected at random, were taken by weight and the greens included sections of leaves and stems. The samples from the larger greens tended to contain a larger proportion of stem material than the samples taken from the smaller greens. Since leaves usually contain a higher concentration of residue than stems, due to sorption through stomata and more direct exposure to spray applications, this might have been the major cause of significant differences among replications.

The interaction between replications and treatments resulted in significantly different levels of DDD, o,p'-DDT, and p,p'-DDT. The remaining amount of DDE was not significantly different.

Greens steam blanched and subsequently thermally processed in different types of cans did not result in significantly different levels

of residues. Greens water blanched three minutes in an unlined kettle and thermally processed in different types of cans resulted in significantly different levels of DDE. However, the mean residue level of DDE was less in the greens that had been processed in plain tin plate cans than in greens processed in enamel lined cans. This indicated that the enamel did not decrease the level of the pesticide in the greens. The levels of residues which remained in greens that had been water blanched three minutes in a lined kettle and subsequently thermally processed in different types of cans, were not significantly different.

Greens blanched by steam or in water did not show a significant difference in the remaining residue levels. However, when the blanching process was followed by thermal processing, a significant difference was observed for the remaining levels of o,p'-DDT and p,p'-DDT. The residue levels of all isomers studied were less in the greens that had been water blanched, but only the levels of o,p'-DDT and p,p'-DDT were significantly less.

Greens which were water blanched three minutes in either an unlined kettle or a kettle lined with enamel showed no significant difference in the residue levels of any of the isomers. However, when the blanching process was followed by thermal processing, significant differences were observed in the remaining levels of DDE and o,p'-DDT. The concentration of both isomers was less in the greens that had been blanched in a kettle lined with enamel. However, it appears unlikely that the enamel lining was responsible for the decrease in residue levels.

Replications were significantly different for all isomers. As mentioned earlier, the lack of uniformity in size of the greens might

have been the major cause of significant differences between replications. The interaction between replications and treatments of greens blanched and thermally processed resulted in significantly different levels of DDE, DDD, and o,p'-DDT.

Both treatments, washing and blanching and thermal processing, decreased levels of all isomers except DDD. DDD was decreased by washing and blanching, but slightly increased by thermal processing. Farrow et al. (25), Lamb et al. (43), and Tressler (80) observed this same trend and reported that during thermal processing, p,p'-DDT is partially converted to DDD.

From the results of this study, it is highly improbable that epoxy-phenolic enamel has an absorptive capacity for DDT or its metabolites. Martin (34) stated that in order for a reaction to occur between the enamel and DDT, it would be necessary for the coating to retain some soluble portion or extractable material. He further stated that a properly cured (baked) enamel would contain practically zero extractables and would be cross linked to the extent that a reaction with DDT would be virtually impossible. It is pointed out, however, that the enamel used in the experiment was not analyzed to determine if it contained any pesticide residue after being utilized in the processing. The conclusion that DDT was not significantly absorbed was based on the analysis of the residues remaining in the turnip greens after blanching and thermal processing.

CHAPTER VI

SUMMARY

This study was conducted to examine the effect of epoxy-phenolic enamel on levels of pesticide residues in turnip greens during processing. The study was based on the recovery of DDE, DDD, o,p'-DDT, and p,p'-DDT. Experimental variables consisted of blanching method (water and steam), blanching time in water (three minutes and five minutes), kettle lining (lined and unlined), and can type (tin plate and enamel lined).

Based on the results of this study, the following conclusions were reached:

1. Low concentrations of o,p'-DDT and p,p'-DDT were present in the greens prior to the first application of pesticide material.

2. Residues in excess of the tolerance level were present in or on the turnip greens at the time of harvest.

3. Water blanching resulted in residue levels slightly lower than steam blanching, but the levels were not significantly different.

4. Kettle linings did not significantly reduce residue levels of the pesticides studied.

5. Blanching times did not produce significantly different residue levels.

6. Enamel lined cans did not significantly reduce residue levels.

From the results of this study, it is evident that epoxy-phenolic enamel does not have absorptive capacity for the pesticides studied and is not an effective means of decreasing residue levels.

