

INSECTICIDAL RESIDUES ON GARDEN CROPS
AND EFFECT OF PROCESSING

By

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Bachelor of Arts

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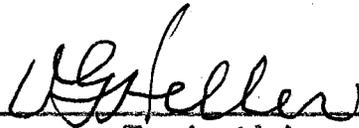
1951

Submitted to the faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
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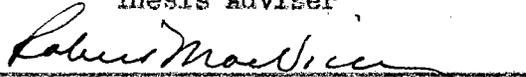
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PREFACE

In the field of agriculture, one of the greatest problems is that of controlling harmful insects. This has been done primarily through the use of insecticides. When these insecticides are applied to soil or plants, there are several factors to be taken into consideration. In applying insecticides to the soil, the concentration must be kept to a minimum to prevent interference with seed germination or even complete sterilization of the soil, yet the quantity must be sufficient to inhibit insect infestation. A further hazard may result from application of insecticide to the soil if it is absorbed into the plant through the roots and translocated, thus rendering the plant toxic to man or animal upon ingestion. In the direct application of the insecticide to the leaves, the concentration must also be limited in order to prevent plant injury, but must be great enough to control the insect pest. The problem is to determine how long the insecticidal residue will remain on the plant, and how much, if any, is absorbed through the leaves or epidermis of the plant. All this must be known before any of the produce can be marketed according to prescribed standards.

In this investigation, the primary interest was to determine the effect of different processing methods of the amount of insecticidal residue present with the view of developed techniques which leave a minimum residue, thus leaving the product less toxic upon ingestion.

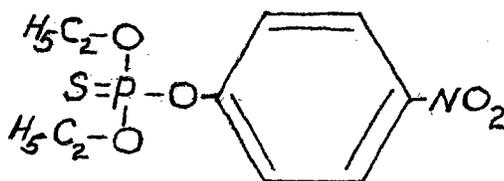
INTRODUCTION

Each year crops estimated to be worth four billion dollars (1) are lost through direct insect destruction and insect borne diseases. This loss could be greatly minimized through the proper application of insecticides. No single insecticide can be pointed out as a "cure all" for this great crop loss, a given compound may exterminate some insects but leave others unaffected by the application. Some insecticides are completely valueless when applied to certain crops, due to the fact that it also severely damages the plant.

The above difficulties all affect the consumer indirectly through the cost of the garden crop. One problem which directly concerns him is how much of the insecticide is on the vegetable when he eats it. How much of the insecticide can be consumed without any ill-effects? Is the insecticide stored in the body? These are some of the questions that are of concern whenever food is encountered that has been treated with insecticides.

Parathion

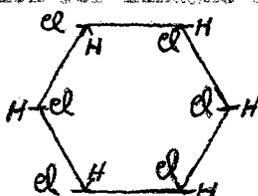
The two insecticides that were especially studied in this research work were "parathion" and "lindane". Parathion (2,3,5) is the generic name of a compound that was first synthesized in Germany where it was designated as E-605. The chemical name of parathion is O,O-diethyl O-p-nitrophenyl thiophosphate. The structural formula for parathion is as follows:



Parathion (4) was first introduced into this country as an insecticide in 1946. It is a high-boiling liquid, often with an onion like odor and with a specific gravity of 1.26. It is only slightly soluble in water, sparingly soluble in petroleum ether, kerosene or refined spray, and completely miscible in acetone, benzene, ethyl ether, cyclohexanone, alcohols, esters and animal or vegetable oils. It is stable as regards hydrolysis, even in hard water containing as much as 650 parts per million of dissolved solids, and is not readily oxidized. It is hydrolyzed in the presence of alkaline materials such as lime, lime-sulfur and Bordeaux mixtures. In the presence of lime, it is hydrolyzed (5) in eight hours. Parathion acts chiefly as a contact poison, but is also a stomach poison and fumigant (3). It is very rapid in its action, although it may be entirely ineffective at temperatures of 50-60° F. The compound is relatively stable, and the spray residues remains somewhat effective for several days. The parathion residues on plants disappear sufficiently to leave no detectable parathion after about thirty days, most of the compound evaporated in the first few days. It has been reported by Lehman (6) that the mean lethal oral dose of parathion is 3.5 mg/kg. body weight, as compared to 250 mg/kg. body weight DDT. Thus parathion is approximately seventy times more toxic than the common insecticide DDT. It is known that parathion has a cumulative (6) action which points toward tissue storage. Lehman, using rats as the experimental animal, proved that the chronic toxicity bears no relation to acute toxicity. Thus the consumer should try and avoid ingesting even the smallest amount of parathion residue.

Lindane

Benzene hexachloride (BHC) can have possibly 16 isomers. Of the sixteen, six are now known (7). BHC was first used as an insecticide in 1935 (8). The gamma isomer is the only one important as an insecticide (6,9). Purified grades of the gamma isomer of BHC, which have a purity of 99 per cent or better, have been given the generic name of "lindane" * (10). Gamma BHC has also been called gammexane. It is a white crystalline substance with a melting point of 124° C (10). The structural configuration for lindane is as follows:



The gamma signifies that it was the third isomer found, and it does not designate the structural relationship. Benzene hexachloride is phytotoxic, but the phytotoxic effects are greatly reduced with the use of lindane (8).

Lindane is very volatile (vapor pressure at 20° C is .03 mm Hg) (9) and therefore has a short residual action. It is practically insoluble in water, and the degree of its solubility in organic solvents is indicated below:

	<u>Gm. per 100 gm. solvent</u>
Acetone	43.5
Benzene	28.9
Carbon tetrachloride	6.7
Chloroform	24.0
Cyclohexane	36.7
Ethyl alcohol	20.8
Ethylene dichloride	28.9
Kerosene, ordinary	3.2
Methyl alcohol	7.4
Xylene	24.7

* After van der Linden who in 1912 discovered the existence of the isomer.

