

Studies on the Fate of 2,4-D and Ester Derivatives in Natural Surface Waters

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Some physical, chemical, and biological factors that might influence the persistence of 2,4-D compounds in natural waters were investigated. The amounts of 2,4-D (sodium salt) of the isopropyl, butyl, and isooctyl esters sorbed on bentonite, illite, and kaolinite ranged from 0.02 to 0.14 mg. per gram. These sorbed amounts are small and insignificant. The solubilities of the calcium and magnesium salts of 2,4-D in distilled water at 25° C. were determined as 4000 mg. per liter and 11,100 mg. per liter, respectively. Ordinary concentrations of calcium and magnesium ions would not remove 2,4-D through precipitation reactions. 2,4-D persisted up to 120 days in lake waters aerobically incubated in the laboratory. Esters of 2,4-D were hydrolyzed biologically to the free acid and corresponding alcohol within 9 days, as measured by manometric techniques. Oxygen uptake data suggested that only the alcohol moiety was oxidized. 2,4-D was decomposed biologically by lake muds, 81 to 85% within 24 hours, but only after extensive microbial adaptation techniques. Ultraviolet irradiation by a mercury lamp decomposed esters of 2,4-D, the rate of which was pH dependent. Photolysis occurred faster at pH 9.0 than at pH 7.0 or 4.0. However, ultraviolet energies from sunlight irradiation are not expected to decompose 2,4-D.

ESTERS of 2,4-dichlorophenoxyacetic acid (2,4-D) are used effectively for control of some species of aquatic weeds in lakes, ponds, and reservoirs that are used frequently for potable water supplies. Also, these compounds may enter surface waters through discharge of industrial waste waters (18). Since nothing is known about the fate of 2,4-D compounds in natural surface waters, some of the physical, chemical, and biological factors that might influence their persistence were examined.

Materials

2,4-Dichlorophenoxyacetic acid, Eastman Kodak's No. 5532 White Label grade. The sodium salt of 2,4-D was prepared by adding the appropriate amount of the acid to an equivalent amount of sodium carbonate in boiling water.

2,4-D isopropyl ester, liquid commercial formulation containing 47.0% of the ester.

2,4-D butyl ester, liquid commercial formulation containing 39.0% of the ester.

2,4-D isooctyl ester, liquid commercial formulation containing 69.0% of the ester.

2,4-Dichlorophenol, Eastman Kodak's No. 1933 White Label grade.

Physical and Chemical Factors

Sorption of 2,4-D Compounds on Clay Minerals. Several investi-

gators (1, 7, 13, 19) reported that 2,4-D was adsorbed in soils and showed that clay components of the soil were among the factors responsible for inactivation of 2,4-D. Since clay minerals constitute the major portion of the turbidity of natural waters, the possible role of these inorganics in affecting the persistence of 2,4-D compounds was evaluated. In this study, the sorption of the sodium salt, isopropyl, butyl, and isooctyl esters of 2,4-D and 2,4-dichlorophenol, which occurs as formulation impurity (10), was determined on three clay minerals: kaolinite, Wyoming bentonite (a montmorillonitic clay), and Fithian illite. These clays were obtained in a 2-micron size, and were freed from exchangeable bases and converted to the standard state (H-saturated) by a process of electrodialysis. The clays were oven-dried to constant weight. The cation exchange capacities of the clays in milliequivalents per 100 grams were as follows: kaolinite, 7.83; illite, 25.0; and bentonite, 86.0.

Procedure. Forty-milliliter aliquots of aqueous solutions that contained increasing concentrations of the 2,4-D compounds were added to 1-gram portions of the clay in 125-ml. flasks. 2,4-D concentrations ranged from 3.7 to 21.0 mg. per liter. The pH values of the clay suspensions were 3.6 with bentonite, 4.6 with kaolinite, and 4.8 with illite. The flasks were agitated continuously on a laboratory shaker at room temperature (25° ± 2° C.) for 24 hours. The clays were separated then

by high speed centrifugation for 20 minutes, and the equilibrium concentration of each 2,4-D compound was determined in the supernatant by an ultraviolet method (2).

Results. Table I shows the amounts of the 2,4-D compounds sorbed by bentonite. Adsorption isotherms were constructed according to the Freundlich equation (12):

$$\log \frac{x}{m} = \log k + 1/n \log C$$

where

x = amount adsorbed, mg.
 m = weight of adsorbent, grams
 C = equilibrium concentration of the solute, mg. per liter.