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APPENDIX

TABLE A-1

PARTS PER MILLION OF PESTICIDE RESIDUES IN GREENS[a]

Sample	DDE	DDD	o,p'-DDT	p,p'-DDT
IPS(A)	0.000	0.000	0.539	0.703
(B)	0.000	0.000	0.471	0.898
(C)	0.000	0.000	1.655	1.059
IIPS(A)	0.000	0.000	1.336	0.825
(B)	0.000	0.000	0.109	0.087
(C)	0.000	0.000	0.100	0.056
IIIPS(A)	0.000	0.000	0.645	1.269
(B)	0.000	0.000	0.225	0.449
(C)	0.000	0.000	2.312	2.615
IPH(A)	4.483	1.131	15.530	26.923
(B)	3.936	0.776	14.962	24.923
(C)	2.515	0.582	10.038	20.923
IIPH(A)	4.264	1.357	14.962	24.359
(B)	3.827	0.905	14.394	23.231
(C)	3.827	0.970	14.773	24.770
IIIPH(A)	6.013	1.260	18.940	26.923
(B)	5.030	0.970	17.614	27.077
(C)	5.467	1.164	19.318	28.000
ISB3(A)	2.776	1.053	7.572	10.552
(B)	2.285	0.778	6.322	9.693
(C)	1.732	0.549	5.240	8.712
IISB3(A)	2.227	0.615	6.543	9.311
(B)	2.818	0.782	8.272	11.259
(C)	1.515	0.559	5.231	10.641
IIISB3(A)	2.208	1.108	6.355	12.881
(B)	3.024	0.997	8.262	14.251
(C)	1.920	0.415	6.419	12.607
IWB3U(A)	2.334	0.686	5.192	7.566
(B)	1.916	0.549	4.615	7.321
(C)	1.843	0.526	4.159	6.544
IIWB3U(A)	2.636	0.838	7.901	10.546
(B)	2.273	0.782	6.914	9.169
(C)	2.591	0.838	7.284	9.929
IIIWB3U(A)	2.256	0.582	5.466	9.154
(B)	1.440	0.415	4.766	9.044
(C)	0.960	0.166	3.559	6.906

TABLE A-1 (continued)

Sample	DDE	DDD	o,p'-DDT	p,p'-DDT
IWB3L(A)	3.391	1.098	8.005	9.939
(B)	3.735	1.373	9.327	12.229
(C)	3.096	1.007	10.072	11.902
IIWB3L(A)	1.818	0.349	5.556	8.551
(B)	1.955	0.349	5.864	9.881
(C)	1.136	0.279	3.333	5.368
IIIWB3L(A)	2.064	0.554	6.165	11.741
(B)	1.920	0.415	5.910	11.401
(C)	1.776	0.415	5.466	10.743
IWB5U(A)	2.948	1.236	6.346	10.123
(B)	2.113	1.281	5.889	6.237
(C)	3.391	0.961	7.043	9.018
IIWB5U(A)	1.955	0.559	5.988	9.169
(B)	2.091	0.698	6.667	0.976
(C)	2.545	0.838	7.778	10.784
IIIWB5U(A)	2.496	0.914	7.181	10.469
(B)	1.872	0.665	5.688	9.099
(C)	2.400	0.997	7.054	10.524
IWB5L(A)	2.285	0.755	5.240	9.693
(B)	2.727	1.121	7.478	10.184
(C)	3.145	1.098	8.269	11.043
IIWB5L(A)	2.136	0.503	6.605	9.739
(B)	1.909	0.279	5.988	9.501
(C)	2.091	0.419	6.173	10.214
IIIWB5L(A)	1.728	0.415	5.783	10.688
(B)	1.680	0.415	5.084	8.989
(C)	1.824	0.415	5.593	9.757
ISB3P(A)	2.555	1.831	5.433	1.391
(B)	1.990	2.197	6.322	1.718
(C)	1.899	1.545	6.346	1.391
IISB3P(A)	1.273	1.131	6.914	1.995
(B)	1.591	1.341	7.407	1.520
(C)	1.273	1.173	7.222	1.853
IIISB3P(A)	1.104	0.914	5.910	1.316
(B)	0.768	0.665	4.957	1.261
(C)	0.816	0.706	5.656	2.083
ISB3E(A)	2.555	2.574	5.769	1.892
(B)	1.622	1.419	4.808	0.818
(C)	1.548	1.739	6.106	1.350
IISB3E(A)	1.455	1.215	7.716	2.942
(B)	1.318	1.173	6.728	2.090

