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Photolysis Rates of (2,4,5-Trichlorophenoxy)acetic Acid and 4-Amino-3,5,6-trichloropicolinic Acid in Natural Waters

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Photoreactions of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) and 4-amino-3,5,6-trichloropicolinic acid (picloram) were studied in distilled water, natural water samples, fulvic acid solutions, and solutions containing iron (III) and/or hydrogen peroxide to determine the effects of dissolved natural substances on the photolysis rates of these herbicides. Most of the experiments were conducted with sunlight as the light source and with dilute solutions of 3,4-dichloroaniline (DCA) as an outdoor actinometer. When reaction quantum yields determined in this study were used, near-surface half-lives for direct photolysis were computed to be 15 days for 2,4,5-T and 2.2 days for picloram during late summer at latitude 40° N, in close agreement with observed values. Humic substances in natural water samples and a commercial fulvic acid enhanced near-surface photolysis rate constants of 2,4,5-T with similar efficiencies, as indicated by the linear dependence of the rate constants on the UV absorbance of the waters. 2,4,5-Trichlorophenol was a major product of the humic-induced photoreactions. Humic substances, even at the highest concentrations usually observed in natural waters, had only a minor enhancing effect on the photolysis rate of picloram. Preliminary studies indicated that photocatalytic processes involving iron species and peroxides may contribute to the sunlight-induced reaction of 2,4,5-T in acidic, weakly absorbing natural waters.

A number of recent studies have examined the influence of natural substances on photochemical transformations in aquatic systems (Draper and Crosby, 1981; Mill et al., 1980; Zepp et al., 1981). Such studies, with varying degrees of success, have considered effects of natural substances on the photoproducts and defined relationships between water composition and photolysis rate. The present work was performed as part of an environmental exchange agreement between the United States and the Soviet Union in an attempt to develop better kinetic equations and to further examine their generality. (2,4,5-Trichlorophenoxy)acetic acid (2,4,5-T) and 4-amino-3,5,6-trichloropicolinic acid (picloram) were chosen for study based on considerations of their direct light absorption rates, analytical methodologies, and previous photochemical data.

Both 2,4,5-T and picloram are herbicides that are used to control undesirable brush and woody plants. Although it has been suggested that sunlight-induced photoreactions of 2,4,5-T (Kenaga, 1974; Crosby and Wong, 1973) and picloram (Hedlund and Youngson, 1972) make a significant contribution to the environmental dissipation of these herbicides, few studies have focused on their photochemical behavior in natural waters.

Previous research has indicated that the photochemistry of 2,4,5-T and picloram may occur by several pathways. The direct absorption of sunlight by these chemicals leads to loss of ring chlorines as well as other reactions (Crosby and Wong, 1973; Glass, 1975; Hall et al., 1968; Mosier and

Guenzi, 1973). Various photosensitizers have been shown to accelerate the photolysis of 2,4,5-T (Crosby and Wong, 1973) and picloram (Glass, 1975), raising the possibility that natural substances in aquatic environments may sensitize the sunlight-induced degradation of these pesticides (Miller et al., 1980). Finally, studies by Mill et al. (1980) have shown that free radicals are generated upon exposure of natural waters to sunlight, and the side chains of substituted phenoxyacetic acids similar to 2,4,5-T are known to be oxidized by attack of free radicals (Brown et al., 1964).

Several studies have appeared concerning the sunlight photolysis rates of 2,4,5-T and picloram in water. Crosby and Wong (1973) reported that 17% of 2,4,5-T photodecomposed upon exposure to sunlight for 4 days, indicating a direct photolysis half-life on the order of 15 days in California summer sunlight. The thorough studies of picloram photolysis by Hedlund and Youngson (1972) indicate that this herbicide undergoes direct photolysis in sunlight about 1 order of magnitude more rapidly than 2,4,5-T. Conflicting studies have appeared concerning the quantum yields for direct photoreaction of picloram, with Mosier and Guenzi (1973) reporting a value of 0.005 at 366 nm and Glass (1975) reporting a quantum yield of 0.04 at 254 and 313 nm. No reports have appeared concerning the reaction quantum yield for 2,4,5-T in water, nor have there been any systematic studies of the photolysis rates of 2,4,5-T or picloram in natural waters.

In this report, we compare kinetic results concerning the photolysis of 2,4,5-T and picloram in distilled water and particle-free natural water samples obtained from several rivers in the United States. Results of these comparisons indicate that 2,4,5-T is considerably more susceptible to photosensitized reaction in natural water than is picloram. Evidence is presented that the humic substances in the

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natural water samples are mainly responsible for the photosensitization.