Lindane is stable in acid solution but decomposes in alkaline solution. It has been reported by Lebman (6) that the mean oral lethal dose of Lindane is 125 mg/kg body weight, as compared with 250 mg/kg body weight DDT; thus Lindane is twice as toxic as DDT. As an insecticide, Lindane has the merit of acting as a stomach poison, as a contact agent, or as a fumigant. It is slow in its killing action and is not an effective knockdown agent (9). It has been found effective against aphids, grasshoppers, wireworms, and several cotton insects including the boll weevil, but not the boll worm (11). Lindane, rather than technical BHC, should be applied to garden crops, since in the manufacture of technical benzene hexachloride (8,11) there is a by-product which gives a pungent, disagreeable odor, which may adversely affect the flavor of the crop. The storage of Lindane in the body is equal to that of the concentration in the diet (6). It differs from other insecticides in that it is stored in the brain. Here again the consumer must be careful not to ingest appreciable amounts of the residual Lindane.

It has been known for a long time that insecticides are toxic to the human. This investigation was undertaken primarily to determine the amount of parathion and lindane deposited on the various crops, and the effects of processing, washing, washing and open-cooking, washing and pressure cooking, and freezing on the amount of insecticide remaining on the various vegetables and fruits studied.

EXPERIMENTAL

Analytical Procedures for Parathion and Lindane

Parathion

Whenever a new insecticide is developed, a quantitative method of analysis must be developed so that the amount of residue on plants and in animal tissue can be determined. This is necessary procedure, since lethal doses per kg. body weight must be determined for each insecticide in order to establish tolerance levels which may be present in foods. Several methods have been developed for the estimation of parathion. The first method, developed by Averell and Norris (12), is a colorimetric procedure based upon the reduction of the nitro group to an amino group with subsequent diazotization and coupling with N-(naphthyl)-ethylene diamine to produce a magenta color that may be measured photocolorimetrically. The second method is a polarographic determination (13). The electrolysis is carried out at 25° C. in an acetone-water solution with 0.05 normal potassium chloride as the electrolyte, and 0.01 % gelatin as the suppressor. The nitro group of parathion is reduced at the dropping mercury electrode, and it is thus quantitatively determined by this means. Another method, not specific for parathion, is an enzymatic determination based upon the ability of organic phosphorus insecticides to inhibit choline esterase (14).

In this investigation the colorimetric method was used for the quantitative determination of parathion residue on plants. The parathion residue was extracted from the plant samples with benzene. The benzene extract was treated with 2 parts attapulgus clay and 1 part hyflosupercel

to remove the organic coloring matter which would interfere with the subsequent color development. Suitable aliquots were taken so that the magenta color would fall into a concentration range that would conform to Beer's Law. The benzene was evaporated, and the parathion taken up with 10 ml. of distilled water, and 2 ml. of 5 normal hydrochloric acid. Five tenths of a gram of zinc dust was added to the solution, and the mixture heated on the steam bath (temperature 85-90° C.) for ten minutes. The mixture was filtered immediately through Whatman's No. 42 filter paper, and washed with 3-seven ml. portions of distilled water. One ml. of 0.25 per cent sodium nitrite was added to the filtrate, and the resulting solution was thoroughly mixed. Exactly ten minutes later, one ml. of 2.5 per cent ammonium sulfate was added with ample mixing of the solution. After another ten minutes, two ml. of N-(1-naphthyl)-ethylene diamine was added and the solution was thoroughly shaken. Ten minutes later, 2 ml. of 3 normal hydrochloric acid, and 50 ml. of 95 per cent ethyl alcohol were added, and the volume was then diluted to 100 ml. The optical densities of the samples were immediately measured with the use of the Coleman Universal Spectrophotometer, model 11, at 555 m μ . A standard curve was first made by using known concentrations of parathion, and this curve was used to determine the amount of parathion present in the plant extract. A correction factor of 1.25 was employed due to incomplete recoveries of added parathion (Average = 80%) by this method.

Lindane

There are a number of methods for the quantitative determination of Lindane; total organic chlorides (15,17), polarographic methods (18,19), infrared spectroscopy (20), a modified partition chromato-

graphic method (21), a cryoscopic method (22), and a mass-isotope dilution method (23). In this investigation, the total organic chloride method was used for the quantitative determination of Lindane residues on plants. This method is based upon the liberation of the chloride by refluxing with metallic sodium, and precipitating the free chloride ions with a known quantity of silver nitrate, and treating the excess silver ions with potassium iodide using a buffered starch solution as an indicator. This method of titration of halides was developed by McLean and Van Slyke (24). The Lindane residue was extracted from the plants with benzene. An aliquot of the extract was chromatographed through a column packed with 2 parts attapulgus clay and 1 part hyflo-supercel, followed by three 15 ml. portions of benzene to thoroughly remove the insecticide from the column. The benzene was evaporated, and the Lindane residue was taken up in isopropanol, and refluxed with sodium for two hours. The solution was acidified with nitric acid, and the chloride ions precipitated with silver nitrate. The silver chloride was removed and the excess silver nitrate titrated with potassium iodide. The amount of Lindane residue on the plants was calculated by the amount of chloride determined.

Effect of Processing on Insecticidal Residues

The most extensive problem of poisoning by insecticides in man and domestic animals arises from the ingestion of residual poisons remaining on agricultural products, on weeds, and in impregnated soil. (25,26) It is because of this that several investigators have undertaken the task of trying to reduce the amount of residue remaining on various foods in order to reduce this hazard.

Brittin and Fairing (27) have shown that when peaches, pears, peas, green beans, and vegetables and beef soups, containing various added insecticides are subjected to the canning process, the insecticides apparently undergo a degree of decomposition. In the range of 0.01-10 ppm added insecticide studied by them, the over-all average loss of parathion was 63.2 per cent, of methoxychlor 95.1 per cent, and of DDT 51.2 per cent.

Hartzell and Storrs (28), using a bioassay method for determining the presence of insecticides, determined spray residues of 10 insecticides in processing foods (strained beans, mixtures of apricots and apples). They used mosquito larvae, Aedes aegypti, as the test organism. The insecticide residues were extracted with benzene, the solvent was evaporated with compressed air and the residues, taken up in acetone, were diluted in tap water to which the larvae were exposed to varying concentrations of residue for 24 hours at 30° C. When added to processed food at 0.5-5.0 ppm, heptachlor, aldrin, dieldrin, chlordan, lindane, 2-nitro-1, 1 bis (p-chlorophenyl) propane (BHP), 2-nitro-1, 1 bis (p-chlorophenyl) butane (BHP), methoxychlor, 2,2 - bis (p-chloro-

phenyl) - 1, 1-dichloroethane (Dihothane), and toxaphene could be readily detected. Their work showed that when the insecticide was present before processing there was a marked reduction in the toxicity to the larvae, which undoubtedly was due to the destruction of the insecticide during the processing. In water solutions of the pure compounds of the insecticide mentioned above, most of these gave 100 per cent kills at 0.10-0.01 ppm. To show how much more toxic parathion is, the mortality to mosquito larvae was 100 per cent at 0.0006 ppm.