By plotting $\frac{x}{m}$ versus C on log-log paper, a straight line is obtained whose slope is $1/n$ and intercept at $C = 1$ is k . k and $1/n$ are parameters for the particular system. Freundlich isotherms are shown in Figure 1 for 2,4-D compounds on bentonite. Similar plots were obtained with illite and kaolinite.

k and $1/n$ values were calculated for the three clay systems and were inserted into the equation

$$\frac{C_0 - C_f}{m} = kC_f^{1/n}$$

where

C_0 = initial concentration of the solute, mg. per liter
 C_f = final concentration of the solute, mg. per liter.

Table I. Sorption of 2,4-D Compounds on Bentonite

Initial Conc'n., C, Mg. per Liter	Equilibrium Conc'n., C, Mg. per Liter	Amount Adsorbed, x, Mg.	$\frac{x}{m}$, Mg. per Gram
2,4-D SODIUM SALT			
5.2	4.0	0.048	0.048
7.2	5.6	0.064	0.064
9.0	7.0	0.080	0.080
14.0	11.0	0.120	0.120
19.0	12.0	0.160	0.160
2,4-D ISOPROPYL ESTER			
5.2	1.7	0.14	0.14
6.0	2.0	0.16	0.16
9.0	4.0	0.20	0.20
12.0	6.0	0.24	0.24
15.0	8.0	0.28	0.28
20.0	12.0	0.32	0.32
2,4-D BUTYL ESTER			
5.0	2.0	0.12	0.12
7.0	3.0	0.16	0.16
9.0	4.0	0.20	0.20
11.0	7.0	0.28	0.28
16.0	8.0	0.32	0.32
21.0	11.0	0.40	0.40
2,4-D ISOCTYL ESTER			
6.0	3.0	0.12	0.12
9.0	5.0	0.16	0.16
12.5	7.5	0.20	0.20
16.0	10.0	0.24	0.24
21.0	14.0	0.28	0.28
2,4-DICHLOROPHENOL			
5.0	3.0	0.08	0.08
8.0	5.0	0.12	0.12
12.0	8.0	0.16	0.16
16.0	11.0	0.20	0.20
19.0	15.0	0.24	0.24

^a Expressed as the acid equivalent.

Consequently, the amount of clay required to reduce the concentration of each compound to any desired level can be computed to obtain an indication of its adsorption capacity.

For comparison, the calculated amounts of each clay to reduce various concentrations of the 2,4-D compounds to 2.0 mg. per liter are shown in Table II. For example, 26.3 grams of kaolinite, 40.0 grams of bentonite, and 34.95 grams of illite were required to reduce the concentration of 2,4-D from 3 mg. per liter to 2 mg. per liter. This shows that the sodium salt of 2,4-D was sorbed in the following decreasing order: kaolinite, illite, and bentonite. A similar comparison for the sorption of the isopropyl, butyl, and isoctyl esters of 2,4-D and 2,4-dichlorophenol revealed that these compounds were sorbed in the following decreasing order: bentonite, illite, and kaolinite. In general, the sorbed amounts of the 2,4-D compounds and 2,4-dichlorophenol were very small since relatively high amounts of each clay were required to remove significant amounts of each compound from the aqueous phase.

Effect of Ultraviolet Irradiation. Ultraviolet irradiation of 2,4-D compounds was studied to evaluate possible oxidative effects from solar radiation on these compounds in natural waters. Also, the use of ultraviolet irradiation was investigated as a potable water treatment method.

PROCEDURE. The ultraviolet light source was a mercury discharge lamp, 660 watt, and a 30-cm. tube. Solutions

were placed in a double-walled quartz jacket surrounding the lamp that had a 90-ml. capacity and a 4-mm. depth. Samples were withdrawn at various times through a side-arm connected to the quartz jacket.

Solutions of the sodium salt, the isopropyl and butyl esters of 2,4-D, and 2,4-dichlorophenol were prepared in distilled water. Approximately 0.26-millimolar solutions of these compounds were prepared also in 0.25M phosphate buffer at pH 7.0. Solutions at pH 4.0 and 9.0 were prepared by the addition of 2.0N phosphoric acid or 2.0N sodium hydroxide to the buffer solution. The irradiated solutions were analyzed periodically for the concentration of the 2,4-D compound (2) and 2,4-dichlorophenol (8), and for the pH.

RESULTS. Irradiation of solutions of the sodium salt, the isopropyl and butyl esters of 2,4-D in distilled water, produced 2,4-dichlorophenol within 20 minutes as shown in Table III. Concentrations of the 2,4-D compounds decreased continuously during the irradiation period. The pH values of all solutions decreased from 7.0 to 3.0.

