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Effects of Halogenated Organic Compounds on Photosynthesis in Estuarine Phytoplankton*

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Chlorine oxidants (chlorine gas, sodium hypochlorite, and calcium hypochlorite) are used as biocides to control fouling in seawater cooled power generating plants and to kill pathogens in sewage effluents entering estuarine waters. Organisms killed include algae, fungi, bacteria, barnacles, oysters, and clams. Use of these chemicals in saline waters results in the formation of halogenated by-products (JOHANNESSON 1958, DUURSMA & PARSI 1976, DOVE 1970).

BEAN et al. (1978), in a study of chlorinated seawater, identified eighteen organic compounds generated by chlorination. Due to rapid substitution of bromine for chlorine, brominated rather than chlorinated by-products are formed (FARKAS et al. 1949, GALAL-GORCHEV & MORRIS 1965, MACALADY et al. 1977, SUGAM & HELZ 1977).

The effects of these chlorinated by-products on estuarine phytoplankton are not known. Experiments in this study examined singly the effects of fifteen commercially available compounds on photosynthesis by estuarine phytoplankton.

METHODS

Seawater containing native phytoplankton was pumped from the North Edisto River at E.P.A. Bears Bluff Laboratory on Wadmalaw Island 26 miles southwest of Charleston, South Carolina ($32^{o}40'N$, $80^{o}18'W$) seven miles inland from the Atlantic Ocean. It was usually turbid, containing 23-37 mg/L dry weight of suspended solids. Taxanomic classes of phytoplankton present during sampling included: Chlorophyceae, Cyanophyceae, and most commonly, Bacillariophyceae. pH was 7.8 ±0.1. Temperature and salinity (2 tests on separate dates) are given on Table 1.

Test apparatus consisted of a 7 ft^2 table located outdoors on which eight 37-L aquaria were arranged in a circle. Water was delivered to the aquaria from a central distribution tower at the rate of 40 L/h through rigid plastic tubing. Mean water

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turnover rate was 1.08 times/h ±2%. Approximate time for 99% replacement of water in the aquaria was 4.5 h (SPRAGUE 1969). Solutions of test compounds at nominal concentrations of 0.5 to 2.0 mg/L were metered into the aquaria using syringe pumps. Control aquaria were maintained with all experiments. After a 24 h exposure period, 100 mL samples were collected from each aquaria on two consecutive days. Larger organisms were removed from samples by straining through 0.110 mm nylon screening.

TABLE 1. Test Compounds and Conditions of Testing.

Compound and Purity	Temp (°C)		Salinity g/L	
	Day 1	Day 2	Day 1	Day 2
chloroform, b.p. 60-61	22	21.5	20	24
bromoform, m.p. 7-8	19.5	21.5	20	24
trichloroethylene, b.p. 85-87	20	19	20	18
tetrachloroethylene, b.p. 119-121	18	18	17.5	16.5
ethylene bromide, m.p. 9-10	23.5	25	21	22
2,4,6-tribromoanisole, m.p. 85-87	1.0	1.5	25	23
4-chlorophenol, unknown	13.5	12.5	23	24
4-bromophenol, unknown	10	7	25	25
2,4,6-trichlorophenol, m.p. 59-64	11	13	20	20
2,4,6-tribromophenol, m.p. 93-95	3.5	1.0	21	21
pentachlorophenol, m.p. 189-191	3.0	5.5	18.5	20
pentabromophenol, m.p. 224-226	5.0	7.0	21	21
phenol, 99.5%	21.5	24	25.5	25.5
haloamines, unknown	27	27.5	27	28.5
chloramine T, 96+%	23	24.5	27	24

Determinations of ^{14}C uptake were conducted in duplicate for each variable and control. Non-photosynthetic ^{14}C was measured in duplicate dark bottles. Samples were mixed by shaking and 25 mL aliquots of seawater were dispensed into 20 X 50 mm borosilicate screw-top culture tubes with teflon cap liners. ^{14}C sodium bicarbonate was added to each tube to give a concentration of 0.04 microcuries/mL and incubated 4 h at 20°C under 2691 lux of incident cool-white fluorescent lighting. After incubation cells were collected on 25 mm diameter 0.45 micrometer pore size membrane filters. Radioactivity was measured with a liquid scintillation spectrometer. Results for each period of sampling were calculated as percent of control uptake of ^{14}C on a net count per min basis.