MATERIALS AND METHODS

Reagents. 2,4,5-T (99+%), picloram (99%), and 2,4,5-trichlorophenol were analytical standards obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC, and were used as received. The fulvic acid used in these studies was purchased from Contech ETC, Ottawa, Canada. γ -Methoxy-*m*-(trifluoromethyl)-butyrophenone (MTB) was synthesized and purified as previously described (Miller and Zepp, 1979a). 3,4-Dichloroaniline (DCA) was purchased from Aldrich Chemical Co. and purified by recrystallization. Natural water samples were obtained from the top meter of the water bodies and were refrigerated at 5 °C prior to use. The water samples and initially prepared solutions of substrates were centrifuged for 1 h at 15 000 rpm in a Sorvall RC-2 centrifuge to remove particulates. Commercial hydrogen peroxide (30%) was used after dilution. Iron(III) was introduced into solution by adding microquantities of a stock solution of ferric sulfate in sulfuric acid or by using H₂O₂-oxidized FeSO₄ solution.

Equipment. Aqueous solutions of DCA, 2,4,5-T, and MTB were analyzed by using a Micromeritics Model 7170 high-pressure liquid chromatograph (HPLC) equipped with a variable-wavelength UV detector and a column packed with ODS-2. Acetonitrile-water mixtures were used as the mobile phase in the case of DCA and MTB; 60% acetonitrile-water buffered at pH 3.5 (0.025 M acetate) was used for the analysis of 2,4,5-T. Picloram solutions were analyzed by employing a Tracor 950 HPLC equipped with UV detector and a Reeve Angel Partisil-10 SAX column that was eluted by an aqueous phosphate buffer. Electronic absorption spectra were obtained by using a Perkin Elmer Model 356 spectrophotometer. A Schoeffel Reaction Chemistry System equipped with a high-intensity monochromator and 1000-W xenon lamp was used in the quantum yield studies.

Experimental Procedures. The quantum yield for reaction of 2,4,5-T at 290 nm (ϕ_r) was determined by using previously described methods (Zepp, 1978). The spectral bandwidth of the light employed was 4 nm, and the molar absorptivity of 2,4,5-T (2280 L mol⁻¹ cm⁻¹) used for computing ϕ_r was averaged over this spectral region.

Outdoor experiments were conducted by exposing air-saturated solutions of the substrates (less than 10 μ M) to sunlight during March and April between 1030 and 1530 EST at Athens, GA. Three separate experiments were conducted in each natural water sample, fulvic acid solution, and distilled water. The solutions were irradiated in 13-mm Pyrex test tubes that were mounted on a rack; weather conditions ranged from clear to overcast with rain, and temperatures were in a range of 15–25 °C. An outdoor actinometer system consisting of 1.0 \times 10⁻⁵ M DCA in distilled water (Miller et al., 1979) was employed. The DCA actinometers were exposed in the same way as the other solutions and were replaced on a daily basis. Generally, three actinometer solutions were analyzed immediately after exposure. Sunlight attenuation effects were estimated by studying the photolysis kinetics of MTB (5.0 \times 10⁻⁶ M) dissolved in the natural water samples. The MTB solutions were irradiated for 30 min on a clear day during late March.

In all cases substrate concentrations in dark controls were carefully monitored during the period of sunlight exposure and no dark reactions were noted.

The chemical yields of 2,4,5-trichlorophenol derived from 2,4,5-T photolysis were determined by direct analysis

of the reaction mixtures using HPLC or by acidification followed by analysis of organic extracts of the mixtures using gas-liquid chromatography (3% SE-30 column).

Treatment of Data. Relative rate constants for the photoreactions $k_{\text{obsd}}(x)$ were computed by using eq 1. In

$$k_{\text{obsd}}(x) = \frac{\ln(C_0/C_t)_x}{\sum \ln(C_0/C_t)_{\text{DCA}}} \quad (1)$$

eq 1 C_0 and C_t represent substrate concentrations initially and after sunlight exposure for time t . The subscripts x and DCA refer to data for the pesticides and for the DCA actinometer, respectively. For exposures of more than 1 day, the denominator of eq 1 was computed by summing the values of $\ln(C_0/C_t)_{\text{DCA}}$ for each day during the exposure.

To assess the error involved in these computations, the standard deviation of $k_{\text{obsd}}(x)$, σ_{obsd} was computed from the standard deviations of the numerator and denominator of eq 1 by using the relation (Strobel, 1960)

$$\sigma_{\text{obsd}} = k_{\text{obsd}}(x)(y^2 + z^2)^{1/2} \quad (2)$$

The symbols y and z represent ratios of the standard deviations of numerator and denominator to their means. In the quantum yield studies for 2,4,5-T, first-order rate constants were computed from the linear regression $\ln(C_0/C_t)$ vs. time by using a program that was written by N. L. Wolfe (1982). A Hewlett-Packard HP-41C calculator was used for the calculations.

Computer Program. Rate constants for the direct photoreactions in sunlight were computed by using a modified version of the SOLAR program that is described by Zepp and Cline (1977). The modified version, which is called GCSOLAR, uses a subroutine based on equations and parameters described by Green et al. (1980) and Baker et al. (1980) to compute the ground-level solar ultraviolet irradiance. The subroutine was provided by Dr. Karen Smith and Dr. Raymond Smith of the University of California—San Diego and Santa Barbara, respectively. The calculations were performed by using a Digital Equipment Corp. PDP 11/70 with an IAS operating system.