Ultraviolet irradiation of the sodium salt, the isopropyl and butyl esters of 2,4-D, and 2,4-dichlorophenol was also studied at pH values of 4.0, 7.0, and 9.0. The sodium salt of 2,4-D was decomposed at different rates at the three pH values within 100 minutes of exposure. 2,4-Dichlorophenol appeared as a decomposition product within 20 minutes at pH values of 4.0 and 7.0.

Figure 2 shows the photodecomposi-

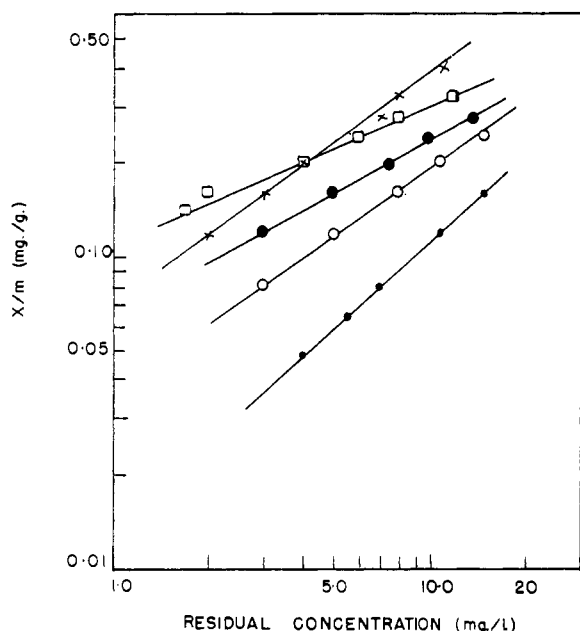


Figure 1. Freundlich adsorption isotherms for 2,4-D compounds on bentonite

- 2,4-Dichlorophenol
- Isopropyl ester
- Isoctyl ester
- 2,4-D (sodium salt)
- × Butyl ester

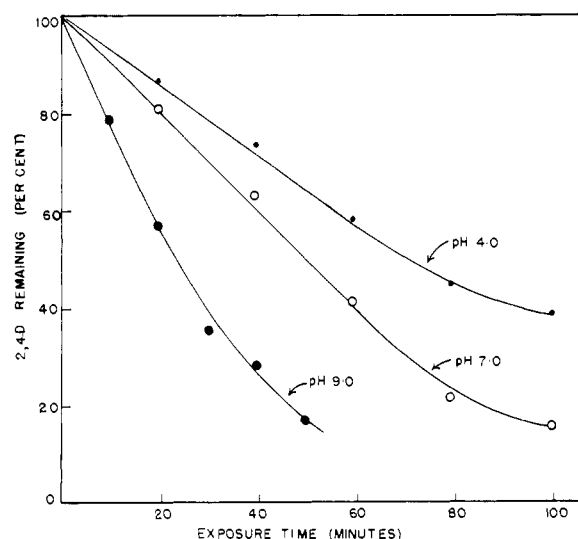


Figure 2. Effect of pH on photolysis of the sodium salt of 2,4-D

Table II. Amount of Clays Required to Reduce the Concentration of 2,4-D Compounds to 2 Mg. per Liter

Initial Concn., C., Mg per Liter	2,4-D, Sodium Salt	2,4-D, Isopropyl Ester	2,4-D, Butyl Ester	2,4-D, Isooctyl Ester	2,4- Dichloro- phenol
KAOLINITE—GRAMS					
3	26.3	37.59	42.73	44.63	96.11
4	52.6	75.18	85.46	89.22	192.22
5	78.9	113.77	128.19	133.89	288.33
6	105.26	150.36	170.92	178.52	384.57
ILLITE—GRAMS					
3	34.95	11.79	13.33	14.16	19.23
4	69.93	23.58	26.66	28.32	38.46
5	104.89	35.37	39.99	42.48	57.69
6	129.86	47.16	53.32	56.64	76.92
BENTONITE—GRAMS					
3	40.0	6.89	8.77	10.31	16.39
4	80.0	13.78	17.54	20.62	32.78
5	120.0	20.67	25.30	30.93	49.18
6	160.0	27.56	35.10	41.24	65.57

tion of the sodium salt of 2,4-D with time. At pH 7.0, photolysis progressed at a decreasing rate with time of exposure. 2,4-Dichlorophenol, 1.2 mg. per liter, appeared within 20 minutes and subsequently disappeared within 60 minutes. At pH 4.0, 2,4-D decomposed at a relatively slower rate than at pH 7.0 and 9.0. A concentration of 7.2 mg. per liter of 2,4-dichlorophenol was produced at pH 4.0 within 20 minutes and decreased to 3.0 mg. per liter within 100 minutes. At pH 9.0, 2,4-D was decomposed relatively faster than at pH 4.0 and 7.0. No 2,4-dichlorophenol was detected at any time at pH 9.0. Similar results were obtained from the photolysis of the isopropyl and butyl esters of 2,4-D.

