Rates and Products of Decomposition of 2,2-Dibromo-3-nitrilopropionamide

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Rates and products of decomposition of 2,2-dibromo-3-nitrilopropionamide (DBNPA), an antimicrobial compound for industrial water treatment, were determined over a range of conditions. Rates of hydrolytic decomposition, determined polarographically at various pH's and temperatures, are consistent with acid- and base-catalyzed amide hydrolysis. Hydrolysis of DBNPA ultimately forms carbon dioxide, ammonia, and bromide ions *via* the following sequence of degradation products: dibromoacetonitrile, dibromoacetamide, dibromoacetic acid, glyoxylic acid, and oxalic acid. DBNPA reacts rapidly with various ions such as bisulfite to form cyanoacetamide. Decomposition under the influence of sunlight also leads to cyanoacetamide. Contact with soil and soil organisms degrades DBNPA. Decomposition of DBNPA by several chemical and biological pathways ensures that the compound will not persist in the environment.

During the last few years, control of bacteria, fungi, and algae in industrial waters of paper mills and cooling towers has become more difficult as more water is recirculated. Industrial compounds for control of microorganisms generally are classified as economic poisons. These biocides are used in low concentrations and can be discharged into the environment. In fact, a source of contamination in many water systems with paper mills, for example, is thought to be phenylmercury derived from phenylmercuric acetate, a very effective biocide that was commonly used until just a few years ago (Kleinert and Degurse, 1971).

2,2-Dibromo-3-nitrilopropionamide (DBNPA, 2,2-dibromo-2-cyanoacetamide) is an effective seed fungicide (Nolan and Hechenbleikner, 1947). More recently, DBNPA has become very useful in controlling microorganisms in cooling tower and paper mill waters (Wolf and Sterner, 1972).

We have evaluated the environmental effects of DBNPA. These studies include the hydrolytic decomposition and the influence of nucleophiles, sunlight, soil, and microorganisms on the decomposition of DBNPA.

EXPERIMENTAL SECTION

DBNPA was prepared by the acid-catalyzed bromination of cyanoacetamide (Hesse, 1896): mp 123–125°; nmr (DMSO-d_6) δ 8.36 (d); ir (Nujol mull) 1710 cm^{-1} (C=O). DBNPA-2-^{14}C was prepared from cyanoacetamide-2-^{14}C (New England Nuclear).

KINETICS

Hydrolyses. Solutions of DBNPA at 40 ppm were prepared in a series of McIlvaine buffers for the pH range of 3.9–8.05. pH 8.9 and 9.7 were attained with borax buffers, and solutions at other pH's were prepared by the use of sodium hydroxide and various strong acids. These solutions were placed in a constant-temperature bath at 25 ± 0.1°. Periodically, aliquots were removed and added to a buffered solution (pH 7) containing about 0.005% gelatin, and oxygen was removed by bubbling nitrogen through the solution for 5 min. The polarogram was recorded between +0.1 and −0.2 V *vs.* Standard Calomel Electrode using a Melabs polarographic analyzer. The sample polarograms in Figures 1 and 2 were obtained using the following settings: cell volts, 1.0; volt range, 0.5; time, 500 or 1000; sensitivity, 3; and chart speed, 1 in./min. The analysis of DBNPA by polarography is complicated by the proximity of the first polarographic wave of DBNPA to the dissolution of mercury. The second wave, which was used for the measurement of the DBNPA concentrations (Figure 1A), is affected by the polarographic wave of dibromoacetonitrile (Figure 1B), which is formed when DBNPA hydrolyzes. Because the waves were ill-defined, the concentration of DBNPA was determined by relating the wave height of the second wave to the wave height of standard samples, as illustrated in Figure 1, A and C.

The disappearance of 1000 ppm of aqueous solutions of dibromoacetonitrile was followed by gc of methylene chloride extracts, using an F&M Scientific 5750 chromatograph, a 10 ft × ⅛ in. column of 10% Apiezon L wax at a temperature of 90°, He flow = 60 cm^3/min.

