Effects and Uptake of Chlorinated Naphthalenes in Marine Unicellular Algae[1]

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Chlorinated naphthalenes (chloronaphthalenes) comprise a group of compounds used for a variety of industrial purposes such as oil additives for cleaning sludge and petroleum deposits from engines, impregnating automobile capacitors, and electroplating stopoff compounds. Goerlitz and Law (1972) stated that these compounds are manufactured for uses similar to those of polychlorinated biphenyls (PCBs). Their chlorine content ranges from monochloronaphthalene to octachloronapthalene, and commercial formulations are composed of mixtures of isomers. They are manufactured under the names Halowax® (Koppers Co., Inc., U.S.A.), Nibron Waxes (Bayer, Inc., West Germany), and Seekay Waxes (Imperial Chemical Industries Ltd., U.K.).

Most toxicity studies on chlorinated naphthalenes have been done on mammals and birds. In general, toxicity is directly related to chlorine content, although octachloronaphthalene has a relatively low toxicity. Penta- and hexachloronaphthalene are usually the most toxic. Cornish and Block (1958) reported that mono- and dichloronaphthalene were metabolized rapidly by rabbits, tetrachloronaphthalene was metabolized slowly, but penta-, hepta-, and octachloronaphthalene were not metabolized. Walker and Wiltshire (1955) reported that two species of soil bacteria degraded monochloronaphthalene while using it as the sole carbon source. Okey and Bogan (1965) reported that mono- and dichloronaphthalene were metabolized by sewage sludge bacteria.

We know of no reports that deal with possible ecological effects, including toxicity to lower organisms or uptake by them. This paper describes studies on effects and uptake of three commercial mixtures of chlorinated napthalenes in marine unicellular algae.

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Materials and Methods

The formulations used here were Halowax 1000, 1013, and 1014. Halowax 1000 is a liquid, consists of 60% mono- and 40% dichloronaphthalene, and contains 26% chlorine by weight. Halowax 1013 is a solid compound of 10% tri-, 50% tetra-, and 40% pentachloronaphthalene. It contains 56% chlorine by weight. Halowax 1014 is a solid compound of 20% tetra-, 40% penta-, and 40% hexachloronaphthalene. Its chlorine content is 62%.

The algae *Dunaliella tertiolecta*, *Chlorococcum* sp. (chlorophytes), *Nitzschia* sp., and *Thalassiosira pseudonana* (bacillariophytes) were exposed to 0.1, 0.5, and 1.0 ppm (parts per million, mg/l) of each chloronaphthalene formulation. All species were obtained from the Culture Collection of Algae, Indiana University, and maintained in stock cultures in nutrient-enriched artificial seawater (Rila Products, Teaneck, NJ) as described by Hollister et al. (1975).

In growth studies, algae were grown in either 25 ml of medium in optically matched test tubes that were shaken at approximately 60 excursions per minute on a New Brunswick Model G2 shaker fitted with an ErlAngle® clamp, or in 50 ml of medium in 125-ml Erlenmeyer flasks shaken at the same rate. In the former method, 0.5 ml of an algal suspension (optical density = 0.100 at 525 nm) was added to 24.5 ml of medium and biomass estimated by optical density each day for seven days thereafter at 525 nm on a Fisher electrophotometer. In the latter method, 1.0 ml of inoculum was added to 49.0 ml of medium and optical density measured after seven days.

Growth medium was artificial seawater of 30 parts per thousand salinity at full- and half-nutrient strengths (Hollister et al. 1975). Temperature was 20°C ± 0.5°C under 5,000 lux illumination from cool, white flourescent tubes with alternating 12-hr periods of light and darkness. Halowax was added in 0.1 ml of pesticide-free acetone; the same volume of acetone was added to control cultures. At least nine separate tests were carried out on each species at each concentration. Effects on growth were expressed as percentage of controls and significance of differences was determined by the t test for two means (Brownlee 1965).

In uptake studies, three cultures of *Chlorococcum* sp. were grown in 100 ml of full-nutrient medium for six days, at which time Halowax, in 0.1 ml of acetone, was added to yield final nominal concentrations of 5, 10, 25, 50, or 100 ppb (parts per billion, µg/l). On the next day, the algae were harvested by centrifugation, washed four times with uncontaminated medium, and analyzed for chloronaphthalenes by gas chromatography. Each value given here is the average of three exposures.

For residue analyses, algal samples were weighed in 125 x 15 mm (OD) test tubes and extracted four times with 5-ml portions of acetonitrile for 30 sec with a model PT10-ST Willems Polytron (Brinkman Instruments, Westbury, NY). The tubes were centrifuged after each extraction and the acetonitrile transferred to a 200 x 25 mm (OD) screw top test tube. Twenty-five ml of 2% aqueous sodium sulfate and 5 ml of hexane were added to the combined acetonitrile extracts. The tubes were sealed with a teflon-lined cap and shaken for one minute. After the solvent phases separated, the upper hexane layer was transferred with a dropping pipette to a 25-ml Kuderna-Danish concentrator tube. The hexane extraction was repeated three more times and the combined extracts concentrated to 0.5 ml by evaporation. This concentrate was transferred with three 1-ml portions of hexane to a 200 x 9 mm (ID) chromatographic column containing 3.0 g of Florisil topped with 2.0 g anhydrous sodium sulfate previously washed with 10 ml hexane. The Halowaxes were eluted from the column with 20 ml of 5% ethyl ether in hexane. Extracts were evaporated to 1.0 ml to remove ether and, if necessary, diluted to appropriate volume for analysis by electron capture gas chromatography.