TABLE A-1 (continued)

Sample	DDE	DDD	o,p'-DDT	p,p'-DDT
(C)	1.591	1.383	7.469	1.995
IIISB3E(A)	0.816	0.706	4.703	1.590
(B)	0.864	0.748	5.084	1.644
(C)	0.960	0.831	6.101	1.809
IWB3UP(A)	1.691	1.347	5.455	0.924
(B)	1.166	0.931	10.364	4.615
(C)	1.166	0.931	4.924	0.862
IIWB3UP(A)	1.455	1.341	6.173	1.758
(B)	1.864	1.718	7.346	1.473
(C)	1.364	1.215	5.432	1.283
IIIWB3UP(A)	0.880	0.955	5.211	0.877
(B)	1.056	0.914	4.512	0.735
(C)	1.056	0.914	4.830	0.877
IWB3UE(A)	2.332	1.810	5.636	0.974
(B)	2.741	2.223	6.546	0.974
(C)	1.983	1.603	5.152	0.769
IIWB3UE(A)	1.773	1.550	5.231	1.900
(B)	1.591	1.425	4.907	1.188
(C)	1.591	1.383	4.583	0.950
IIIWB3UE(A)	1.056	0.789	4.639	0.987
(B)	1.008	0.872	5.021	1.206
(C)	1.008	0.872	4.830	0.822
IWB3LP(A)	1.516	1.293	5.091	0.821
(B)	1.341	1.086	4.424	0.974
(C)	1.341	1.086	4.667	1.128
IIWB3LP(A)	0.636	1.397	3.009	0.950
(B)	0.955	0.838	3.333	0.808
(C)	0.955	3.603	6.481	2.423
IIIWB3LP(A)	0.624	0.540	3.559	0.548
(B)	0.720	0.623	3.750	0.603
(C)	0.624	0.195	3.368	0.548
IWB3LE(A)	1.574	1.293	4.909	0.974
(B)	1.399	1.138	4.546	1.180
(C)	1.516	1.241	4.485	0.769
IIWB3LE(A)	0.955	0.769	3.102	0.903
(B)	0.909	0.838	3.241	0.950
(C)	1.515	1.047	3.935	1.330
IIIWB3LE(A)	1.200	0.997	5.338	0.877
(B)	1.200	1.034	5.466	1.151
(C)	1.104	0.872	4.703	0.877

TABLE A-1 (continued)

[a]Roman numeral indicates replication or plot. Letters indicate process level from which the sample was taken: PS, prespray; PH, pre-harvest; SB, steam blanch; WB, water blanch; L, lined kettle; U, unlined kettle; P, plain can; E, enamel lined can. Arabic numbers indicate blanching time in minutes. Letters in parentheses indicate observations.

VITA

James G. Fair was born in Houston, Missouri, on April 7, 1945. He attended both elementary and high school in Houston and graduated in May of 1963. The following September he entered Southwest Missouri State College located in Springfield, Missouri. In May of 1968, he received a Bachelor of Science degree in Biology and was commissioned a Lieutenant in the United States Army Medical Service Corps.

After returning from an assignment in Southeast Asia, he entered The University of Tennessee Graduate School and received a Graduate Research Assistantship in Food Technology. From July, 1970, to June, 1972, he has been working to complete the requirements for the degree, Master of Science in Food Technology.

He and his wife, the former Miss Carla Sue Tanner, were married on November 16, 1968, and they have one son, Marc James, age six months.