plant system. When paraoxon residues were calculated as percentages of total residue remaining at each posttreatment interval, as determined by the PNBP method, they steadily increased with time and represented about 25% of the remaining residue on glass surfaces and 10% of the total plant residue at 10 days. Other anticholinesterase degradation products were detected in trace amounts in the internal extracts, and evidence suggests that they accumulate at least partially through cuticular absorption of surface degradation products. Relative amounts of paraoxon arising from photochemical oxidation processes on the leaves and from enzymatic oxidation within the plant system are not known and, indeed, would be difficult to determine. Parathion carefully injected into the vascular bundle region of the stem gave rise to paraoxon in the plant, but that does not eliminate the possibility of photochemical oxidation within the cells of the leaves following translocation.

The root absorption studies demonstrated that parathion was absorbed readily into roots of bean plants from nutrient solution, but very little was translocated to aerial parts. The rate of parathion absorbed by roots of bean plants in this study would be expected to be much higher than by plants grown in soil, since several workers have demonstrated such a relationship (*3*). Water absorption by the roots may be enhanced further by action of the insecticide. Tietz (*16*) found that demeton stimulated water uptake by *Phaseolus vulgaris* roots during the first few hours of treatment.

Paraoxon accumulated in the roots and, to a much lesser extent, in the aerial plant parts. However, the ratio of paraoxon to parathion in aerial parts was lower, suggesting that paraoxon is formed more rapidly in aerial parts of the plant or is translocated to a larger extent than parathion because of its greater water solubility.

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HERBICIDE METABOLISM

The Metabolism of Carbon-14 Diphenamid in Strawberry Plants

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Diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) is a selective pre-emergence herbicide, which is registered for use on many horticultural and agronomic crops. A residue of diphenamid was detected in strawberry fruit harvested from plants grown in diphenamid-treated soil. To determine the nature of the total residue concentration and the metabolic products of the herbicide, bearing strawberry plants were grown in greenhouse soil treated with 3.83 pounds per acre of diphenamid labeled with carbon-14 in the carbonyl position. Unaltered diphenamid was found as the major product incorporated in strawberry fruit and plants. *N*-methyl-2,2-diphenylacetamide, present in much lower concentration, was identified as the major diphenamid metabolite.

Diphenamid (Dymid, Elanco Products Co., a division of Eli Lilly and Co.) is a selective, pre-emergence herbicide whose properties were discovered by Eli Lilly and Co. Research Laboratories (*8*). This compound is useful in the pre-emergence control of a wide variety of annual grasses and broadleaf weeds. Tolerant crops include direct-seeded and transplanted tomatoes and peppers, peanuts, Irish potatoes, sweet potatoes, and nonbearing strawberry plants.

When diphenamid applications are made to established strawberry plants from 1 to 5 months prior to producing strawberry fruit, residues of diphenamid in the range of 0.05 to 0.5 p.p.m. were found in the strawberry fruit. The presence of a residue indicated that diphenamid was absorbed by the strawberry plants from treated soil, suggesting that the strawberry plant would be useful in studying the metabolism of diphenamid. The purpose of this investigation was to detect and identify the metabolites of diphenamid in the strawberry fruit and the strawberry plant.

Material and Methods

Labeled Diphenamid. Diphenamid labeled with carbon-14 in the carbonyl group was prepared in the Lilly Research Laboratories (*6*). The radiochemical

purity of this compound, as determined by thin-layer chromatography on silica gel G in a system of benzene–ethyl acetate (3 to 1), was greater than 99%. Its specific activity was 3.64 μc. per mg.

Soil Sample Preparation. Labeled diphenamid (146.6 mg.) was dissolved in acetone. One-ninth of the solution was added to each of nine glass jars containing 325 grams of air-dried greenhouse soil (one part sand and one part silty clay loam). The jars were then rotated on a mechanical roller for 30 minutes. The covers were removed from the jars until the odor of acetone was no longer detectable. The contents of each jar were then mixed with 1200 grams of air-dried greenhouse soil.