Since 2,4-dichlorophenol was found in all pH 4.0 and 7.0 systems and none was found at pH 9.0, an investigation of the effect of pH on the photodecomposition of this phenol was conducted to explain the accumulation in the irradiated solutions of 2,4-D compounds. Figure 3 shows that the photodecomposition of 2,4-dichlorophenol progressed at decreasing rates as the pH value decreased similar to that observed with the 2,4-D compounds.

The times required for 50% decomposition of the various 2,4-D compounds and 2,4-dichlorophenol were estimated graphically. These data are given in Table IV. Photodecomposition of all compounds proceeded at relatively slow rates at pH 4.0, faster at pH 7.0, and reached a maximum at pH 9.0. 2,4-Dichlorophenol decomposed quicker than the 2,4-D compounds.

Evaluation of the Solubilities of the Calcium and Magnesium Salts of 2,4-D. Knowledge of the solubilities of the calcium and magnesium salts of 2,4-D would give valuable data concerning

the fate of this herbicide in hard surface waters. A survey of the chemical literature did not reveal any pertinent information on the solubilities of the 2,4-dichlorophenoxyacetates of calcium and magnesium.

The solubilities and solubility products for $\text{Ca}(2,4\text{-D})_2$ and $\text{Mg}(2,4\text{-D})_2$ were determined in distilled water and in KCl solutions ranging from 0.001M to 0.1M. The solubilities of calcium and magnesium 2,4-dichlorophenoxyacetates were found to be $9.05 \times 10^{-8}M$ (4000 mg. per liter as the acid equivalent) and $2.51 \times 10^{-8}M$ (11,100 mg. per liter as the acid equivalent), respectively, in distilled water. The average pK values at 25° C. for $\text{Ca}(2,4\text{-D})_2$ and $\text{Mg}(2,4\text{-D})_2$ were determined as 5.96 and 4.81, respectively (9).

DISCUSSION. Sorption of the sodium salt and esters of 2,4-D and 2,4-dichlorophenol on kaolinite, bentonite, and illite was generally small. The sorbed amounts ranged between 0.02 to 0.14 mg. per gram of clay at applied concentrations of 5.0 mg. per liter. Kaolinite sorbed more of the 2,4-D anion than bentonite and illite. Yoe (19) suggested that the 2,4-D molecule is attracted to numerous positively charged receptive sites on the basal planes and broken edges of the lattice structure of the kaolinite. Bentonite and

Table III. Effect of Ultraviolet Irradiation on 2,4-D Compounds in Distilled Water

Time, Min.	Concn., Mg per Liter	% Remaining	2,4-DCP, ^a Mg per Liter	pH
2,4-D SODIUM SALT				
0	70.0 ^b	..	0.0	7.0
20	59.2	84.5	9.0	4.0
40	47.6	68.0	9.0	4.0
60	35.7	51.0	7.0	3.7
80	27.3	39.0	4.2	3.3
100	21.0	30.0	3.8	3.0
2,4-D ISOPROPYL ESTER				
0	90.0 ^b	..	0.0	7.0
20	65.0	72.2	10.0	5.3
40	43.0	47.8	10.0	4.2
60	30.0	33.3	7.6	3.0
80	20.0	22.2	6.2	3.0
100	15.0	16.7	5.6	3.0
2,4-D BUTYL ESTER				
0	70.0 ^b	..	0.0	7.0
20	53.0	75.7	4.0	..
40	40.0	57.1	5.4	..
60	32.0	45.7	6.2	3.7
80	27.0	38.6	5.6	3.6
100	23.0	32.8	3.0	3.0
2,4-DICHLOROPHENOL				
0	36.0	7.0
10	24.0	94.4
20	27.0	75.0	..	5.3
40	17.0	47.2	..	4.3
60	8.0	17.4	..	3.7
80	4.4	12.2	..	3.5

^a 2,4-Dichlorophenol. ^b Expressed as the acid equivalent.

illite have numerous negatively charged receptive sites and relatively few positive sites.