Field tests show that there is a reduction in the amount of insecticidal residue on sprayed and dusted crops a few days after the application. Table I contains experimental data on the reduction of insecticidal residues with time. In growing plants, this is not all due to evaporation, oxidation, or hydrolysis of the insecticide. Sloan et al (29) have reported that fourteen days after the last application of parathion on lettuce, growth of plants had reduced the apparent residue by 73 per cent. Since residues are expressed relative to plant weight, any increase due to growth would result in a dilution of the insecticide even though no loss had occurred. Loss due to weathering caused a decrease of the parathion spray deposit by more than 99 per cent.

In processing, the reduction of the insecticidal residue is actual and not apparent because there is no outside factor involving the increased weight of the plant. In fact, extraction of water soluble constituents might be assumed to actually effect a concentration of any residual material which was water insoluble.

This work was undertaken to determine the effect of various cooking and storage practices on the amount of insecticidal residue remaining in or on the food. Washing, washing and open-kettle cooking, washing and

Table I
Residues of Lindane and Parathion
Recovered From
Spinach, Swiss Chard, Beans, Peas, and Crowders

Date of Test	Formulation	Period after treatment	Pounds Toxicant applied per acre	PPM			
				Toxicant: Lindane	Recovered Parathion		
			Spinach	% Loss	% Loss		
11-25-51	Dust	1 hour	0.7--0.9	64.7	----	23.8	----
11-25-51	Dust	4 days	0.7--0.9	9.6	85.2	3.5	87.9
11-25-51	Dust	9 days	0.7--0.9	5.1	92.1	2.4	91.7
11-25-51	Emulsion	1 hour	1.4--1.9	69.2	----	11.3	----
11-25-51	Emulsion	4 days	1.4--1.9	11.4	83.5	6.3	44.3
11-25-51	Emulsion	9 days	1.4--1.9	6.9	90.0	1.4	87.6
11-25-51	Wettable powder	1 hour	1.2	67.1	----	24.0	----
11-25-51	Wettable powder	4 days	1.2	29.6	55.9	5.6	76.7
11-25-51	Wettable powder	9 days	1.2	----	----	0.9	96.3
5-9-52	Dust	1 hour		9.8	----	5.8	----
5-9-52	Dust	3 days		1.4	85.7	0.3	94.3
5-9-52	Dust	6 days		0.3	96.9	0.2	96.6
5-9-52	Dust	9 days		----	----	0.0	100.0
5-9-52	Emulsion	1 hour		9.9	----	25.0	----
5-9-52	Emulsion	3 days		2.2	77.3	7.1	69.1
5-9-52	Emulsion	6 days		0.5	95.0	0.4	98.3
5-9-52	Emulsion	9 days		0.6	93.9	0.0	100.0

Table I, Continued
 Residues of Lindane and Parathion
 Recovered From
 Spinach, Swiss Chard, Beans, Peas, and Crowders

Date of Test	Formulation	Period after treatment	Pounds Toxicant applied per acre	PPM Toxicant Recovered		
				Lindane	Parathion	
			Spinach	% Loss	% Loss	
5-9-52	Wettable powder	1 hour	9.8	----	15.6	----
5-9-52	Wettable powder	3 days	1.0	89.8	1.9	87.8
5-9-52	Wettable powder	6 days	0.2	98.0	0.0	100.0
5-9-52	Wettable powder	9 days	0.0	100.0	0.1	99.4

pressure cooking, and deep freezing were carried out in the laboratory on vegetables previously treated with lindane and parathion emulsions, wettable powders, and dusts. In most of the experiments, emulsions of parathion and lindane were used, because these were found to adhere longer to the plants in the field than did the wettable powders and dusts.

The vegetables processed in an open-kettle were first washed three times with distilled water, and then cooked for 30 minutes. The results of these analyses are recorded in table 4. Spinach, mustard greens, Swiss chard, and Crouder peas were used for this experiment.

There were definite trends in the reduction of the two insecticides on the vegetables in open-kettle cooking. High concentrations of insecticidal residues of parathion and lindane on the vegetables were more effectively reduced than lower concentrations which had been on the vegetables a period of time. The concentration of parathion and lindane on all four vegetables was markedly reduced by open-kettle cooking. In most cases the residue was reduced by 80 per cent or better on those vegetables which were freshly dusted or sprayed and then processed by open-kettle cooking. The lower concentrations of insecticidal residues were also effectively reduced by this method of processing. The least reduction, about one-third, was effected with lindane dust; the cooked spinach still contained 0.2 ppm. The average reduction of the two insecticidal residues by open-kettle cooking was found to be 76.4 per cent for lindane, and 74.6 per cent for parathion.

Apples that were processed similarly to the vegetables were cooked in an open-kettle for 1 hour instead of 30 minutes. The data obtained on this experiment are found in table 5. The reduction of the insecticidal residue on this fruit was as marked as it was on the vegetables.

Processing Vegetables by Washing

The vegetables were washed three times with distilled water, then air dried until the wash water had evaporated. Spinach, mustard greens, Swiss chard, beans, and crowder peas were used as the experimental plant material for the application of the two insecticides. The results of this experiment are tabulated in table 2. The higher quantities of insecticide were removed from the vegetables more readily than were the residues that were lower in concentration but has been on the vegetable for longer periods of time. Washing reduced the amount of residue from 0 to 100 per cent, the mean reduction was 52.6 per cent for lindane, and 57.3 per cent for parathion. Table 3 contains the experimental data for the washing of apples previously sprayed with DDT and parathion wettable powders. This table shows that there was a marked reduction on the residue left upon the apple after washing, but not enough analyses were run to obtain the average per cent reduction. This experiment, however, does indicate that the size of the apple is of importance in determining insecticidal residue, presumably because the chemical is restricted almost solely to the epidermis. If the amount of residue on the apple is calculated on the basis of surface area rather than by weight, the apparent concentration is greatly increased. It is apparent therefore, in expressing residue concentrations, factors involved in preparing the food for consumption must be considered. Peeling apples should, therefore, be assumed to effectively reduce residues very substantially.

