Investigations on Dursban Insecticide. Metabolism of O,O-Diethyl O-3,5,6,-Trichloro-2-pyridyl Phosphorothioate and 3,5,6-Trichloro-2-pyridinol in Plants

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Studies on the absorption, translocation, and metabolism of [^{36}Cl] Dursban, [^{14}C] Dursban, [^{36}Cl] 3,5,6-trichloro-2-pyridinol, and [^{14}C] 3,5,6-trichloro-2-pyridinol in plants show that Dursban is not absorbed into the plant, although it accumulates on the surface of the roots. Once it enters the plant it appears to be metabolized to form primary hydrolysis products where the phosphorus is still attached to the 3,5,6-trichloro-2-pyridinol. The rate at which the 3,5,6-trichloro-2-pyridinol enters the plant depends on the pH of the solution. The sodium salt enters the plant more than five times faster than the free pyridinol. The pyridinol undergoes metabolism with the liberation of chloride and the formation of several decomposition products.

I n previous studies on the uptake and translocation of O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate (Dursban, trademark of The Dow Chemical Co.) in plants (Smith et al., 1967) the compound did not penetrate either the roots or leaves, but only metabolites entered the plant. In previous investigations the amount of radioactive compound found inside the plant was in the order of 0.5 to 2% of the applied dose. Under these circumstances it was difficult to determine if the plant actually metabolized the compound or if the breakdown products found were formed outside the plant by ultraviolet light, in the case of foliar treatments, or by soil bacteria.

A series of experiments was conducted in which a continuous supply of Dursban was available to the plant, with the hope of increasing the quantity of compound within the plant.

Soil studies (Theigs, 1965) showed that 3,5,6-trichloro-2-pyridinol formed by the hydrolysis of Dursban was also present in the soil. The uptake and metabolism of this compound were also studied.
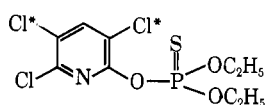
METHODS

In these investigations, both the [^{36}Cl] chlorine and the [^{14}C] carbon-labeled Dursban and 3,5,6-trichloro-2-pyridinol were used.

The [^{36}Cl] chlorine-labeled Dursban and 3,5,6-trichloro-2-pyridinol were prepared by the New England Nuclear Corp., using a procedure based on the synthesis method developed by Rigterink and Kenaga (1966). The [^{36}Cl] 3,5,6-trichloro-2-pyridinol was prepared by the direct chlorination of 6-chloro-2-pyridinol using HCl^{36} and H$_2$O$_2$. Part of the resulting [^{36}Cl] 6-chloro-2-pyridinol was then converted to [^{36}Cl] Dursban. The pyridinol-[^{36}Cl] and [^{36}Cl] Dursban had a specific activity of 29 μc. per mmole and were labeled in the 3,5-position as indicated.
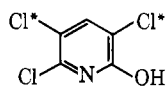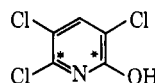
[^{14}C] 3,*5,*6-Trichloro-2-pyridinol

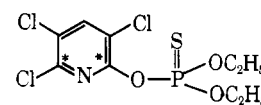O,O-Diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate

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The purity of both compounds was checked by infrared analysis and paper chromatography. As far as could be ascertained, at least 97% of the radioactivity was associated with the original compounds. There were traces of impurities in both compounds. The amount of parent compound and the impurities in the samples were determined by locating the spots on the chromatographic strips by autoradiographs, removing the radioactive areas, and counting them in a scintillation counter.

The ring-labeled- [^{14}C] pyridinol and [^{14}C] Dursban were prepared by Muelder and Wass (1967) to give compounds labeled in the 2,6-positions of the ring. The specific activity of the [^{14}C] Dursban was 1.5 mc. per mmole and the pyridinol- [^{14}C] was 0.973 mc. per mmole.

[^{14}C] 3,5,6-Trichloro-2-pyridinol

[^{14}C] O,O-Diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate

Analysis indicated that the pyridinol and the phosphorothioate had a radiochemical purity of at least 98%. There were traces of impurities in both preparations.

In the nutrient culture experiments the plants were grown in half-strength Hoagland's solution for one week prior to the introduction of the compound. Two hundred milliliters of fresh nutrient solution was used for each plant at the time the compound was added. The solution was placed in a climatizer and aerated continuously during the growing period.

