Mirex and Marine Unicellular Algae: Accumulation, Population Growth and Oxygen Evolution[1]

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Many organochlorine compounds are toxic to algae. SODERGREN (1967) demonstrated that less than 0.3 parts per billion (ppb) of DDT inhibited growth of a fresh water species of Chlorella. WURSTER (1968) reported that DDT reduced the rate of photosynthesis in five species of marine algae. de la CRUZ and NAQVI (1973) showed that one part per million (ppm) of mirex reduced net photosynthesis by 55% in a fresh water species of Chlamydomonas.

Uptake of organochlorine compounds by algae is well documented. VANCE and DRUMMOND (1969) reported that selected cultures of green and blue-green algae concentrated DDT an average of 210 x, aldrin 188 x, and endrin 215 x the exposure concentration of 1 ppm. RICE and SIKKA (1973) showed that various marine algae accumulated dieldren from 1,000 to 16,000 x the exposure concentration of 1.7 ppb.

Mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cylobuta (cd) pentalene) is a persistant organochlorine insecticide used to control the imported fire ant, Solenopsis richteri Forel, in the southeastern United States. Fire ant infestations are often located in areas that drain into estuarine marshlands and embayments. Mirex applied to coastal areas and upland watersheds can enter estuaries (BORTHWICK et al. 1973).

This study was initiated (1) to determine effects of mirex, if any, on population growth and oxygen evolution by selected estuarine unicellular algae under various conditions of salinity and nutrient concentration and (2) to determine if mirex can be accumulated by the algae.

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METHODS

Population growth. Algae were exposed to mirex in artificial sea water[3] supplemented with trace elements and vitamins to determine its effects on population growth. Three nutrient concentrations were tested: one-tenth, one-half, and full-strength. Full-strength medium was that which yielded maximal growth in untreated cultures and it contained, per liter, 30 mg Na_2EDTA, 14 mg $FeCl_2 \cdot 6H_2O$, 34 mg H_3Bo_3, 4 mg $MnCl_2 \cdot 4H_2O$, 2 mg $ZnSO_4 \cdot 7H_2O$, 6 mg K_3PO_4, 100 mg $NaNO_3$, 40 mg $Na_2SiO_3 \cdot 9H_2O$, 5 µg $CuSO_4$, 12 µg $CoCl_2$, 50 µg thiamine hydrochloride, 1 µg vitamin B_{12}, and 0.01 µg biotin. Salinities were 5, 15, and 30 parts per thousand (ppt) and pH ranged between 7.9 and 8.1. The medium was sterilized by autoclaving at 121 C for 15 minutes.

WALSH (unpubl.) showed mirex to have low solubility in seawater, 0.2 ppb being the highest concentration obtainable. This concentration was used in growth studies.

Algae tested were the chlorophytes Chlorococcum sp., Dunaliella tertiolecta Butcher, and Chlamydomonas sp.; the bacillariophytes Nitzschia sp. (Indiana strain 684) and Thallasiosira pseudonana Hasle and Hundal, and the rhodophyte Porphyridium cruentum (Ag.) Naeg. Algae were obtained from the culture collections of the Woods Hole Oceanographic Institution, Scripps Institution of Oceanography, and Indiana University.

Stock cultures, grown in and acclimated to various salinities and nutrient concentrations for one week, were diluted with appropriate medium to the absorbance of 0.100 at 525 nm on a Fisher electrophotometer. The diluted algal suspensions were used as inocula for growth tests. One ml of the appropriate suspension was added to culture flasks that contained 49 ml of test medium. Treated cultures contained 0.2 ppb technical mirex that was added in acetone carrier. Untreated control flasks contained an identical amount of acetone (0.01% of the volume).

All cultures were grown on rotary shakers at 20 C under 6,000 lux illumination with alternating 12-hr periods of light and darkness. Triplicate flasks were

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[3] From Rila Products, Teaneck, New Jersey. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

analyzed at each salinity and nutrient concentration and each test was performed twice. After seven days, growth was measured spectrophotometrically at 525 nm on a Fisher electrophotometer.

