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THE EFFECTS OF COMMERCIAL AND HOME PROCESSING TECHNIQUES ON INSECTICIDE RESIDUES IN FOOD

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Marilyn Anne Nielsen

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Nature and scope of the work

Organic pesticides have been used on a world-wide basis for about 25 years to protect human health and to increase the yield of agricultural products. Unfortunately, the benefits derived from pesticides are partially offset by broad dispersion in and detrimental effects on the environment.

Concern has centered recently on the organochlorine pesticides because of their persistence and threat to human health. They accumulate in water and soil and enter the food chain. Translocation of these pesticides is greatest into root vegetables and oilseed crops. The pesticide residues which are present in most food in varying amounts are the major source of pesticides absorbed by humans.

Much research has been carried out on the extent of pesticide residues in raw food. However, most food is processed commercially or domestically before being eaten. Common processing methods such as washing, peeling and boiling of vegetables or the refining of edible vegetable oils may have a significant effect on pesticide residues in food. If a reduction in pesticide residues is achieved by various processing methods then any possible hazard that the pesticide may constitute to the consumer is decreased. Consequently, there is a need for more information about the

effect of processing techniques on pesticide residues in many foods.

The object of this thesis was to investigate the following:

1. To determine the effect of home processing techniques such as peeling, boiling and baking on chlordane residues in potatoes, beets, carrots and turnips. Chlordane was selected because its use may increase substantially as a replacement for other more persistent organochlorine pesticides, especially for the control of wireworms.
2. To determine the effect of simulated commercial vegetable oil processing techniques on the DDT and lindane residues in rapeseed oil. Lindane and DDT were selected because they are commonly used for pest control on rapeseed and also because they represent two extremes in volatility which could be a factor in their reduction during processing.

1.2 Pesticide risks versus benefits

Since DDT was discovered in 1939 the number and uses of chemical pesticides have increased rapidly (Metcalf, 1965). The efficiency and economic value of pesticides in the production of food and fiber and in controlling arthropod borne diseases such as

malaria, plague, typhus, and African sleeping sickness is well documented.

The increasing world population requires an increased rate of food production. It is estimated that one-half of the world's population already exists on a suboptimal diet of less than 2200 calories per person per day. Although the food deficit is likely to become more acute, the use of pesticides is a major factor in increasing agricultural crop yields. For instance, farm output in the United States has increased 2.5 times since 1945, due in part to the use of pesticides (West, 1966).

In addition to increasing food production, pesticides are sometimes required to control agricultural or medical emergencies such as locust plagues and typhus epidemics (West, 1966).

Some time after the use of pesticides had become widespread the residual characteristic of some types was recognized. They accumulated and dispersed in the environment thus contaminating water, air, soil, crops, animals and people. It is very difficult to assess the long-term significance of pesticide residues in the environment and man, but it is generally conceded that the proper use of pesticides usually results in residue concentrations that are far below the levels judged hazardous to human health by present medical standards (West, 1966).

Several non-insecticidal methods of pest control such as the use of attractants and repellents, sterilization, and biological controls are currently being investigated. Much more

Table 1.1 Classification of pesticides

Insecticides		
	(a) Organochlorine	aldrin BHC chlordane DDT dieldrin, endrin heptachlor, heptachlor epoxide lindane methoxychlor
	(b) Organophosphorous	diazinon guthion malathion parathion
	(c) Carbamate	carbaryl
Fungicides		captan maneb zineb
Herbicides		dalapon diuron trifuralin 2,4-D

In the United States, insecticides accounted for almost one-half of the 750 million pounds of pesticides used in 1964. Herbicides and fungicides accounted for most of the balance. The use of fungicides and organochlorine insecticides has apparently reached a peak while the use of organophosphorus insecticides and herbicides is increasing. It has been predicted that the use of herbicides will far surpass that of insecticides within a few years (Mitchell, 1966).

While Canada uses only about four percent of the amount of pesticides used in the United States, the same trend seems to be operating. As shown in Table 1.2, the value of all pesticides used in Canada was 39 million dollars in 1965, but this had risen to 66 million dollars by 1968 (Dominion Bureau of Statistics, 1966, 1969).

Table 1.2 Volume and value in millions of pounds and dollars of pesticides used in Canada in the years 1965 and 1968

Pesticide	1965		1968		Percent increase in dollars
	Pounds	Dollars	Pounds	Dollars	
Insecticides	6	5	8	7	33
Fungicides	5	5	5	5	0
Herbicides	22	17	36	35	64
Others	-	12	-	19	52
Total		39		66	40

1.4 Persistence in soil

In the past 25 years increasing quantities of very stable pesticides have reached agricultural soils either by direct application or indirectly as a result of foliar application run-off or by drift. Although the organochlorine insecticides account for only about 15% of all pesticides used in Canada, they are responsible for almost all the persistence and residue problems. They are not readily biodegradeable as are the fungicides, the organophosphorus insecticides and most of the herbicides (Saha, 1968).

The persistence of organochlorine insecticides has received a great deal of attention from numerous investigators, but the reported estimates lack uniformity. This variation in results most likely reflects soil and environmental variations as well as different sampling techniques and analytical methods (Van Middelem, 1966). DDT was found to be the most persistent by Allen et al. (1954), Lichtenstein et al. (1960), and Roberts et al. (1962). However, Kincaid et al. (1960) reported endrin to be more persistent than DDT. Foster and Boswell (1956) found endrin to be very persistent, reporting a recovery of almost 100% of applied endrin after two years. Edwards (1964) in an attempt to clarify the persistence of organochlorine insecticides has calculated regressions for their rate of disappearance from all available data. These are summarized in Table 1.3

Table 1.3 Persistence of some organochlorine insecticides in soil

Chemical	Average dosage active ingredient, pounds/acre*	Years for 95% disappearance**
DDT	1 - 2½ (10)	4 - 30 (10)
Dieldrin	1 - 3	5 - 25 (8)
Lindane	1 - 2½ (5)	3 - 10 (6½)
Chlordane	1 - 2 (6)	3 - 5 (4)
Telodrin	¼ - 1	2 - 7 (4)
Heptachlor	1 - 3	3 - 5 (3½)
Aldrin	1 - 3	1 - 6 (3)

*Bracketed figures are doses which may be used for particular pests in unusual circumstances.
**Bracketed figures are average persistence.

In general, DDT is the most persistent, about 80% remaining one year after application as compared with 75% of dieldrin, 60% of lindane, 55% of chlordane, 45% of heptachlor and 26% of aldrin. Three years after application, the residues remaining are about 50% for DDT, 40% for dieldrin, 15% for chlordane, 10% for heptachlor and 5% for aldrin (Edwards, 1964).

1.4.1 Characteristics of the insecticide

The chemical structure of the insecticide is the most important factor in determining persistence, although climate and

soil; type may also affect the rate of breakdown (Bollen et al., 1958; Young and Rawlins, 1958). Pest control requires insecticides to be stable and persist until they destroy the pest. The effective persistence of these chemicals is influenced by the nature of the eventual breakdown products, which may or may not have insecticidal properties. Some such as lindane, degrade to compounds that are not toxic to insects (Edwards et al., 1957; Lichtenstein and Schulz, 1959a). Others such as DDT yield a mixture of products, some of which are insecticidal and others not (Edwards, 1966). Other insecticides such as aldrin and heptachlor break down to yield dieldrin and heptachlor epoxide which are more persistent and more toxic than the parent compounds (Gannon and Biggar, 1958; Lichtenstein and Schulz, 1960; Lichtenstein et al., 1964; Wilkinson et al., 1964).

Organochlorine insecticide persistence is determined not only by chemical breakdown, but also by volatility. There is considerable evidence to indicate that even the relatively stable insecticides volatilize. Edwards (1966) has indicated that insecticides with greater vapour pressures usually disappear faster from soil. This is illustrated in Table 1.4.

Table 1.4 Insecticide vapour pressure and disappearance from soils

Insecticide	Average % insecticide in soil after one year	Vapour pressure at 20°C. (mm. Hg.)	
DDT	80	1.0×10^{-7}	
Dieldrin	75	1.0×10^{-7}	slightly volatile
Lindane	60	9.4×10^{-6}	
Aldrin	26	6.0×10^{-6}	moderately volatile
Chlordane	55	1.0×10^{-5}	
Heptachlor	45	3.0×10^{-4}	volatile

Reports by Harris (1961) and Harris et al. (1961) agree with the results obtained by Edwards, with the exception that they called heptachlor and chlordane moderately volatile and DDT non-volatile. Other investigations show that volatilization is one of the major factors involved in the disappearance of insecticidal residues from the soil (Barthel et al., 1960; Lichtenstein et al., 1962).