RESULTS AND DISCUSSION

Direct Photoreactions. As discussed above, direct absorption of sunlight by 2,4,5-T and picloram leads to various photoreactions in water. In the environment these direct photoreactions compete with indirect photochemical reactions that involve natural substances. Here we present quantitative results concerning direct photolysis rates in pure water. These results are compared with kinetic results observed in natural waters later in the paper.

The direct photochemistry of ionizable pesticides such as 2,4,5-T and picloram is linked to the various species that are present in aqueous solution. Both 2,4,5-T and picloram exist as two or more equilibrated species in water, with the distribution of the various forms dependent on hydrogen ion activity (Figure 1). Previously obtained data indicate that the pK_a of 2,4,5-T is 2.88 (Nelson and Faust, 1969) and that of the picloram zwitterion is 1.97 (Osteryoung and Whittaker, 1980). With hydrogen ion activities that range from pH 5 to pH 9, a range that is commonly found in natural waters, the anions I and II (Figure 1) account for over 99% of the dissolved species present in pure water. Accordingly, we focused our efforts on quantitating the direct photolysis rates of these anionic species.

The kinetic data for direct photolysis of weakly absorbing solutions (absorbance less than 0.1) of 2,4,5-T and picloram can be described by first-order rate expressions, as expected from earlier studies (Zepp, 1978; Hedlund and

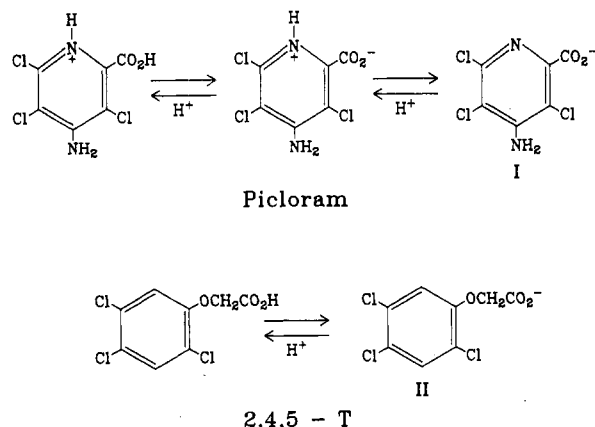


Figure 1. Various forms of picloram and 2,4,5-T in aqueous solution.

Table I. Molar Absorptivities (ϵ_λ) and Reaction Quantum Yields (ϕ_r) for Direct Photolysis of 2,4,5-T, Picloram, and 3,4-Dichloroaniline (DCA)

	2,4,5-T	picloram	DCA
ϕ_r	0.015 ± 0.001^a	0.066 ± 0.008^b	0.052 ± 0.001^c
	$\epsilon_\lambda, M^{-1} cm^{-1}$		
wavelength, nm	2,4,5-T	picloram	DCA
297.5	2140	667	1660
300	1170	502	1620
302.5	494	361	1520
305	201	257	1370
307.5	98	181	1170
310	46	124	928
312.5	24	83	767
315	16	57	602
317.5	14	43	465
320	13	31	342
323.1	11	21	237
330		12	58
340		4.7	21

^a ϕ_r (II) was obtained by using monochromatic (290 nm) light in phosphate-buffered solution at pH 7. ^b ϕ_r (I) was estimated from the sunlight photolysis rate constant in distilled water by using eq 3. ^c Taken from Miller et al. (1979).

Youngson, 1972). The first-order rate constant is proportional to the specific light absorption rate k_a and the reaction quantum yield ϕ_r .

The reaction quantum yield, ϕ_r (II), for the longest wavelength electronic absorption band of the 2,4,5-T anion II was determined at pH 7.0 by using monochromatic light (290 nm) and a procedure that is described elsewhere (Zepp, 1978). From three separate determinations, a value of 0.015 ± 0.01 was calculated (Table I). To ascertain the applicability of these results to outdoor conditions, solutions of 2,4,5-T in distilled water were exposed to sunlight along with outdoor actinometers consisting of DCA in distilled water. The rate constant for this actinometer, averaged over a full year at latitude $40^\circ N$, is 1.7 day^{-1} (Miller et al., 1980). When eq 1 was used, values of $k_{\text{obsd}}(\text{II})$, the rate constant for the photoreaction of II ratioed to that observed for the DCA actinometer, were computed. The mean value from three experiments was 0.014 ± 0.002 . By use of the spectral data and reaction quantum yields for 2,4,5-T and for DCA (Table I), rate constants were calculated for direct photolysis of these substrates employing the GCSOLAR computer program. A theoretical value of $k_{\text{obsd}}(\text{II})$, computed by using GCSOLAR outputs for spring in Athens, GA, was 0.015, in almost exact agreement with the experimental $k_{\text{obsd}}(\text{II})$. By use of GCSOLAR, the near-surface half-life for direct photolysis

Table II. Properties of Natural Water Samples and Fulvic Acid Solutions Used in the Studies

no.	water body	pH	a_{312}, m^{-1}	organic C, mg/L
1	distilled water	5.5	0.12	<1
2	Wakulla River, Wakulla Springs, FL	8.21	5.1	6
3	Oconee River, Athens, GA	4.95	6.7	
4	Williamson River, Chiloquin, OR	7.25	41	13
5	Aucilla River, Lamont, FL	6.80	109	21
6	Econfina River, Perry, FL	4.2	191	33
7	Fenholloway River, Foley, FL	7.55	476	69
1	fulvic acid (Contech)	6.0	135	17
2	fulvic acid (Contech)	6.0	405	55

of II during midsummer at Davis, CA, was calculated to be 15 days, in close agreement with the half-life of 15 days computed from data previously reported by Crosby and Wong (1973) for this time and place.

