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Photosensitizing Compounds in Extracts of Drinking Water

Abstract. *By means of a photodynamic bioassay, with* Paramecium caudatum, *photosensitizing compounds have been demonstrated in extracts of finished drinking water in the United States. These findings are of interest in view of a demonstrated association between photodynamic toxicity and carcinogenicity. Neither the origin nor the identity of these compounds has been determined.*

Using a series of defined polycyclic compounds, we have demonstrated a significant positive association between photodynamic toxicity to *Paramecium caudatum* and carcinogenicity (*1*). From such data, a photodynamic bioassay was developed for purposes of large-scale screening, as a rapid and sensitive presumptive index, for carcinogenicity attributable to polycyclic compounds (*2*). The assay has been applied to organic extracts and fractions of particulate atmospheric pollutants from numerous urban and nonurban sources in the United States (*3*). We now report significant concentrations of photosensitizing compounds, as judged by the photodynamic bioassay, in extracts of finished drinking water.

Extracts from finished drinking water are routinely collected by the Water Supply Section of the Division of Environmental Engineering of the U.S. Public Health Service for the identification and measurement of organic chemicals. For this purpose, large volumes of water are filtered through columns of activated carbon, at a flow rate of 0.25 gallon (0.94 liter) per minute by standardized procedures (*4*). The carbon filters are subsequently extracted with chloroform and then with 95 percent ethanol. Such extracts, collected in 1961 from finished drinking water of four cities with predominantly nonindustrial watersheds in the northeastern United States, were used in our studies (Table 1). With the exception of city D, the concentrations of these chloroform extracts are less than the recommended Public Health Service standard of 200 μg/liter (*5*).

Fine aqueous suspensions of solvent-free water extracts, freshly prepared over a range of 1000 to 1.56 μg/ml, were incubated with suspensions of *P. caudatum* in the log-phase of growth, and then irradiated with long-wave ultraviolet light from a high-pressure mercury vapor tube with a peak at 360 mμ (*2*). The endpoints of photosensitizing effects were the times required for immobilization of 90 percent of the motile cells (LT_{90}), as determined with a stereoscopic microscope through which all wells were scanned, with an arbitrary observational limit of 1.5 hours. Mean LT_{90} values (Table 2) were determined from a minimum of three independent pairs of replicate assays, and dose-response curves were prepared for each set of extracts. As a standard, benzo-[*a*]pyrene (BP) was simultaneously assayed at tenfold dilutions from 1.0 to 0.001 μg/ml; this compound is a convenient standard in such nondilution types of photodynamic assays, and the results may properly be expressed with reference to the standard without implying the obligate presence of BP in the test materials assayed. A composite dose-response curve based on eight paired replicate assays of BP is presented in Fig. 1, together with dose-response curves for water extracts of city A. These extracts have steep, approximately parallel dose-response curves, and a similar relation obtains for the extracts from the other three cities.

The photodynamic activities of the BP standard and each pair of extracts were determined by interpolation of the respective concentrations (C_{30}) yielding LT_{90} values of 30 minutes (Fig. 1). Relative photodynamic potencies were determined from the C_{30} values and computed both on a weight basis, as microgram equivalents of BP per gram of extract, and on a volume basis, as micrograms of BP equivalents per gallon of water (Table 3). On a weight basis the potency of the chloroform extracts exceeds that of the corresponding alcohol extracts, although these potencies span only a tenfold range. With the exception of the sample from city A, which has a disproportionately low concentration of

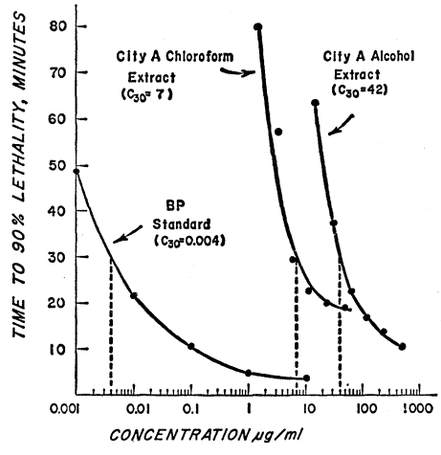

Fig. 1. Photodynamic assay results and method of interpolation for concentrations (C_{30}) producing 90-percent lethality (LT_{90}) within 30 minutes.

Table 1. Sources and yields of water extracts. CE, chloroform extract; AE, alcohol extract. (The same abbreviations are used in Tables 2 and 3.)

City	Type of water	Volume of water filtered (gal)	Weight of extracts (g)		Extract yield (μg/gal)	
			CE	AE	CE*	AE
A	Surface	5040	0.223	1.776	44	352
B	Surface and springs	5000	1.984	3.096	397	619
C	Surface	2400	1.481	2.207	617	920
D	Surface	5205	4.331	2.282	832	438

* Corresponding values for cities A, B, C, and D are 11.6, 104.9, 163.0, and 219.8 respectively.

Table 2. Photodynamic activities of water extracts. DT, extracts toxic to cells during dark-incubation and before irradiation.