The water solubility of an insecticide can also influence its persistence in the soil. Since most of the chlorinated hydrocarbon insecticides have water solubilities of less than ten parts per million (p.p.m.), it is unlikely that these residues will be leached from the soil. This was demonstrated by Lichten-

stein (1958) who found that the largest proportion of the applied materials remained in the top three inches of soil under leaching conditions. Harris (1964) postulated that the bioactivity of an insecticide in soil is inversely related to its solubility. Water insoluble materials such as aldrin, heptachlor, dieldrin and DDT are the most effective.

The concentration of the insecticide in the soil is also a factor determining its persistence. Lichtenstein and Schulz (1959a) found that the disappearance of an insecticide residue from the soil depends on the rate of application; the lower the concentration in the soil, the more rapidly it disappears.

The formulation of the insecticide also has some effect on its persistence. Granules are the most persistent followed by emulsions and then miscible liquids (Lichtenstein et al., 1964; Mulla, 1960; Young and Rawlins, 1958).

1.4.2 Characteristics of the soil

Soil type has been found to have a great influence on the persistence of insecticidal residues second in importance only to the characteristics of the insecticide. Soil type is determined by features such as the proportion of sand, silt and clay, the mineral content, pH, and organic matter content (Edwards, 1966). Heavier soils with higher clay and or organic matter retain insecticides longest, probably due to adsorption. This renders the

insecticide less toxic to pests. Early reports by Appleman and Sears (1946), Chulski (1948) and Foster and Boswell (1956) indicated that crops grown on soils treated with very large dosages of organochlorine insecticides were damaged by the insecticides more in light sandy soils than in heavy clay, muck or peat soils. These results were later confirmed by toxicity and residue experiments by Edwards et al. (1957), Harris and Lichtenstein (1961), Lichtenstein (1959), Lichtenstein and Schulz (1959b), Roberts (1963), and Young and Rawlins (1958).

Soil type also affects the rate of biodegradation of some insecticides. Lichtenstein and Schulz (1959a) found that most aldrin was converted into dieldrin in a sandy soil, less in a silt loam, and least in a muck soil. This was verified in further work (Lichtenstein and Schulz, 1960), although Harris and Lichtenstein (1961) showed that more dieldrin was formed in a given time in silt loam soils than in sand or sandy loam, possibly due to increased vaporization from the lighter soils.

1.4.3 Environmental features

Environmental factors affecting insecticide persistence include such variables as the soil microflora, temperature, soil moisture, plant cover and cultivation.

The effect of the microbial soil population in the degradation of insecticides has been investigated by Bollen et al.

(1958), Lichtenstein and Schulz (1960) and Menn et al. (1960). They found that insecticides persisted longer in sterile soils than in non-sterile soils.

Temperature affects the rates of bacterial decomposition, volatilization and chemical degradation. Higher temperatures increase these rates, thereby hastening the disappearance of the insecticide from the soil. Edwards (1964) and Lichtenstein and Schulz (1959b) found that no significant losses occurred in frozen soil. More rapid disappearance was observed at higher temperatures by Kiigemagi et al. (1958) and Lichtenstein and Schulz (1959b). Higher temperatures also increase the rate of conversion of insecticides to other compounds. Much more aldrin was found to be converted to dieldrin in soil at temperatures of 26°C. and 46°C. than at seven degrees C. (Lichtenstein and Schulz, (1959a). The rate of evaporation of volatile compounds is also increased by higher temperatures. Harris and Lichtenstein (1961) found that the volatilization of aldrin from soil increased with an increase in temperature.

Soil moisture content is closely related to rainfall, and can greatly affect the availability and toxicity of an insecticide by influencing the adsorption on various soil fractions. Water competes with the insecticide for adsorption sites, so in dry soils more insecticide is adsorbed than in wet ones. This fact was reported by Harris and Lichtenstein (1961) and Swanson et al.

(1954). Harris (1964) estimated that the toxicity of heptachlor and DDT may vary two to three times within the range of normal field moisture conditions. He also reported that they were more toxic in moist sand and muck than in dry, but dry muck did not inactivate the insecticides to the extent that the mineral soil did.

Plant cover on soils can increase insecticide persistence. Lichtenstein et al. (1962) found that a dense cover crop of alfalfa considerably increased the persistence of aldrin and heptachlor residues in soil. Three years after 25 pounds of aldrin had been applied to soil plots, 9.1 p.p.m. remained in the soil plots with an alfalfa cover, while bare plots contained only 4.8 p.p.m. Bare soil is likely exposed to greater extremes of temperature, rainfall and wind movement. The effect of air movement was also investigated (Harris and Lichtenstein, 1961). Very little insecticide was lost in still air, possibly due to slower volatilization.

Cultivation also may influence persistence since insecticides left on the soil surface disappear more rapidly than those incorporated into the soil (Lichtenstein and Schulz, 1961; Mulla, 1960). While this initial incorporation of insecticides into the soil increases persistence, Lichtenstein and Schulz (1961) reported that regular weekly cultivation of soil after insecticide application decreased persistence.

1.5 Absorption and translocation by crops

When pesticides are applied directly to the plant surface the amount absorbed depends mainly on the nature of the host plant. Cook (1965) found that methoxychlor applied to tomatoes at a rate of three pounds per acre and to alfalfa at a rate of two pounds per acre resulted in residues of only two p.p.m. in tomatoes, but 200 p.p.m. in alfalfa immediately after application. The amount of insecticide residue absorbed by plants grown in treated soil depends on the soil type, the crop grown (Harris and Sans, 1967; Lichtenstein, 1959), the nature of the insecticide (Lichtenstein, 1959), and climatic conditions (Harris and Lichtenstein, 1961).

Soil type exerts a profound influence on the amount of insecticide residue found in crops. Crops absorb least residue from muck soil, an intermediate amount from clay soil, and most from sandy soil. Lichtenstein (1959) found less lindane, aldrin and DDT translocated into crops in muck soils than in silt, and most in sandy soils. This was later confirmed by Harris and Sans (1967). The effect of soil type on the amount of lindane residues found in carrots at harvest is illustrated in Table 1.5 as reported by Lichtenstein (1959).

Table 1.5 The effect of soil type on uptake of lindane by carrots

Soil type	Soil p.p.m.	Carrots p.p.m.	p.p.m. crop/ p.p.m. soil
Sandy loam	0.96	5.99	6.24
Silt loam	0.90	2.41	2.68
Muck	6.66	0.40	0.06

The amount of residue absorbed from the soil by crops also depends to a great extent on the type of crop grown (Marth, 1965). Crop residue absorption may also vary from one variety to another within the same crop (Lichtenstein et al., 1965). Root crops absorb the most residues, carrots being the worst offender. Carrots may accumulate as much or more residue as the soil itself (Lichtenstein, 1959). Oilseed crops, forage crops and cereals absorb decreasing amounts of insecticide (Harris and Sans, 1967; Lichtenstein, 1959). The influence of the type of crop on the amount of residue present at harvest is shown in Table 1.6. The residue concentrations indicated in this table can only be considered as approximations as the values will depend on other factors such as the soil type, climate, application rate,etc. and may vary quite widely.

Table 1.6 The effect of crop type on absorption of aldrin and dieldrin residues from soil

Crop	Aldrin and dieldrin in p.p.m.			Reference
	Soil	Crop	p.p.m. crop/ p.p.m. soil	
Carrots	0.98	0.83	0.85	Lichtenstein (1959)
Beets	0.98	0.06	0.06	ibid.
Potatoes	0.98	0.15	0.15	ibid.
Radishes	0.98	0.18	0.18	ibid.
Turnips	0.98	0.10	0.10	ibid.
Cucumbers	0.98	0.14	0.14	ibid.
Onions	0.59	0.01	0.02	Harris and Sans (1967)
Peanuts	0.33	0.46	1.39	Morgan et al. (1967)
Soybeans	1.00	0.14	0.14	Bruce and Decker (1966)
Wheat	1.80	0.03	0.02	Saha and McDonald (1967)
Alfalfa	0.07	0.02	0.29	Moubray et al. (1967)

Oilseed crops absorb considerable amounts of residues which tend to accumulate in the fat portion of the seed. Morgan et al. (1967) reported residues of aldrin and dieldrin in peanut meats of 0.57 p.p.m. Bruce and Decker (1966) found 0.04 p.p.m. of aldrin in soybeans from crops grown in soil treated at a rate of one pound per acre. Bruce et al. (1966) reported a direct relationship

between oil content of seeds and residue content of crops grown on aldrin and heptachlor treated soil.