The climatizer was of modern design which permitted cycling of the air (70° to 92° C.), relative humidity (30 to 95%), soil temperature (70° to 95° C.), and light intensity (0 to 2000 foot candles) over a 24-hour period. The spectrum of the light also was varied with maximum quantity of the low wavelength light (range 3600 A.) during the maximum light intensity period. The air was circulated, passing through a water wash each time it went through the chamber to remove volatile chemicals in the air (Figure 1).

The plants were harvested at various time intervals and analyzed for radioactivity. The plants containing [^{36}Cl] chlorine-labeled compounds were burned and analyzed by the method of Smith (1966). The plants containing [^{14}C] carbon-labeled material were burned and counted using the procedure described by Smith et al. (1964).
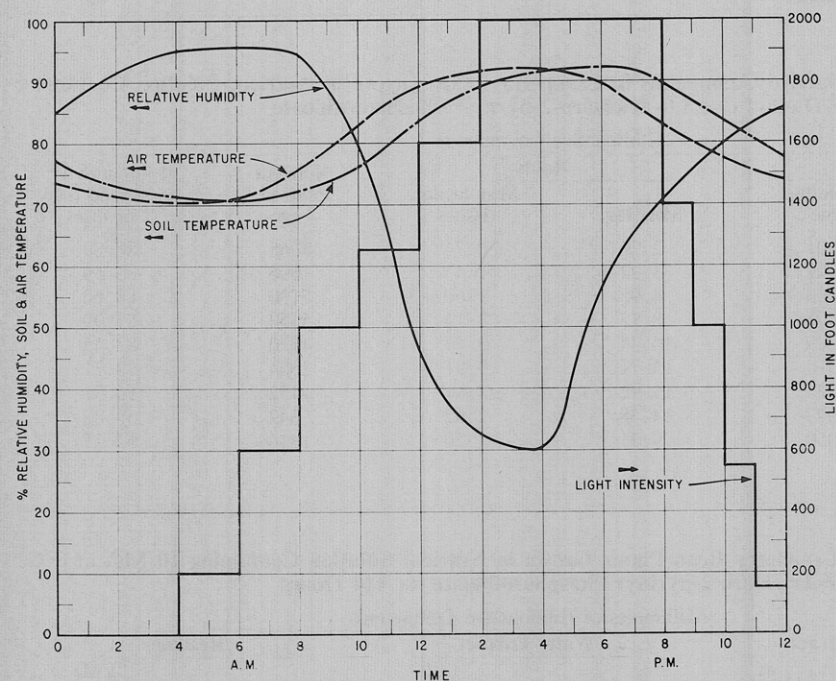
Figure 1. Climatic conditions

Attempts were made to get larger concentrations of Dursban and the pyridinol into plants by introducing it directly into the plant via the string technique (Figure 2). By using this technique, 1 ml. of solution could be introduced into the plant over a 4-hour period.

The isolation and identification of the radioactive compounds in the plants were performed by homogenizing the sample in acetone and extracting the sample in a Soxhlet extractor for 4 hours. The residue was extracted again with water for 4 hours, air-dried, and analyzed for radioactivity.

The acetone and water extracts were concentrated to a few milliliters, spotted on Whatman No. 1 paper, and chromatographed by the descending technique using several solvent systems (Smith, 1966). The areas of radioactivity were located on the strips by scanning in a Nuclear Chicago Actigraph Model III. The radioactive areas were cut out and counted directly in a Nuclear Chicago Scintillation Counter Model 725.

The acetone extraction removed Dursban and the first hydrolysis products in which the phosphate moiety is still connected to the pyridinol. The water extraction removed the pyridinol and dehalogenated and oxidized products of the pyridinol.