Reaction with Ions. A modification of the polarographic method was used because of the speed of these reactions. Buffered solutions (pH 7.2) of 50 ppm of DBNPA were placed in a polarographic cell. The limiting current of DBNPA was obtained and a potential was chosen sufficiently cathodic so that anodic interference by the ion under study could not take place. Thus, the only current was due to the electrochemical reduction of DBNPA. To the solution was added, by means of a microsyringe, a solution of the desired ion. The time for the disappearance of the limiting current was determined.

Contact with Soil. A 50-ppm solution of DBNPA in water (100 ml) was placed in stoppered flasks containing 20 g of soil. The flasks, at room temperature, were shaken occasionally, and the supernatant liquid was analyzed polarographically at selected intervals.

PRODUCT STUDIES

Hydrolyses. To a 1-l. solution of the desired pH was added 1–12 g of DBNPA. The solution was stirred magnetically for the desired time and aliquots were withdrawn for bromide analysis. The remainder of the solution was acidified to pH ≈2 with 48% hydrobromic acid, and nitrogen was bubbled through the solution to entrain carbon dioxide in an Ascarite train (Kolthoff and Sandell, 1952). The aqueous solution was extracted with an equal volume of diethyl ether in three portions, the combined ether layer was dried (anhydrous sodium sulfate), and the desiccant was filtered. The ether was evaporated and the remaining mixture was analyzed by ir, nmr, and mass spectral methods. Once tentative identification was made, the components of the mixture were confirmed by spectral comparison with authentic samples. Nmr (DMSO-d_6) and ir data (Nujol mull) for the decomposition products of DBNPA are: DBNPA, δ 8.36 (d), 1710 cm^{-1} (C=O); $NCCHBrCONH_2$, δ 7.90 (s), 5.42 (s), 1680 cm^{-1} (C=O); $CHBr_2CN$, δ 6.87 (s); $CHBr_2CONH_2$, δ 6.20 (s), 1675 cm^{-1} (C=O); $CHBr_2CO_2H$, δ 6.37 (s), 1730 cm^{-1} (C=O, CCl_4); $NCCH_2CO_2NH_2$, δ 7.47 (d), 3.56 (s), 1680 cm^{-1} (C=O); $NCCH_2CO_2H$, δ 3.68 (s), 1730 cm^{-1} (C=O).

Reaction with Sodium Bisulfite. A solution of 6.00 g

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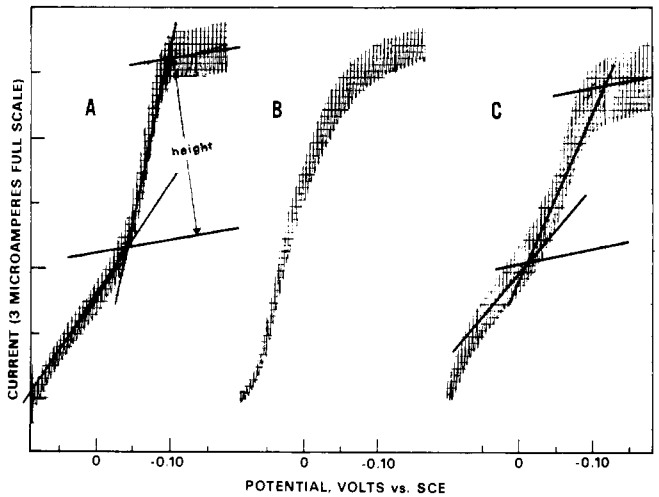


Figure 1. Polarograms of: (A) 40 ppm of DBNPA; (B) 40 ppm of dibromoacetonitrile; (C) a mixture of 30 ppm of DBNPA and ~15 ppm of dibromoacetonitrile.