The crop variety also determines the amount of insecticide residue absorbed from the soil. Lichtenstein et al. (1965) investigated the uptake of aldrin and heptachlor by five different carrot varieties and found considerable difference in the rate of absorption. Residues varied from 22 to 80% of the concentration in the soil. Yellow carrots contained 70 to 86% of the total residue within the peel, while the white Belgian variety contained only 50% of the residues within the peel.

The quantity of insecticidal residue in crops is also determined partially by the nature of the insecticide used in soil treatment. Lichtenstein (1959) determined that carrots absorbed less DDT than lindane or aldrin when grown on the same soil.

Translocation of insecticide residues will occur within the plant (Finlayson and MacCarthy, 1965), as demonstrated in studies on wheat, alfalfa, and pea plants. Wheeler et al. (1967) found that dieldrin was translocated by wheat from the root system into the aerial part of the plant where it localized in varying amounts, the most being in the stem and the least in the top. King et al. (1966) detected more residue translocated into the crown of alfalfa than the rest of the aerial portion of the plant when grown in soil treated with heptachlor. Lichtenstein et al. (1967) related the translocation of aldrin and lindane in pea plants to their water solubilities, which are 10.0 p.p.m. for lindane as

compared to 0.01 p.p.m. for aldrin. Four times as much lindane was translocated into the aerial portions of the pea plants as aldrin.

Lichtenstein et al. (1967) also found that the pea plant converted aldrin to dieldrin. There was little conversion of aldrin to dieldrin in the soil, only one percent of the total soil residue consisted of dieldrin. The amount of dieldrin in the roots was 11 to 12% of the total aldrin and dieldrin residue recovered from the roots. However, 93% of the total residue recovered from the greens was in the form of dieldrin. Saha and McDonald (1967) found no residues in the seed of wheat grown in soil treated with eight pounds of aldrin and endrin per acre, although there were detectable residues in the remainder of the plant.

1.6 Translocation into meat, milk and eggs

Organochlorine insecticide residues may be translocated into meat, milk and eggs as a result of the consumption of contaminated feed by animals or direct application of the insecticide to barns (Cummings et al., 1966; Saha, 1969). Henderson (1965) reported widespread contamination of milk and other dairy products with organochlorine insecticides in the United States. A market survey conducted by Duggan (1969) revealed that 20% of the animal tissue samples analyzed contained over 0.5 p.p.m. pesticide residues. Numerous studies have implicated insecticide contaminated animal feeds as an important cause of meat and milk residues

(Saha, 1969). Cole et al. (1966) detected DDT residues in 54% of forage and 62% of grain and commercial feed supplement samples in a recent study. Saha et al. (1968) found that 80% of legume crop samples taken from fields in Saskatchewan contained 0.01 p.p.m. or more dieldrin. This is sufficiently high, in the authors' opinion, to cause appearance of low levels of dieldrin in meat or milk of animals fed these contaminated forage crops as their entire ration.

The amount of organochlorine insecticides stored in the body fat or excreted in the milk of animals fed contaminated feed follows a definite order (Saha, 1969); heptachlor epoxide > aldrin ≥ dieldrin > endrin > γ-BHC > heptachlor > methoxychlor. The relative rates of detoxification of these insecticides by animals should follow the reverse order.

1.7 Regulations

Pesticide residues on foods are subject to legal restrictions in Canada as determined by the Food and Drug Directorate of Canada. Internationally, the FAO/WHO Expert Committee on Pesticides draws up recommendations for acceptable daily intake. This is the daily dosage which, during an entire lifetime appears to be without appreciable risk. The acceptable daily intake is normally derived from the no-effect level in the most susceptible animal species divided by 100 to provide a margin of safety (Fitzhugh, 1965). The permissible residue on the food, or

"tolerance", is calculated from the need for use of the pesticide on the agricultural product, the acceptable daily intake, the average fraction of the total diet made up by the food, and the average weight of the consumer.

There is no legal tolerance for cyclodiene insecticides such as aldrin, dieldrin, chlordane, heptachlor and endrin in milk in Canada or the United States. There is also no legal tolerance for cyclodiene insecticides in animal tissues or eggs. Four to seven parts per million of lindane are allowed in animal fat, the amount depending on the type of fat. Tolerances on a wide range of non-animal foods including many fruits and vegetables are: 0.1 p.p.m. for aldrin and dieldrin, 1.0 to 3.5 p.p.m. for DDT depending on the particular product, 0.3 p.p.m. for chlordane, and 10.0 p.p.m. for lindane.

1.8 Residue reduction in food

It is desirable to reduce unavoidable residues in foods as much as possible before consumption. The contribution of various food groups to the chlorinated hydrocarbon insecticide residues in the diet were reported by Martin and Duggan (1968) as: dairy products 17.5%, meat, fish and poultry 35%, grains and cereals 7.5%, potatoes 1.3%, leafy vegetables 2.5%, garden fruits 10%, fruits 22.5%, and oils, fats and shortening 2.5%.

Residues in animal products are generally associated

with the lipid material while in plants the residues may be found on the surface in the waxy layer of the skin or in the inner tissue (Liska and Stadelman, 1969). The method and success of deliberate residue reduction in food products is determined not only by the location of the residue, but also by the type of residue and the particular food involved. Some commercial and home processing techniques have been found to remove or reduce residues while the use of special techniques for residue reduction have gained little support.

1.8.1 Residue reduction in animal products

Organochlorine insecticide residues which are concentrated in fatty portions of animal products may be materially reduced by physical separation of the fat. This may be accomplished by rendering or trimming of meat, separation of milk into skim milk and cream, and eggs into white and yolk. Heat treatment during processing also reduces residue levels in some cases (Liska and Stadelman, 1969).

A review of the literature by Lidka and Stadelman (1969) indicates that pasteurization has little effect on DDT residues in milk, but separation of fat leaves the skim milk almost residue free. Processing of the cream into butter oil removes up to 53% of the DDT present but high temperature vacuum distillation is required to remove all the DDT, dieldrin and heptachlor epoxide from the butter oil. Up to 50% of the heptachlor epoxide, chlordane,

dieldrin and methoxychlor in milk are eliminated during condensing and sterilization operations. Spray or drum drying of milk resulted in a reduction of as much as 83% of the DDT, lindane, dieldrin, heptachlor, heptachlor epoxide, and chlordane residues. Methoxychlor was not affected.

Meat is relatively resistant to insecticide loss during cooking. Only frying and pressure cooking removed noticeable amounts of DDT from beef (Carter et al., 1948). Chicken is more amenable to residue removal. Reports indicate a loss of 50% of the DDT during frying and baking (Ritchey et al., 1967), 40 to 50% of DDT, dieldrin, heptachlor, lindane and chlordane by simmering, and up to 95% of these insecticides by pressure cooking (Liska et al., 1967; McCaskey et al., 1968).

1.8.2 Residue reduction in vegetables

Insecticide residues in raw garden vegetables are more easily removed or reduced than in animal products. Few studies have shown that normal commercial and home preparative procedures result in complete or very significant residue removal (Liska and Stadelman, 1969).

A study conducted by the National Canners Association (1967) investigated the effects of commercial and home processing techniques on several pesticide residues in tomatoes, green beans, brocolli, potatoes and spinach. The pesticides studied were DDT,

parathion, malathion and carbaryl. In general, carbaryl was found to be the most easily removed, DDT and malathion intermediate, and parathion the most difficult.