Determinations were performed on Varian Aerograph Models 2100 and 1400 gas chromatographs equipped with 182 x 2 mm (ID) glass columns packed with 2% SP2100 and 0.75% SP2250: 0.97% SP2401 on 100/120 mesh Supelcoport. The operating parameters were: oven temperature 185°C; injection temperature 200°C; detector temperature 215°C; nitrogen carrier gas flow rate 25 ml/l/min.

The recovery rate of Halowax from fortified algae was greater than 85%. Residue concentrations were calculated on a wet-weight basis without correction for percentage recovery. All samples were fortified with an internal standard prior to analysis to evaluate the integrity of the results.

Results

None of the Halowax formulations was highly toxic (Table 1). The least chlorinated formulation, Halowax 1000, was most toxic, but a concentration as high as 1.0 ppm did not inhibit growth by as much as 50%. The table shows that 0.1 ppm reduced growth of *D. tertiolecta* significantly and that 0.5 and 1.0 ppm reduced growth of all species significantly.

Halowax 1013 did not inhibit growth of any species at 0.1 ppm, or of *D. tertiolecta*, *T. pseudonana*, or *Chlorococcum* sp. at 0.5 ppm. There was significant inhibition of growth of *Nitzschia* sp. at 0.5 ppm and *T. pseudonana* at 1.0 ppm.

Halowax 1014, the most highly chlorinated formulation, had no significant effect on growth of any species at any concentration.

TABLE 1

Growth of marine unicellular algae, as percentage of controls, in three chloronaphthalene formulations. Degrees of freedom are given in parentheses.

Test Concentration ppm	Percentage of Control Growth Halowax 1000	1013	1014
D. tertiolecta			
0.1	89.0(40)[a]	101.4(58)	114.2(40)
0.5	81.4(40)[b]	98.0(58)	105.4(40)
1.0	57.0(31)[b]	91.8(58)	105.4(40)
Chlorococcum sp.			
0.1	94.0(40)	96.7(64)	109.9(46)
0.5	88.3(40)[a]	95.0(64)	100.3(46)
1.0	54.2(31)[b]	89.1(64)	101.1(46)
Nitzschia sp.			
0.1	90.9(21)	95.4(52)	99.5(46)
0.5	82.9(22)[a]	86.8(52)[a]	94.4(46)
1.0	57.7(19)[b]	83.4(52)[b]	91.1(46)
T. pseudonana			
0.1	94.5(16)	105.9(52)	117.1(46)
0.5	78.7(16)[b]	94.1(52)	117.0(46)
1.0	51.6(16)[b]	92.9(52)[a]	109.2(46)

[a] Significantly different from control at 0.05 level.
[b] Significantly different from control at 0.01 level.

These data are in contrast to those reported for mammalian toxicity of chloronaphthalenes. In mammals, mixtures of penta- and hexachloronaphthalene are generally the most toxic formulations; toxicity decreases as chlorine content increases or decreases (Bennett et al. 1939, Greenburg et al. 1939, Shakhnovskaya 1953, Deadrick et al. 1955, Hambrick 1957, Shelly and Kligman 1957, Link et al. 1958, Weil and Goldberg 1962, Crow 1970.

Degree of bioaccumulation, in contrast to toxicity, appeared directly related to chlorine content (Table 2). However, bioaccumulation was not great, and the highest accumulation factor (concentration in algae/concentration in medium) was 140. It is possible that low concentrations of Halowax 1000 in algal cells were due to dechlorination of mono- and dichloro compounds by the cells, since degradation by organisms is known to occur (Cornish and Block 1958, Walker and Wiltshire 1955).

TABLE 2

Chlorinated naphthalene residues associated with Chlorococcum sp. after 24-hr exposure. Accumulation factors are given in parentheses.

Exposure Concentration ppb	Halowax Concentration in Algae, ppm[a]		
	1000	1013	1014
5	ND[b]	0.3(60)	0.7(140)
10	ND	0.6(60)	1.1(110)
25	ND	2.1(84)	3.1(124)
50	1.2(25)	4.3(86)	5.5(110)
100	3.2(32)	12.0(120)	12.0(120)

[a] Lower limit of detectability: Halowax 1000 - 0.5 ppm; Halowax 1013 - 0.25 ppm; Halowax 1014 - 0.5 ppm.

[b] Not detected.

Conclusions

A chlorinated naphthalene formulation that contained a mixture of mono- and dichloro isomers was more toxic to marine algae than were formulations of tri-, tetra-, penta-, and hexachloro isomers. However, uptake was directly related to chlorine content. The penta- and hexachloronaphthalenes are generally the most toxic to animals, and if algae were exposed to these compounds in nature, it is possible that they could serve as vehicles for transfer to aquatic animals. Transfer through food webs could begin with algae and result in effects at higher trophic levels.

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