Investigations on Dursban Insecticide. Uptake and Translocation of [^{36}Cl] *O,O*,-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate and [^{14}C] *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate by Beans and Corn

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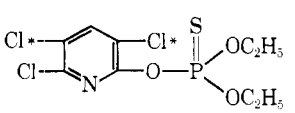
The uptake and translocation of [^{36}Cl] Dursban and [^{14}C] Dursban by leaves and corn plants were studied. Both root and leaf absorption were insignificant. Only a small percentage of the radioactivity (1 to 2%) was translocated into the plant. These were largely breakdown products such as the 3,5,6-trichloro-2-pyridinol. In foliar spray treatment, most of the radioactivity is lost from the leaf by volatilization. Since absorption and translocation of Dursban or its decomposition products are insignificant, the only place a significant amount of radioactivity could be found is on the treated area.

The new Dursban insecticide (Dow Chemical Co.) is being tested for the control of soil and foliar insects. The active ingredient of this insecticide is *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate and will hereafter be designated Dursban, for purposes of clarity and brevity. Metabolism studies were initiated to determine if this insecticide was absorbed into the plant via either the leaves or roots, and if the compound would translocate and metabolize. In these investigations, radioactive Dursban was employed. In the first series of experiments, [^{36}Cl] Dursban was employed because of the ease and cost of preparing this material. In those experiments in which [^{36}Cl] Dursban was employed, traces of [^{36}Cl] chloride were found in the plants. This information suggested that dehalogenation of the pyridinol was occurring. Under these conditions it would be impossible to determine the fate of the pyridinol ring. Ring-labeled [^{14}C] Dursban then was prepared and used in all the later investigations.

Experimental Procedures

Chemicals. The radioactive [^{36}Cl] Dursban was prepared by New England Nuclear Corp. based on a synthesis procedure developed by Rigterink and Kenaga (*2*). The [^{36}Cl] chloride-labeled 3,5,6-trichloro-2-pyridinol was prepared by the direct chlorination of the 6-chloro-2-pyridinol using HCl^{36} and H_2O_2. The resulting 3,5,6-trichloro-2-pyridinol, therefore, was labeled in the 3,5-positions. The 3,5,6-trichloro-2-pyridinol then was converted to Dursban. The Dursban had a specific activity of 29 μc. mmole and was labeled in the 3,5-position as indicated.



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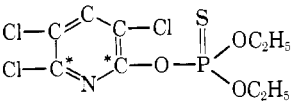
The purity of the compound was checked by infrared analysis and paper chromatography. As far as could be ascertained, at least 97% of the radioactivity was associated with the labeled Dursban. It is difficult to determine the absolute purity of pyridinol compounds as they form hydrochloric acid salts which are difficult to dissociate completely. If the hydrochloric acid is ^{36}Cl-chloride labeled, part of the activity present in the pyridinol molecule then could be associated with hydrochloric acid and not the chlorines on the pyridinol molecule. In the preparation of the [^{36}Cl] 3,5,6-trichloro-2-pyridinol, the compound was recrystallized from alcohol and water, then dissolved in sodium hydroxide solution. The pyridinol was reprecipitated by addition of hydrochloric acid. This procedure was repeated three times to minimize the possibility of having a [^{36}Cl] hydrochloric acid salt of the pyridinol. The purity of the compound then was checked by paper and thin-layer chromatography (*5*) and infrared analysis.

In the initial studies, [^{36}Cl] Dursban was used and data were obtained which indicated that the compound might be undergoing dehalogenation. Ultraviolet light studies indicated that dehalogenation could be induced by light (*4*). Similarily, enzymatic dehalogenation also can occur with this type of compound (*8*). Therefore, a small quantity of the ring-labeled Dursban was prepared so that the dehalogenated products of the pyridinol could be detected.

The ring-labeled pyridinol was prepared by Muelder (*1*) to give Dursban which was labeled with carbon-14 in the 2,6-positions as indicated.



The compound was reported to have a radiochemical purity of at least 99% as determined by infrared analysis and thin-layer chromatography.