Planting and Growing Conditions. Four strawberry plants of the variety Pocahontas, which would bear fruit in spring, were shallowly transplanted in each of nine two-gallon glazed crocks ($8^1/_2$-inch diameter × $8^1/_2$-inch depth) having a surface area of 0.37 square foot. The soil containing the labeled diphenamid, equivalent to an application rate of 3.83 pounds per acre, was then spread around the plants to a depth of $1^1/_4$ inches. The final planting depth resulted in soil being located next to the crown.

The strawberry plants were maintained in a greenhouse with a day length of 10 to 12 hours and a temperature range of 70° to 90° F. until mature strawberry fruits were available. Twenty-seven days elapsed from planting time until the first berry harvest. At each harvest, the calyx was removed from the strawberry fruit, and both the calyx and fruit were frozen until analyzed. The strawberry fruits were harvested over a period of approximately 2 weeks. After the last harvest, the plants were removed from the crocks, washed, and separated into roots, crowns, and leaves plus petioles. All parts were frozen until assayed. The roots, crowns, and calyxes were analyzed for total radioactivity only.

Counting Procedure. All counting of radioactivity was done with a Packard Tri-Carb liquid scintillation spectrometer, Model 314 EX. Plant and fruit tissues were dried for 16 hours at 50° to 60° C. and combusted using a modified Schöniger technique (*4*). The combustion products were absorbed in a mixture of 2-aminoethanol and methylcellosolve (30 to 70), diluted with toluene scintillator solution [toluene containing 0.5% 2,5-diphenyloxazole (PPO) and 0.01% 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP)] and counted. Liquid samples, such as extracts, were dissolved in an appropriate scintillation solution and counted directly. The zones or spots, corresponding to the standard substances on silica gel GF chromatoplates, were removed, placed in the scintillation vials, and eluted with approximately 0.2 ml. of methanol. Scintillation solution was added, and the samples were counted. All counting efficiencies were determined by using toluene-1-C^{14} as an internal standard. Counting times ranged from 10 to 30 minutes depending on the level of radioactivity in the sample.

Extraction Procedure. The strawberry fruits and leaves including petioles (hereafter referred to as leaves) were investigated for metabolic products. Since direct solvent extraction resulted in the formation of emulsions, dialysis was used to isolate the radioactive components. Samples were homogenized with an Omni-Mixer. A 50-gram sample of each homogenate was diluted with 50 ml. of distilled water, transferred to a dialysis casing (Visking Size 20 DC, width 63/64 inches, wall thickness (0.0008-inch), sealed, and placed in a 500-ml. Erlenmeyer flask containing 100 ml. of distilled water. All sample flasks were agitated on a rotary shaker for 5 hours at room temperature. The dialyzate was recovered, replaced with 100 ml. of distilled water, and dialysis continued as above until six individual dialyzates were obtained from the berries, and ten individual dialyzates were obtained from the leaves.

The dialyzates 1 to 5 and 6 to 10 obtained from the leaves and dialyzates 1 to 6 obtained from the berries were combined and extracted four times with 600-ml. portions of solvents with increasing polarity at room temperature. The solvent order used was: 1, chloroform; 2, ethyl acetate; 3, chloroform with the aqueous phase adjusted to pH 1.5; 4, ethyl acetate—aqueous phase pH 1.5; 5, ethyl acetate—aqueous phase adjusted to approximately pH 0.15; and 6, the aqueous phase, approximately pH 0.15, was heated under refluxed condition for 1 hour, then extracted as before with ethyl acetate. The extracts of each type solvent were combined evaporated to dryness in a rotary evaporator, and dissolved in a known volume, usually 10 ml. of the same respective solvent.

The impermeates were extracted four times with 600-ml. portions of chloroform and ethyl acetate. As before, like extracts of each impermeate were combined, evaporated to dryness, and dissolved in a small volume of the same respective solvent.