2,4-D esters and the phenol, on the other hand, were sorbed on the clays in the following decreasing order: bentonite, illite, and kaolinite. The sorbed amounts were directly proportional to the specific surface area of the clays: the expanding lattice clay, bentonite, had the highest, followed by the nonexpanding lattice clays, illite and kaolinite. The amounts sorbed on kaolinite, bentonite, and illite are so small that 7.0 to 96.0 grams of the clays are required to remove 1.0 mg. per liter of 2,4-D compounds from water.

Frissel and Bolt (11) showed that adsorption of organic ions by clay

Table IV. Irradiation Time Required for 50% Decomposition of 2,4-D Compounds and 2,4-Dichlorophenol

Compounds	Irradiation Time, Min.		
	pH 4.0	pH 7.0	pH 9.0
2,4-D sodium salt	71	50	23
Isopropyl ester	48	31	9
Butyl ester	54	42	9
2,4-Dichlorophenol	34	5	2

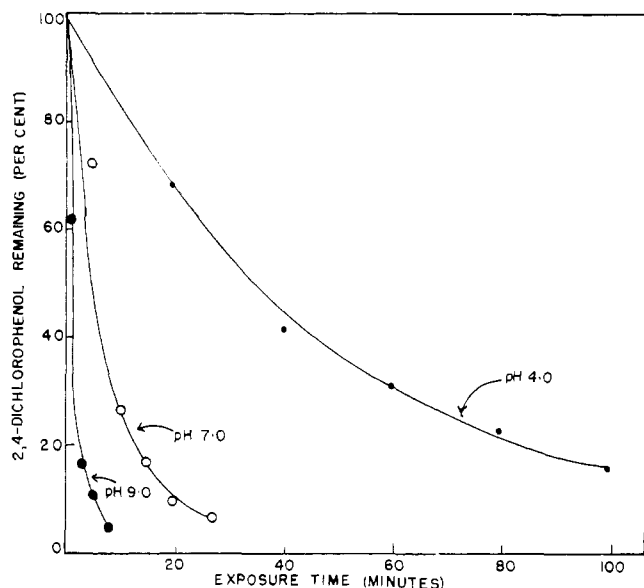


Figure 3. Effect of pH on photolysis of 2,4-dichlorophenol

minerals is strongly dependent upon pH and electrolyte concentration of the system. 2,4-D showed negative adsorption with montmorillonite at low salt concentrations and above pH 4.0. Negative adsorption on illite occurred at pH values higher than 7.0. Positive adsorption occurred at lower pH values and high salt concentrations. Natural waters, in general, have low salt concentrations and have pH values ranging between 6 and 8. These pH values are considerably higher than the H-saturated clay systems in this investigation. Therefore, suspended clays in surface waters will not remove significant amounts of these compounds.

Results from the ultraviolet irradiation of aqueous solutions of 2,4-D compounds suggest that the initial pH value influences the degree of decomposition of each compound. Decomposition was relatively slow at low pH values and increased with the increase of pH. Irradiation of 2,4-D compounds resulted in cleavage at the ethereal linkage, and 2,4-dichlorophenol was produced with some free acids as evidenced by a decrease of pH value in the distilled water systems. These results are in agreement with Bell (4). 2,4-Dichlorophenol was decomposed also at a relatively slow rate at low pH values, but the rate was much faster than the 2,4-D compounds at all pH values. Accumulation of the phenol at pH 4.0 in the 2,4-D systems is explained by its relatively slow rate of photolysis under these conditions. On the other hand, no phenol is detected in the irradiated 2,4-D solutions at pH 9.0 because it is decomposed as fast as it is produced.

The solar ultraviolet spectrum ranges from 292 to 400 m μ . The mercury lamp used in this investigation emits light of

shorter wave lengths with the peak at 253.7 m μ . Since the energy per photon increases as wave length decreases, the shorter ultraviolet irradiation might cause reactions which would not occur in aqueous solutions exposed to sunlight (74). In addition, the presence of suspended matter and organic matter in surface waters will greatly reduce any effect of solar radiations. Therefore, ultraviolet irradiation from the sun is expected to be an insignificant factor in the decomposition of 2,4-D compounds in surface waters.

The observations that 2,4-D compounds and 2,4-dichlorophenol are photodecomposed, together with the fact that these rays have bactericidal properties, make it reasonable to suggest the use of ultraviolet irradiation as a treatment method for the removal of these compounds from natural waters.

The calcium and magnesium salts of 2,4-D have relatively high solubilities in water. Consequently, the removal of 2,4-D from natural waters through precipitation reactions is not likely to occur.