RESULTS AND DISCUSSION

The movement of radioactive compounds into cranberry beans grown in nutrient solution containing 50 p.p.m. of [^{36}Cl] Dursban is shown in Table I. In these studies the nutrient solution contained a total of 10 mg. of [^{36}Cl] Dursban. On this basis, in 72 hours only about 0.1% of the radioactivity had entered the plant, while almost 30% of the radioactivity had plated out on the surface of the roots (Figure 3). The small amount of material in the top of the plant could easily be accounted for in terms of impurities in the original sample of [^{36}Cl] Dursban. There was some evidence that [^{36}Cl] Dursban was lost from the

Figure 2. String technique used to introduce chemicals into plants

Table I. Uptake and Distribution of Radioactive Compound in Cranberry Beans Grown in Nutrient Solutions Containing 10 Mg. of [^{36}Cl] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate

| Time of Harvest, Hours after Treatment | Radioactive Compounds | | | |
| | Top of Plant | | Roots | |
	Mmole	Mmole/kg. tissue	Mmoles	Mmoles/kg. tissue
1	0.0010	0.0003	1.29	0.59
2	0.0056	0.0024	1.25	0.59
4	0.0079	0.0028	2.02	1.36
8	0.010	0.0080	4.35	2.00
16	0.014	0.0077	9.44	4.67
24	0.012	0.0081	8.52	4.42
48	0.031	0.0133	9.02	5.75
72	0.034	0.0215	8.46	5.74

Table II. Uptake and Distribution of Radioactive Compounds in Cranberry Beans Grown in Nutrient Solution Containing 10 Mg. of [^{14}C] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate

Time of Harvest, Hours after Treatment	Radioactive Compounds				Nutrient solution, μg.	Radioactivity accounted for, % of total
	Top of Plant		Roots			
	Mmole	Mmole/kg. tissue	Mmoles	Mmoles/kg. tissues		
1	0.0004	0.0001	2.20	0.74	8066	88.39
2	0.0004	0.0002	3.22	1.43	7696	88.59
4	0.0010	0.0003	4.95	1.60	7130	88.65
8	0.0013	0.0005	8.12	3.14	5350	81.99
16	0.0027	0.0009	10.34	3.47	4620	82.45
24	0.0055	0.0010	13.71	3.91	2564	73.71
48	0.0112	0.0025	11.92	3.41	1452	56.31
72	0.0132	0.0027	14.38	2.97	642	56.82
144	0.0188	0.0050	9.31	1.79	1316	52.17

Table III. Distribution of Radioactivity in Cranberry Bean Plants Grown in Nutrient Solution Containing 10 Mg. of [^{14}C] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate for 144 Hours

Tissue	Millimoles of Radioactive Compounds					
	Acetone Extract		Water Extract		Residue	
	Mmoles	% of total activity	Mmole	% of total activity	Mmoles	% of total activity
Roots	7.06	75.87	0.10	1.08	2.15	23.05
Top of plant	0.0001	6.06	0.01	54.54	0.0074	39.39

Table IV. R_f Value of Radioactive Compounds Isolated from Cranberry Beans Grown in Nutrient Solution Containing 10 Mg. of [^{14}C] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate for 144 Hours

Tissue	R_f Value	% of Total Radioactivity Extracted	Identification of Compound
Roots			
Acetone extract	0.00	4	
	0.18	4	
	0.38	2	
	0.44	11	3,5,6-Trichloro-2-pyridyl phosphate
	0.59	Trace	Ethyl 3,5,6-trichloro-2-pyridyl phosphate
	0.69	27	3,5,6-Trichloro-2-pyridinol
	0.83	Trace	O-Ethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
	0.95	49	O,O-Diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
Roots			
Water extract	0.00	1	
	0.03	15	
	0.16	12	
	0.22	2	
	0.31	11	
	0.46	4	3,5,6-Trichloro-2-pyridyl phosphate
	0.64	42	3,5,6-Trichloro-2-pyridinol
	0.89	13	
Top of plant			
Acetone extract	0.64	95	3,5,6-Trichloro-2-pyridinol
Water extract	0.71	97	3,5,6-Trichloro-2-pyridinol

Solvent system: 80 % acetonitrile
2 % concd. NH$_3$OH
18 % water

Table V. Uptake and Distribution of Radioactivity in Cranberry Bean. [14C] *O,O*-Diethyl *O*-3,5,6-trichloro-2-pyridyl Phosphorothioate Introduced into Stem of Plant via String Technique