The study found that washing the vegetables easily removed surface residues of DDT. Up to 91% of the DDT residue was removed from tomatoes, somewhat less from beans and spinach and a small amount from potatoes. Residue not found on the surface was tightly held by the plant waxes. Hemphill et al. (1967) on the other hand, found that washing of green beans removed only five percent of the DDT residues. Carbaryl was readily removed from tomatoes, green beans, spinach and broccoli by washing, as it is partially water soluble and has little affinity for plant waxes (N.C.A., 1967). Washing easily removed surface residues of malathion from tomatoes and green beans. Kilgore and Windham (1970) however, reported that washing caused only a small reduction in malathion residues in broccoli. The National Canners Association reported that parathion residues in spinach and broccoli were resistant to removal by washing.

The report (N.C.A., 1967) indicated that blanching was less effective in removing residues than washing. They did find that washing plus blanching removed significant amounts of residues. Commercial blanching methods were reported to be more effective than home methods.

They also found that peeling of tomatoes and potatoes

was the most effective method of residue removal. Over 90% of the DDT, malathion and carbaryl residues were removed from tomatoes by peeling, and a similar amount of DDT was removed from potatoes by this method. Other reports indicate a high concentration of insecticide residues in vegetable peelings (Lichtenstein et al., 1965; Saha and Stewart, 1967).

Heat processes such as boiling or canning were effective in further reducing insecticide residues so that most of the vegetables were almost residue free after treatment. Carbaryl and parathion were less affected by heat processing than DDT or malathion. Lichtenstein et al. (1965) found aldrin residues in carrots were unaffected by boiling, but heptachlor residues were reduced. Saha and Stewart (1967) reported that one-half of the heptachlor residues in turnip peels were removed by boiling. Boiling also removed the small amount of residue remaining in the pulp of the turnip after peeling.

The effects of processing on the DDT residues in several vegetables are illustrated in Table 1.7. Most of these processes resulted in significant reduction of insecticide residues.

Table 1.7 The effects of processing on the DDT residues in vegetables

Crop	Residue p.p.m.	Treatment	Percent loss
Tomatoes[a]	7.0	Commercial wash, juiced, canned	99
	4.0	Home wash, peeled, stewed	85
Potatoes[a]	0.5	Commercial wash, peeled, canned	99
	0.5	Home wash, unpeeled, boiled or pressure cooked	0
	0.5	Wash, peeled	91
Spinach[a]	25.0	Commercial wash, blanched, canned	95
	25.0	Home washed, blanched, boiled	39
Green beans[a]	4.0	Commercial blanched, canned	80
	13.0	Home wash, canned	89
	13.0	Home wash, blanched, frozen	77
	13.0	Home wash, boiled	52
	13.0	Home wash, pressure cooked	66
Beans[b]	3.6	Washed, blanched, frozen	60
Beans[c]	0.67	Washed, trimmed, boiled	46
	0.67	Washed, trimmed, pressure cooked	63
	0.67	Washed, trimmed, electronic cooked	48
Green Pepper[d]	5.8	Washed, canned	60

[a] National Canners Association (1967)
[b] Carlin et al. (1966)
[c] Hemphill et al. (1967)
[d] Menzer et al. (1960)

1.8.3 Residue reduction in vegetable oils

Organochlorine insecticide residues have been found to concentrate in the oilseed portion of such crops as soybeans, peanuts and cotton (Bruce and Decker, 1966; Morgan et al., 1967). Since organochlorine insecticides are oil soluble, they are removed with the oil during the extraction process. This results in less residue in the meal, but a higher concentration in the crude oil.

Two studies carried out in the past have shown that some of the organochlorine insecticides can be removed from soybean and cottonseed oils in the refining process (Gooding, 1966; Smith et al., 1968). Gooding (1966) found that 14 organochlorine insecticides were removed simultaneously from cottonseed oil in the deodorization step of vegetable oil refining. Smith et al. (1968) confirmed these findings in a study of cottonseed and soybean oils containing seven organochlorine insecticides.

While the present study was in progress, Mounts et al. (1969) reported that 96% of the endrin-^{14}C was removed from soybean oil during deodorization.

The hydrogenation step of vegetable oil refining was reported by Gooding (1966) to remove trace amounts of chlordane and DDT. Smith et al. (1968) found that endrin residues were reduced to very low levels during hydrogenation.

These findings lend support to the statement by Gooding (1966). "Although no tolerances have been established in the U.S.A.

for processing edible vegetable oils, it appears that none may be required since the processing of these oils inherently involves steps which result in food products free of chlorinated organic pesticide residues."

2. MATERIALS AND METHODS

2.1 The effect of processing methods on pesticide residues in vegetables

The first part of this thesis involved a study of the effect of home processing methods such as boiling, peeling and baking on chlordane residues in selected root crops. Chlordane was chosen as the organic pesticide for this research because its use is increasing and few studies have been done on the effect of processing methods on the chlordane residues in vegetables. Since root crops absorb a greater amount of organic pesticides from the soil than other vegetables; potatoes, carrots, beets and turnips were selected for this research.

The potatoes were grown at the Canada Department of Agriculture experimental farm at Scott, while the other three root crops; carrots, beets and turnips were grown at the Saskatoon experimental farm. Test plots were treated with chlordane immediately before seeding the vegetables. Analyses for chlordane were carried out on samples of the treated soil and on the unprocessed and processed vegetables using well established methods.

The analytical methods consisted essentially of extracting the sample with a suitable solvent, then purifying the extract in a chromatographic cleanup column. Analysis of the purified extract was carried out by gas chromatography.

2.1.1 Soil treatments

Chlordane is commonly used as a soil dressing for wireworm control in root crops. The recommended rate of application for wireworm control is ten pounds of active chlordane per acre. The insecticide is normally applied to the soil before seeding in the form of granules or as an emulsifiable concentrate and incorporated into the upper five or six inches of the soil by discing. For effective wireworm control, the chlordane treated soil must be in direct contact with the root crops.

In this investigation, soil treatments with chlordane were conducted in the usual manner for field application of this insecticide as outlined above. However, the rate of application was varied for the purposes of this study.

2.1.1.1 Potato plots

Potatoes were grown in soil treated at various levels of chlordane application. Three clay loam plots, each ten by 20 feet, were treated with 25% chlordane granules at a rate of 5.0, 7.5 and 10.0 pounds per acre, respectively, of the active ingredient. A fourth plot of the same size which was not treated with insecticide served as the control. The chlordane was applied and incorporated into the soil by discing on June 30, 1967. On the same day, two rows of Norland potatoes were seeded lengthwise in each plot. The potatoes were harvested on September 28, 1967.

2.1.1.2 Carrot, beet and turnip plots

Carrots, beets and turnips were grown in soil treated with chlordane. Three plots of a heavy clay soil each 40 by 100 feet were sprayed with an emulsifiable concentrate of chlordane at a rate of 15 pounds of active chlordane per acre. The toxicant was applied to the soil surface and incorporated into it by discing on June 9, 1968. Each plot was then subdivided into three sub-plots each 40 by 30 feet with five foot buffer strips on both sides of the center plot.

On the day the chlordane was applied to the soil, the plots were seeded with carrots, beets and turnips. One sub-plot in each of the three main plots was seeded with Tenderlong Imperator carrots. A second sub-plot in each of the main plots was seeded with Crosby beets. Swede Canadian Gem turnips were seeded in the third sub-plot. The crops were harvested at maturity on September 29, 1968.

2.1.2 Soil and crop sampling

The soil on which the vegetables were grown was sampled for chlordane analysis on the seeding and harvesting dates. Soil samples were taken from each plot and consisted of ten cores, each two inches in diameter and six inches long. The soil samples were air-dried at room temperature, screened through a 20 mesh sieve, mixed thoroughly and stored in sealed plastic bags at -18°C. (0°F.)

until they were analyzed.

Each type of vegetable grown in soil treated with the same level of chlordane was pooled and a ten pound sample taken for processing and analysis. The samples were first washed with cold water to remove adhering soil. The tops of the carrots, beets and turnips were cut off one inch above the root. These were stored separately in sealed plastic bags at -18°C. until they were analyzed. Each root crop sample was divided into three equal parts. One-third of each sample was diced into small pieces, while another one-third of the sample was peeled. The peels and the pulp were cut into small pieces and stored separately. The remainder of the sample was left whole. All samples were stored in sealed plastic bags at -18°C. until they were analyzed or processed.