(0.025 mol, 0.167 *M*) of DBNPA and 5.40 g (0.050 mol) of sodium bisulfite in 150 ml of water was stirred for 75 min. An aliquot contained 0.330 *M* bromide ion. The water was removed at the water pump. The remaining oily solid was extracted for 2 hr with ether in a Soxhlet extractor. The yellow solid was cyanoacetic acid, 1.70 g (81%).

Photodegradation Products. A solution of 3.20 g (1.32×10^{-2} mol) of DBNPA in 800 ml (1.65×10^{-2} *M*) of pH 4 buffer solution was divided into two portions. One (400 ml) was placed in a 500-ml quartz tube fitted with a pressure release and the other was put in a closed, brown bottle. The two vessels were placed on the roof of the building. After 28 days, [DBNPA] = 1×10^{-4} *M*, [Br^-] = 3.0×10^{-2} *M* in the quartz; in the dark, [DBNPA] = 1.5×10^{-2} *M*, [Br^-] = 1.5×10^{-3} *M*.

In a second experiment, 1.0 g of DBNPA in 500 ml of water (8.3×10^{-3} *M*) in a quartz tube was exposed to a G.E. sunlamp at a distance of 50 cm for 3 days: [DBNPA] = 1.74×10^{-3} *M*, [Br^-] = 1.24×10^{-2} *M*. The yellow solution (320 ml) was extracted with 100 ml of ether, which yielded no material. Evaporation of the water yielded a residual oil which contained cyanoacetic acid, cyanoacetic acid amide ($HO_2CCH_2CONH_2$), malonic acid, and oxalic acid by ir and mass spectral analysis.

Biochemical oxygen demand was determined by the azide modification method of the Winkler titration (American Public Health Association, 1971).

RESULTS

Hydrolysis Rates of DBNPA. Relative concentrations of DBNPA can be determined by an empirical, polarographic method. With this method, the rate of disappearance of DBNPA can be followed for the first two half-lives of the hydrolysis reaction. These hydrolysis rates are pseudo-first-order at low concentrations of DBNPA. The reaction rates are unaffected by changes in the buffer concentration from 0.02 to 0.2 *M*.

Figure 3 illustrates the effect of pH on the hydrolysis rate. The compound is stable under acidic conditions, but the rate of disappearance increases by a factor of about 450 in going from pH 6, essentially neutral, to pH 8.9, slightly basic. At pH 11.3, the half-life ($\tau_{1/2}$) for the disappearance of DBNPA is 25 sec, essentially instantaneous. Table I summarizes kinetic data over the pH range of 4–10, the range of conditions commonly encountered in cooling towers, paper mills, and the environment.

Hydrolysis Products. The extraction procedure generally recovered 70–80% of the products, the nature of which was unaffected by pH. At high acid or base concentrations, however, faster hydrolysis allowed a more convenient determination of the hydrolysis products of the first breakdown products.

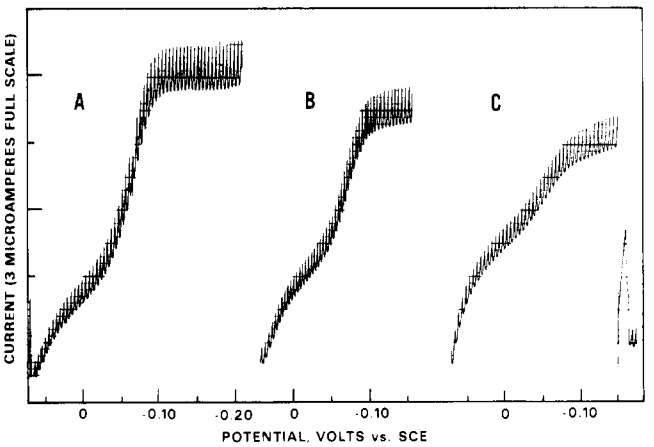


Figure 2. Polarograms of a 40-ppm solution of DBNPA at pH 8.05: (A) 25 min; (B) 85 min; (C) 172 min.