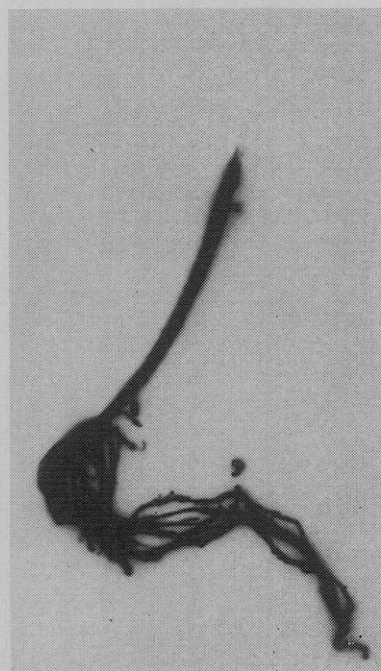| Time of Harvest, Days after Treatment	Mmoles of Radioactive Compounds		
	Acetone extract	Water extract	Tissue residue
	Top of Plant		
2	0.089	0.0098	0.0016
8	0.094	0.0035	0.0027
14	0.026	0.0022	0.0080
18	0.014	0.0024	. . .
	Roots		
2	0.096	0.011	0.0005
8	0.013	0.0033	0.0003
14	0.0038	0.0023	0.0002
18	0.0009	0.0009	. . .
	Soil		
2	0.0048	0.0007	. . .
8	0.0051	0.0003	. . .
14	0.0016	0.0003	. . .
18	0.0025	0.0002	. . .
	Cup		
2		0.52	
8		0.38	
14		0.30	
18		0.22	

Figure 3. Radioautograph of bean plant grown in nutrient solution containing [36Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate

Table VI. R_f Value of Radioactive Compounds Isolated from Cranberry Beans. Dursban Introduced via String Technique

Tissue	R_f Value	% of Total Radioactivity	Identification of Compound
Roots			
Acetone extract	0.94	95	Dursban
Water extract	. . .	. . .	. . . .
Top of plant			
Acetone extract	0.46	23	3,5,6-Trichloro-2-pyridyl-phosphate
	0.61	62	Ethyl *O*-3,5,6-trichloro-2-pyridyl phosphate
	0.71	6	3,5,6-Trichloro-2-pyridinol
	0.96	10	Dursban
Water extract	0.21	3	
	0.33	< 1	
	0.44	< 1	
	0.73	< 2	3,5,6-Trichloro-2-pyridinol
	0.86	91	
	0.97	2	Dursban
Soil			
Acetone extract	0.96	98	Dursban
Water extract	. . .	. . .	. . . .

Solvent system: 80% acetonitrile
2% concd. NH4OH
18% water

nutrient solution as the plants were being grown. There was a continuous aeration of the nutrient solution which would result in the loss of some of the [36Cl] Dursban.

A similar experiment conducted with [14C] Dursban showed a lower level of radioactivity in the tops of the plants (Table II). This was not surprising, as the [36Cl] Dursban contained more impurities than the [14C] Dursban. One of these impurities in the [36Cl] Dursban was chloride-Cl36. In the [14C] Dursban experiments, the amount of radioactivity in the top of the plant was equal to about 7 μg. of [14C] Dursban, or about 0.07% of the applied dose. Again the roots were coated heavily with radioactive compounds, reaching as much as 5 mg. in 72 hours. From the analysis of the nutrient solution it was apparent that the total quantity of [14C] Dursban in the nutrient solution was decreasing with time. The apparent increase in the amount of radioactivity between the 72-hour and 144-hour sampling was due to decomposition of the
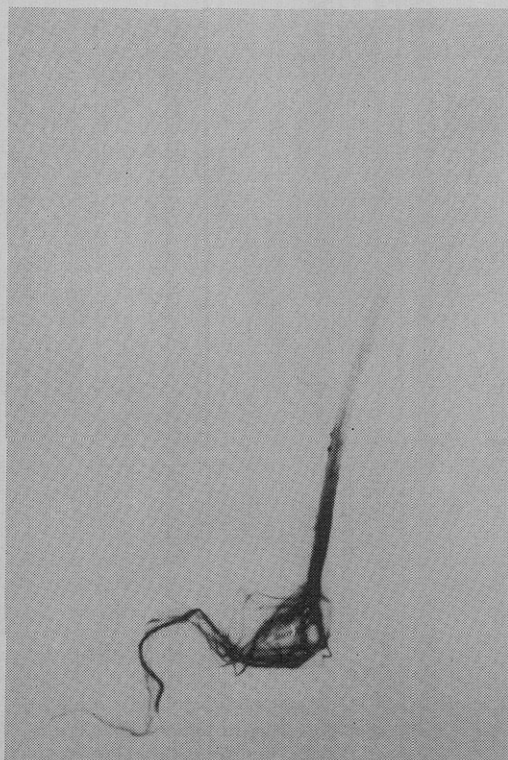
Figure 4. Radioautograph of bean plant grown in nutrient solution containing [^{36}Cl] 3,5,6-trichloro-2-pyridinol