In all the plant studies, Dursban was applied as a

water emulsion to permit maximum spread of the chemical over the surface of the leaf or in the soil. The composition of the concentrate is shown in Table I. This formulation was diluted with various amounts of water depending on the amount of Dursban to be applied.

Treatment of Plants. In the initial experiments with [^{36}Cl] Dursban, cranberry bean (Taylor strain dwarf horticultural bean) and corn were grown in a climatizer which was set to have a 16-hour light period and 8 hours of darkness with a temperature of 92° F. during the light period and 76° F. during the dark period. The relative humidity was approximately 45 to 50%. The [^{36}Cl] Dursban was applied as a water emulsion to one of the basal leaves of the bean and to one of the outer leaves of the corn.

The leaves were held in a vertical position with a wire frame made of stainless steel. The emulsion was applied with a lambda pipet and spread over the surface of the leaf. The formulation was allowed to dry on the leaf before the plant was moved, thus minimizing the possibility of cross-contamination.

At various time intervals, the plants were harvested and divided into roots, treated leaf, and remaining parts of the top. All samples were then analyzed for radioactivity by combusting the sample, collecting the resulting silver chloride, and counting it in a scintillation counter. The combustion procedure and the counting method have been described previously (*3*).

With the [^{14}C] Dursban, the bean plants were covered with plastic so only the two cotyledonous leaves were exposed. The plants then were sprayed in a special chamber designed for the use of radioactive materials. The formulation then was allowed to dry on the leaves, and the plants were returned to the climatizer. In this case, a new type of climatizer was employed which permitted regular cycling of the air temperature, light, humidity, and soil temperature. The air temperature was cycled from 95° to 75° F., the lights from 0 to 2000 foot candles, humidity from 35% daytime to 95% at night. The soil temperature varied from 75° to 95° F. In this climatizer, the air supply in the chamber is changed approximately five to seven times per minute. As the air is recycled, it passes through a water wash so that any volatile material present in the air is removed. This decreases the chance of any cross-contamination of plants with volatile radioactive material.

Assay for Radioactivity. In those experiments in which [^{36}Cl] Dursban was employed, the samples were analyzed for [^{36}Cl] activity by the method previously reported by Smith (*3*). In this procedure the sample is combusted in a mixture of 10% nitric and 90% sulfuric acid. The resulting chloride is trapped as silver chloride, dissolved in a scintillation solution, and counted in a standard scintillation counter. With the [^{14}C] Dursban, the samples were combusted and counted using the procedure described by Smith *et al.* (*6*). In this procedure, the sample is combusted in Van Slyke reagent, and the resulting carbon dioxide trapped in ethanol amine and counted in a scintillation counter. All samples were counted in a Nuclear Chicago Scintillation Counter Model 725.

Table I. Per Cent Composition of Emulsifiable Formulation of *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate

Atlox 8816T	2.5
Emcol P10-20P	2.5
Chlorothene[a]	25.0
Xylene	47.4
[^{36}Cl] Dursban or [^{14}C] Dursban	22.6

[a] Dow Chemical Co.

Results and Discussion

In the initial experiments with [^{36}Cl] Dursban, the formulation of [^{36}Cl] Dursban was diluted with water to give an emulsion that contained 10 mg. of Dursban per ml. One hundred lambda of this solution containing 1 mg. of [^{36}Cl] Dursban was spread over one of the cotyledonous leaves of the bean plant. The leaves were held in a vertical position until the formulation had dried on the leaf. At various time intervals the plants were sacrificed and analyzed for radioactivity.

Typical results obtained with bean plants are shown in Tables II and III. Table II has a summary of the distribution of radioactivity between the treated leaf, roots, and top of the plant and accounts for the amount of radioactivity remaining in or on the plant of the original 1000 μg. applied to the plant. Table III gives a more detailed picture of the distribution within the individual plants at five and seven days.