Extract concentration (μg/ml)	Mean time to 90 percent lethality (min) of chloroform and alcohol extracts							
	City A		City B		City C		City D	
	CE	AE	CE	AE	CE	AE	CE	AE
1000		DT		DT		DT		DT
500		11	DT	9	DT	17	13	19
250		14	15	14	DT	19	17	21
125		17	17	16	16	20	19	25
100	DT							
62.50		22	18	23	17	32	23	33
50.00	19							
31.25		38	21	33	24	55	41	57
25.00	20							
15.62		64	32	60	37	80	73	79
12.50	22							
7.81			72		59		>90	
6.25	30							
3.12	57							
1.56	80							

chloroform extract, a similar relation also obtains when potency is expressed on a volume basis (Table 3). As seen, the sum of the photodynamic potencies for both extracts ranges from 0.06 to 0.16 μg of BP equivalent per gallon of water (16 to 42 μg/m^3 or 0.016 to 0.042 μg/liter). Thus, on the basis of an average photodynamic potency of 0.12 μg of BP equivalents per gallon of water, and if the average water consumption is 0.5 gallon daily, then the consumption of photosensitizing compounds, expressed for convenience as BP equivalents, would be of the order of 22 μg/yr.

While these data are too restricted for formal analysis, no association is apparent between photodynamic potencies and concentrations of extracts in water (Tables 1 and 3). We have recently demonstrated a similar independence between photodynamic potencies and concentrations of organic pollutants in urban air (*3*).

As judged by thin-layer chromatography and fluorescence spectroscopy, there was no BP in the water extracts; however, compounds with fluorescence maxima at 398 and 418 mμ were detected in both chloroform and alcohol extracts, and at higher concentrations in the former. Although these compounds have not been identified, solubility and spectroscopic characteristics indicate their polycyclic nature. Further, these compounds were probably responsible for photodynamic activity in the water extracts, such activity being generally associated with polycyclic structures (*1*). While the origin of these compounds is unknown, it is likely that a relatively stagnant mass of reservoir water acts as a catchall for polycyclics derived from a variety of sources, including soil (*6*), fallout from polluted air, and domestic or industrial pollution of raw water (*7*). When one considers the ubiquitous distribution of BP in the environment, its absence from water extracts is, perhaps, surprising. The possibility was further considered that the photosensitizing compounds detected in these assays may have originated, not from the water itself, but from the activated carbon in the filters, as this is not extracted by solvents before use. For this reason, in a blank experiment, 100 g of activated carbon, as routinely used in the filters, was successively extracted with chloroform and 95-percent ethanol. No polycyclic compounds were detected in the alcohol extract, although traces of unidentified monoazapolycyclic compounds were detected in the chloroform extract by thin-layer chromatography and fluorescence spectroscopy. In view of the similar order of magnitude in the photodynamic potencies of the extracts it is unlikely that such activity is due to polycyclics originating from the carbon filters.

That there are high concentrations of photosensitizing compounds in extracts of finished drinking water is of additional interest in view of the statistical association between photodynamic toxicity and carcinogenicity. However, photodynamic toxicity affords only a presumptive index of carcinogenicity attributable to polycyclic compounds (*1*), and the photodynamic assay does not obviate the need for direct carcinogenicity tests.

Polycyclic compounds, including carcinogens, have been found in raw or unfinished water (*7*), but apparently not in small samples of finished drinking water (*8*). Weak carcinogenicity has been reported in extracts of industrially polluted and unfinished water, and still less carcinogenicity in extracts of finished water (*9*). More recently, both chloroform and alcohol extracts were tested separately by repeated subcutaneous injection in neonatal mice and found to be noncarcinogenic; it was, however, necessary to administer these pollutants in small doses because of their toxic effects (*10*). Whether polar solvating agents in alcohol extracts, such as synthetic detergents (*4*), may increase the cell uptake of, or in some other way interact with, potentially biologically active agents in chloroform extracts has not been determined.

Table 3. Apparent photodynamic potency of water extracts and derived from C_{30} values. Photodynamic potency is expressed as micrograms of benzo[*a*]pyrene equivalent per gram of extract or per gallon of water.

City	C_{30} values of extracts (μg/ml)		Apparent photodynamic potency of extracts			
			Weight basis (μg/g)		Volume basis (μg/gal)	
	CE	AE	CE	AE	CE	AE
A	7.0	42.0	571	95	0.025	0.033
B	17.5	35.0	229	114	.091	.071
C	22.5	80.0	178	50	.110	.046
D	40.0	90.0	100	44	.083	.019

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Biodegradation of the Gamma Isomer of Benzene Hexachloride in Submerged Soils