roots of the plants. The large amount of chemical present on the roots apparently caused the roots to die, as they turned brown and tended to fall apart. The root fragments remained in the nutrient solution and were counted as part of the nutrient solution.

In both studies with [^{36}Cl] Dursban and [^{14}C] Dursban such a small amount of the chemical entered the plant that it was difficult to isolate enough material for chromatographic identification of all the fractions obtained in the extraction procedure (Table III). However, several pieces of information were significant in the extraction data. When the roots were extracted with acetone, 76% of the activity could be removed, while only 1% could be removed by water. This would indicate that most of the activity in the roots was associated with Dursban or primary hydrolysis product. The 3,5,6-trichloro-2-pyridinol may or may not be extracted by the acetone, depending on the pH of the system. The sodium salt of the pyridinol is very water-soluble with very limited solubility in acetone. The free pyridinol is soluble in acetone but insoluble in water. At the normal pH of tissue (about 6.8 to 7.0) part of the pyridinol would exist as the salt and part as the free pyridinol. Titration curve of the 3,5,6-trichloro-2-pyridinol indicated that one half of the pyridinol would exist as the sodium salt at pH 6.50.

The low activity in the water phase indicates small quantities of the breakdown products of the 3,5,6-trichloro-2-pyridinol.

These assumptions were confirmed by the chromatographic data (Table IV). In the solvent system employed, the dehalogenated and oxidized products of the 3,5,6-trichloro-2-pyridinol have R_f values less than 0.50, while
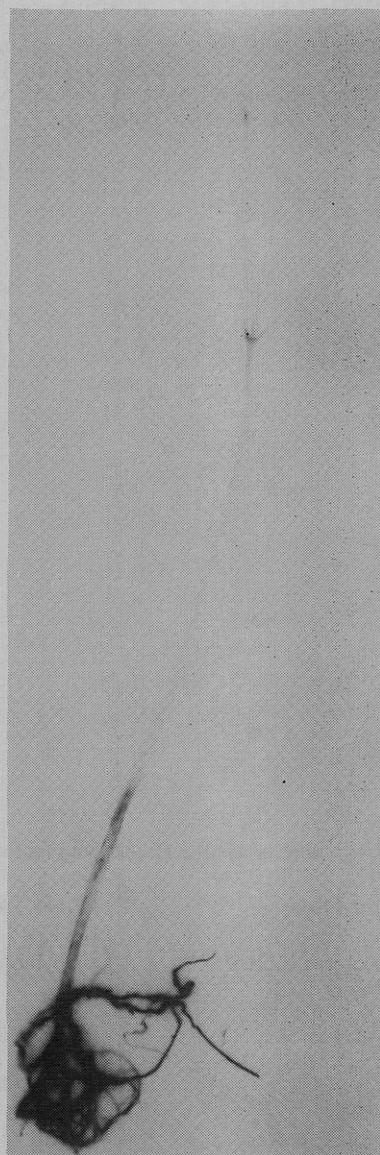

Figure 5. Radioautograph of bean plant grown in nutrient solution containing [^{36}Cl] 3,5,6-trichloro-2-pyridinol sodium salt

most of the primary hydrolysis products of Dursban with the phosphorus still attached to the pyridinol ring have R_f values above 0.50.

There are two possible suggestions as to the nature of the material remaining in the tissue residue. Ultraviolet light decomposition studies, which will be described in detail in a later paper, show that dehalogenation of the 3,5,6-trichloro-2-pyridinol could occur rapidly with the formation of diols and triols. These compounds are very reactive and could react easily with various constituents of the plant to form products which would not be extracted by water or acetone. Furthermore, once the diols and triols have been formed, they can undergo chemical cleavage of the ring with liberation of radioactive carbon dioxide, which could be used to synthesize natural components of the plant tissue.