Encouraged by the successful application of GCSOLAR to the computation of outdoor kinetic results for 2,4,5-T, we used the program to calculate a reaction quantum yield, $\phi_r(\text{I})$, for the direct photolysis of picloram species I in sunlight. Equation 3 was used for the calculation (Zepp, 1978; Dulin and Mill, 1982):

$$\phi_r(\text{I}) = \frac{k_a(\text{DCA})\phi_r(\text{DCA})k_{\text{obsd}}(\text{I})}{k_a(\text{I})} \quad (3)$$

The ratio of the specific sunlight absorption rates, $k_a(\text{DCA})/k_a(\text{I})$, was computed by using the GCSOLAR program with the molar absorptivities of the pesticides as inputs (Table I). For spring in Athens, GA, the computed ratio is 7.3 during the 5-h period centered at midday, the time when our sunlight experiments were conducted. The reaction quantum yield for DCA, $\phi_r(\text{DCA})$, is known to equal 0.052 (Miller et al., 1979). The relative photolysis rate constant for picloram species I, $k_{\text{obsd}}(\text{I})$, was computed to be 0.17 ± 0.02 by using eq 1 and the kinetic data obtained in the exposures of dilute solutions of I and DCA to sunlight. When these various kinetic parameters and eq 3 were used, a value of 0.066 ± 0.008 was calculated for $\phi_r(\text{I})$. This value is about 65% larger than the value of 0.04 reported by Glass (1975) but over 1 order of magnitude greater than the value of 0.005 reported by Mosier and Guenzi (1973). The difference may in part be ascribable to uncertainties in the solar irradiance values used by GCSOLAR. On the other hand, the previous studies were conducted by using picloram concentrations that were 100 times larger than those used here, and it is possible that the reaction quantum yield for picloram may be reduced by self-quenching at higher concentrations.

Employing GCSOLAR with the estimated $\phi_r(\text{I})$ of 0.066 and the spectral data in Table I as inputs, we computed that the photolysis half-life for picloram at shallow depths during late summer in Walnut Creek, CA, is 2.2 days, in approximate agreement with experimental results (half-life of 2.3 days) reported by Hedlund and Youngson (1972) for late August.

Natural Water Studies. Photoreactions of 2,4,5-T and picloram were studied in particle-free natural water samples and in solutions of a commercially obtained fulvic acid. Physical and chemical properties of these water samples are summarized in Table II. The dissolved substances in natural waters have been previously shown to influence photolysis rates through light attenuation, a retarding

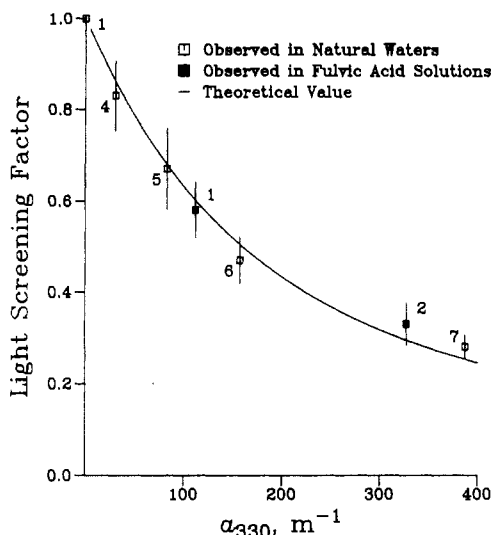


Figure 2. Comparison of theoretical calculation and experimental data for the light attenuation effect on photolysis rates of MTB in natural waters. Bars indicate standard error. Numbers refer to the water samples listed in Table II.

effect, and through mediation of indirect photoprocesses, an accelerating effect (Miller et al., 1980). In addition, humic substances are known to retain or form complexes with certain organic chemicals (Carter and Suffet, 1982; Guy and Narine, 1980; Parris, 1980; Khan, 1973) and such interactions may alter the photochemistry of pesticides in natural waters.