Table 2
The Effect Three Separate Washings have on
the Amount of Lindane and Parathion Residue
Removed from Spinach, Mustard Greens, Swiss
Chard, Beans, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on Vegetable <u>Before Washing</u>		PPM Insecticide on Vegetable <u>After Washing</u>				
			Lindane	Parathion	Spinach		Lindane	Parathion	% Loss
11-25-51	Dust	1 hour	64.7	28.8	-----	-----	8.1	71.9	
11-25-51	Dust	4 days	9.6	3.5	-----	-----	2.3	34.4	
11-25-51	Dust	9 days	5.1	2.4	-----	-----	1.0	58.3	
11-25-51	Emulsion	1 hour	69.2	11.3	-----	-----	9.6	15.0	
11-25-51	Emulsion	4 days	11.4	6.3	-----	-----	5.1	19.0	
11-25-51	Emulsion	9 days	6.9	1.4	-----	-----	0.5	64.3	
11-25-51	Wettable powder	1 hour	67.1	24.0	-----	-----	-----	-----	
11-25-51	Wettable powder	4 days	29.6	5.6	-----	-----	5.3	5.4	
11-25-51	Wettable powder	9 days	-----	0.9	-----	-----	0.1	88.9	
5-9-52	Dust	1 hour	9.8	5.8	2.7	72.5	2.0	65.5	
5-9-52	Dust	3 days	1.4	0.3	1.4	0.0	0.0	100.0	
5-9-52	Dust	6 days	0.3	0.2	0.3	0.0	0.1	50.0	
5-9-52	Dust	9 days	-----	0.0	0.4	-----	-----	-----	
5-9-52	Emulsion	1 hour	9.9	23.0	3.4	65.7	6.1	73.5	
5-9-52	Emulsion	3 days	2.2	7.1	0.0	100.0	2.0	71.8	
5-9-52	Emulsion	6 days	0.5	0.4	0.45	10.0	0.5	-----	
5-9-52	Emulsion	9 days	0.6	0.0	0.4	33.3	-----	-----	

Table 2, Continued
 The Effect Three Separate Washings have on
 the Amount of Lindane and Parathion Residue
 Removed from Spinach, Mustard Greens, Swiss
 Chard, Beans, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on Vegetable <u>Before Washing</u>		PPM Insecticide on Vegetable <u>After Washing</u>		Lindane	Parathion	% Loss	% Loss
			Lindane	Parathion	Lindane	Parathion				
Spinach										
5-9-52	Wettable powder	1 hour	9.8	15.6	1.7	82.7	3.3	78.9		
5-9-52	Wettable powder	3 days	1.0	1.9	0.7	30.0	0.6	68.4		
5-9-52	Wettable powder	6 days	0.2	0.0	0.4	-----	0.1	-----		
5-9-52	Wettable powder	9 days	0.0	0.1	-----	-----	-----	-----		
Mustard Greens										
12-13-51	Dust	1 hour	12.9	5.5	0.6	95.4	2.5	54.6		
12-13-51	Emulsion	1 hour	25.3	5.5	5.6	77.9	3.5	36.4		
12-13-51	Wettable powder	1 hour	1.1	24.5	0.0	100.0	5.0	79.6		
Swiss Chard										
6-16-52	Emulsion	1 hour	-----	271.2	-----	-----	65.8	75.7		
6-16-52	Emulsion	3 days	-----	165.3	-----	-----	30.0	81.9		
6-16-52	Emulsion	9 days	-----	128.8	-----	-----	32.2	75.0		
Beans										
6-16-52	Emulsion	1 hour	-----	246.8	-----	-----	-----	-----		
6-16-52	Emulsion	3 days	-----	67.8	-----	-----	6.7	90.1		
6-16-52	Emulsion	9 days	-----	*	-----	-----	*	-----		

* Bean plants died from the use of the high concentration of parathion

Table 2, Continued
 The Effect Three Separate Washings Have on
 the Amount of Lindane and Parathion Residue
 Removed from Spinach, Mustard Greens, Swiss
 Chard, Beans, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on vegetable <u>Before Washing</u>		PPM Insecticide on vegetable <u>After Washing</u>			
			Lindane	Parathion	Lindane	Parathion	% Loss	% Loss
			Crowders				% Loss	% Loss
7-15-52	Emulsion	1 hour	67.32	29.0	8.14	87.9	4.25	85.3
7-15-52	Emulsion	3 days	4.94	1.30	3.86	21.9	0.99	23.9
7-15-52	Emulsion	7 days	3.22	0.35	3.03	5.9	0.20	42.9
8-15-52	Emulsion	1 hour	26.01	12.50	8.47	67.4	8.75	30.0
8-15-52	Emulsion	3 days	5.35	2.30	5.35	0.0	1.53	33.5
8-15-52	Emulsion	6 days	3.80	0.36	0.96	74.7	-----	-----

Table 3
The Effect Washing has on the Amount
of DDT and Parathion Residues removed
from Different Varieties of Apples.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on apples before washing		PPM Insecticide on apples after washing			
			DDT	Parathion	DDT	Parathion	% Loss	% Loss
			Holland				% Loss	% Loss
6-30-52	Wettable powder	2 hours	14.50	1.03	3.48	76.0	0.61	40.8
6-30-52	Wettable powder	4 days	-----	0.30	-----	-----	0.0	100.0
			-----*	1.31	-----	-----	*0.0	100.0
7-22-52	Wettable powder	2 hours	2.27	-----	-----	-----	-----	-----
			*10.46	-----	-----	-----	-----	-----
			Ada Red					
6-30-52	Wettable powder	2 hours	101.25	0.90	0.18	98.2	0.75	16.7
6-30-52	Wettable powder	4 days	-----	0.25	-----	-----	0.0	100.0
7-22-52	Wettable powder	2 hours	3.0	-----	2.42	19.3	-----	-----
			*11.3	-----	*9.31	17.6	-----	-----
			Yellow Transparent					
6-30-52	Wettable powder	2 hours	-----	0.12	-----	-----	0.10	16.7

* PPM insecticides per peel and not PPM per apple as the other numbers designate.