The nature of the residual radioactivity in the tissues is now being investigated.

Table VII. Absorption and Translocation of Radioactivity in Corn Plants Grown in Soil and Sand Containing 1 Lb. of [³⁶Cl] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate or [³⁶Cl] 3,5,6-Trichloro-2-pyridinol

| Time after Treatment, Days | [³⁶Cl] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate | | | | [³⁶Cl] 3,5,6-Trichloro-2-pyridinol | | | | Sodium Salt of [³⁶Cl] 3,5,6-Trichloro-2-pyridinol | | | |
| | Sand | | Soil | | Sand | | Soil | | Sand | | Soil | |
	Mmole per kg. of tissue	Fresh wt. of plant, grams	Mmole per kg. of tissue	Fresh wt. of plant, grams	Mmole per kg. of tissue	Fresh wt. of plant, grams	Mmole per kg. of tissue	Fresh wt. of plant, grams	Mmole per kg. of tissue	Fresh wt. of plant, grams	Mmole per kg. of tissue	Fresh wt. of plant, grams
1	0.0001	5.1	0.00003	7.0	0.0007	3.7	0.0004	5.9	0.0004	3.6	0.0001	8.3
	0.0001	5.9	0.00006	7.0	0.0006	4.7	0.0003	5.8	0.0005	3.6	0.0001	7.9
	0.0001	5.0	0.00003	8.7	0.0005	5.0	0.0002	7.1	0.0004	4.8	0.00005	9.8
	0.0001	4.7	0.00003	7.2	0.0004	5.1	0.0002	7.4	0.0004	4.1	0.00005	8.3
2	0.0002	5.7	0.00003	9.2	0.0042	5.1	0.0002	9.5	0.0020	4.6	0.0002	7.8
	0.0003	4.5	0.00003	9.1	0.0023	5.0	0.0002	8.1	0.0018	4.2	0.0003	7.7
	0.0003	5.5	0.00003	8.3	0.0031	4.5	0.0002	6.8	0.0021	4.2	0.0002	9.1
	0.0003	4.9	0.00006	8.3	0.0025	5.1	0.0002	7.4	0.0014	4.4	0.0003	7.1
3	0.0005	5.0	0.00009	9.0	0.0047	4.8	0.0008	10.6	0.0034	5.4	0.0003	10.3
	0.0007	5.5	0.00009	11.4	0.0041	5.3	0.0006	10.2	0.0070	4.9	0.0006	8.2
	0.0006	6.1	0.00011	9.7	0.0041	5.7	0.0006	9.7	0.0034	6.2	0.0005	9.9
	0.0007	4.6	0.00009	11.3	0.0072	4.7	0.0006	9.6	0.0053	5.9	0.0004	10.9
8	0.0024	5.1	0.00037	11.4	0.026	4.0	0.0016	13.3	0.016	7.1	0.0021	18.6
	0.0032	3.6	0.00034	14.6	0.015	6.6	0.0017	14.6	0.018	4.4	0.0021	21.8
	0.0019	6.3	0.00023	9.3	0.024	4.8	0.0015	13.8	0.016	4.8	0.0018	18.9
	0.0026	3.8	0.00051	8.4	0.021	3.8	0.0018	12.3	0.012	4.8	0.0020	18.3
16	...	...	...	...	...	...	...	...	0.015	8.2	0.0034	27.5
	...	...	...	...	...	...	...	...	0.018	8.4	0.0033	30.8
	...	...	...	...	...	...	...	...	0.016	7.1	0.0032	29.1
	...	...	...	...	...	...	...	...	0.016	10.2	0.0034	25.2

Table VIII. Absorption and Translocation of Radioactivity in Bean Plants Grown in Nutrient Solution Containing 10 Mg. of [³⁶Cl] 3,5,6-Trichloro-2-pyridinol at pH 5.5 and 7.5

| Time after Chemical Added to Solution, Hours | Mmole of Radioactivity per Kg. of Fresh Tissue—Tops of Plants[a] | |
	Nutrient solution pH 5.5	Nutrient solution pH 7.5
12	0.0029	0.0022
24	0.026	0.019
48	0.028	0.093
72	0.036	0.227

[a] Average of four to six analyses.