Both 2,4,5-T and picloram absorb solar radiation in the middle-UV region (Table I), and past studies have indicated that middle-UV light (280–340 nm) is most effective at promoting photosensitized oxidations in natural waters (Zepp and Schlotzhauer, 1981a). Therefore, for the purpose of estimating light attenuation effects in the natural waters studied here, it was assumed that middle-UV radiation was mainly responsible for inducing the photoreactions observed in sunlight. To quantitate light attenuation effects, the phenyl ketone, MTB, was irradiated by sunlight in distilled water, in the natural water samples, and in the fulvic acid solutions employing the same experimental conditions that were used with the studies of picloram and 2,4,5-T. Previous studies have shown that MTB absorbs sunlight most strongly at about 330 nm (Miller and Zepp, 1979b). Direct photolysis of MTB is so rapid (half-life less than 30 min of midday sunlight) that competing indirect photoreactions make an insignificant contribution to its photoreaction in natural water samples (Miller and Zepp, 1979a). The light screening factor (S) was assumed to equal the ratio of the first-order rate constant for MTB photolysis in the natural waters or fulvic acid solutions to that observed in distilled water. The light attenuation effect also was computed for comparison by using eq 4:

$$S = \frac{1 - e^{-\alpha_{330}l}}{\alpha_{330}l} \quad (4)$$

In eq 4 α_{330} is the (Napierian) absorption coefficient of the water at 330 nm and l is the light path length, which was assumed to equal 1.00 cm for the tubes used in our sunlight experiments. The experimental and theoretical values of S were found to be in agreement within experimental error (Figure 2). The relative rate constants for photolysis of 2,4,5-T and picloram, $k_{\text{obsd}}(x)$, were approximately corrected for the light attenuation effect by using eq 5, where

$$k_{\text{surf}} = k_{\text{obsd}}(x)/S \quad (5)$$

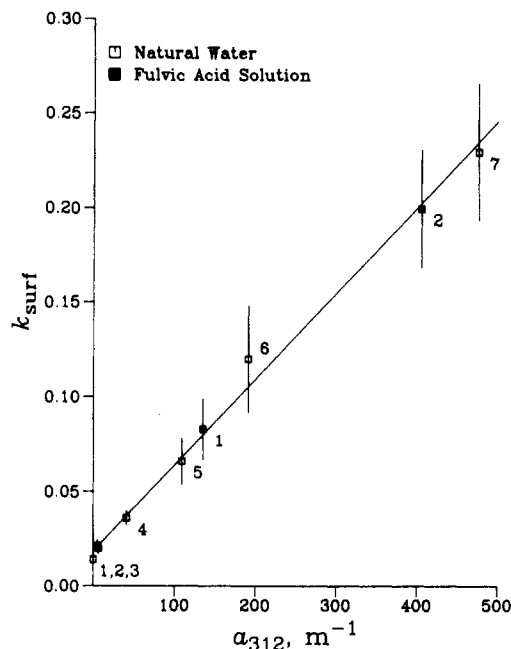


Figure 3. Relative rate constants for photoreaction of 2,4,5-T in aqueous solutions exposed to sunlight. Bars indicate accumulated standard deviation, and numbers correspond to water samples in Table II.

k_{surf} is the photolysis rate constant that applies to an infinitely thin layer of the natural water solution. A similar approach to correcting for light attenuation has been previously discussed by Miller et al. (1980).

Comparisons of the photolysis kinetic data and the properties of the natural waters and fulvic acid solutions indicated that k_{surf} for both 2,4,5-T and picloram increased with increasing light absorption by the solutions in the UV region (300–400 nm). In the case of 2,4,5-T the k_{surf} values were linearly related to the absorption coefficients of the water at 312 nm, α_{312} (Figure 3). This wavelength was selected to facilitate comparisons to other natural waters as discussed below; linear relations were obtained for other wavelengths in the UV as well. By applying a linear least-squares fit to the data for the natural waters, an excellent empirical relation was derived (eq 6):

$$k_{\text{surf}} = 0.018 + (4.5 \times 10^{-4})\alpha_{312} \quad r^2 = 0.993 \quad (6)$$

The intercept of the plot corresponds within experimental error to the value of $k_{\text{obsd}}(\text{II})$, the relative direct photolysis rate constant for the 2,4,5-T anion. Kinetic data for photoreaction of 2,4,5-T in fulvic acid solutions fell almost exactly on the line that described the natural water data (Figure 3). These results are consistent with the hypothesis that the dissolved fulvic acids in the natural waters were accelerating some sunlight-induced reaction of 2,4,5-T in competition with the direct photoreaction. The excellent correlation between k_{surf} and α_{312} , coupled with other results that indicate close similarities in the spectroscopic properties of the humic substances in several of the waters studied here (Zepp and Schlotzhauer, 1981b), provide further evidence that the photochemical effects of humic substances from various locations are very similar (Zepp et al., 1981).