Table 4
The Effect Washing and open kettle cooking
for 30 minutes has on the amount of Lindane
and Parathion Residues removed from Spinach,
Mustard Greens, Swiss Chard, and Cressions.

Date	Formu- lation	Period after treat- ment	PP. Insecticide on vegetable before washing and open cooking		PP. Insecticide on vegetable after washing and open cooking			
			Lindane	Parathion	Lindane	Parathion	%Loss	%Loss
			Spinach				%Loss	%Loss
11-29-51	Dust	1 hour	64.7	23.2	-----	-----	0.1	94.5
11-29-51	Emulsion	1 hour	59.2	11.3	-----	-----	1.6	85.8
11-29-51	Wettable powder	1 hour	67.1	24.8	-----	-----	1.6	93.3
5-9-52	Dust	1 hour	9.3	5.3	1.0	89.0	0.5	91.4
5-9-52	Dust	3 days	1.4	0.3	0.0	100.0	0.1	66.6
5-9-52	Dust	6 days	0.3	0.2	0.2	33.3	0.1	50.0
5-9-52	Dust	9 days	-----	0.0	0.1	-----	0.0	-----
5-9-52	Emulsion	1 hour	9.9	23.0	1.3	86.9	1.3	92.2
5-9-52	Emulsion	3 days	2.2	7.1	0.0	100.0	0.6	91.6
5-9-52	Emulsion	6 days	0.5	0.4	0.0	100.0	0.7	-----
5-9-52	Emulsion	9 days	0.6	0.0	0.4	33.3	0.0	-----
5-9-52	Wettable powder	1 hour	9.2	15.6	1.0	89.0	0.9	91.2
5-9-52	Wettable powder	3 days	1.0	1.9	0.5	50.0	0.1	87.4
5-9-52	Wettable powder	6 days	0.2	0.0	0.4	-----	0.0	-----
5-9-52	Wettable powder	9 days	0.0	0.1	0.4	-----	0.0	-----

Table 4, Continued
 The Effect Washing and Open Kettle cooking
 for 30 minutes has on the amount of Lindane
 and Parathion Residue removed from Spinach,
 Mustard Greens, Swiss Chard, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on vegetable before washing and open cooking		PPM Insecticide on vegetable after washing and open cooking		%Loss	%Loss
			Lindane	Parathion	Lindane	Parathion		
			Mustard Greens					
12-13-51	Dust	1 hour	12.9	5.5	0.0	100.0	0.5	90.9
12-13-51	Emulsion	1 hour	25.3	5.5	0.8	96.8	2.0	63.6
12-13-51	Wettable powder	1 hour	1.1	24.5	0.4	63.6	4.5	81.6
			Swiss Chard					
6-16-52	Emulsion	1 hour	---	271.2	---	---	45.6	83.2
6-16-52	Emulsion	3 days	---	165.3	---	---	19.2	83.4
6-16-52	Emulsion	9 days	---	128.8	---	---	19.0	85.3
			Crowders					
7-15-52	Emulsion	1 hour	67.32	29.0	5.52	91.8	1.34	95.4
7-15-52	Emulsion	3 days	4.92	1.30	3.03	38.4	0.30	76.9
7-15-52	Emulsion	7 days	3.22	0.35	1.14	65.6	0.20	42.9
8-15-52	Emulsion	1 hour	26.01	12.50	11.20	56.9	7.50	40.0
8-15-52	Emulsion	3 days	5.35	2.30	0.29	94.6	0.60	73.9
8-15-52	Emulsion	6 days	3.80	0.36	0.08	97.9	0.29	19.4

Table 5
The Effect Washing and the Open Cooking
for 1 hour has on the amount of DDT and
Parathion Residue Removed from different
varieties of apples.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on apple before washing and open cooking		PPM Insecticide on apples after washing and open cooking		% Loss	% Loss
			DDT	Parathion	DDT	Parathion		
			Holland					
6-30-52	Wettable powder	2 hours	145.0	1.03	0.15	99.0	0.0	100.0
6-30-52	Wettable powder	4 days	-----	0.30	-----	-----	0.0	100.0
			-----	*1.31	-----	-----	*0.0	100.0
7-22-52	Wettable powder	2 hours	2.27	-----	1.34	41.0	---	-----
			10.46	-----	*8.23	20.8	-----	-----
			Ada Red					
6-30-52	Wettable powder	2 hours	101.25	0.90	0.17	98.3	0.13	85.6
6-30-52	Wettable powder	4 days	-----	.25	-----	-----	0.0	100.0
7-22-52	Wettable powder	2 hours	3.0	-----	2.36	21.3	-----	-----
			*11.3	-----	*9.85	12.8	-----	-----

* PPM Insecticide per peal and not PPM per apple
as the other numbers designate.

Table 6
 The Effect Washing and Pressure Cooking
 (15 lbs. pressure per square inch at 120° C)
 for 15 minutes has on the amount of Lindane
 and Parathion residue removed from Spinach,
 Mustard Greens and Crowders.

Date	Formu- lation	Period after treat- ment	PPH Insecticide on vegetable before washing and pressuring		PPH Insecticide on vegetable after washing and pressuring				
			Lindane	Parathion	Lindane	Parathion	% Loss	% Loss	
			Spinach						
11-25-51	Dust	1 hour	64.7	23.8	----	----	0.5	94.2	
11-25-51	Emulsion	1 hour	69.2	11.3	----	----	0.5	94.6	
11-25-51	Wettable powder	1 hour	67.1	24.0	----	----	1.5	93.4	
5-9-52	Dust	1 hour	9.8	5.8	0.4	95.9	0.8	86.2	
5-9-52	Dust	3 days	1.4	0.3	0.0	100.0	0.0	100.0	
5-9-52	Dust	6 days	0.3	0.2	----	----	0.0	100.0	
5-9-52	Dust	9 days	----	0.0	----	----	0.1	----	
5-9-52	Emulsion	1 hour	9.9	23.0	0.3	97.0	1.5	93.5	
5-9-52	Emulsion	3 days	2.2	7.1	0.7	68.2	1.0	85.9	
5-9-52	Emulsion	6 days	0.5	0.4	----	----	0.1	25.0	
5-9-52	Emulsion	9 days	0.6	0.0	----	----	0.1	----	
5-9-52	Wettable powder	1 hour	9.8	15.6	0.4	95.9	0.8	94.9	
5-9-52	Wettable powder	3 days	1.0	1.9	0.7	30.0	0.3	84.2	
5-9-52	Wettable powder	6 days	0.2	0.0	----	----	0.1	----	
5-9-52	Wettable powder	9 days	0.0	0.1	----	----	0.1	0.0	

Table 6, Continued
 The Effect Washing and Pressure Cooking
 (15 lbs. pressure per square inch at 120° C)
 for 15 minutes has on the amount of Lindane
 and Parathion residue removed from Spinach,
 Mustard Greens and Crowders.