Table IX. Uptake and Distribution of Radioactive Compounds in Cranberry Beans Grown in Nutrient Solution Containing 10 Mg. of Sodium Salt of [¹⁴C] 3,5,6-Trichloro-2-pyridinol

| Time after Treatment, Hours | Mmoles of [¹⁴C] 3,5,6-Trichloro-2-pyridinol per Kg. of Sample | | |
	Top of plants	Roots	Nutrient solution
1	0.022	0.23	110.07
2	0.052	0.51	107.78
4	0.30	0.92	105.70
16	0.36	1.30	102.34
24	0.43	2.88	102.03
48	0.50	6.70	97.16

In the top of the plant the majority of the activity was in the water phase (55%) or in the residue (39%). Analysis of the radioactivity isolated from the plant showed only 3,5,6-trichloro-2-pyridinol (Table IV). Other products, because of the low level of radioactivity present, could not be detected.

In the nutrient culture experiments, a considerable amount of Dursban was deposited on the surface of the roots, but very little entered the plant. It was difficult, therefore, to determine the extent to which Dursban could be metabolized inside the plant. Attempts were made to introduce [¹⁴C] Dursban directly into the stem of the plant by the string technique.

In these studies 500 μg. of [¹⁴C] Dursban in an emulsifiable formulation (Table V) was introduced into the cup in 1 ml. of water. This solution was allowed to pass into the plant and 1 ml. of water again was added to the cup. At various time intervals, plants were harvested and divided into tops, cup area, and roots. The cup area was taken as that portion of the stem 1 inch above and below the cup. The use of the string technique has two disadvantages with Dursban. Dursban in an emulsified water suspension does not easily migrate up the string; the water moves up, but the chemical stays behind. Furthermore, considerable Dursban is lost by volatility. About 50% of the chemical actually enters the plant under these conditions.

The typical results obtained are shown in Table V. There was considerably more radioactivity in the plant via the string technique than in the nutrient culture experiments. This could be extracted and chromatographed

Table X. Uptake and Distribution of Sodium Salt of [14C] 3,5,6-Trichloro-2-pyridinol in Bean Plants Treated via String Technique

3,5,6-Trichloro-2-pyridinol, Mmole per Kg. of Tissue

Time of Harvest, Days after Treatment	Top			Roots			Soil		Residue in cup	% of Total Pyridinol Accounted For
	Acetone extract	Water extract	Residue	Acetone extract	Water extract	Residue	Acetone extract	Water extract		
1	0.58	0.11	0.037	0.078	0.0030	0.014	0.022	0.0030	3.32	82.77
2	2.22	0.66	0.037	0.066	0.037	0.012	0.015	0.0020	1.03	81.44
3	2.16	0.56	0.023	0.065	0.059	0.020	0.020	0.0010	1.17	81.19
6	2.09	0.58	0.042	0.051	0.067	0.0086	0.017	0.0040	1.48	86.08
9	2.48	0.60	0.058	0.066	0.076	0.0060	0.044	0.0045	0.97	85.63
12	2.04	0.67	0.089	0.15	0.13	0.0070	0.039	0.0025	0.92	80.86

Table XI. R_f Value of Radioactive Compounds Isolated from Cranberry Beans Treated with 3,5,6-Trichloro-2-pyridinol-C^{14} via String Technique