Chromatographic Procedure. The various extracts were examined by thin-layer chromatography (TLC). Twenty by twenty centimeter glass plates coated with a standard 250-micron layer of silica gel GF (Brinkmann No. 7730) and activated at 105° C. for 1 hour were employed (*10*, *11*).

Two solvent systems were used for development: System I, benzene-formic acid (95 to 5) nonsaturated chamber and System II, benzene–diethylamine (95 to 5) saturated chamber.

Six compounds of known related structure were selected as model substances. A solution containing these six model substances was always applied as a separate lane on the one-dimensional plates to aid in zone identification. In two-dimensional chromatography, employing Systems I and II, known standards were cochromatographed with the radioactive sample. The model compounds were located on the chromatogram by scanning the plates with an ultraviolet source.

Thin-Layer Radioautography. Radioautographs (*1*, *9*), employing 8 × 10 inch Kodak Medical X-Ray Film (Eastman Kodak Corp., Rochester, N. Y.) were prepared of representative TLC plates of the chloroform extract. Exposure times ranged from a few days to a month.

Gas Chromatographic Measurement. The separated zones on preparative TLC plates (500-micron absorbent layer) were removed, placed in a small glass column, and eluted with chloroform. The resulting chloroform solutions were analyzed by gas chromatography (*5*) with a Jarrell-Ash, Model 26-700 instrument, utilizing a hydrogen flame-ionization cell.

Reverse Isotope Dilution Analysis. The remainder of the material eluted from the preparative chromatoplates was used for reverse isotope dilution analysis (*2*, *3*, *7*, *12*). To each remaining material, obtained from zones 1 and 2 of the chloroform extract of the leaves, was added 100 mg. of cold diphenamid and 100 mg. of cold *N*-methyl-2,2-diphenylacetamide, respectively. Each mixture was recrystallized repeatedly from *n*-hexane–acetone, and the specific activity was determined after each recrystallization.

Results

Total radioactivity in the various pooled plant parts, expressed as disintegrations per minute (d.p.m.) in the wet tissue, is shown in Table I. This table represents an average of all data accumulated on the various plant parts from the replicates in this experiment.

Table II shows the distribution of radioactivity in the individual dialyzates obtained from fruit and leaves, respectively. The percentage values are based on total radioactivity in the original samples.

The recovery of radioactivity from the dialyzates by exhaustive solvent extraction is presented in Table III. Although leaf dialyzates 1 to 5 and 6 to 10 were extracted separately, their similar behavior in the different solvents permitted combining the results as shown in Table III.

The names and formulas of the six model compounds, used as references in the TLC examination of the extracts, are illustrated in Table IV. The be-

Table I. The Distribution of Radioactivity in Pooled Wet Strawbe ry Plant Parts

(Average of four replicate samples)

	D.P.M./ Gram Wet Tissue	Coefficient of Variation, %
Fruits	3,620	±1.9
Roots	26,640	±2.9
Leaves and petioles	240,780	±1.1
Calyxes	76,560	±0.4
Crowns	40,540	±1.1

havior of the reference compounds in the two TLC systems is shown in Figure 1. System I, benzene–formic acid (95 to 5), readily separated 2,2-diphenylacetic acid from the other model compounds. System II, benzene–diethylamine (95 to 5) distinguished diphenamid, *N*-methyl-2,2-diphenylacetamide, from the more polar products.