Biological Factors

Biological breakdown of 2,4-D on soils has been reported by several investigators, with the concurrent isolation of various species of bacteria (3, 16, 77). In this study, biological degradation of 2,4-D compounds in lake waters and in bottom muds was investigated.

Manometric Studies. PROCEDURE. Solutions of 50 mg. per liter of the butyl ester and 100 mg. per liter of the isopropyl ester, and suspensions of 100 mg. per liter of formulations of these esters were prepared in mineralized biochemical oxygen demand (B.O.D.) dilu-

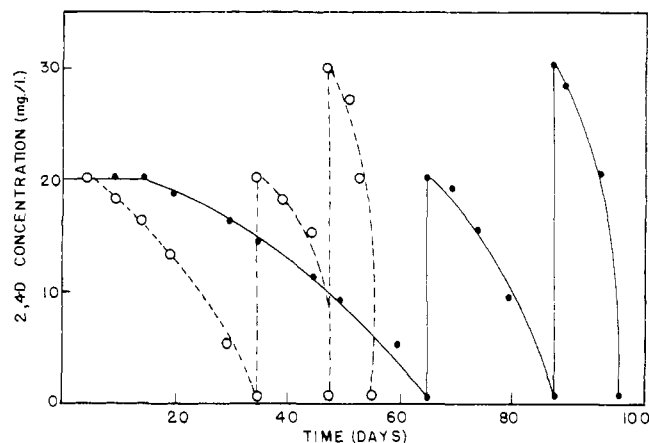


Figure 4. Biological degradation of 2,4-D in lake mud

● Untreated lake mud; ○ treated lake mud

tion water (15). Each system was seeded with 5% settled domestic sewage by volume. Oxygen uptake for each ester was measured by a manometric technique (15). The concentration of each ester was determined by an ultraviolet technique (2) after each manometric run.

RESULTS. Oxygen that was utilized in the flasks containing the 2,4-D esters exceeded that of the control (Table V). Each ester concentration, however, was not changed after 9 days, suggesting biological hydrolysis into the free 2,4-D acid and the corresponding alcohol. Apparently, oxygen uptake was due to biological degradation of the alcohol moieties.

The theoretical amounts of oxygen required for complete oxidation of alcohols that might be produced from the hydrolysis of 50 mg. per liter of the butyl

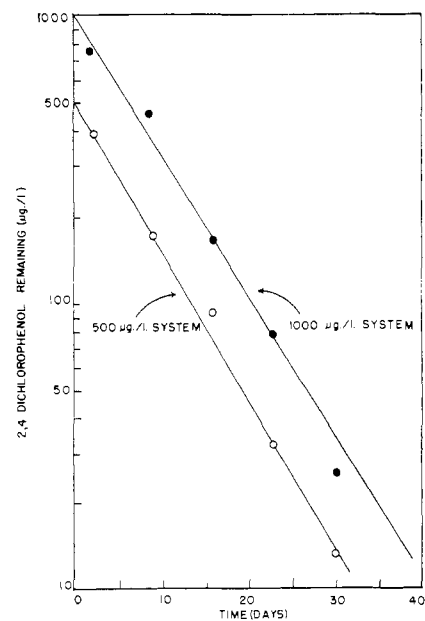


Figure 5. Disappearance of 2,4-dichlorophenol in an aerated and buffered lake water

Table V. Oxygen Uptake for 2,4-D Esters and Formulations

Time, Days	Oxygen Uptake, ^a Mg. per Liter			
	Isopropyl ester	Butyl ester	Isopropyl ester formulation	Butyl ester formulation
1	3	7	3	11
2	3	36	56	73
4	3	39	132	206
5	3	40	145	224
7	8	43	163	252
8	43	43	169	261
9	65	..	173	270
Concn. after 9 days ^b	100	50	100	100

^a Corrected for control. ^b Expressed as acid equivalent, milligrams per liter.

ester and 100 mg. per liter of the isopropyl ester were calculated to be 44.0 mg. per liter for butyl alcohol and 66.0 mg. per liter for isopropyl alcohol. Table V shows that 97.7% of the butyl alcohol was oxidized within 7 days. In the case of the isopropyl ester, there was a lag period of 7 days, after which oxygen uptake increased until 98.5% of the theoretical amount of isopropyl alcohol was oxidized within 9 days.

Oxygen uptake in the case of the two formulations was much higher than theoretical amounts required for oxidation of the hydrolyzed alcohols. Ester concentrations as the acid equivalent were not changed at the end of the run. This indicates that the emulsifier or carrier in both formulations was decomposing biologically.

