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Toxicity of Arsenic and PCB to a Green Alga (*Chlamydomonas*)

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High arsenic and PCB concentrations have recently been reported for Green Bay, Lake Michigan (VEITH 1972, ANDERSON et al. 1978, HOLM 1979). The source of arsenic has been localized to a chemical company producing arsenic herbicides in Marinette, Wisconsin. While the production ceased several years ago, the arsenic pollution persists because of ground water infiltration of leachates from storage piles of arsenic-containing wastes. The sources of PCBs are less well known. The sources are probably non-point in nature, and originate in part from local dumpings of PCBs previously used as dielectrics and hydraulic fluids.

The levels of these contaminants, e.g., up to 10 µg As/L and 0.45 µg PCB/L could present a hazard to the biota. The effect of arsenic and PCB on algae have been studied for each of these toxicants acting separately, but not in combination. PCB at 10-100 µg/L inhibit chlorophyll production and RNA synthesis (KEIL et al. 1971, SINCLAIR et al. 1977). On the other hand, arsenate at 1 µM behaves as an antimetabolite occupying sites for phosphate (PLANAS & HEALEY 1978). Based on the different toxic mechanisms for the two compounds, it is hypothesized that they will show independent joint action (FINNEY 1971), i.e., that they will not form a particular toxic combination. The purpose of this study was to establish toxic thresholds and possible interactive effects of arsenic and a PCB (Aroclor 1248) for the green alga Chlamydomonas isolated from Lake Michigan.

MATERIALS AND METHODS

A slightly modified version of the culture medium of GUILLARD (1975) was used as growth medium for Chlamydomonas. Major nutrients, EDTA, and vitamins were added as specified, as well as all trace elements except boron which was added as boric acid at a final concentration of 0.6 mg/L, a factor ten lower than required by the recipe. This lower boron level was chosen so as to better corespond to the levels specified in other standard media and to concentrations found in natural waters.

The test alga Chlamydomonas was obtained from the University of Michigan Great Lakes and Marine Waters Center at Ann Arbor.

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The alga was isolated from Lake Michigan by Richard Steinberger. The mean diameter was found to be about 4.9 μm using an electronic particle counter. A stock of actively growing Chlamydomonas was maintained by weekly transfers to fresh nutrient media.

Algal assays were carried out essentially according to the EPA procedure: Algal Assays Procedure Bottle Test (U.S. EPA 1978). The adopted procedure included maintenance of the cultures at 15°C in incubators with continuous 20 W "cool-white" fluorescent lighting. Aseptic techniques were applied throughout the bioassays. The algae were kept in autoclaved, cotton-plugged Erlenmeyer flasks filled with 100 mL nutrient solution. The flasks were shaken every 24 hours to maintain the cultures in homogeneous suspension.

First, two screening experiments were conducted to establish the toxicity ranges of the two toxicants using dibasic sodium arsenate and PCB (Aroclor 1248). Next, a combination experiment was set up based on these ranges. For fortifications, aqueous solutions of dibasic sodium arsenate were prepared, while the PCB was diluted with hexane, acetone, and water, successively. As an example, the 111 μg/L PCB flasks contained 711 mg acetone/L and 69 mg hexane/L, while the other PCB flasks contained proportionately lower concentrations of hexane and acetone. Control flasks with these solvents, but without PCB, were also set up.

After fortification, the flasks were inoculated with 5×10^6 cells/L. Algal growth was monitored at least once a week for about four weeks. Algal population and size distribution were measured by means of a Particle Data Inc. Electrozone/Celloscope, Model 112 LTH, coupled to a PDP 8 computer and a Teletype terminal.

Data analysis, based on maximum standing crop was performed at the 95% confidence level using a two-tailed t-test for the independent joint action hypothesis (CHRISTENSEN et al. 1979).

RESULTS AND DISCUSSION

The results of the algal assays are plotted in Fig. 1. The screening tests (Figs. 1a, b) with PCB and arsenic added singly were carried out first to establish the toxic ranges of these compounds. Next, the combination experiment (Fig. 1c) was conducted based on these levels. Interactions could not be excluded outside the toxic ranges of the individual toxicants, but were considered most likely to occur there.

From Fig. 1a, PCB depresses growth at concentrations between 11 and 111 μg/L. This is consistent with the levels of 10-100 μg/L found by KEIL et al. (1971) to inhibit chlorophyll production and RNA synthesis of a marine diatom (Cylindrotheca