Date	Formu- lation	Period after treat- ment	P/M Insecticide on vegetable before washing and pressuring		P/M Insecticide on vegetable after washing and pressuring			
			Lindane	Parathion	Lindane	Parathion	% Loss	% Loss
			Mustard Greens				% Loss	% Loss
12-13-51	Dust	1 hour	12.9	5.5	-----	-----	0.4	92.7
12-13-51	Emulsion	1 hour	25.3	5.5	-----	-----	0.6	90.9
12-13-51	Settable powder	1 hour	1.1	24.5	-----	-----	0.6	97.6
			Crowders					
7-15-52	Emulsion	1 hour	67.32	29.0	5.85	91.3	3.00	89.7
7-15-52	Emulsion	3 days	4.92	1.30	3.73	24.9	0.58	55.4
7-15-52	Emulsion	7 days	3.22	0.35	1.14	64.6	0.33	5.7
8-15-52	Emulsion	1 hour	26.01	12.50	1.15	55.8	1.26	89.9
8-15-52	Emulsion	3 days	5.25	2.30	0.29	94.5	0.83	63.9
8-15-52	Emulsion	6 days	3.80	0.36	0.29	92.4	0.25	30.6

Processing Vegetables by
Washing and Pressure Cooking

The vegetables (spinach, mustard greens, and Crowder peas) were washed three times with distilled water and pressure cooked at 15 pounds pressure per square inch (120° C.) for 15 minutes. The reduction of the insecticidal residue by this process, as shown in Table 6, ranged from 0 to 100 per cent, average reduction for lindane being 75.9, and for parathion 69.1 per cent. The higher residue concentrations were the more effectively reduced in this process, than were the lower concentrations that had been on the vegetables for a longer period of time. Ada Red apples that were previously sprayed with DDT wettable powder, when pressure cooked under the above conditions, showed a reduction of 77.0 per cent of the applied spray residue. The results of this study is found in Table 7.

Table 7
The Effect washing and then Pressure Cooking
(15 lbs. per square inch at 120° C. for 15
minutes) has on the amount of DDT residue on
Ada Red Apples.

Date	Formu- lation	Period after treat- ment	PPM DDT on apple before washing and pressure cooking	PPM DDT on apple after washing and pressure cooking	%Loss
7-22-52	Wettable powder	2 hours	3.0 per apple	0.69 per apple	77.0
			11.3 per peel	3.30 per peel	70.8

The Storage of Vegetables by Deep-freezing

Some of the vegetables treated with the insecticide were washed three times with distilled water before canning and storage in the deep-freeze, others were not washed before they were frozen. Spinach, mustard greens, and crowder peas were used in this experiment. The results of these analyses are compiled in Table 8. In all analyses there was a

Table 8
The Effect Washing and then Freezing has on
the amount of Lindane and Parathion Residue
on Spinach, Mustard Greens, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on vegetable before washing and freezing		PPM Insecticide on vegetable after washing and freezing			
			Lindane	Parathion	Lindane	Parathion	% Loss	% Loss
			Spinach					
11-25-51	Dust	1 hour	64.7	28.8	-----	-----	-----	-----
11-25-51	Emulsion	1 hour	69.2	11.3	-----	-----	11.0	-----
11-25-51	Wettable powder	1 hour	67.1	24.0	-----	-----	7.0	70.8
5-9-52	Dust	1 hour	9.8	5.8	*1.3	86.7	*1.9	67.3
5-9-52	Dust	3 days	1.4	0.3	0	100.0	0.1	66.6
5-9-52	Dust	6 days	0.3	0.2	-----	-----	-----	-----
5-9-52	Dust	9 days	-----	0.0	-----	-----	0.1	-----
5-9-52	Emulsion	1 hour	9.9	23.0	*5.7	42.4	*12.8	44.4
5-9-52	Emulsion	3 days	2.2	7.1	0.0	100.0	1.6	77.5
5-9-52	Emulsion	6 days	0.5	0.4	-----	-----	-----	-----
5-9-52	Emulsion	9 days	0.6	0.0	-----	-----	0.1	-----
5-9-52	Wettable powder	1 hour	9.8	15.6	*3.6	63.3	*7.2	53.9
5-9-52	Wettable powder	3 days	1.0	1.9	0.0	100.0	0.5	73.7
5-9-52	Wettable powder	6 days	0.2	0.0	-----	-----	-----	-----
5-9-52	Wettable powder	9 days	0.0	0.1	-----	-----	0.1	0.0

* Samples unwashed before freezing.

Table 8, Continued
 The Effect Washing and then Freezing has on
 the amount of Lindane and Parathion Residue
 on Spinach, Mustard Greens, and Crowders.

Date	Formu- lation	Period after treat- ment	PPM Insecticide on vegetable before washing and freezing		PPM Insecticide on vegetable after washing and freezing				
			Lindane	Parathion	Lindane	Parathion			
Mustard Greens									
12-13-51	Dust	1 hour	12.9	5.5	-----	-----	* 3.1	43.6	
12-13-51	Emulsion	1 hour	25.3	5.5	-----	-----	-----	-----	
12-13-51	Wettable powder	1 hour	1.1	24.5	-----	-----	*19.6	20.0	
Crowders									
7-15-52	Emulsion	1 hour	67.32	29.0	11.05	83.6	1.12	96.1	
7-15-52	Emulsion	3 days	4.92	1.30	3.22	34.6	-----	-----	
7-15-52	Emulsion	7 days	3.22	0.35	-----	-----	0.16	54.3	
8-15-52	Emulsion	1 hour	26.01	12.50	*15.52	40.3	*7.25	42.0	
8-15-52	Emulsion	3 days	5.35	2.30	* 0.0	100.0	*1.80	21.7	
8-15-52	Emulsion	6 days	3.80	0.36	* 0.0	100.0	*0.30	16.7	

* Samples unwashed before freezing.

reduction of the amount of the insecticidal residues of parathion and Lindane present on the vegetables sprayed in the field after they were frozen and then thawed. The residual lindane decreased on an average by 72.2 per cent; parathion decreased on an average by 44.7 per cent.