Table II. The Total Dialyzate Radioactivity and the Distribution of Radioactivity in Individual Dialyzates Obtained from Strawberry Fruits and Leaves

	Original Radioactivity, %	
Dialyzate	*Fruits*	*Leaves*
1	48.0	19.2
2	23.0	15.0
3	11.0	11.6
4	5.4	9.2
5	2.1	6.8
6	0.8	5.8
7		4.0
8		3.5
9		2.4
10		1.9
Total dialyzate	90.3	79.4
Impermeate	9.7	20.6

Table III. Recovery of Radioactivity by Successive Solvent Extraction of Pooled Dialyzates and Impermeate Obtained from Strawberry Fruits and Leaves

	Original Radioactivity, %	
	Fruits	*Leaves*
Pooled dialyzates	90.3	79.4
Chloroform	62.5	66.2
Ethyl acetate	10.1	...
Chloroform, pH 1.5	2.3	1.5
Ethyl acetate, pH 1.5	3.6	...
Ethyl acetate, pH 0.15	1.0	1.0
Ethyl acetate, pH 0.15 (reflux)	2.0	...
Total of dialyzate extracted	81.5	68.7
Impermeate	9.7	20.6
Chloroform and ethyl acetate	3.2	6.5
Total radioactivity extracted	84.7	75.2

The solvent extracts of the dialyzates obtained from fruits and leaves, referred to in Table II, were examined by these two TLC systems. Based on the amount of radioactivity originally in the system, Table V summarizes the distribution of radioactivity after TLC of the dialyzate extracts. Radioautographs of the TLC plates are shown in Figures 2 to 4.

Spots 1 and 2 (Figure 1) were eluted from preparative TLC plates and individually compared with three model compounds by gas chromatographic analysis. After the initial comparison of the individuals, the material from spots 1 and 2 were combined. The gas chromatographic pattern of the combination is shown in Figure 5.

The results of reverse isotope dilution analysis on diphenamid and *N*-methyl-2,2-diphenylacetamide isolated from preparative TLC plates of the leaf extracts are shown in Table VI. The respective products, initially isolated as described for gas chromatographic measurement, were diluted with unlabeled compound, and the specific activity was determined after each successive recrystallization.

Discussion

The radioactivity in the strawberry plant parts is not distributed uniformly when grown in soil containing C^{14} diphenamid. Table I shows the very high concentration of radioactivity in the leaves and calyxes as compared with the strawberry fruits. On the basis of this difference, the investigation was centered on leaves and the strawberry fruits.

Recovery of the radioactivity from these plant tissue homogenates by progressive dialysis indicated the radioactivity in the strawberry fruits is readily dialyzed with a 10% greater yield than that obtained with leaves (Table II). Subsequent exhaustive extraction of the impermeates (Table III) further suggests that the radioactivity in the leaves may be conjugated to endogenous material which does not appear to occur to the same degree in the strawberry fruit.

Radioactivity in the solvent extracts after TLC (Table V) is most prominently distributed in designated zones with R_f

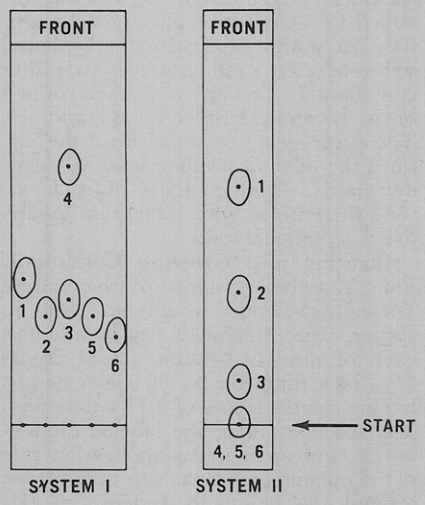


Figure 1. TLC of the model compounds (Table IV) on silica gel GF

System I: Benzene–formic acid (95 to 5)
System II: Benzene–diethylamine (95 to 5)

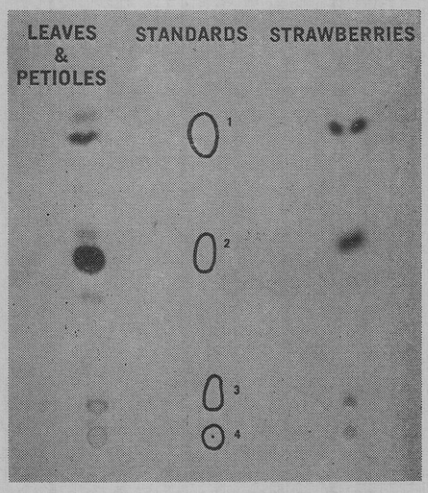
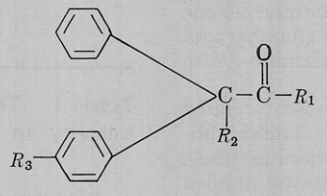