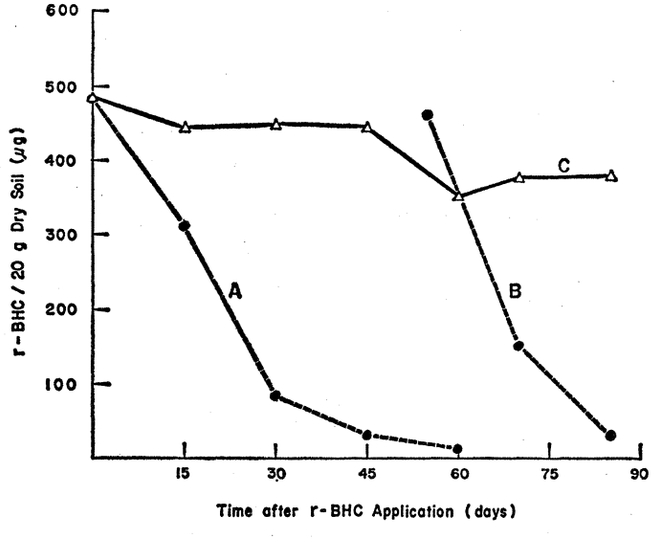
Abstract. *Determination of the residual gamma isomer of benzene hexachloride (γ-BHC) by gas chromatography showed that the insecticide persisted longer in sterilized flooded soils than in nonsterilized flooded soils. A second addition of γ-BHC to one of the nonsterilized soils. (55 days after the first application) disappeared more rapidly than the first addition. These results strongly indicate biodegradation of γ-BHC in flooded soils.*

The gamma isomer of benzene hexachloride (γ-BHC) has been shown to be effective in controlling the rice stem borer *Chilo suppressalis* (Walker) when the insecticide is directly applied to the standing water in a rice field (*1*). Widespread use of γ-BHC to control this major insect pest of rice could lead to substantial increases in yields of this crop in the tropics. However, because of the prolonged persistence reported for this insecticide in unflooded soils (*2–5*) it is essential that information be obtained on the fate of γ-BHC in flooded soils before widespread use of the insecticide is advocated. Information obtained by employing bioassay techniques, as well as by a nonspecific colorimetric procedure for determining residual γ-BHC, indicates that the insecticide persists in unflooded soils for periods ranging from 3½ to 11 years (*2–5*). This insecticide has thus been described as highly recalcitrant (*6*).

To evaluate the significance of biodegradation of γ-BHC in flooded soils, we compared its persistence in sterilized samples of Philippine rice soil to its persistence in nonsterilized samples (*7*). The test tubes containing the soil samples were flooded, treated with γ-BHC (*8*) and incubated in the greenhouse. At appropriate intervals, three replicate tubes of each soil were removed, the γ-BHC was extracted, and the amount of insecticide present was determined quantitatively by gas chromatography (*9*). Identification of γ-BHC was based on R_T (retention time) values, and the amount present was calculated by relating the peak heights to those of analytical standards.

The γ-BHC disappeared more rapidly from nonsterilized flooded soil samples than from sterilized flooded soil samples (Figs. 1 and 2). The γ-BHC disappeared more quickly from the nonsterilized Luisiana clay than from the nonsterilized Maahas clay (compare curve *A*, Fig. 1, with Fig 2). The greater loss of insecticide from the nonsterilized flooded soils indicated that there was active participation of the microflora in the degradation of γ-BHC.

Loss of insecticide from the sterilized soils (Figs. 1 and 2) can be attributed to volatilization. To check this point, at the end of the incubation period we analyzed the cotton plugs which were used to seal the tubes; we found 45 to 64 μg of γ-BHC in the plugs from the tubes of sterilized soil and from 18 to 19 μg in the plugs from



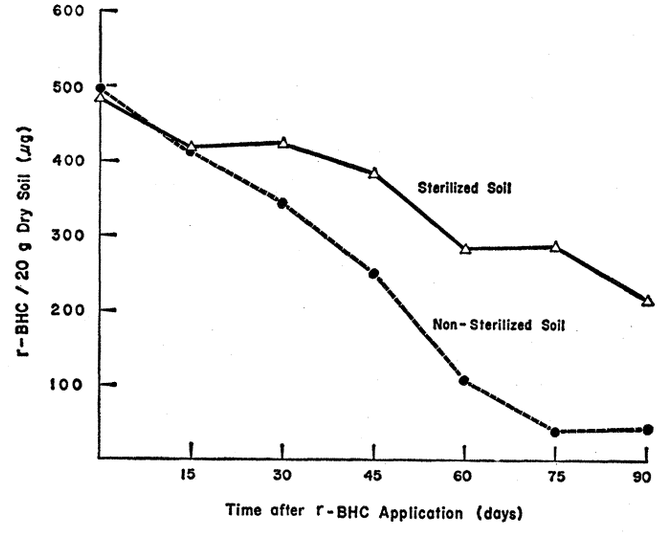


Fig. 1 (left). Persistence of γ-BHC in submerged Luisiana clay. *A*, Nonsterilized soil, first application of γ-BHC; *B*, nonsterilized soil, second application of γ-BHC; *C*, sterilized soil. Fig. 2 (right). Persistence of γ-BHC in sterilized and nonsterilized submerged Maahas clay.