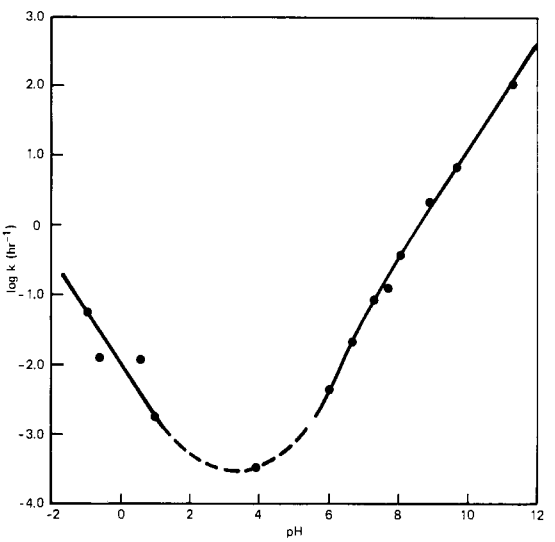


Figure 3. pH profile for the hydrolysis of DBNPA at 25°.

Table I. Rates of Hydrolysis of DBNPA

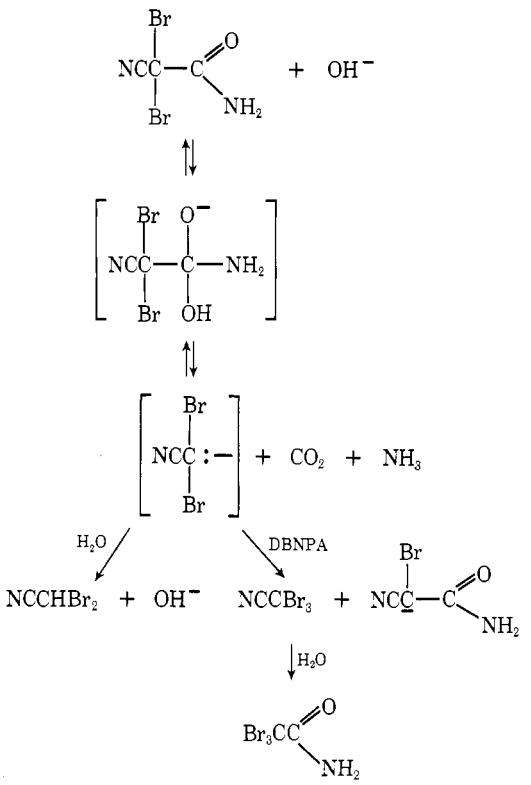
pH	$\tau_{1/2}$, hr	Temp, °C
3.9	2,140	23
6.0	155	25
6.7	37.0	25
7.3	8.8	25
7.7	5.8	25
7.7	145	0
8.0	2.0	25
8.9	0.34	25
9.7	0.11	25
9.7	1.5	0

Table II summarizes the experimental results from some of the product isolation studies. Under mild hydrolysis conditions, experiments 1–3, the major products were the starting material, DBNPA, and dibromoacetonitrile. As the reaction time increased, DBNPA decreased and dibromoacetonitrile increased, confirming hydrolysis of DBNPA to dibromoacetonitrile. More stringent reaction conditions (experiments 4 and 5) yielded dibromoacetic acid and 90–100% of theoretical carbon dioxide. The appearance of dibromoacetamide in experiment 3 indicates that dibromoacetonitrile, the first degradation product, was hydrolyzed to the amide. Hydrolysis of dibromoacetic acid yielded bromide ions. Formation of bromide ions is consistent with formation of glyoxylic acid.

Salts present in the water layers were determined by X-ray procedures and consisted of ammonium and sodium bromides. No cyanides were detected above the sensitivity limit of *ca.* 10 ppb.