2.1.3 Reagents

High purity solvents were required for the extraction and cleanup procedures to eliminate interferences in the subsequent gas chromatographic analysis. Reagent grade hexane and petroleum ether (b. 30° to 60°C.) were heated under reflux with sodium-lead alloy and distilled through a Vigreux column to remove traces of contaminants. The first 100 ml. and the last 250 ml. were discarded when distilling a two liter sample. Nanograde acetonitrile, benzene, and isopropyl alcohol were used without further purification.

The cleanup column packing materials were aluminum oxide for the potato extract and 4:1 magnesia-celite for the other vegetable extracts. Woelm aluminum oxide grade III was used without further purification. Magnesia (Sea Sorb 43) was heated at 130°C. for three hours prior to use. Celite 545 was used without further purification.

Reference samples of analytical grade cis- and trans-chlordane, heptachlor, heptachlor epoxide and technical chlordane were obtained from Velsicol Corporation, Chicago, Ill. Analytical grade endrin was obtained from Shell Chemical Company, Modesto, California. The purity of all pesticides was checked by electron capture gas chromatography and were found to be at least 99% pure.

2.1.4 Extraction and cleanup of soil extract

The stored soil samples were mixed thoroughly just prior to analysis. Duplicate ten gram samples of air-dried soil were mixed with three ml. of water and allowed to stand at room temperature for 15 minutes. The mixture was then agitated in a mechanical shaker for one hour with 50 ml. of 1:1 hexane-isopropyl alcohol. The supernatent liquid was filtered and the soil was re-extracted twice in the same way with 50 ml. of the same solvent mixture and shaken for ten minutes only. The combined filtrate was diluted with 500 ml. of a two percent solution of sodium chloride in a separatory funnel and the hexane layer withdrawn. The aqueous

layer was extracted twice with 75 ml. portions of petroleum ether. The combined petroleum ether layers were dried with anhydrous sodium sulfate, filtered and concentrated to about ten ml. The concentrated solution was then chromatographed on a three inch aluminum column and eluted with 250 ml. of 15% benzene in hexane. The eluate was concentrated to about 10 ml. and examined by gas chromatography after the addition of endrin, the internal standard.

2.1.5 Vegetable processing techniques

In determining the home preparative methods to be employed in this study, primary consideration was given to the methods most often used by the housewife. The most common home processing methods for potatoes are baking and boiling with or without the peel. Mature carrots and turnips are normally prepared by peeling and then boiling. The usual home processing procedure for beets is to boil them with the peel on to minimize "bleeding" and to then remove the skins after they are cooked. Occasionally beets are prepared by removing the peel before boiling. Carrots and turnips are sometimes eaten raw, with or without the peel.

The processing methods used for potatoes were baking, and boiling with and without the peel. Peel was also boiled. Samples of unpeeled potatoes, peeled potatoes and potato peel from each level of chlordane treatment were boiled separately for 25 minutes in covered enamel saucepans in sufficient water to cover

the material. Whole potatoes from each level of chlordane treatment were baked for one hour at 204°C. (400°F.).

Duplicate samples of unpeeled and peeled carrots, beets and turnips as well as their peels were boiled separately for ten, 15 and 20 minutes respectively, in covered stainless steel saucepans in sufficient water to cover the vegetables.

2.1.6 Extraction and cleanup of vegetable extract

Fifty gram samples of raw and boiled unpeeled potatoes and peeled potatoes were analyzed from each level of chlordane treatment. Twenty gram samples of raw and boiled potato peel from each level of chlordane treatment were also analyzed. One whole baked potato from each level of chlordane treatment was analyzed.

Duplicate twenty gram samples of raw and boiled unpeeled carrots, beets and turnips were analyzed. Duplicate twenty gram samples of raw and boiled peeled carrots, beets and turnips were also analyzed. Analyses were also carried out on duplicate twenty gram samples of the raw and boiled peels from these vegetables. Duplicate twenty gram samples of the carrot, beet and turnip tops were analyzed.

Each sample was finely chopped and macerated in a Virtis blender at high speed for ten minutes with 75 ml. of acetonitrile. The mixture was filtered under suction and the filtrate was diluted in a separatory funnel with 200 ml. of two percent

sodium chloride solution. This was partitioned into 100 ml. of petroleum ether and the petroleum ether layer drawn off. The aqueous layer was re-extracted twice with 50 ml. portions of petroleum ether and the combined petroleum ether extracts were dried with anhydrous sodium sulfate. After filtration, the extract was concentrated to about ten. ml. The concentrated solution was then chromatographed on a ten gram aluminum oxide column deactivated with six percent water in the case of potatoes, and on a ten gram, 4:1 magnesia-celite column for carrots, beets and turnips. The columns were eluted with 250 ml. of 15% benzene in hexane. The eluate was concentrated to about ten ml. and examined by gas chromatography using endrin as the internal standard.

2.1.7 Gas chromatography

An Aerograph Hy-Fi Model 600-D gas chromatograph with a tritium electron capture detector was used as the analytical instrument. The five foot by one-eighth inch i.d. aluminum column was packed with four percent SE-30 on 80- to 100-mesh Chromosorb W. Operating conditions were: carrier gas, oxygen-free nitrogen; flow rate, 60 ml. per minute; temperatures, column 175°C., injector 200°C. and detector 200°C.; electrometer range and sensitivity, one and four, respectively.

The identity of some of the components of technical chlordane was confirmed by the use of standards. An identical

retention time for the unknown peak and the standard was considered positive identification of the unknown. Trans-chlordane and heptachlor standards were available for identification of these two peaks and they are reported separately. Individual standards for the other components of technical chlordane were unavailable, therefore all other peaks are reported in terms of trans-chlordane. This introduces a small but unavoidable source of error, as the electron affinity factor, 'f' value, for these compounds would not be identical with the 'f' value of trans-chlordane. Technical chlordane itself could not be used as a standard since the relative peak heights change during weathering in the field. Neither could radioactive labeled technical chlordane be used for soil treatment and later measurement in the vegetables as labeling of all the components of technical chlordane is not possible.

The weight of each pesticide in each sample was determined by the internal standard method of Chang and Karr (1959). An internal standard was selected whose peak did not interfere with any peak appearing in the chromatogram of the sample to be analyzed. The electron-affinity factor, 'f' value, of each component of technical chlordane to be estimated was calculated from the chromatogram of a synthetic mixture containing known weights of the internal standard and the component to be estimated, using Equation 2.1 The 'f' value of the internal standard is, by definition, unity.

$$f_p = (W_s \times A_p)/(W_p \times A_s) \qquad (2.1)$$

$$W_p = (W_s \times A_p)/(f_p \times A_s) \qquad (2.2)$$

where f_p = electron-affinity factor of the pesticide to be determined, W_s and W_p = the weight of the internal standard and the pesticide, respectively, and A_s and A_p = the peak heights of the internal standard and pesticide, respectively. The total amount of a particular pesticide may then be calculated from equation 2.2.

When analyzing the vegetable extracts, a known quantity of endrin was added as an internal standard to the sample to be analyzed. A two to four µl. aliquot of the sample was then chromatographed under the same conditions as was used for the synthetic mixture. It was not necessary for the volume of the orginal solution or of an injection to be precise because the ratio of the weights and peak heights was used. Volumes were adjusted to obtain gas chromatographs whose peak heights were of a convenient size for measurement. An average 'f' value was calculated from three injections of one µl. each of the synthetic mixture, and was recalculated each time the gas chromatograph was used. The amounts of heptachlor and trans-chlordane were then calculated from equation 2.2. The other components of technical chlordane were not identified individually and were calculated as trans-chlordane. This was done by using the total of their peak heights and the 'f'

value for trans-chlordane in equation 2.2.

2.2 The effect of commercial processing on pesticide residues in rapeseed oil

The second part of this thesis involved the study of the effect of commercial processing methods on DDT and lindane residues in rapeseed oil. The processing methods used were adapted from those used by the Saskatchewan Wheat Pool's rapeseed oil processing plant in Saskatoon. DDT and lindane were selected for this study since they are both used as a field application for pest control in rapeseed production and they have quite different volatilities. Lindane is more volatile than DDT and this was considered to be a possible factor influencing their rate of disappearance during the processing operation. The use of radioactive labeled DDT and lindane eliminated extensive extraction and cleanup procedures. The analytical method consisted merely of removing suitable aliquots of the oil after each processing step and counting the radioactivity in a scintillation counter.