From the data given in Table II and III, certain general trends concerning the absorption and translocation of the radioactive compounds in the plant and the loss of activity from the surface of the treated leaves seem apparent. Initially, 1000 μg. of [^{36}Cl] Dursban

Table II. Uptake and Translocation of Radioactive Compounds (Mmole/Kg. of Tissue) in Bean Plants Following Treatment of a Single Leaf of the Plant with [^{36}Cl] Dursban

Days after Treatment	Treated Leaf	Plant	Roots	% of Total Dosage
1	0.48 ± 0.13	0.0010 ± 0.0002	0.0004 ± 0.0002	32.2 ± 3.0
2	0.36 ± 0.04	0.0010 ± 0.0001	0.0036 ± 0.0002	23.1 ± 3.2
3	0.28 ± 0.04	0.0014 ± 0.0004	0.0025 ± 0.0005	20.3 ± 2.7
4	0.31 ± 0.06	0.0013 ± 0.0004	0.0034 ± 0.0012	19.4 ± 3.9
5	0.20 ± 0.03	0.0011 ± 0.0000	0.0031 ± 0.0008	15.7 ± 3.0
7	0.20 ± 0.04	0.0010 ± 0.0001	0.0005 ± 0.0002	14.8 ± 6.2

was applied to each plant. There was a considerable loss of radioactivity during the first three days. In the order of 80% of the activity had disappeared during this period. This was presumed to be due to the volatility of the compound from the surface of the leaf. The activity remaining on the leaf was confined largely to the treated area. After seven days, less than 1% of the activity applied to the plant was found in the nontreated area. The small amount of radioactivity which entered the plant appeared to be translocated to the tops of the plants as well as to the roots. There did not appear to be any significant translocation of the compound from the treated basal leaf to the opposite basal leaf or to the growth tip. With easily translocated material such as dalapon, the compound is generally translocated from one basal leaf to the other and to the growth tip.

From the data obtained, it must be concluded that, for all practical purposes, [^{36}Cl] Dursban is not translocated in plants. The small amount of activity found in the plant could be accounted for easily in terms of trace quantities of radioactive impurities present in the original [^{36}Cl] Dursban preparation, or small amounts of breakdown products of Dursban which could be formed by enzymatic or ultraviolet light degradation of the insecticide.

Similar results were obtained with corn plants given a foliar treatment with [^{36}Cl] Dursban (see Table IV). The majority of the 1000 μg. of [^{36}Cl] Dursban applied to the leaves was lost by volatilization. Of that remaining in or on the plant, the majority was confined to the treated area.

Attempts were made to identify the radioactivity found in the plants. For convenience the plants were divided into the nontreated and treated areas. The nontreated areas contained less than 1% of the applied radioactivity while the treated areas contained between 15 to 20% of the applied radioactivity.

In the initial studies with [^{36}Cl] Dursban, a significant percentage of the radioactivity in the nontreated portion of the plant could be removed by a water extraction and could be precipitated with silver nitrate from an acid solution. Apparently, radioactivity was present as [^{36}Cl] chloride. This indicates that dehalogenation of the pyridinol has taken place. Other studies on the dehalogenation of Dursban have shown that hydrolysis of the compound must occur before dehalogenation can occur (*4*). This would mean the Dursban had been hydrolyzed to the pyridinol and the pyridinol had undergone dehalogenation with the formation of diols

Table III. Distribution of Radioactivity in Various Parts of Bean Plant after Applying 1000 μG. of [^{36}Cl] Dursban to One of the Basal Leaves