An experiment on deep-freezing vegetables was carried out in the laboratory using lettuce as the host material for the two insecticides. The lettuce leaves were dipped into a solution containing the dissolved insecticides, and then allowed to dry in the open air before deep-freezing. The data obtained from this experiment is compiled in Table 9.

Table 9
The Reduction of Parathion and Lindane
on Lettuce by Deep-freezing (1-2-53)

<u>Lettuce</u>	<u>Ave. PPM parathion</u>	<u>Ave. PPM lindane</u>
Unfrozen	17.5	21.8
Frozen + H ₂ O extractions	12.7	15.2
H ₂ O extract with ether	6.6	7.6

These data indicate that some of the insecticidal residue is washed off the plant material when the vegetable water is released upon thawing of the vegetables. The water from the lettuce was extracted with ether in order to obtain quantitative data on the total amount of insecticidal residue present, after freezing and thawing of the vegetable.

There is apparently no parathion hydrolyzed in this process, because it was fully recovered after the vegetable had been thawed. Lindane, on the other hand, was not fully recovered. Examination of the data reveals a net loss of 6.6 ppm. Part of this discrepancy may have been due to incomplete extraction of lindane from the wash water. Also part of the gamma-BHC may have been hydrolyzed to hydrochloric acid and benzene trichloride. This inorganic chloride would not be extracted by ether from the water solution.

The data in Table 5 shows that more lindane is lost from the vegetable upon thawing than parathion, and the laboratory experiment with lettuce, residual lindane was also decreased the most. Vegetables that were frozen would, however, be cooked before being served, and hence still further decrease must be expected.

Translocation of parathion in plants has been investigated by several scientists. Work done by Granger and Lisle (3) in nasturium seedlings indicated that parathion in the soil did not adversely affect germination. A slight retardation of growth during the first two weeks after planting was indicated where parathion was used, but after 11 weeks, checks and parathion plots were the same. The peak of toxicity of the plant to the black bean aphid was 6 weeks after the seed was planted. The toxicity then decreased to zero between the 9th and 11th weeks. They also showed that the higher the concentrations of the insecticide used in the soil, the greater were the kills of the aphids on the plant.

The foliage of a number of different crops was painted with parathion (31) to determine the amount of parathion, if any, that would be translocated from the leaves to the edible portions (roots, leaves, fruits) of certain vegetables. In the greenhouse they used a parathion preparation containing 0.2 lb. of 25 per cent wettable powder per 100 gal. of water on the different vegetables. Their results of the greenhouse tests are as follows: Parathion was not found in shelled Lima beans or in tomatoes, and less than 0.10 ppm in cucumber, pepper, and radish; 0.04 ppm and 0.51 ppm in snap beans and beets; 0.13 to 0.34 ppm in cabbage, Chinese cabbage, Swiss chard, collards; and in kale 0.15 ppm. In their field tests, snap beans, cucumbers, summer squash, peas, and

strawberries contained 0.2 - 0 ppm of parathion. The results of their greenhouse and field tests indicated that in some vegetables, parathion may be translocated from treated foliage, but in only minute quantities. It must be noted that they did not preclude the chance of absorption of parathion vapors.

Quastel and Comins (32) have shown that corn planted in soil treated with parathion is toxic to the European corn borer, Pyrausta nubilalis, when they were confined on the leaves and stalks of the corn. In 24-48 hours 94-100 per cent of the larvae died. In another experiment performed by them, the soil was watered with a suspension of parathion sufficient to give 2 grams of parathion per 5-inch flower pot. Sections of leaves and stalks taken from the treated plots were fed one and two weeks after treatment to newly hatched larvae. Leaves one week after treatment were non-toxic, but the stalks killed 96 per cent of the larvae. The leaves and stalks two weeks after treatment killed 100 per cent of the larvae.

Genzle and Allen (33) have pointed out that parathion is translocated either up or down in plants in insecticidal amounts, and that the toxicity to insects is not due to a fumigating effect caused by the evaporation of the insecticide from the soil. Starnes (34), using bioassay tests and chemical analyses on potatoes grown in soil containing 25 pounds of parathion per acre, has indicated that the toxicant is present at harvest in the plant above the ground in the tubers and in the soil. It has been shown that parathion enters the leaf tissue and moves a few ms. in Lycopersicon tomentosum (35). The presence or absence of parathion was tested by the measurement of the areas where phytophagous died or survived.

The primary interest of the work undertaken in this problem was to see how much, if any, of the parathion which had been mixed with the soil, would be present in the vegetables at the time of harvest. It was also of interest to obtain some data on the concentrations of parathion that could be used effectively without killing or slowing the germination of the seeds.

The furrows were treated with 10, 30 and 50 per cent concentrations of parathion wettable powder respectively at the time of planting the beans. The furrows were then covered with top soil. The soil was watered frequently, so that growth would not be hampered because of the lack of moisture in the soil.

It must be kept in mind that this experiment was not done to prove or disprove that parathion is translocated through the roots of plants, but to obtain data on the amount of the insecticide present at the time of harvest from vegetables grown in soil treated with parathion. A second objective was to determine if the parathion found to be present could be reduced by cooking in an open kettle.

The 50 per cent concentration of parathion put into the soil at the time of planting killed the germination of the bean plants. The 30 per cent concentration of parathion slowed down germination of the bean seeds, and it also killed germination of some of the seeds. The 10 per cent concentration of parathion did not seem to have much effect on the rate that the seeds germinated compared to the controls.

Bean seeds that did germinate in the row treated with 30 per cent parathion contained 0.26 ppm parathion in the bean plant forty days after planting. The row treated with a 10 per cent concentration of parathion at the time of planting contained 0.21 ppm parathion forty days

after planting.