2.2.1 Reagents

Lindane-^{14}C (specific activity 129 μCi per mg.) and p,p'-DDT-^{14}C (specific activity 12.9 μCi per mg.) were purchased from the Radiochemical Centre, Amersham, England. Both the compounds were analyzed by thin-layer and gas chromatography and were found

to be at least 99% pure. Lindane-^{14}C was diluted with cold lindane to give a specific activity of 13 μCi per mg.

The counting solution was scintillation grade 2,5 diphenyloxazole (PPO) in toluene at a ratio of four grams of PPO per 1000 ml. toluene. Ten ml. of the PPO solution was used for counting the aliquots of oil.

Rapeseed oil, V. L. Colza brand, manufactured by Vinegars Limited, Saskatoon, was purchased at a local retail store. Rapeseed flakes and the activated bentonite-charcoal mixture that was used to bleach the rapeseed oil were obtained from the Saskatchewan Wheat Pool.

Redistilled hexane, as described in section 2.1.3, was used.

2.2.2 Scintillation counter

Radioactivity in the samples were determined in a Beckman Model LS-100 scintillation counter using the channel ratio method. A known quantity of lindane-^{14}C (four mμCi) was added to 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml. of rapeseed oil and the samples were counted in ten ml. of 0.4% PPO in toluene solution. The counting efficiency for each sample was calculated from the observed counts per minute in channel one and the actual amount of radioactivity present in the sample. A correlation curve was drawn between the ratios of counts per minute in channels one and

two and percent efficiency. Counting efficiencies of unknown oil samples were determined from this correlation curve and the observed ratios of counts per minute in channel one and channel two. The actual amounts of radioactivity present in an aliquot of the processed oil was then determined from the observed count per minute in channel one and the counting efficiency as determined from the correlation curve.

2.2.3 Processing of rapeseed

Processing methods used for rapeseed and rapeseed oil were adapted from those used by the Saskatchewan Wheat Pool's rapeseed oil processing plant in Saskatoon. In the local plant the rapeseed was flaked in a roller crusher and the resulting rapeseed flakes were cooked for 30 minutes in a five stage cooker where they reached a final temperature of about 105°C. The cooked flakes were then extracted with hot hexane at 60°C. for 30 minutes in a continuous solvent extractor. The hexane extract was then passed into an evaporator-stripper where desolventization occurred by heating to 105°C. The last traces of solvent were removed by heating the oil to 127°C. at a vacuum of 17 mm. Hg. The desolventized oil was then subjected to an alkali-refining treatment by vigorous agitation in the presence of aqueous five percent sodium hydroxide at 71° to 82°C. for 15 minutes, followed by two washings with water. The refined oil was then bleached by stirring it with 2.4 to 5.0%

activated bentonite-charcoal mixture at 82° to 105°C. for about 30 minutes followed by filtration. The bleached oil was finally deodorized by vacuum steam distillation of the volatile components. In this operation the oil was heated in batches at 232° to 260°C. and at a vacuum of five to seven mm. Hg. for 3.5 to 5.0 hours while steam was passed through the oil at a rate per hour of less than ten percent of the oil being processed.

2.2.3.1 Fortification of rapeseed flakes and oil with lindane- and DDT-^{14}C

Rapeseed flakes were obtained from the Saskatchewan Wheat Pool's oil processing plant for use in this investigation. Twenty-five gram samples of the rapeseed flakes were fortified with lindane or p,p'-DDT-^{14}C in pentane solution at a rate of four mμCi per gram (0.3 p.p.m.) and the pentane removed in a film evaporator. The fortified rapeseed flakes were stored in stoppered bottles for two weeks at room temperature.

The total radioactivity was determined in samples of the rapeseed flakes containing each of the insecticides by extraction of the oil and pesticides and subsequent measurement. Duplicate one gram samples of the rapeseed flakes were extracted in a ball mill with 20 ml. of petroleum ether for one hour. The supernatent liquid was filtered and the residue re-extracted twice by shaking with ten ml. portions of petroleum ether. The combined

filtrate was concentrated to two ml. and transferred quantitatively to counting vials. To extract any remaining insecticide, the residual meal was extracted with 1:1 chloroform-methanol mixture in a Soxhlet apparatus for eight hours. The Soxhlet extract was then concentrated to two ml. and transferred quantitatively to counting vials. Radioactivity was determined in a scintillation counter.

Since lindane and DDT are fat soluble, the processing methods involving only oil could be studied more conveniently using fortified rapeseed oil. For this purpose, 25 ml. samples of rapeseed oil were fortified with lindane- or p,p'-DDT-^{14}C in hexane solution at a rate of four mμCi per gram (0.3 p.p.m.). The total radioactivity present in the oil was determined by counting an aliquot in a scintillation counter.

2.2.3.2 Cooking of rapeseed flakes and oil extraction

Duplicate ten gram samples of the fortified rapeseed flakes and 75 ml. of hexane were placed in a three-neck round-bottomed flask fitted with a condenser and a stirrer. The contents of the flask were stirred and heated on a boiling water bath for 30 minutes and filtered under suction. The filtrate was made up to 100 ml. in a volumetric flask and 0.5 and 1.0 ml. aliquots were counted in a scintillation counter.

The residual rapeseed meal was extracted in a Soxhlet apparatus with 1:1 chloroform-methanol for eight hours in order to

determine the amount of insecticide retained by the meal. The Soxhlet extract was made up to 25 ml. and 0.5 and 1.0 ml. aliquots were counted in a scintillation counter.

2.2.3.3 Desolventization of rapeseed oil

One hundred ml. of hexane was added to duplicate 25 ml. samples of the lindane- and DDT-^{14}C fortified rapeseed oil and mixed thoroughly. Desolventization was carried out by distillation over an oil bath at 100°C. for 30 minutes followed by heating at 127°C. and 17 mm. Hg. for ten minutes. Aliquots of 0.5 and 1.0 ml. were taken for counting in a scintillation counter.

2.2.3.4 Refining of rapeseed oil

Duplicate ten ml. samples of the fortified oil from the desolventizer process were vigorously stirred with five ml. of five percent sodium hydroxide solution at 70° to 80°C. for 15 minutes. The mixture was then centrifuged, washed twice with water and 0.5 and 1.0 ml. aliquots were counted in a scintillation counter.

2.2.3.5 Bleaching of rapeseed oil

Duplicate ten ml. samples of the fortified rapeseed oil were mixed with 0.5 grams of bentonite-charcoal adsorbent and stirred vigorously over a boiling water bath for 30 minutes. The mixture was filtered by suction and 0.5 and 1.0 ml. aliquots were

counted in a scintillation counter.

2.2.3.6 Deodorization of rapeseed oil

Duplicate ten ml. samples of the fortified rapeseed oil were heated at 230° to 260°C. at six mm. Hg. for four hours while a slow current of live steam was introduced into the bottom of the flask. The deodorized oil was cooled to room temperature and 0.5 and 1.0 ml. aliquots were counted in a scintillation counter.

3. RESULTS AND DISCUSSION

3.1 The effects of processing on chlordane residues in vegetables

3.1.1 The components of chlordane

A gas chromatogram of the ether extract of carrots grown in chlordane treated soil, as illustrated in Figure 3.1, is typical of the gas chromatograms of all the vegetables investigated. This gas chromatogram gives about 14 peaks which are essentially the same as those obtained by Saha and Lee (1969) in a gas chromatogram of the ether extract of technical chlordane granules shown in Figure 3.2. Although the relative peak heights are not identical due to weathering of the chlordane in the field, the number of peaks are the same. The relative retention times of the peaks in the two chromatograms are similar but not identical since the samples were chromatographed on two different machines with varying conditions.