Days after Treatment	Plant Part	Weight of Plant Part, G.	Radioactive Compounds Found in Tissue, Mmole/Kg.
5	Treated basal leaf	2.11208 ± 0.39581	0.21 ± 0.03
	Untreated basal leaf	2.18756 ± 0.09954	0.0011 ± 0.0002
	1st trifoliated leaves	3.25756 ± 0.39038	0.0011 ± 0.0000
	2nd trifoliate leaves	2.86656 ± 0.22189	0.0011 ± 0.0002
	3rd trifoliate leaves	1.86068 ± 0.27628	0.0009 ± 0.0000
	Growth tip	0.72347 ± 0.23178	0.0009 ± 0.0002
	Stem	3.85146 ± 0.45149	0.0011 ± 0.0002
	Roots	0.37825 ± 0.02606	0.0031 ± 0.0008
7	Treated basal leaf	2.00014 ± 0.40743	0.20 ± 0.04
	Untreated basal leaf	1.89928 ± 0.07485	0.0017 ± 0.0006
	1st trifoliate leaves	3.14594 ± 0.30034	0.0017 ± 0.0005
	2nd trifoliate leaves	3.08706 ± 0.20182	0.0013 ± 0.0007
	3rd trifoliate leaves	2.50759 ± 0.26941	0.0009 ± 0.0006
	4th trifoliate leaves	0.92239 ± 0.30836	0.0010 ± 0.0007
	Stems	3.91250 ± 0.67220	0.0013 ± 0.0003
	Roots	3.83233 ± 0.66935	0.0005 ± 0.0006

Table IV. Uptake and Translocation of Radioactive Compounds from Foliar Treatment of Single Leaf of Corn with [^{36}Cl] Dursban—Concentration in Mmole/Kg. of Tissue

Days after Treatment	Treated Leaf	Plant	Roots	% of Total Dose Remaining per Plant
3	0.23 ± 0.07	0.0009 ± 0.0005	0.0006 ± 0.0003	13.9 ± 2.2
4	0.42 ± 0.09	0.0014 ± 0.0003	0.0028 ± 0.0000	17.2 ± 2.1
5	0.23 ± 0.10	0.0019 ± 0.0005	0.0029 ± 0.0016	12.8 ± 4.8
6	0.17 ± 0.06	0.0013 ± 0.0006	0.0033 ± 0.0004	11.4 ± 1.8
7	0.17 ± 0.06	0.0009 ± 0.0003	0.0028 ± 0.0010	10.7 ± 2.4
8	0.24 ± 0.07	0.0018 ± 0.0011	0.0034 ± 0.0016	11.6 ± 2.3
9	0.24 ± 0.07	0.0021 ± 0.0002	0.0054 ± 0.0016	14.6 ± 4.1
10	0.19 ± 0.04	0.0015 ± 0.0006	0.0039 ± 0.0008	7.8 ± 1.6
11	0.17 ± 0.06	0.0023 ± 0.0003	0.0071 ± 0.0026	9.6 ± 2.0
12	0.23 ± 0.01	0.0016 ± 0.0005	0.0039 ± 0.0011	10.6 ± 4.6

and triols. Depending on the number of chlorine atoms removed, the diols and triols would be either nonradioactive or of low specific activity.

Analysis of the treated leaves showed the presence of traces of [^{36}Cl] Dursban with significant quantities of [^{36}Cl] 3,5,6-trichloro-2-pyridinol and [^{36}Cl] chloride. This would further suggest that hydrolysis and dehalogenation was occurring at the site of application, and the decomposition products were being translocated into the plant. In these experiments, [^{36}Cl] Dursban was employed, and once the labeled chlorine had been removed from the ring, it was impossible to follow the metabolism of the ring.

When [^{14}C] Dursban became available, some of the experiments reported above were repeated. In these cases, the bean plants were covered with plastic so that only the two basal leaves were exposed. The plants then were sprayed with [^{14}C] Dursban in an emulsified solution at the rate of 1 lb. per acre. The plants were allowed to dry, the plastic was removed, and the plants were returned to a climatizer. The plants were harvested at various time intervals and divided into leaves and tops. The results of the radiochemical analysis are shown in Table V.

The results obtained in these investigations with [^{14}C] Dursban are similar to those obtained with the [^{36}Cl] Dursban. There was a significant loss of [^{14}C] Dursban from the leaf during the first two days. This amounted to about 75 to 80% being lost from the plant. Again the amount entering the plant was small, less than 2%. The plant appeared to be growing faster than the radioactive compounds were entering the plant, so there was a decrease in residue per unit weight in the top of the plant with time. At the end of 14 days, the treated leaves were showing the effects of the chemical and had turned yellow. Once the treated leaves had dropped from the plant, there would be a significant drop in the total residue as the plant became larger.