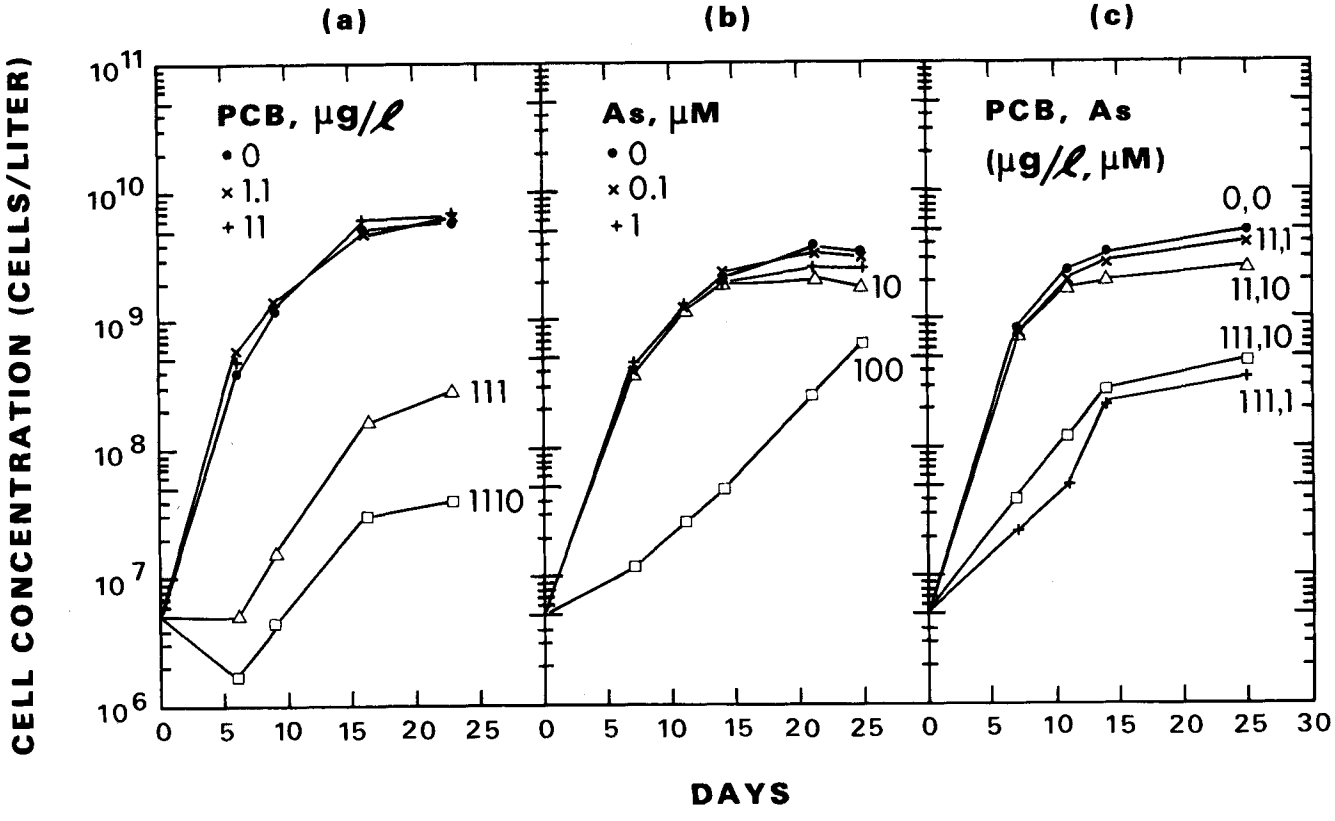

Fig. 1 Growth response of Chlamydomonas fortified with (a) PCB (Aroclor 1248), (b) dibasic sodium arsenate, and (c) these toxicants in combination.

closterium) and with similar findings of SINCLAIR et al. (1977) who measured the respiration rate of Chlorella vulgaris. None of the control flasks with hexane and acetone, but without PCB, showed any significant growth depression due to these solvents.

From Fig. 1b, arsenate is inhibitory to growth at a concentration of 1 µM. By comparison, PLANAS & HEALEY (1978) demonstrated growth inhibition for five different algae, including Chlamydomonas reinhardtii, at arsenate concentration between 1 and 100 µM.

The combination experiment conducted with fortifications of PCB and As was performed at the levels indicated in Fig. 1c. No interactive effects are immediately apparent based on this figure. Note that despite the antiseptic techniques used throughout the experiments, a contaminant organism dominated at any concentrations of or above 111 µg PCB/L and 100 µM As, added singly or in combination. The organism was unicellular, clear, flagellated, and slightly longer and narrower than Chlamydomonas. The organism may have been present in the original cultures received from Ann Arbor, Michigan.

A statistical analysis of data from the combination experiment is given in Table 1. The hypothesis tested is independent joint action, that is a product model for the combined yield. It is evident that this hypothesis can not be disproved at any of the two levels, since the associated t-values are less than 4.30 for a p equal to 0.05. Consequently, PCB and As act independently in the above sense, and do therefore not form a particularly toxic combination. Since the actual concentrations in Green Bay of these contaiminants are less than or equal to about 10 µg As/L and 0.45 µg PCB/L it would therefore appear that current levels of PCB and As present little, if any, hazard to the algae in this area. However, several factors not considered here could conceivably change this situation. These factors include: differential species sensitivity to these toxicants, geographic differences in phytoplankton sensitivity to PCB (FISHER et al. 1973), differences in toxicity depending on levels of major nutrients, and possibly greater upconcentration of PCB and As in the algal cells under long-term continuous culture conditions.

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TABLE 1

Statistical analysis of cell concentration (10^6 cells/L) of Chlamydomonas on day 25 in the PCB - arsenic combination experiment.

Level*	Test	Fortification	Replicate 1	Replicate 2	Mean	Normalized mean	Predicted normalized mean, product model	t*
	a	None	5830	5680	5760±110	1.00±0.02		
	b		2630	4080	3360±1030	1.00±0.31		
	c		4470	5060	4770±420	1.00±0.09		
1	a	PCB	6030	6920	6480±630	1.13±0.11		
	b	As	1720	2050	1890±230	0.56±0.18		
	c	PCB,As	3200	1680	2440±1080	0.51±0.23	0.63±0.21	-0.54
2	a	PCB	290	296	293±4	0.051±0.001		
	b	As	1720	2050	1890±230	0.56±0.18		
	c	PCB,As	291	600	466±218	0.094±0.047	0.029±0.0093	1.92

*PCB: 11 µg/L (1), 111 µg/L (2)
As: 10 µM

**t(2): 4.30, p = 0.05

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