In determining the hydrolysis products of DBNPA, the concentrations of DBNPA (*ca.* 12,000–15,000 ppm) were, of necessity, considerably higher than those of use conditions, *ca.* 1 ppm. As the elucidation of the hydrolysis pathway progressed and as analytical techniques for compounds such as dibromoacetonitrile became accessible, effective hydrolysis studies could be carried out at lower concentrations. Descriptions of this work are listed in experiments 10, 12, and 13. In 12 and 13, analyses were made for remaining DBNPA, carbon dioxide, bromide ions, and dibromoacetonitrile. Based on reacted DBNPA, the following data were obtained (Table III).

In experiments 12 and 13, vpc analysis suggested the presence of an unidentified compound, a compound which was identified as tribromoacetonitrile by vpc–mass spectrometry (*m/e* 275, 3 Br). In addition tribromoacetamide, ir (mull) 1686 cm^{-1} (C=O), was also isolated from these same solutions. The formation of tribromoacetonitrile accounts for the discrepancy in the yields between carbon dioxide and dibromoacetonitrile. Formation of tribromoacetonitrile also accounts for the fact that only about 80% of carbon dioxide is obtained, since tribromoacetonitrile must arise *via* a bimolecular reaction as shown in the following scheme.



Thus, each mole of tribromoacetonitrile formed required 2 mol of DBNPA, with the formation of only 1 equiv of carbon dioxide and monobromonitrilopropionamide. Since monobromonitrilopropionamide yields bromide ion readily at pH 7.4, the reaction path also accommodates the formation of bromide ions.

The formation of tribromoacetonitrile gives a satisfactory material balance for the product studies. However, tribromoacetonitrile is formed *via* a bimolecular reaction because of the high concentrations used in the product studies. At lower concentrations (112 ppm) little bimolecular reaction is observed, since portions of the 7% bromide ions that are formed may also arise from light-catalyzed reactions. At the expected use levels of 1–10 ppm of DBNPA, no tribromoacetonitrile should be formed by hydrolysis.

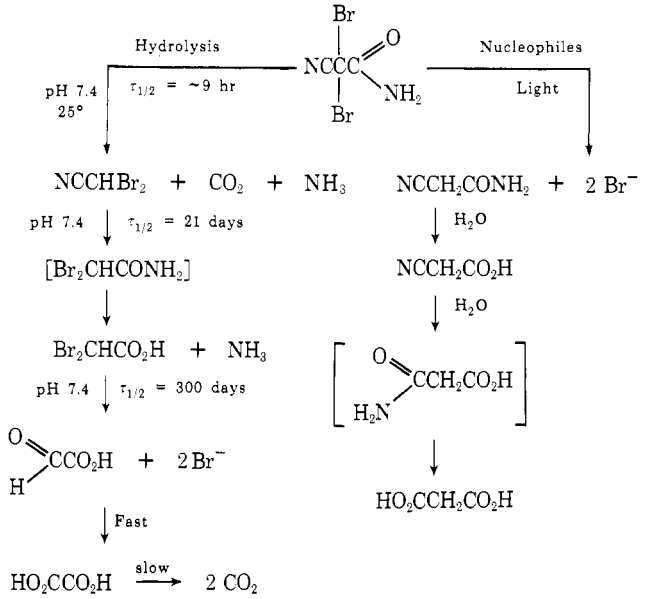


Figure 4. Decomposition pathways of DBNPA.

Reactions of DBNPA. Under use conditions and in the environment, DBNPA may encounter a variety of organic and inorganic species. DBNPA is reduced by a number of sulfur-containing species such as sulfite, bisulfite, sulfide, thiosulfate, and xanthate ions, and by phenyl mercaptan. The reaction occurs within seconds even at very low concentrations (<100 ppm). Reduction by sodium bisulfite yields cyanoacetic acid and 2 equiv of bromide ions. Iodide ions are oxidized quantitatively by DBNPA, a reaction which is the basis for an iodometric analytical method.