At 14 days after treatment, five plants were collected and divided into treated leaves, top of the plant, and roots. The samples were homogenized in acetone and extracted for 4 hours in a Soxhlet extractor with acetone. The residue then was extracted with water. The residue and extracts were analyzed for radioactivity (see Table VI). In this extraction procedure, the acetone will remove the Dursban, its oxygen analog, and any of the pyridinols or partially hydrolyzed products which are present as the free pyridinol or acids. It will not remove any of the pyridinols or partially hydrolyzed products which are present as salts. These will be removed by the water extraction. Only the acetone extracts showed significant quantities of radioactivity, and these were chromatographed. The results from chromatographing the extract of the treated leaves are shown in Table VII. There appears to be a variety of hydrolyzed products of the Dursban. The main products formed were the ethyl-3,5,6-trichloro-2-pyridyl phosphate, 3,5,6-trichloro-2-pyridyl phosphate, and the 3,5,6-trichloro-2-pyridinol. In this case, it would be impossible to determine if chloride had been produced since

Table V. Uptake and Translocation of [^{14}C] Dursban Sprayed on Basal Leaves of Cranberry Beans at Rate of 1 Lb./Acre, Mmole/Kg. of Plant Tissue

Days after Treatment	Basal Leaves A	Basal Leaves B	Remaining Plant
0	0.60 ± 0.12	0.64 ± 0.34	0.0083 ± 0.0035
1	0.22 ± 0.05	0.23 ± 0.06	0.0115 ± 0.0051
2	0.13 ± 0.04	0.14 ± 0.05	0.0066 ± 0.0014
3	0.42 ± 0.08	0.13 ± 0.01	0.0064 ± 0.0011
4	0.11 ± 0.02	0.13 ± 0.03	0.0056 ± 0.0013
8	0.13 ± 0.02	0.11 ± 0.04	0.0044 ± 0.0006
11	0.10 ± 0.02	0.09 ± 0.03	0.0030 ± 0.0004
12	0.11 ± 0.03	0.15 ± 0.06	0.0026 ± 0.0007

Table VI. Distribution of Radioactivity in Various Parts of Cranberry Bean Treated with [^{14}C] Dursban

Tissue	Radioactivity, Mmole	%
Treated leaves	0.263	100.0
Acetone extract	0.231	87.8
Water extract	0.016	6.1
Tissue residue	0.012	4.6
		98.5
Plant	0.0195	100.0
Acetone extract	0.0150	76.9
Water extract	0.0009	4.6
Tissue residue	0.0031	15.9
		97.4
Roots	0.0046	100.0
Acetone extract	0.0023	50.0
Water extract	0.0003	6.5
Tissue residue	0.0017	37.0
		93.5

Table VII. R_f Values of Radioactive Compounds Isolated from Cranberry Beans Sprayed with [^{14}C] Dursban

Sample	R_f Value	Radioactivity, %
Leaf-acetone extract	0.35	3
	0.46	19
	0.60	38
	0.69	10
	0.79	8
	0.96	6
Plant-acetone extract	0.32	2
	0.47	15
	0.59	42
	0.82	6
	0.98	2
Root-acetone extract	0.60	
	0.97	
Sodium bicarbonate-C^{14}	0.00	
3,5,6-Trichloro-2-pyridyl phosphate	0.45	
Ethyl-3,5,6-trichloro-2-pyridyl phosphate	0.59	
3,5,6-Trichloro-2-pyridinol	0.70	
Ethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate	0.78	
O,O-Diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate	0.96	

Solvent system: Acetonitrile 80%; Concd. NH_4OH 2%; Water 18%

Table VIII. Uptake of Radioactive Compounds by Corn Plants Following Soil Treatment with [^{14}C] Dursban at Rate of 2 Lb./Acre