Test compounds were technical grade (Eastman Kodak Co., Rochester NY) except phenol which was reagent grade (Mallinckrodt Chemical Works, St. Louis, MO; Table 1). Haloamines were synthesized in the aquaria by metering molar solutions of NaOCl into the intake water pipes and molar solutions of NH_4Cl into the aquaria. This resulted in a mixture of halogenated amines composed of mostly dibromoamine and tribromoamine (JOHNSON 1977). Stock solutions of the aliphatic compounds were prepared in

J.T. Baker Chemical Co. Polyglycol 200.® The amines and phenolic compounds were dissolved in neutral deionized water or alkaline deionized water.

Data presented in the tables of the results section were evaluated by multiple t-tests for statistical significance (α=0.05). Numbers represent the mean of two replicate samples.

RESULTS

The halogenated aliphatic hydrocarbons chloroform, bromoform and ethylene bromide caused no statistically measurable effects on ^{14}C uptake by estuarine phytoplankton. Trichloroethylene stimulated ^{14}C uptake at 0.5 to 1.0 mg/L. Tetrachloroethylene inhibited ^{14}C uptake only at 2.0 mg/L. Data are summarized on Table 2.

TABLE 2. Effects of Halogenated Aliphatic Hydrocarbons on Estuarine Phytoplankton Expressed as Percent of Control.

Compound	mg/L			Control cpm
	0.5	1.0	2.0	(100%)
chloroform	101	103	99	2132
bromoform	112	107	104	2300
trichloroethylene	127*	127*	118	1423
tetrachloroethylene	98	95	87*	2150
ethylene bromide	113	103	97	2383

* Significant at α=0.05

Phenol, 4-bromophenol and 2,4,6-trichlorophenol produced no significant effects at the concentrations tested while 2,4,6-tribromoanisole, 2,4,6-tribromophenol and 4-chlorophenol inhibited ^{14}C uptake 6 to 24 percent of control. The most inhibitory compounds in this group were pentachlorophenol and pentabromophenol (Table 3).

TABLE 3. Effects of Halogenated Phenolic Compounds on Estuarine Phytoplankton Expressed as Percent of Control.

Compound	mg/L			Control cpm
	0.5	1.0	2.0	(100%)
2,4,6-tribromoanisole	81*	79*	76*	3322
4-bromophenol	107	108	96	1088
4-chlorophenol	97	94*	94	2513
2,4,6-tribromophenol	96*	88*	89*	1930
2,4,6-trichlorophenol	106	95	103	6536
pentachlorophenol	39*	16*	2*	4731
pentabromophenol	4*	-1*	-1*	3282
phenol	103	99	93	1477

* Significant at α=0.05.

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Additional ^{14}C uptake tests were performed on the two pentahalophenolic compounds at a broader range of concentrations of 0.125 to 2.0 mg/L. Data from these tests were compared to data from an earlier static screening study (ERICKSON & FREEMAN 1978) and evaluated for statistical significance. In the screening study, laboratory clones were grown under static conditions in enriched seawater under standard conditions of light and temperature (20°C, 5382 lux) and exposed to 0.125 to 2.0 mg/L of test compound. Cell division was measured by optical density. The results from the two systems were similar with the ^{14}C method appearing to be slightly more sensitive (Tables 4,5).

TABLE 4. Comparison of the Effects of Pentachlorophenol on Phytoplankton from the N. Edisto River Estuarine Water and Static Culture of Laboratory Clones Expressed as Percent of Control.

Organism	Pentachlorophenol mg/L				
	0.125	0.25	0.5	1.0	2.0
Glenodinium halli	93	83	65	12*	12*
Skeletonema costatum	103	92	89	71*	15*
Thalassiosira pseudonana	97	93	44*	9*	7*
Isochrysis galbana	100	45*	4*	5*	2*
Mixed phytoplankton from flowing seawater	69*	51*	39*	16*	2*

Control optical density, G. halli= 0.298, S. costatum= 0.275, T. pseudonana= 0.211, I. galbana= 0.207. Control cpm mixed phytoplankton= 4731. * Significant at α=0.05.