Figure 2. A 26-day exposure radioautograph of a TLC plate

Developed with solvent system II, benzene–diethylamine (95:5), of the chloroform extracts obtained from dialyzates of strawberry leaves and fruits. The doublet observed in zone 1 for strawberries is due to a defect in the TLC plate

Table IV. Model Compounds



Model Compound No.	Name	R_1	R_2	R_3
1	Diphenamid	$-N(CH_3)_2$	—H	—H
2	*N*-Methyl-2,2-diphenylacetamide	$-N(H)-CH_3$	—H	—H
3	2,2-Diphenylacetamide	$-NH_2$	—H	—H
4	2,2-Diphenylacetic acid	—OH	—H	—H
5	*p*-Hydroxy-2,2-diphenylacetic acid	—OH	—H	—OH
6	α-Hydroxy-2,2-diphenylacetic acid	—OH	—OH	—H

Table V. The Distribution of Radioactivity after TLC of the Solvent Extracts as Compared with TLC of the Model Compounds

Model Compound	Original Radioactivity, %	
	Fruits	*Leaves*
1	27.4	9.7
2	36.8	51.2
3	4.5	7.4
4	4.0	2.1
5 and 6	5.2	1.0

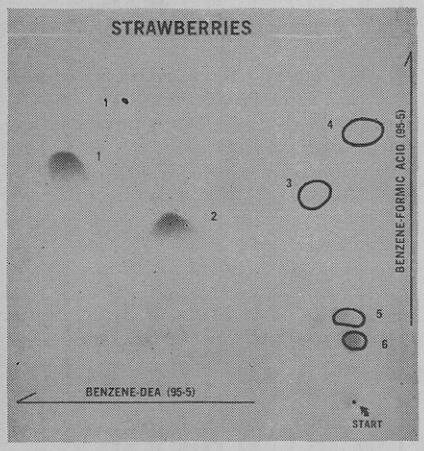


Figure 3. A 24-day exposure radioautograph of a two-dimensional TLC plate

Chloroform extract obtained from dialyzates of strawberry fruit

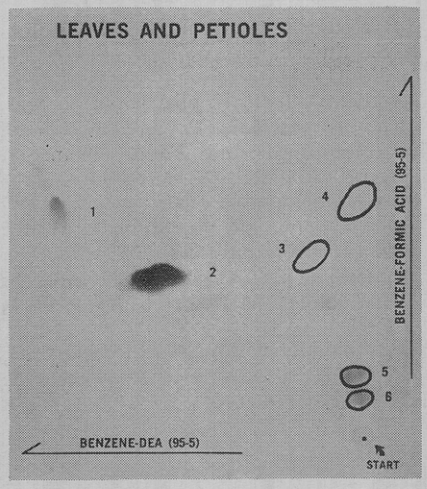


Figure 4. A 24-day exposure radioautograph of a two-dimensional TLC plate

Chloroform extract obtained from dialyzates of strawberry leaves

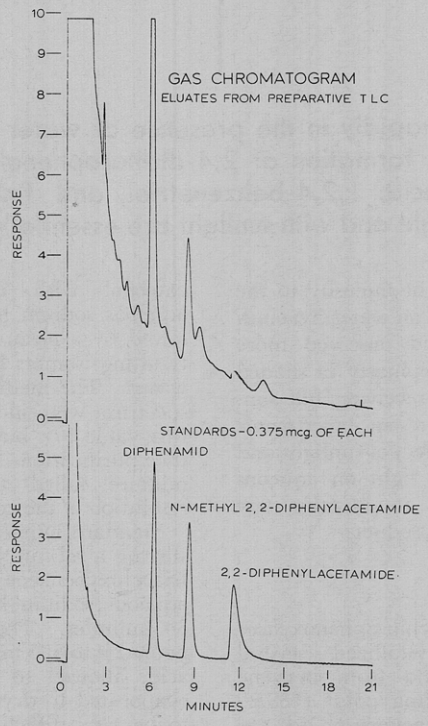


Figure 5. Gas chromatographic analysis of recombined spots 1 and 2

Obtained from one-dimensional preparative TLC plates compared with three model compounds

values similar to model compounds 1 and 2. The amount of radioactivity in zones 1 and 2 is significantly different from the extract of leaves and the strawberry fruits pointing to selective difference in distribution of the radioactive material or type of metabolite formed. The more polar zones, corresponding to R_f values of model compounds 4, 5, and 6 contain a very small fraction of the total radioactivity.

Radioautographs of the TLC plates (Figures 2 to 4) confirm the distribution of radioactivity in the leaves and strawberry fruits. Inasmuch as spots 1 and 2 correspond with the R_f values of model compounds, diphenamid and *N*-methyl-2,2-diphenylacetamide, respectively, provide the basis for identification of these spots. Similarly, spots 3 and 4 (shown better on two-dimensional radioautographs), which contain a very small amount of radioactivity, agree with the location of known diphenylacetamide and diphenylacetic acid. Spots 5 and 6, corresponding to known *p*-hydroxy-2,-2-diphenylacetic acid