Some of these bean plants from those two rows were soaked in an open kettle for 15 minutes, and the parathion was reduced 0.09 ppm in the bean plants. However, this is not proof that the parathion content was due to translocation. Its presence may be due to evaporation from the soil and absorption by the leaves.

Analysis of bean pods produced 55 days after planting of the seeds showed that there was 0.00 ppm of parathion present in them at that time. This information points toward the translocation of parathion through the roots and its subsequent storage in the bean pods. If this is not the case, the application of parathion to the soil was sufficient to have fumigating quantities two months after application to the soil. The parathion vapors may have been absorbed by the leaves and later moved to the developing bean pods.

There has been some work done on gamma benzene hexachloride in regard to translocation from the roots of plants to the foliage. Santa Maria (36) has shown that oat plants, grown from seeds immersed for 5 minutes in a solution of 0.3 per cent gamma benzene hexachloride in 65 per cent ethanol, were toxic for the aphid, Schizanthus graminis, for 30 days. The degree of toxicity varied with the variety of oats, the temperature, and environmental factors. He stated that the toxic action was due to lindane in the plant sap, not to fumigation nor to contact poisoning by lindane. Longer immersion times, higher concentrations of gamma benzene hexachloride, or uses of technical benzene hexachloride affected, in some instances, the growth of the oats.

An experiment performed by Starnes (34) showed that the growth in potatoes was retarded in soil containing 100 pounds per acre of gamma benzene hexachloride. The rate of sprouting and growth was retarded

proportionally as higher concentrations of lindane were used. Aqueous suspensions and benzene extracts of the potato foliage and tubers from these potatoes grown in soil containing gamma benzene hexachloride, were highly toxic to Aedes larvae. The presence of a toxicant was also indicated when these tubers were infested with larvae of the potato tuber worm. He also pointed out that there was a large amount of the toxicant present in the soil at the time of harvest.

The primary interest in this investigation was to determine the amount of lindane that would be present in vegetables at the time of harvest that were grown in soil containing lindane. It was also an academic interest to see if lindane was actually translocated through the roots of the plants in any large enough amount that could be detected chemically.

The lindane was injected into the soil of the potted plants, in equal concentrations, after the plants were growing. Some of the plants were sealed off with cellophane and wax to prevent any evaporation of the insecticide. Lindane was also injected into the soil without sealing off the plant from the soil to prevent evaporation of the insecticide. Controls with no injections of lindane were also run.

Lindane was injected into the soil of potted onions and two weeks later the tops were analyzed to determine the amount of the insecticide present. Table 10 contains the results.

Table 10
Experimental Data on the Translocation
of Lindane in onions.

<u>Onion Tops</u>	<u>Chloride calculated</u> <u>in PPM Lindane</u>
Control	7.57
Unsealed	12.35
Sealed	6.22

The onion analysis points out that the amount of lindane found on the onions is not due to translocation, but to either evaporation or by coming in direct contact with the soil containing lindane. The onion tops sealed off from the soil contained less chloride than did the controls, and the onion tops unsealed and injected contained almost twice as much chloride as either the control or the sealed onion tops. This would indicate that some of the chloride on the onion tops is due to the direct contact of the tops with the inorganic chloride salts in the soil. The remainder of the chloride, in terms of lindane, on the onion tops is then due to the direct contact with lindane in the soil or by evaporation of the lindane from the soil. A similar experiment was performed using tomato plants as the host, and the results were the same as the experiment using onion tops.

In the field, a 25 per cent emulsion of lindane was injected around the roots of crowders on August 13, and three days later the pods were analyzed. At this time they were found to contain 4.35 ppm of lindane. Ten days after the injection, they contained 1.26 ppm. This all points toward a fumigating action of lindane from the soil on the plants.

Conclusions

There were definite trends in the reduction of the two insecticides on the vegetables in all processing. The high concentrations of insecticidal residues of parathion and lindane were more effectively reduced than those concentrations which were lower, but had been on the vegetables over a period of time. This is attributed to an adherence of these organic insecticidal molecules which are in direct contact with the plant, or molecules which have penetrated into the plant by absorption.

Care must be taken in the application of these insecticides, since they are phytotoxic when applied either to the soil or on the plant at high concentrations.

It is clearly shown that plants cannot store an overabundance of these insecticides; therefore, one would not ingest large quantities of an insecticidal residue unless the plant was harvested a few hours after application of the insecticide. If a longer period elapsed after the insecticidal application, and the vegetables died, they would not then be harvested.

Thus, the toxicity of these insecticides is such that we cannot discuss the possible dangers of indiscriminate use. However, this data would indicate that under proper spray conditions, harvesting and processing of such foods, there would be very small probabilities of the existence of any dangerous quantities of spray residues.

Summary

1. Vegetables sprayed with moderate concentrations of parathion and lindane can be harvested 9 days after application of the insecticide without the presence of any appreciable residue.
2. High concentrations of parathion sprayed on Swiss chard and beans is phytotoxic to these plants.
3. Processing vegetables and apples by washing with water effectively removed some of the insecticidal residue. The average lindane reduction on the vegetables amounted to 52.6 per cent; the parathion to 57.3 per cent.
4. Processing vegetables and apples by washing and open-kettle cooking effectively removed some of the insecticidal residue. The average lindane reduction on the vegetables amounted to 76.7 per cent; the parathion to 74.6 per cent.
5. Processing vegetables and apples by washing and pressure cooking effectively removed some of the insecticidal residue. The average lindane reduction on the vegetables amounted to 75.9 per cent; the parathion to 69.1 per cent.
6. Storage of vegetables by deep-freezing had no effect on the reduction of residual parathion, when the plant water was separately extracted to account for the apparent loss. Lindane was not fully recovered, hence, some of it may have been hydrolyzed to hydrochloric acid and trichlorobenzene.
7. Bean seeds planted in soil containing a 10 per cent solution of parathion contained 0.03 ppm of this insecticide in the bean pods 52 days after planting.
8. Concentrations much higher than 10 per cent solutions of parathion applied to the soil hindered germination of the seeds.
9. Lindane is not translocated through the roots of onions to their tops, at least in any detectable quantities.

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