Oxygen uptake was measured on solutions of 20, 80, and 150 mg. per liter of the sodium salt of 2,4-D prepared in 50% and 100% settled domestic sewage by volume. At all 2,4-D concentrations, the oxygen uptake was not different from the control over a period of 9 days. Thus 2,4-D was not biologically degraded under these conditions.

Lake Mud Studies. PROCEDURE. Bottom muds were obtained from an untreated lake and from a lake that was treated with 2,4-D the previous summer. These muds were wet-sieved to remove coarse particles and decaying vegetation. Aliquots of mud suspensions in distilled water containing about 8 grams (dry weight) were added to 250 ml. of 2,4-D solutions of 20.0 mg. per liter in unseeded B.O.D. dilution water and were placed in 500-ml. Erlenmeyer flasks. A parallel series of flasks with 2,4-D and lake mud was treated to a final concentration of 0.01%, by weight, with sodium azide to inhibit microbial transformations. All flasks were placed on a laboratory shaker at 20° ± 0.5° C. Samples were taken from each flask at different intervals and centrifuged at 12,000 r.p.m. for 10 minutes for separation of mud particles. 2,4-D concentration was determined in the supernatant by an ultraviolet method (2).

RESULTS. Figure 4 shows the decrease in concentration of 2,4-D with time for the two mud samples. 2,4-D disappeared completely from the untreated lake mud within 65 days, and from the treated lake mud within 35 days. Subsequent additions of 2,4-D disappeared in shorter periods of time in both systems. That 2,4-D did not disappear from the samples treated with sodium azide indicated that disappearance was not due to sorption on the mud particles.

By repeated additions of increasing concentrations of 2,4-D to the mud samples, an adapted population of microorganisms was obtained. Oxygen utilization was measured for 2,4-D in concentrations of 55, 160, and 200 mg. per liter in the presence of the adapted mud flora using the manometric technique (7-7). Table VI shows that 81 to 85% of all concentrations of 2,4-D were biologically decomposed within 24 hours.

Lake Water Studies—2,4-D. Lake water solutions of the sodium salt of 2,4-D, 3 mg. per liter, were prepared at pH values of 5.0, 7.0, and 8.0. Ten-liter portions of each solution were placed in five-gallon carboys and kept at 20° = 0.5° C. These systems were kept aerobic through bubble aeration and were examined periodically over 120 days for 2,4-D and 2,4-dichlorophenol. No change in the initial concentration of 3 mg. per liter of 2,4-D nor production of phenol was observed over entire period.

Lake Water Studies—2,4-Dichlorophenol. PROCEDURE. Solutions of 2,4-dichlorophenol in concentrations of 100,

500, and 1000 µg. per liter were prepared in natural lake waters. These systems were adjusted to a pH of 7.0 with a phosphate buffer. Fifteen liters of each solution were placed in five-gallon carboys, and a continuous supply of oxygen was maintained by bubble aeration. All solutions were kept at room temperature, 25° ± 2° C. Samples were withdrawn periodically for determination of 2,4-dichlorophenol (8) and the pH.

RESULTS. Table VII shows that 2,4-dichlorophenol disappeared completely within 9 days from the 100 µg. per liter system, and 97.5% disappeared from the 500 and 1000 µg. per liter systems within 30 days. Phenol concentration was unchanged in a distilled water control solution over 30 days. By plotting residual phenol concentration in the 500 and 1000 µg. per liter solutions versus time on semilog paper, parallel straight lines were obtained, as shown in Figure 5. In each case, 50% of the phenol was decomposed at 6 days.

To simulate the effect of excessive amounts of decaying organic matter on the biological decomposition, ethanolic solutions of 2,4-dichlorophenol were added to an unbuffered lake water to final concentrations of 100, 500, and 1000 µg. per liter. These waters were kept un-aerated and periodic samples were withdrawn for determination of pH and residual phenol concentration (8).

Table VII shows that 2,4-dichlorophenol persisted over 43 days in the three systems. The pH of the 500 and 1000 µg. per liter solutions dropped within 3 days to 5.1 and 4.1, respectively. Anaerobic conditions prevailed in these systems as evidenced by the presence of hydrogen sulfide.

DISCUSSION. 2,4-D was biologically broken down after incubation with bottom muds obtained from lakes. Repeated additions of 2,4-D to the bottom muds resulted in the buildup of microbial flora capable of decomposing relatively high concentrations of this compound. The disappearance of 2,4-D under these conditions was attributed to biological activity as indicated by loss of the aromatic moiety of the molecule, as evidenced by the decrease in absorbance in the ultraviolet region; uptake of oxygen, as measured by the manometric technique; and persistence of the compound in the presence of the bacterial inhibitor, sodium azide.