and α-hydroxy-2,2-diphenylacetic acid, contain slightly more radioactivity than spots 3 and 4. However, the amount of radioactivity is insufficient to verify the identity of these two components. In addition, trace amounts of radioactivity are observed in other spots which do not correspond to available model compounds. These unidentifiable spots might represent some hydroxylated derivatives or conjugated diphenamid endogenous products.

The verification of diphenamid and *N*-methyl-2,2-diphenylacetamide in the strawberry plant determined by gas chromatography (Figure 5) adds credence to this identification. Final proof of identity was obtained by reverse isotope dilution analysis (Table VI). For compounds 1 and 2, a variation of only 2% in the specific activity was found from the first to the fifth or sixth recrystallization. This denotes a very high degree of purity of compounds 1 and 2 and further substantiates their identity.

This investigation reveals the interesting distribution of radioactivity in strawberry plant leaves and fruit from soil-incorporated diphenamid. In addition to the direct assimulation of diphenamid in the strawberry plant, the principal metabolite of diphenamid occurring in strawberry leaves was identified as *N*-methyl-2,2-diphenylacetamide. The nature of other minor metabolites, 2,2-diphenylacetamide, 2,2-diphenylacetic acid, *p*-hydroxy-2,2-diphenylacetic acid, and α-hydroxy-2,2-diphenylacetic acid, is implied but not established conclusively.

Table VI. Specific Activity after Reverse Isotope Dilution Analysis of Diphenamid and *N*-Methyl-2,2-diphenylacetamide Isolated from Chloroform Leaf Extract by Preparative Thin-Layer Chromatography

Recrystallization	*Diphenamid, D.P.M./Mg.*	*N-Methyl-2,2-diphenylacetamide, D.P.M./Mg.*
1	4761	10,270
2	4822	9,575
3	4878	10,535
4	5004	10,270
5	4891	9,780
6	5018	
Observed specific activity	4896 ± 41.1	10,086 ± 177
Theory	5310	9,800
Purity, %	92.1	102.9

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HERBICIDES

Photodecomposition of 2,4-Dichlorophenoxyacetic Acid

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2,4-Dichlorophenoxyacetic acid decomposes rapidly in the presence of water and ultraviolet light. This decomposition results in the formation of 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and, finally, polymeric humic acids. The results with artificial light and with sunlight are essentially identical.