Oxygen evolution. To determine effects of mirex on oxygen evolution, 10 ml samples of Chlorococcum sp. and Chlamydomonas sp. from growth studies were centrifuged gently and resuspended in fresh medium to the absorbance of 0.100 at 525 nm. Four ml of each cell suspension were placed in reaction vessels of a Gilson photosynthesis-model differential respirometer. The vessels contained CO_2 buffer in the wells (UMBRIET et al. 1964). After equilibration at 20 C for 20 min, oxygen evolution was measured for 60 min. Flasks were analyzed in triplicate at each salinity and nutrient concentration and each test was performed twice.

Accumulation. Fifty ml of stock algal cultures, in the logarithmic phase of growth and diluted to the absorbance of 0.100 at 525 nm, were added to 950 ml of sterile medium in 2,800 ml Erlenmeyer flasks. The algae were Chlorococcum sp., Chlamydomonas sp., D. tertiolecta, and T. pseudonana. The test medium contained the full-strength concentration of nutrients and salinity was 15 ppt. Stock solutions of mirex were prepared in acetone and added to the medium to give a concentration of 10, 25, or 50 parts per trillion (pptr) in accumulation studies. Incubation was similar to that for growth studies except the flasks were not shaken. After seven days exposure, the cells were harvested by centrifugation at 4,200 x g for 10 min, resuspended in mirex-free medium, and centrifuged again. This procedure was repeated three times to remove mirex in interstitial water of the algal pellet or bound loosely to the cells. Samples were then stored in a dessicator until analyzed.

To determine the amount of mirex accumulated, each sample was weighed and placed in a Dual1® tissue grinder and extracted with three 2.0 ml portions of acetonitrile. The combined acetonitrile extract was diluted with 6 ml of 2% (w/v) Na_2SO_4 in distilled water, shaken and extracted with three 2.0 ml portions of hexane. The combined hexane extract, concentrated to 0.5 ml by evaporation with a Snyder column, was transferred to a size "B" Chromaflex® column containing 1.5 g of Florisil and 1.5 g of anhydrous Na_2SO_4. The extract was eluted from the column with 20 ml of 1% (v/v) ethyl ether in hexane. The eluate was adjusted to an appropriate volume for

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analysis by gas chromatography using a Varian Aerography model 1400 gas chromatograph equipped with an electron capture detector and a 182 cm x 2 mm (ID) glass column packed with 2% OV-101 on Gas Chrom Q. Operating parameters were: injector temperature, 210 C; column temperature, 192 C; detector temperature, 210 C; and gas flow, 25 ml/min. Mirex was quantitated by comparison with the peak height of a known-concentration standard.

Student's "t" test was used to analyze differences between means of treated and control cultures.

RESULTS

Population growth. Figure 1 shows population densities of the six algal species after growth for seven days in 0.2 ppb mirex and three nutrient concentrations.