The large number of peaks present in the gas chromatogram is due to technical chlordane being a complex mixture of many components which are produced in the manufacturing process. Technical chlordane is manufactured in a two stage process (Riemschneider, 1963). First, hexachlorodicyclopentadiene (chlordene) is prepared by the Diels-Alder addition of hexachlorocyclopentadiene to cyclopentadiene, as in equation 3.1. In the second step the hexachloro-

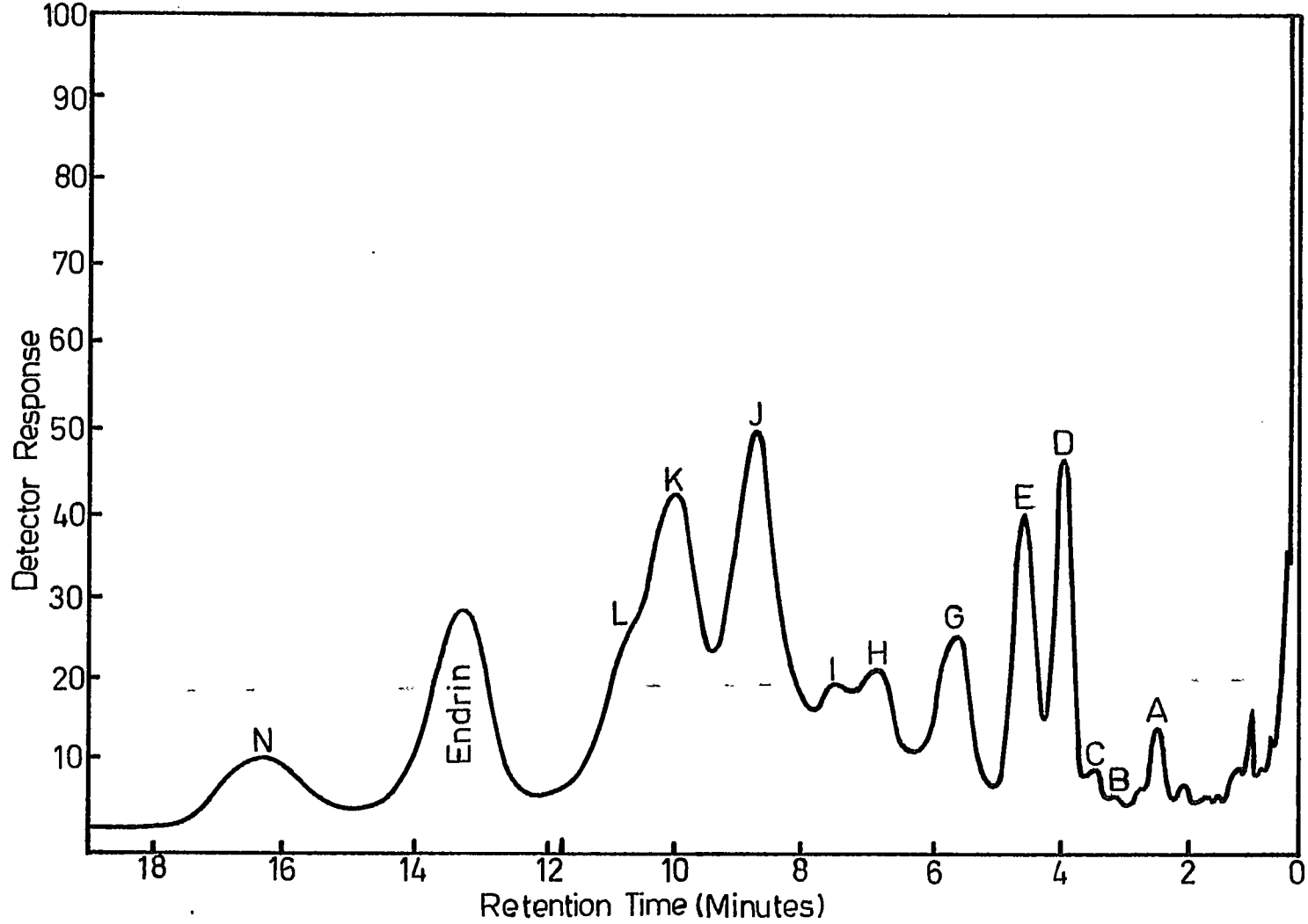


Figure 3.1 Gas Chromatogram of carrot extract. Column 5ft.x1/8in. 5% SE-30 on Chromasorb W at 175°C. Injector 200°C. Detector 200°C. Nitrogen 60ml/min.

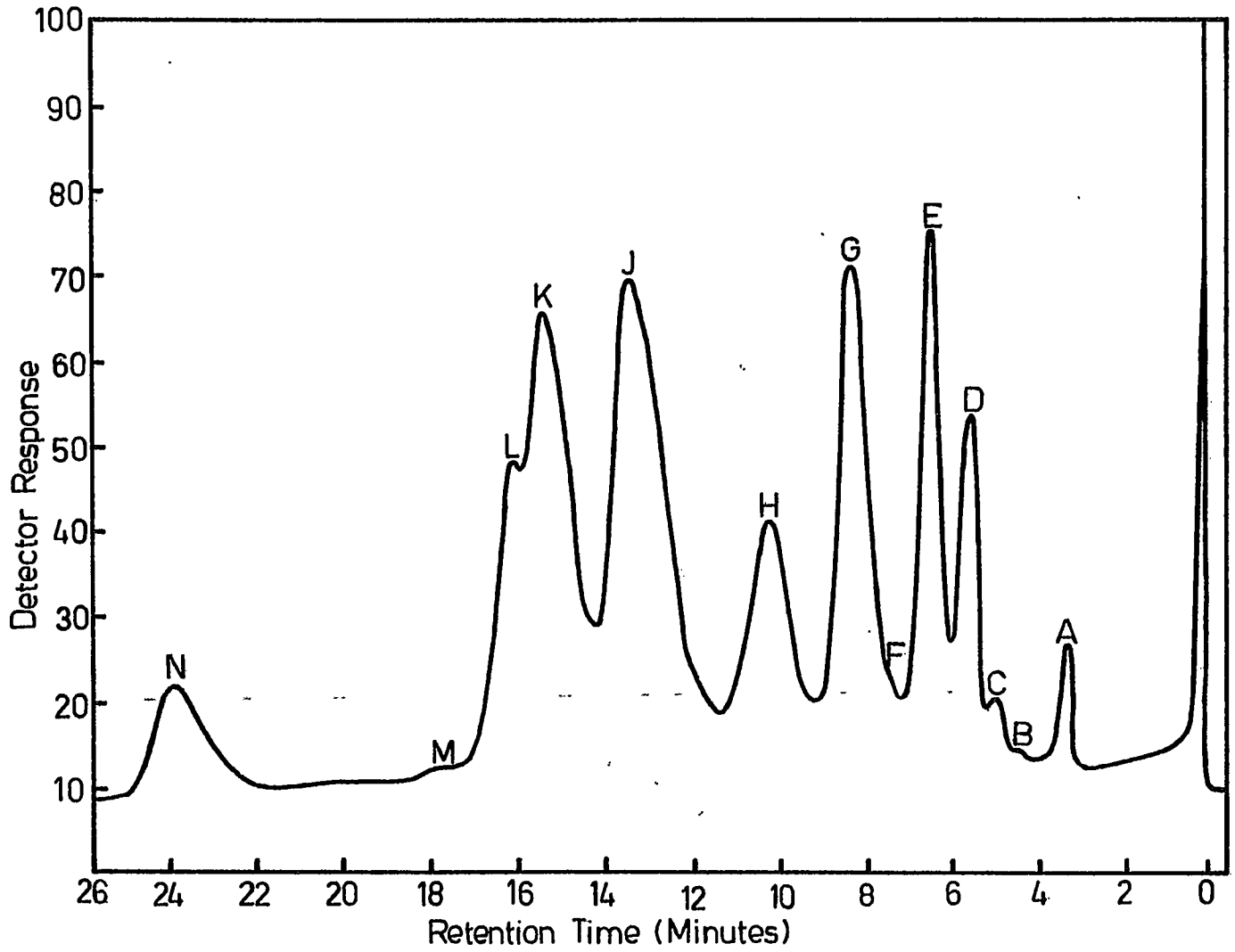


Figure 3.2. Gas Chromatogram of commercial chlordane (dust). Column: 5 ft. x 3/16 in. 5% SE-30 on Chromosorb W at 180°C. Injector: 230°C. Detector: 250°C. Helium: 60 ml/min. Reference Saha & Lee (1969)

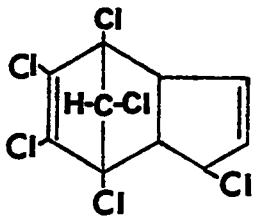
dicyclopentadiene is then chlorinated to give a mixture of chlorination products, as shown in equation 3.2. The two principal products of this latter reaction are endo-cis- and endo-trans-chlordane, however, some heptachlor is also formed along with many heptachloro-, octachloro-, and nonachlorodicyclopentadienes.