Results obtained with picloram were more difficult to interpret (Figure 4). As with 2,4,5-T, a ragged trend of increasing k_{surf} values with increasing water absorption was evident. The k_{surf} values for the natural waters, however, fell into a much narrower range for picloram than for 2,4,5-T, with a minimum of 0.17 and a maximum of 0.25. This result provides more extensive confirmation of earlier

Table III. Estimated Contribution of Humic Substance Mediated Photoreactions to Photolysis Rate of Picloram and 2,4,5-T in Selected Natural Waters

water body	a_{312}, m^{-1}	2,4,5-T		picloram	
		k_{surf}	fraction mediated by humic substances	k_{surf}	fraction mediated by humic substances
Delaware Bay	1.6 ^a	0.018	0.04	0.18	<0.01
Chesapeake Bay	2.8 ^a	0.019	0.07	0.18	<0.01
Patuxent River	7.7 ^a	0.021	0.16	0.18	<0.01
Lake Superior	1.2 ^a	0.018	0.03	0.18	<0.01
Lake Michigan	1.3 ^a	0.018	0.03	0.18	<0.01
Lake Huron	1.0 ^a	0.018	0.03	0.18	<0.01
Lake Erie	2.2 ^a	0.019	0.05	0.18	<0.01
Douglas Lake, MI	10 ^a	0.023	0.20	0.18	<0.01
Lake Herrington, KY	7.5 ^a	0.021	0.15	0.18	<0.01
Okefenokee SW, GA	113	0.069	0.74	0.20	0.10
Quincy Bog, NH	128	0.076	0.76	0.20	0.11
Protva River, USSR	25	0.029	0.38	0.18	0.02
Luzha River, USSR	23	0.028	0.36	0.18	0.02
Volga River, USSR	22	0.028	0.35	0.18	0.02

^a Computed from the field data of Calkins (1975) by assuming the mean wavelength of the Robertson meter is 312 nm (Smith and Calkins, 1976).

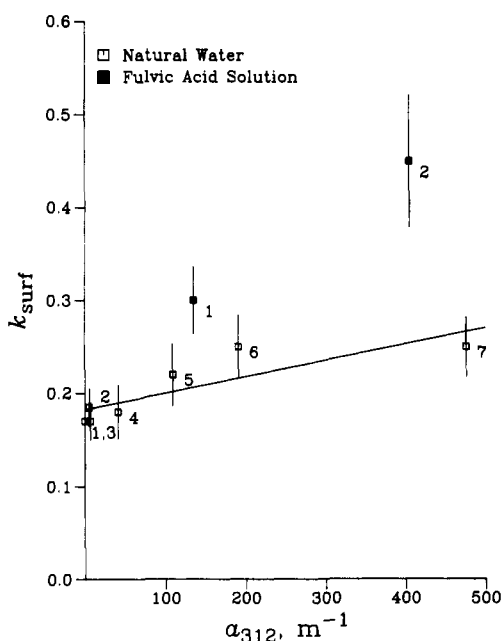


Figure 4. Kinetic data for photolysis of picloram in natural waters and fulvic acid solutions. Bars indicate cumulative standard error. Numbers refer to the water samples in Table II.

findings that the photolysis rate constant of picloram is not strongly influenced by the substances in natural waters (Hedlund and Youngson, 1972). A linear least-squares treatment of the natural water data yielded the relation shown in eq 7, clearly a much poorer fit than was observed

$$k_{\text{surf}} = 0.18 + (1.8 \times 10^{-4})a_{312} \quad r^2 = 0.714 \quad (7)$$

with the 2,4,5-T data. The intercept, which agreed closely with the relative direct photolysis rate constant of picloram, was 1 order of magnitude larger than that observed with 2,4,5-T (eq 6). The slope of the picloram plot, however, was similar to that for 2,4,5-T, indicating that the component of k_{surf} attributable to photolysis involving the natural substances was about the same for both herbicides.

An interesting difference between the kinetic results for picloram and 2,4,5-T is revealed in comparisons of the fulvic acid sensitized reactions of the two pesticides. Although the fulvic acid sensitized the 2,4,5-T reaction with almost the same efficiency as the substances in the natural waters (Figure 3), the fulvic acid was about 3 times more efficient in the case of the picloram reaction (Figure 4).

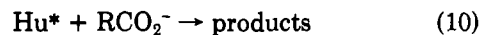
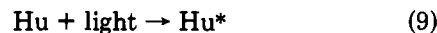
(The slope of k_{surf} vs. a_{312} was 6.7×10^{-4} for the fulvic acid solutions.). Natural waters contain dissolved substances other than fulvic acids, and the simplest explanation of these data is that these other substances somehow inhibit the fulvic-mediated photoreaction.

The empirical relations (eq 6 and 7) derived from these studies are used in Table III to estimate some typical contributions of photoreactions mediated by humic substances to the photolysis rate constants of picloram and 2,4,5-T in various water bodies of the United States and the USSR. In computing the humic component, the following equation was used where β is the slope in Figures 3 and 4.

$$\text{fraction mediated by humic substance} = \frac{\beta a_{312}}{k_{\text{surf}}} \quad (8)$$

The results indicate that the humus concentrations in most natural waters are too low to significantly affect the photolysis rate of picloram. The competing process, direct photolysis, is too rapid. On the other hand, the estimates indicate that the photolysis of 2,4,5-T can be significantly accelerated by humic substances in some natural waters of the United States and USSR. In large lakes, e.g., the Great Lakes, and bays, humic-mediated photoreactions of 2,4,5-T play a minor role in its photochemistry.

Mechanism. Two general mechanisms can explain the experimental results. The first mechanism involves photosensitization by the humic substances, Hu, in the water (eq 9 and 10).