Esters of 2,4-D showed higher oxygen uptake than the control when incubated with domestic sewage microorganisms. The concentration of 2,4-D, however, was not changed at the end of the incubation period. This suggested that the esters were hydrolyzed to the free acid and the corresponding alcohol and that the latter was decomposed biologically. The hydrolyzed acid was not decom-

Table VI. Oxygen Uptake for 2,4-D with Adapted Bottom Mud Flora in 24 Hours

Concentration, Mg. per Liter	Oxygen Uptake, Mg. per Liter	Theoretical Oxygen, Mg. per Liter	Oxidation, %
55	51	64	81.0
160	157	185	84.5
200	197	232	85.0

Table VII. Persistence of 2,4-Dichlorophenol in Lake Waters

Time, Days	100 µg. per Liter			500 µg. per Liter			1000 µg. per Liter		
	pH	Concn.	Oxid., %	pH	Concn.	Oxid., %	pH	Concn.	Oxid., %
AERATED AND BUFFERED									
0	7.4	100	0.0	7.4	500	0.0	7.4	1000	0.0
2	7.3	64	36.0	7.6	390	22.0	7.6	760	24.0
9	7.3	0	100.0	7.5	170	66.0	7.4	460	54.0
16	6.9	0	100.0	7.1	92	81.6	7.2	165	83.5
23	7.5	32	93.6	7.5	78	92.2
30	7.3	13	97.5	7.3	25	97.5
UNAERATED AND UNBUFFERED									
0	7.3	100	0.0	7.3	500	0.0	7.3	1000	0.0
3	6.2	80	20.0	5.1	390	22.0	4.1	780	22.0
7	6.1	70	30.0	6.1	380	24.0	6.0	770	23.0
14	6.1	620	38.0
17	7.9	40	60.0	6.5	253	49.4	6.3	560	44.0
24	..	40	60.0	..	192	61.6	..	540	46.0
43	..	20	80.0	..	192	61.6	..	506	49.4

posed under these conditions. This finding is in agreement with the observations of other investigators that 2,4-D esters were hydrolyzed by microbial action in soils (5) or during absorption by plant leaves (6).

The lake bottom mud and manometric studies indicate that 2,4-D is biologically decomposed in a relatively short period of time with adapted microorganisms. However, 2,4-D is applied usually once every 1 or 2 years to a surface water for aquatic plant control. Consequently, the development of adapted microorganisms may take a relatively long time under such unfavorable anaerobic conditions that may develop when dead aquatic plants decompose. Since some 2,4-D-treated surface waters serve as continuous potable water supplies, this herbicide may persist for sufficient periods of time

to warrant its removal at the treatment plant.

2,4-Dichlorophenol, on the other hand, was biologically broken down in the lake water indicating the presence of microorganisms capable of decomposing this compound in natural waters. Concentrations of the phenol up to 1000 µg. per liter did not affect the rate of oxidation since the two lines in Figure 5 were parallel and reached 50% removal level at the same time. Under unfavorable environmental conditions that may result from the decomposition of excessive amounts of organic matter in lake waters, the pH value will drop, anaerobic conditions prevail, and the phenol will persist for longer periods of time.

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PESTICIDE PURITY MEASUREMENT

Cryoscopic Analysis of Organic Phosphate Pesticides—Malathion, Dimethoate, O, O-Diethyl O-2-Pyrazinyl Phosphorothioate, and Phorate

It was necessary to establish the purity of specially purified samples of malathion, dimethoate, O, O-diethyl O-2-pyrazinyl phosphorothioate, and phorate. Organic phosphate compounds may be analyzed by vapor phase chromatography, ultraviolet spectroscopy, infrared spectroscopy, polarography, and various chemical methods such as bromination, hydrolysis,

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silver titration, etc. However, any of these methods requires a primary standard or comparison compound and in addition is frequently subject to interference from other compounds present. In the case of large molecules, these difficulties are more pronounced. Therefore the cryoscopic method, which is not subject to interference from other compounds and does not require a standard, was selected. The cryoscopic method consists of measuring the equilibrium temperature of a solid-liquid system as

the compound being analyzed is slowly frozen or melted (7, 2, 4-8). The method is particularly useful in the analysis of a compound approaching 100% purity because it directly measures the amount of impurity present without requiring knowledge of the nature of any impurity.

Experimental

Instrumentation and Apparatus. A Thwing-Albert constant temperature differential cryoscope with a controller

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