TABLE 5. Comparison of the Effects of Pentabromophenol on Phytoplankton from the N. Edisto River Estuarine Water and Static Culture of Laboratory Clones Expressed as Percent of Control.

Organism	Pentabromophenol mg/L				
	0.125	0.25	0.5	1.0	2.0
Glenodinium halli	106	104	86	11*	14*
Skeletonema costatum	90	82	90	47*	8*
Thalassiosira pseudonana	85	80*	86	11*	11*
Isochrysis galbana	53*	35*	12*	17*	13*
Mixed phytoplankton from flowing seawater	57*	35*	4*	-1*	-2*

Control optical density, G. halli= 0.185, S. costatum= 0.270, T. pseudonana= 0.207, I. galbana= 0.157. Control cpm mixed phytoplankton= 3270. * Significant at α=0.05.

The haloamines produced by metering NaOCl and NH_4Cl into the aquaria were more inhibitory than NaOCl or NH_4Cl separately. Chloramine T, a stable cyclic compound, was not inhibitory to photosynthesis over a range of 0.5 to 1.0 mg/L. These compounds are compared on Table 6.

A further test was performed to determine if by-products remained which influenced photosynthesis after the initial

TABLE 6. Effects of NaOCl, NH_4Cl, Haloamines and Chloramine T on Estuarine Phytoplankton Expressed as Percent of Control.

Compound	Concentration mg/L			Control cpm
	0.25	0.5	1.0	(100%)
NaOCl	78*	65*	54*	9325
NH_4Cl	109	110	95	4253
Haloamines	40*	17*	1*	4655
Chloramine T	101	95	97	774

* Significant at α=0.05.

oxidative process was completed. Seawater dosed with 1.0 mg/L of haloamines was compared to an untreated sample. The treated sample was split into two fractions. One fraction was treated with sodium thiosulfate to neutralize the oxidative action of the haloamines. A control and the two fractions were inoculated with 1.5 X 10^4 cells/mL of I. galbana, a marine chrysophyte, and incubated 5 h under conditions described in methods. Uptake in the control and the thiosulfate treated fractions were approximately equal. The untreated portion was greatly inhibited (^{14}C uptake=8% of control).

DISCUSSION

Relatively high concentrations of the test compounds were required to inhibit uptake of ^{14}C by N. Edisto River phytoplankton in flowing seawater. Pentabromophenol was the most inhibitory compound followed by the haloamines and pentachlorophenol. The remaining compounds were either ineffective or caused less than 25% stimulation or inhibition. Except in the immediate area of a spill or an outfall, concentrations used in this study are not likely to occur in the environment.

Not all of the tested compounds are known to be generated by the chlorination of seawater. Pentachlorophenol and pentabromophenol are commercially synthesized products used for wood preservation and could enter the environment on treated wood, by spillage or used as anti-fouling agents.

Haloamines may be produced in quantity as a result of chlorination of ammonia containing sewage effluents. In this study haloamines were more effective as a biocide than sodium hypochlorite. This group of compounds needs specific identification and further study of the individual compounds.

When halogenated compounds of similar structure were compared (example pentachlorophenol and pentabromophenol), the brominated compound was the most inhibitory.

Bromoform has been reported as the principal by-product of the chlorination of seawater (HELZ & HSU 1978, CARPENTER & SMITH 1978, BEAN et al. 1978). No significant effects on phytoplankton were observed in this study. Since bromoform is

an animal carcinogen, it may be of importance to marine animals if carried through food chains.

The use of native phytoplankton in flowing seawater under existing conditions of light, temperature, turbidity, salinity and pH allowed testing under conditions that resemble the environment. A further advantage was that several concentrations of each compound was tested in a relatively small system without contamination of large areas of the surrounding estuary. Although no control of physical conditions was possible, results were similar but more sensitive than static tests using laboratory clones.

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