This mechanism could include direct reaction between the substrate and the electronically excited sensitizer, Hu^* , or reaction between substrate and some oxidizing species derived from Hu^* . As discussed in earlier work, theory predicts and experiments have confirmed that the rate constants for photosensitized reactions under conditions employed in these studies are proportional to sensitizer concentration and thus to the UV absorbance of the solutions (Zepp and Schlotzhauer, 1981a,b).

A second possibility involves reversible formation of a photoreactive complex between the humic substances and the herbicides (eq 11):



Assuming that complex formation and dissociation are

rapid compared to its photoreaction, the second mechanism would require the rate expression

$$k_{\text{surf}} = \frac{Kk_c[\text{Hu}]}{1 + Kk_c[\text{Hu}]} \quad (12)$$

This expression predicts that the k_{surf} value would exhibit a hyperbolic relationship with the humic concentration in the water, [Hu], plateauing with increasing [Hu]. When only a small fraction of the pesticide is complexed and $Kk_c[\text{Hu}] \ll 1$, however, k_{surf} would be proportional to [Hu] and thus to a_{312} . The data for picloram (Figure 4) do appear to be hyperbolic rather than linear, but there is too much experimental error to clearly distinguish these possibilities. Further research is required to determine which of these possible mechanisms is operative with these herbicides.

The close similarity of the slopes of all the plots in Figures 3 and 4 points to a common mechanism for both herbicides. Both herbicides are carboxylic acids, suggesting that photochemical reactions centered on the carboxylate group may be involved. Product studies indicated that 2,4,5-trichlorophenol (TCP) is a major product of the sunlight-induced photoreaction of 2,4,5-T in natural waters and fulvic acid solutions in which over 75% of the photoreaction was mediated by humic substances. The chemical yield of TCP was considerably higher (80% yield) in a fulvic acid solution ($a_{312} = 135 \text{ m}^{-1}$) than in the natural water samples obtained from the Aucilla River, the Econfinia River, and the Fenholloway River (<10% yield). The other products that formed were inseparable from the fulvic acid under the HPLC conditions that were used. Studies by Brown et al. (1964) have shown that phenoxyl radicals decarboxylate to form the corresponding phenols in water. Thus, the humic-sensitized reaction of 2,4,5-T may involve electron transfer to the humus (or a reactive species formed therefrom) to form (trichlorophenoxy)acetoxyl radicals as intermediates as shown in eq 13 and 14:



The variation in yields of TCP may reflect differences in the behavior of the free radical intermediates in fulvic acid solutions and the natural water samples.

Photocatalytic Reactions of 2,4,5-T. Most of the studies reported in preceding sections considered sunlight-induced reactions that involve organic constituents in natural waters. It is known, however, that many natural waters contain inorganic substances that may enhance photolysis rates of chemicals in natural waters (Draper and Crosby, 1981, 1983; Zika, 1980; Sinelnikov, 1976).

Preliminary kinetic data indicate that iron(III) species, at concentrations that have been observed in natural waters, can induce rapid transformations of 2,4,5-T, particularly in the presence of trace concentrations of hydrogen peroxide (Table IV). The catalysis by trivalent iron was slowed in the natural waters, but even so, large enhancements were observed in the weakly absorbing acidic natural waters. The iron-induced photoreaction was completely inhibited in the strongly alkaline Wakulla River water. The photocatalytic effect of the iron species is probably attributable to oxidation of the 2,4,5-T by oxygen-containing free radicals that are generated upon sunlight absorption by hydroxy or humic complexes of the iron(III) (Balzani and Carrasiti, 1970). The retardation of the reactions in natural waters can be attributed to changes in the speciation of the iron(III) or to scavenging

Table IV. Influence of Trivalent Iron Species and Hydrogen Peroxide on Photolysis Rates of 2,4,5-T in Water

water type	pH	added [Fe(III)] _{tot} , M	[H ₂ O ₂], M	k_{obsd}
distilled water	5.5	0	0	0.015
distilled water	3.8	10 ⁻⁵	0	4.0
Oconee River	4.9	0	0	0.020
Oconee River	4.9	10 ⁻⁵	3 × 10 ⁻⁵	0.75
Aucilla River	6.9	0	0	0.06
Aucilla River	6.9	10 ⁻⁵	3 × 10 ⁻⁵	0.24
Fenholloway River	7.6	0	0	0.23
Fenholloway River	7.6	10 ⁻⁵	3 × 10 ⁻⁵	0.47
Wakulla River	8.3	0	0	0.020
Wakulla River	8.3	10 ⁻⁵	3 × 10 ⁻⁵	0.02

of the free radicals by natural substances such as fulvic acids and carbonate.

CONCLUSION

Results of these studies indicate that the photolysis rates of 2,4,5-T and picloram in sunlight are enhanced in various natural water samples compared to distilled water. The 2,4,5-T rate is more strongly influenced by humic substances in natural waters than is the rate of picloram photoreaction. Humic substances from various natural waters and a commercial fulvic acid photosensitized reaction of 2,4,5-T with similar efficiencies, as indicated by the linear dependence of the photolysis rate constant on the UV absorbance of the natural water. It is estimated that humic-induced photoreactions of 2,4,5-T account for the majority of its photoreaction in natural waters with concentrations of humic substances that exceed 15 mg of organic carbon/L.