Within 3 to 5 days, none of the following species affect DBNPA: pyridine, Br^-, NO_2^-, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, and Cu^{2+}.

Effect of Sunlight on DBNPA in Water. A solution of DBNPA (4000 ppm) was exposed to sunlight for a period of 28 days. After this time, less than 1% DBNPA remained and 92% of theoretically possible bromide ion had formed. During the same period, a control sample contained 91% of the original DBNPA and less than 5% bromide ion. Analysis of the aqueous solution from the light-catalyzed reaction yielded no isolatable organic material other than buffer constituents.

In a similar experiment, a 2000-ppm solution of DBNPA in water was exposed to irradiation from a G.E. sunlamp for 3 days. At the end of this period, 20.5% of DBNPA remained and 90% of the theoretical bromide ion was obtained. By ir and mass spectrometry, the remaining organic material was identified as a mixture of cyanoacetic acid, the amide derived from cyanoacetic acid, malonic acid, and oxalic acid.

DBNPA in Presence of Soil. When solutions of DBNPA were contacted with soil, DBNPA disappeared at a much faster rate than in aqueous solutions of similar pH. Table IV summarizes the rate of disappearance of DBNPA in the presence of various types of soil. In one case, soil sample No. 70 was contacted with distilled water overnight. The supernatant liquid was filtered, and DBNPA was added to the filtrate. DBNPA in this solution disappeared at essentially the same rate as samples in the presence of soil.

DISCUSSION

Hydrolysis. The polyfunctional molecule, DBNPA, can be envisioned to decompose *via* several probable pathways: amide hydrolysis; α-elimination of a bromonium ion; an abnormal Hoffmann arrangement in analogy to similar α-haloamides (Stevens, *et al.*, 1963); and nitrile

Table II. Products from the Room Temperature[a] Hydrolysis of DBNPA and Degradates under Various Reaction Conditions

Experiment	Starting material	Condition		Product (% yield)
		pH[b]	Time, hr	
1	DBNPA	7.4	30	DBNPA (63), $CHBr_2CN$ (5) MBNPA[c] (2)
2	DBNPA	8.4	2.5	DBNPA (40), $CHBr_2CN$ (10) MBNPA (2)
3	DBNPA	7.4	88	DBNPA (26), $CHBr_2CN$ (22) $CHBr_2CONH_2$ (2), MBNPA (2)
4	DBNPA	0.25 N NaOH	138	$CHBr_2CO_2H$ (45), CO_2 (90)
5	DBNPA	0.25 N NaOH	244	$CHBr_2CO_2H$ (42), CO_2 (103) $CHBr_2CN$ (10)
6	$CHBr_2CN$	0.25 N NaOH	42	$CHBr_2CO_2H$ (66), CO_2 (<4)
7	$CHBr_2CO_2H$	0.25 N NaOH	288	$CHBr_2CO_2H$ (81), Br^- (5%) CO_2 (<2%)

[a] Temp = 20–23°. [b] pH did not remain constant throughout the reaction. [c] MBNPA = $NCCHBrCONH_2$.

Table III

Experiment	DBNPA, ppm	% CO_2	% CH_2Br_2CN	Br^-
12	1000	81	53	22
13	2420	77	43	32
10	112			7

hydrolysis. The hydrolysis pathway and the rates of the various decomposition steps at 25° and a pH of 7.4 are summarized in the left-hand side of Figure 4. The first step in the hydrolysis involves the hydrolytic decarboxylation of DBNPA to dibromoacetonitrile, with formation of carbon dioxide and ammonia. This hydrolysis of an amide containing three highly electro-negative groups on the α-C atom is analogous to decarboxylations of esters of trichloroacetic and dibromocyanoacetic acid (Parham and Schweizer, 1959; Wilt, 1956). However, it is different from the hydrolysis of the similarly activated dibromomalononitrile, which loses positive bromine in the presence of base (Freeman, 1969).