Days after Treatment	Weight of Plant, G.	Radioactive Compounds/Kg. of Tissue, Mmole
3	0.97 ± 0.34	0.00061 ± 0.00010
5	1.82 ± 0.18	0.00082 ± 0.00002
8	2.81 ± 0.08	0.00111 ± 0.00004
10	3.97 ± 0.04	0.00126 ± 0.00004
14	5.93 ± 0.67	0.00135 ± 0.00014
19	23.75 ± 0.69	0.00099 ± 0.00002
24	57.77 ± 0.92	0.00064 ± 0.00014
34	128.35 ± 1.06	0.00060 ± 0.00008

Table IX. R_f Values of Radioactive Compounds Isolated from Corn Growth in Soil Containing [^{14}C] Dursban

Sample	R_f Value	Radioactivity, %
Acetone extract of corn leaves	0.00	3
	0.48	2
	0.58	6
	0.74	8
	0.91	76
	0.97	5
3,5,6-Trichloro-2-pyridinol	0.74	
O,O-Diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate	0.96	
Ethyl-3,5,6-trichloro-2-pyridyl phosphate	0.56	
3,5,6-Trichloro-2-pyridyl phosphate	0.45	
Sodium bicarbonate	0.00	
Solvent system: Acetonitrile	80%	
Concd. NH_4OH	2%	
Water	18%	

the chlorine atoms were nonradioactive. Once the diols and triols were formed, they could undergo ring cleavage readily with the formation of radioactive carbon dioxide. This could be utilized to form tissue components. A future paper in this series will demonstrate that ring cleavage does occur with the liberation of radioactive carbon dioxide which can be utilized by the plants (*4*, *8*).

The uptake and translocation of [^{14}C] Dursban and its breakdown products from the soil also are very limited (see Table VI). In the soil, the [^{14}C] Dursban is hydrolyzed slowly with the formation of [^{14}C] 3,5,6-trichloro-2-pyridinol. The half-life of [^{14}C] Dursban in the soil is in the order of 80 to 90 days. No significant amount of the Dursban is translocated into the plant via the roots. The amount of 3,5,6-trichloro-2-pyridinol that will enter the plant depends on the pH value of the soil. If the soil is acid—pH 6 or below—most of the pyridinol will be present as the free pyridinol. Under these circumstances, very little of the pyridinol will enter the plant. If the soil is pH 7 or above, the pyridinol will exist as the salt which is soluble in water and will enter the plant. With the Dursban being broken down slowly in the soil, there will be a continuous supply of pyridinol which can be taken up by the plants. Since the rate of growth of the plants will be generally faster than the rate at which the pyridinol can enter the plant, there will be a gradual decrease in the residue in the plant per unit weight (see Table VIII).

In the first experiments with [^{36}Cl] Dursban, attempts were made to isolate the radioactivity from the plants and to identify the compounds present. The results again indicated significant quantities of [^{36}Cl] chloride. Other compounds were present, but it was difficult to obtain definite identification of these products. Attempts were then made to identify the radioactive compounds present in the tops of the plants described in Table VIII. The results obtained are shown in Table IX. In this case, all the radioactivity in the top of the plant had entered via the roots. The results indicated that the majority of the radioactivity was present in a fraction with an R_f value of 0.91. This compound has not yet been identified but there are some indications that it may be derived from 3,5,6-trichloro-2-pyridinol. As will be discussed in a later paper, this compound is formed when the 3,5,6-trichloro-2-pyridinol is introduced into a plant, but is not formed by ultraviolet breakdown of the pyridinol. In ultraviolet decomposition of the pyridinol, the chlorines are removed with the formation of diols and triols all of which are more water soluble than the pyridinol. In the solvent system described in Table VII, they all give lower R_f values than the 3,5,6-trichloro-2-pyridinol.

In all of these investigations on the identification of the radioactive compounds in plants, there appear to be traces of the *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, ethyl-3,5,6-trichloro-2-pyridyl phosphate, and 3,5,6-trichloro-2-pyridyl phosphate. Because of the low level of radioactivity present in these plants, it is impossible to obtain definite proof that these compounds are present and no great emphasis should be placed on these compounds as possible chemical residues. From the data presented, it is impossible to ascertain if the plant produced these products by enzymatic action or if they represent ultraviolet breakdown products formed on the surface of the leaves.

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