Analysis of the kinetic data indicates that humic substances accelerate reactions of picloram with almost the same efficiency as observed with 2,4,5-T. Direct photolysis of picloram, however, is about 1 order of magnitude faster in sunlight than direct photoreaction of 2,4,5-T. Consequently, humic substances, even at the highest concentrations usually observed in natural waters, have only a minor enhancing effect on the photolysis rate of picloram. It is proposed that the photoreactions induced by humic substances involve electron transfer from the carboxylate group to the humic substance or to some oxidizing species derived therefrom.

Preliminary studies indicate that photocatalytic processes involving iron species and naturally occurring peroxides may contribute to the sunlight-induced reaction of 2,4,5-T in acidic, weakly absorbing natural waters. Additional research is required to determine the importance of iron in photoreactions of 2,4,5-T and other pesticides in the environment.

Registry No. 2,4,5-T, 93-76-5; picloram, 1918-02-1; 2,4,5-trichlorophenol, 95-95-4; water, 7732-18-5.

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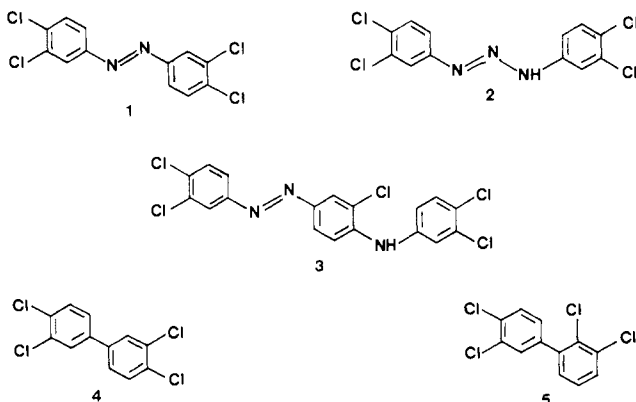
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Reductive Transformations of Nitrate with 3,4-Dichloroaniline and Related Compounds by *Escherichia coli*

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The transformation of 3,4-dichloroaniline (3,4-DCA) in partly anaerobic cultures of *Escherichia coli* in the presence of nitrate ions affords tetrachloroazobenzene, tetrachlorobiphenyls, and dihydroxy-tetrachlorobiphenyls, all in yields <1%. Major products are 3,4-dichlorophenol and a bis(dichlorophenyl)triazene. All products are rationalized in terms of an intermediate diazonium cation. Labeling studies using $\text{Na}^{15}\text{NO}_3$ and $[^{14}\text{C}]$ -3,4-DCA support this mechanism. When 3,4-dichloronitrobenzene is the substrate, it is reduced to tetrachloroazoxybenzene and tetrachloroazobenzene in both the absence and presence of added NaNO_3 .

The fate of chlorinated anilines in the environment has been the subject of much interest because of the widespread use of herbicides containing a chloroaniline moiety. These anilines, principally 4-chloroaniline and 3,4-dichloroaniline (3,4-DCA), are formed in soil when the herbicides are hydrolyzed; they are then transformed further by microbial action. From 3,4-DCA the transformation products in soil include 3,3',4,4'-tetrachloroazobenzene (1) (Bartha and Pramer, 1967; Bartha, 1968;



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Chisaka and Kearney, 1970; Sprott and Corke, 1971), 1,3-bis(3,4-dichlorophenyl)triazene (2) (Plimmer et al., 1970), and 3,3',4'-trichloro-4-(3,4-dichloroanilido)azobenzene (3) (Linke, 1970). Interest in these systems revived when Poland et al. (1976) noted the structural similarity of azo compound 1 and 2,3,7,8-tetrachlorodibenzo-dioxin and reported that both compounds are potent inducers of liver aryl hydrocarbon hydroxylase [see also Sundström et al. (1978) and Bunce et al. (1979)].

Pure cultures of bacteria transformed 3,4-DCA into 1, 2, and the isomeric tetrachlorobiphenyls 4 and 5, in addition to other unidentified materials. These processes took place only when nitrate was present and when the bacteria possessed a nitrate reductase enzyme (Corke et al., 1979; Shushan et al., 1981). It was proposed that these transformations involve the microbial reduction of NO_3^- to NO_2^- and that diazotization of the aniline then occurs [Corke et al., 1979; see also Minard et al. (1977)]. This was shown by intercepting the diazonium ion with 2-naphthol, and the formation of products 1, 2, 4, and 5 was suppressed. Diazotization appears to be a purely chemical, as opposed to a biological, process because the rate of diazotization from preformed NO_2^- and the aniline is the same whether or not the cells are present (Lammerding et al., 1982).

In this paper we present further studies on the mechanism of the transformation of 3,4-DCA by a representative bacterial species (*Escherichia coli*) used in the previous