The acid and base catalysis of DBNPA shown in the pH profile in Figure 3 is consistent with general amide hydrolysis (O'Connor, 1970). The linearity of the curve in the 6–11 pH range shows approximate first-order-rate dependence on hydroxide ion concentration, with an average rate constant of $(3 \pm 1) \times 10^5\ M^{-1}\ hr^{-1}$. The activation energy at pH 7.7 is *ca.* 19 kcal $deg^{-1}\ mol^{-1}$.

Hydrolysis of dibromoacetonitrile proceeds *via* dibromoacetamide to dibromoacetic acid, at a half-life of 21 days at pH 7.4, and 19 hr at pH 9.0. The intermediate, dibromoacetamide, exists only in small quantities since amide hydrolyses are much faster than corresponding nitrile hydrolyses (Hammett, 1940; Krieble and Noll, 1939).

The most stable of the degradation products is dibromoacetic acid. It hydrolyzes to glyoxylic acid at a half-life of about 300 days at 25°, pH 7.4. This decomposition path, which is indicated by the formation of bromide ions, is the same as for dichloroacetic acid (Larsson, 1946). Larsson was unable to isolate the glyoxylate ion, presumably due to rapid oxidation to oxalic acid. Oxalic acid slowly decarboxylates to carbon dioxide (Dinglinger and Schroer, 1937, 1938). Thus, the hydrolytic decomposition of DBNPA proceeds *via* dibromoacetonitrile, dibromoacetamide, dibromoacetic acid, glyoxylic acid, and oxalic acid to carbon dioxide, ammonia, and bromide ions.

Reaction with Nucleophiles and Light. DBNPA, like the brominated malononitrile (Freeman, 1969), reacts rapidly with a number of reducing nucleophiles such as I^-, HS^-, HSO_3^-, $S_2O_3^{2-}$, and SO_3^{2-} to form cyanoacetamide. Cyanoacetamide is hydrolyzed during workup to cyanoacetic acid, its amide, and malonic acid. These products are also observed on exposure to light.

DBNPA in Presence of Soil. The disappearance of DBNPA solutions in the presence of soil (Table III) could be due to hydrolysis, adsorption, chemical degradation, or microbial degradation. Hydrolysis in slurries of pH less than 7 is negligible. For example, at pH 6, the half-life for hydrolysis is 155 hr, but the disappearance in soil slurries of pH 5.8 is between 6 and 15 hr. Disappearance by adsorption on soil is possible; however, adsorption is negligible in at least one case, No. 70 in Table III, since the filtrate from an aqueous suspension of soil No. 70 decomposed DBNPA at the same rate as the original soil slurry. Thus, the decomposition of DBNPA in the presence of soil probably is due to either chemical or microbial degradation.

Fate in the Environment. The data on the rates and products of DBNPA allow an assessment of the fate of DBNPA in the environment. The hydrolysis of DBNPA is very rapid above pH 6, and the degradation products are less toxic to microorganisms than DBNPA. The most stable of the hydrolysis products, dibromoacetic acid, is about 100 times less toxic to fish than DBNPA. In addition, dibromoacetic acid is biologically degraded, and similar compounds are destroyed by soil bacteria (Jensen, 1960). Sunlight degrades DBNPA in water at rates which become significantly fast, relative to hydrolysis, at a pH less than 5. Rapid reaction occurs with a variety of nucleophilic reagents to form cyanoacetamide, a compound which is biodegradable and which can hydro-