ULTRAVIOLET LIGHT has been shown to exert drastic changes in many pesticides under laboratory conditions (*7*). If similar action were to take place in sunlight under field conditions, the result would be of major importance to the environmental stability and practical use of these substances, and the detailed chemistry and toxicology of the resulting decomposition products would be significant to both agriculture and public health.

The effect of ultraviolet light on the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been examined by several investigators. Although Mitchell (*7*) did not observe any breakdown, Payne and Fults (*8*) reported that irradiation of 2,4-D solutions increased their stimulation of cell elongation in the pea slit-stem test, and Bell (*3*) noted that 2,4-D solutions were decomposed to unidentified "phenolic" substances. Aly and Faust (*2*) examined the effect of ultraviolet light on aqueous solutions of 2,4-D salts and esters, demonstrated the rapid disappearance of the herbicide, and provided colorimetric evidence for the presence of phenols in the irradiated solutions. The effect of riboflavin on this decomposition also was studied by Bell (*3*) and others (*5, 6*), but no conclusive evidence for the chemical identity of any of the breakdown products has been reported.

Practical tests also indicate that sunlight has an effect on 2,4-D in the field. Penfound and Minyard (*9*) investigated the relationship of light intensity to the effect of the herbicide on water hyacinth and kidney bean and observed more necrosis and greater epinasty in shaded plants than in those receiving full sun. The purpose of the present experiments was to compare the effect of sunlight and laboratory ultraviolet light on aqueous 2,4-D solutions and to identify any major decomposition products.

Experimental

Materials. 2,4-Dichlorophenoxyacetic acid was recrystallized several times from water and from benzene until a constant melting point (138.2–138.8° C.) and homogeneity to gas chromatography and thin-layer chromatograph were attained.

4-Chlorocatechol, commercially available (Aldrich Chemical Co.) as a dark powder, was crystallized from benzene to remove insoluble resin and then sublimed at 40° to 50° C. (0.1 mm.) to provide the pure compound as white crystals.

2-Chlorohydroquinone (J. T. Baker Chemical Co.) was purified in the same way.

2,4-Dichlorophenol, 1,2,4-triacetoxybenzene, and other chemicals were used in commercially available form.

2-Chloro-4-hydroxyphenoxyacetic acid was prepared from *p*-methoxyphenol by the method of Brown and McCall (*4*) and exhibited a melting point and infrared spectrum identical with those reported in the literature.

2-Hydroxy-4-chlorophenoxyacetic acid was prepared by reaction of 4-chlorocatechol with chloroacetic acid in aqueous sodium hydroxide solution followed by a clean separation of the two resulting isomers by crystallization from water. The melting point and infrared spectrum were identical with the literature value (*4*), but an attempt to repeat the purification of the crude isomer mixture failed to provide adequate resolution of the constituents.

Standard humic acid was prepared by stirring a mixture of 2.5 grams of 1,2,4-triacetoxybenzene and 40 ml. of 10% aqueous sodium hydroxide solution for 30 minutes. The dark mixture was poured into 500 ml. of 20% hydrochloric acid, allowed to stand overnight, and evaporated to dryness (filtration proved to be very difficult). The residue was extracted with acetone, the extract was filtered to remove salt, and the filtrate was evaporated to dryness. The residue was dried under vacuum at 50° C. over solid sodium hydroxide and phosphorus pentoxide to provide a good yield of fine, almost black powder.

Irradiation. Standard solutions for irradiation were prepared by dissolving 440 mg. of 2,4-D and 170 mg. of sodium bicarbonate per liter of distilled water to provide a concentration of 2×10^{-3} *M*. The solution (10 liters) was stirred vigorously in a large glass tank and externally cooled in ice to maintain a temperature of 20° to 25° C. Irradiation time varied from 5 to 12 hours, although the rate of photodecomposition slowed markedly after about 8 hours. Solution depth in these instances was 8.5 cm.

Laboratory irradiations employed mercury arc lamps which produced light