				Days after Treatment			
1		2		3		6	
R_f value	% of total radioactivity	R_f value	% of total radioactivity	R_f value	% of total radioactivity	R_f value	% of total radioactivity
Acetone Extract of Plant Top							
0.12	5	0.16	10	0.16	5	0.14	10
0.38	10	0.38	25	0.38	35	0.34	35
0.45	5	0.47	5	0.48	10	0.45	5
0.65	30	0.66	40	0.65	40	0.65	40
0.72	10						
0.80	40	0.80	Trace	0.80	5	0.81	5
0.92	Trace	0.92	2	0.92	5	0.93	5
Water Extract of Plant Top							
0.00	10	0.00 0.05	50	0.00 0.03	50	0.00 0.02	50
0.11	Trace			0.10	Trace		
0.25	Trace	0.25	Trace	0.24	Trace	0.22	Trace
				0.35	5	0.32	5
		0.48	Trace	0.49	5	0.46	5
0.64	90	0.65	50	0.66	40	0.66	40
		0.75	Trace	0.76	Trace	0.72	Trace
Acetone Extract of Roots							
...	...	0.00	Trace	0.00	Trace	0.00	Trace
...	...	0.14	40	0.13	5	0.14	Trace
...	...	0.24	Trace	0.20	Trace	0.22	Trace
...	...	0.34	25	0.33	15	0.32	5
...	...	0.47	Trace	0.50	Trace	0.50	Trace
...	...	0.54	15	0.57	25	0.54	40
0.65	95	0.64	10	0.64	5	0.65	Trace
...	...	0.80	5	0.80	30	0.79	30
...	...	0.91	5	0.91	20	0.92	20
Water Extract of Roots							
...	...	...	...	0.00	10	0.00	10
...	...	...	...	0.10	5	0.10	5
...	...	...	...	0.63	80	0.56	80
...	...	...	...	0.79	5	0.80	5

(Table VI). There appeared to be Dursban in all parts of the plant and the soil. In the plant the major products were the primary hydrolysis products of Dursban—i.e., the 3,5,6-trichloro-2-pyridyl phosphate, ethyl-3,5,6-trichloro-2-pyridyl phosphate and the 3,5,6-trichloro-2-pyridinol. The water extract showed a significant quantity of a compound which had an R_f value of 0.86. The nature of this compound is unknown, but preliminary data suggest that it might be a conjugate of the 3,5,6-trichloro-2-pyridinol.

In the studies on Dursban it was apparent that the compound was hydrolyzing to the 3,5,6-trichloro-2-pyridinol, which is more susceptible to ultraviolet light decomposition, enzymatic dehalogenation. and oxidation than is Dursban (Smith, 1966). It therefore seemed desirable to study the uptake, translocation, and metabolism of the pyridinol in plants.

In the first experiments corn was grown in sand and soil. The [36Cl] Dursban or [36Cl] 3,5,6-trichloro-2-pyridinol either as the sodium salt or the free pyridinol

was added to the soil or sand. At various time intervals the plants were harvested and analyzed for [³⁶Cl] chlorine activity. The results shown in Table VII indicate that the pyridinol enters the plant faster than Dursban. Here again the amount in the plant was very low and it was hard to detect the nature of the compounds present.

Bean plants, therefore, were grown in nutrient solution to which the 3,5,6-trichloro-2-pyridinol was added. Two series of experiments were conducted—one in which the pH of the solution was maintained at pH 5.5, and the other in which the pH was maintained at pH 7.5. Under these conditions the pyridinol existed as the free pyridinol at pH 5.5 and as the salt at pH 7.5. The difference in the rate of uptake of the pyridinol is shown in Table VIII. About five times more pyridinol entered the plant at pH 7.5 than at pH 5.5 (Figures 4 and 5).

When the extracts of these plants were chromatographed, the [³⁶Cl] 3,5,6-trichloro-2-pyridinol and [³⁶Cl] chloride could be detected. This suggested that dehalogenation could be occurring and other derivatives of the pyridinol might be present, but these could not be detected because they were no longer radioactive.

Additional plants, therefore, were grown in nutrient solution containing [¹⁴C] 3,5,6-trichloro-2-pyridinol (Table IX). In this case there was a considerable amount of radioactivity in the tops of the plants, most of it associated with the roots, and it was mainly the 3,5,6-trichloro-2-pyridinol.

As the sodium salt of the pyridinol is soluble in water, it seemed desirable to introduce the compound into the plant via the string technique to get sufficient quantities of the breakdown products to permit their identification by paper chromatography. Typical results obtained with the string technique are shown in Table X.

The activity in the plants harvested at various time intervals was extracted and chromatographed (Table XI). The 3,5,6-trichloro-2-pyridinol apparently is metabolized by the plant to form a number of breakdown products. The water-soluble components increase with time, as does the quantity of material which has an R_f value of 0.00 to 0.05, the R_f value of [¹⁴C] sodium carbonate.

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