Table IV. Disappearance of DBNPA by Contact with Soil

Soil sample number	pH of aqueous slurry	Percent organic carbon	% sand	% silt	% clay	Description	$\tau_{1/2}$, hr
21	~7.5	0.46	72.4	23.2	4.4	Sandy loam	4
29	4.8	2.37	38	41	21	Loam	12
37	5.8	2.26	13.6	64	22.4	Silty loam	15
45	6.5	1.16	59	28	13	Sandy loam	15
70	5.8	5.7	83	11.6	5.4	Loamy sand	6
80	5.1	1.68	10	62	28	Silty clay loam	25
83	4.8	1.86	28.6	47	24.4	Loam	15

lyze further to biodegradable materials such as malonic acid (Krieble and Noll, 1939; Malaney and Gerhold, 1969). Chemicals or microorganisms in soil can decompose DBNPA. Microbial degradation of DBNPA has been demonstrated by the use of tracer techniques. For example, DBNPA-2-^{14}C yielded 40% $^{14}CO_2$ after 2 weeks in the presence of unaerated waste treatment sludge (Wolf, 1971). Thus, hydrolytic, photolytic, chemical, and microbial decomposition of DBNPA and its degradates suggests that these compounds will not persist in the environment.

The question of bioconcentration of DBNPA in the environment must be considered along with the persistence of the compound. Metabolic studies on rats (Rose, 1971) with DBNPA-2-^{14}C indicate that DBNPA is cleared rapidly from the body. This fact and the low lipid affinity of DBNPA ($P_{octanol/H2O}$ = 6.1) suggest a low potential for bioconcentration (Wolf, 1971).

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Persistence of Endothall in Aquatic Environment as Determined by Gas-Liquid Chromatography

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A gas chromatographic method was used to determine the residues of endothall in both the water and hydrosoil of a farm pond and of laboratory aquaria. The bulk of endothall added to the aquaria remained in the water during the course of the experiment. Both in the pond and in the aquaria, the herbicide persisted in the hydrosoil for a longer period than in the water. In the pond treated with approximately 2 ppm of endothall, the herbicide could not be detected in the water and top 1 in. of the hydrosoil 36 and 44 days after treatment, respectively. In the aquaria treated with 2 and 4 ppm, endothall was reduced to nondetectable levels in the water within 7 days after treatment. It took 2 and 4 weeks for the herbicide in the hydrosoil to reach a level of less than 0.1 ppm in the aquaria treated with 2 and 4 ppm, respectively. The rate of endothall dissipation in the aquaria was similar at both application rates.

The herbicide endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) has been found to be effective in controlling certain submersed aquatic weeds (Frank *et al.*, 1961; Walker, 1963). In cases where the treated water is to be consumed by humans and/or livestock or used for irrigation, it becomes imperative that we have information on the persistence of the herbicide in the aquatic environment. Hiltibran (1962) studied the persistence of endothall in pond water, both in aquaria and under field conditions. In the field, endothall applied at 0.3 to 10 ppm could not be detected after an average of 2.5 days and a maximum of 4 days. In aquaria, endothall at these rates was detectable for a much longer period. The rate of disappearance of endothall varied directly with the amount of organic material and organisms present in the water. In studies dealing with the disappearance of the di-*N,N'*-dimethylcocoamine salt of endothall, Walker (1963) found that detectable residues of the herbicide had disappeared within 8 days following application of 0.3 ppm and within 2 weeks for 0.6 ppm. However, 1 to 3 ppm of the herbicide took up to 25 days to disappear. Frank and Comes (1967) observed that in a pond treated with 1 ppm of the di-*N,N'*-dimethylcocoamine salt of endothall, the herbicide could not be detected 24 days after treatment. Yeo (1970) reported that in some ponds treated with the dipotassium salt of endothall, the initial concentrations of 0.3 to 1.4 ppm dissipated to less than 0.03 ppm in 8 to 20 days, while in others the average dissipation was about 71% during the same period. In growth pools treated with 0.5 to 4 ppm of endothall, about half the initial concentration had disappeared within 12 days.

In most of the above studies, endothall residues were determined using a flaxseed bioassay method first described by Hiltibran (1962). This method has the limitations inherent in a bioassay, namely it is indirect, and is more time consuming, less precise, and less quantitative than a chemical assay. Frank and Comes (1967) used a

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