The components of technical chlordane have been isolated and tentatively identified by Saha and Lee (1969). The compounds representing the peaks in Figure 3.2 are as follows:

Peaks A, C, I - not identified

B Chlordene (III)

D Hexachlorodicyclopentadiene, chlordene analog (IX)

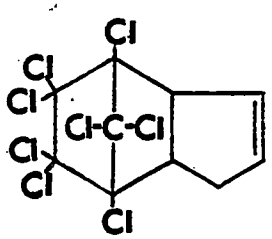


(IX)

E Heptachlor (VII) and an isomer of chlordane (VI)

F Chlordane isomer (VI)

G Heptachlor isomer + chlordane analog (X)



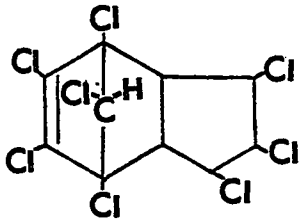
(X)

H Chlordane isomer (VI)

J Endo-trans-chlordane (V)

K Endo-cis-chlordane (IV)

L Chlordane analog (XI) and nonachlor (VII)

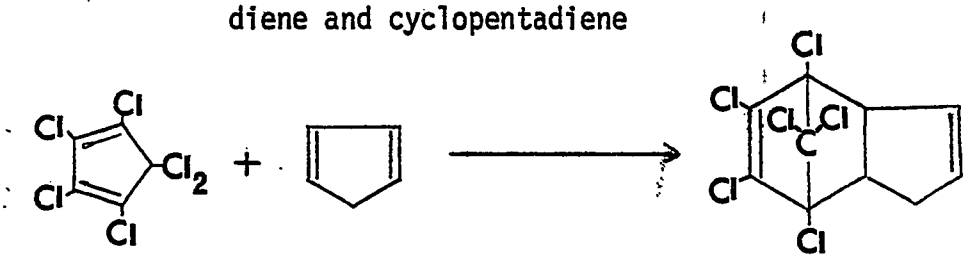


(XI)

M Chlordane isomer (VI)

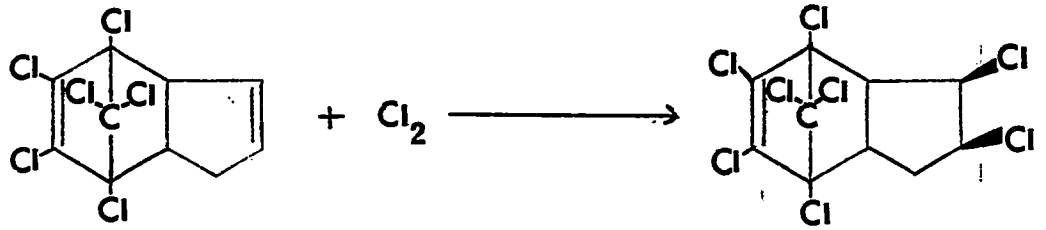
N Nonachlor isomer (VIII)

Equation 3.1 Diels-Alder addition reaction of hexachlorocyclopentadiene and cyclopentadiene



Hexachlorocyclopentadiene(I) — Cyclopentadiene (II) — Hexachlorodicyclopentadiene (III)

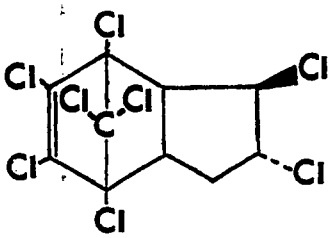
Equation 3.2 Some of the chlorination products formed in the chlorination of hexachlorodicyclopentadiene (chlordene)



Hexachlorodicyclopentadiene (III)

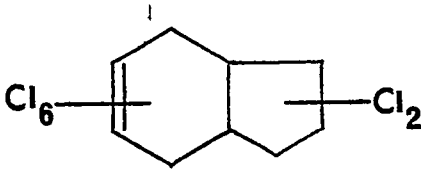
cis-chlordane (IV)

+



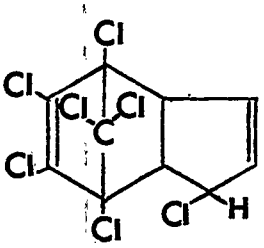
trans-chlordane (V)

+



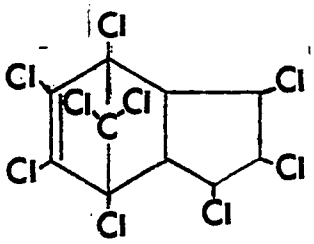
Chlordane isomers (VI)

+



Heptachlor (VII)

+



Nonachlor (VIII)

+ related compounds

The chlordane formulation used in this study consisted of approximately 48% cis- and trans-chlordane, however, there was about nine percent heptachlor present. The application of technical chlordane at the recommended rate of ten pounds per acre for wireworm control would result in the application of almost one pound of heptachlor as well. The use of heptachlor has been restricted recently and chlordane has been suggested as an alternate chemical because of its lower toxicity to mammals. Interest was therefore focused on the presence of trans-chlordane and heptachlor and heptachlor epoxide in the root crops and the soil in which they were grown.

3.1.2 Evaluation of pesticide extraction methods

The quantitative determination of organochlorine insecticides in plants and soils involves three main steps. Firstly, the insecticide present in the soil or plant is extracted by a suitable solvent or a mixture of solvents in suitable equipment such as a blender, mechanical shaker, or in a Soxhlet apparatus. Secondly, the extracted insecticide is subjected to a "cleanup" procedure where the co-extractives from the plants and soils are removed by partitioning into two immiscible solvents and/or by thin layer or column chromatography. Finally, the amount of insecticide present in the "cleaned up" extract is determined by electron capture gas chromatography, where a few picograms (10^{-12} gm.) of organochlorine

insecticides can be detected and estimated.

While much work has been done in the past on the cleanup of extracts and the final determinative techniques, very little attention has been given to the evaluation of extraction efficiency. Since extraction is virtually the first step in the analysis of pesticide residues, the efficiency of extraction will play a decisive role in obtaining quantitative reproducible results. Residues not extracted will not be estimated. Even if all procedures employed after extraction are satisfactory and the final determination is carried out by an advanced and sophisticated instrumentation, results obtained from inconsistent and inefficient extractions will be of little value (Chiba, 1969).

It is also essential to know the absolute recovery of the insecticide present in the sample. The extraction efficiency of a given method has been expressed usually as a percentage recovery of a specific insecticide from a fortified (spiked) sample. It has been pointed out, however, that such fortification studies do not give an accurate measure of the ability of solvents to extract field applied pesticides (Gunther, 1962; Wheeler and Frear, 1966). The use of radioactive labeled compounds is perhaps the best way to determine the absolute extraction efficiency of a given process (Gunther, 1962; Saha et al., 1969; Wheeler and Frear, 1966).

Although numerous solvents and methods of extraction have been used in the past to recover organochlorine insecticides

from soils (Chiba and Morley, 1968), very few extraction procedures have been evaluated with radioactive labeled compounds. Saha et al. (1968) conducted such an evaluation using dieldrin-^{14}C. The absolute extraction efficiencies of various solvents under a variety of conditions from eight types of soils were determined. They reported that common extraction solvents such as acetonitrile, hexane-isopropanol, and hexane-acetone mixtures extracted only 19 to 77% of the dieldrin from air-dried soils. However, the addition of 20% water to the air-dried soil immediately before extraction by 1:1 mixtures of hexane-isoporpanol, hexane-acetone, and benzene-methanol gave 92 to 98% recovery of the dieldrin, irrespective of the soil type or the solvent system involved.

The method used in the present study for soil extraction was among those evaluated by Saha (1968) as outlined above. This method involved the use of a 1:1 mixture of hexane-isopropanol as the extraction solvent and the addition of 20% water to the air-dried soil. Thus, this method is able to extract up to 98% of the total dieldrin in the soil.

The extraction efficiency of this method when used to extract chlordane residues from soil was evaluated by Saha (1970b). It was not possible to investigate the absolute extraction efficiency of this method using radioactive labeled technical chlordane, as it is impossible to label technical chlordane and still retain the identical chemical composition. Saha did