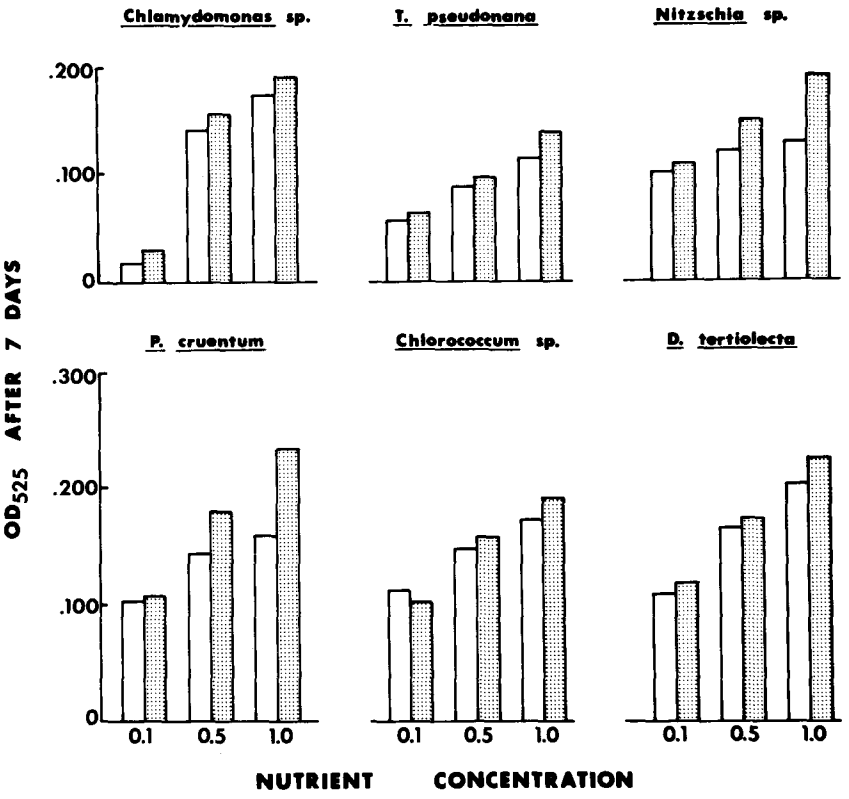

Figure 1. Comparison of cell densities at three nutrient concentrations of untreated cultures (solid bar) and those grown in 0.2 ppb mirex (hatched bar).

There were no statistically significant differences ($p = 0.05$) in final population density when any species was grown in 5, 15 or 30 ppt salinity. Therefore, population densities for all salinities were combined and only differences in density between nutrient concentrations are shown in Fig. 1.

In all cases, except Chlorococcum sp. at 1/10 strength nutrients, treated cultures exhibited higher absorbances than did control cultures, regardless of nutrient concentration, but the differences were not statistically significant at the 0.05 level.

Oxygen evolution. Tables 1 and 2 compare oxygen evolution by control and treated cultures of Chlorococcum sp. and Chlamydomonas sp. in the growth study. Cultures grown at 1/10 strength nutrient concentration did not contain a sufficient number of cells to make an adequate comparison. No significant differences in rates of oxygen evolution were found between control and treated cultures of either species at any salinity or nutrient concentration ($p = 0.05$).

TABLE 1

Oxygen evolved by Chlorococcum sp. in control cultures and in cultures exposed to 0.2 ppb of mirex for seven days at three salinities and two nutrient concentrations.

	Salinity ppt	0.5 nutrient strength, µl/hr	Full nutrient strength, µl/hr
Control	5	17.8	18.9
Exposed		20.4	18.6
Control	15	23.6	19.2
Exposed		18.2	19.8
Control	30	19.8	20.4
Exposed		16.8	20.8

Table 2

Oxygen evolved by Chlamydomonas sp. in control cultures and in cultures exposed to 0.2 ppb of mirex for seven days at three salinities and two nutrient concentrations.

	Salinity ppt	0.5 nutrient strength, µl/hr	Full nutrient strength, µl/hr
Control	5	41.0	42.6
Exposed		45.8	42.4
Control	15	39.5	45.6
Exposed		44.4	43.2
Control	30	45.4	46.6
Exposed		44.2	46.8

Accumulation. Figure 2 shows accumulation of mirex by algae exposed to three low concentrations of the pesticide for seven days at a salinity of 15 ppt. Chlorococcum sp., D. tertiolecta and Chlamydomonas sp. showed a significant linear relationship between amounts accumulated and mirex concentrations in the medium. Chlorococcum sp. was most efficient in uptake, accumulating 88% of the mirex present in the medium. Dunaliella tertiolecta and T. pseudonana removed approximately 79% whereas Chlamydomonas sp. took up 55%. Chlorococcum sp. concentrated the pesticide 7,300 x,

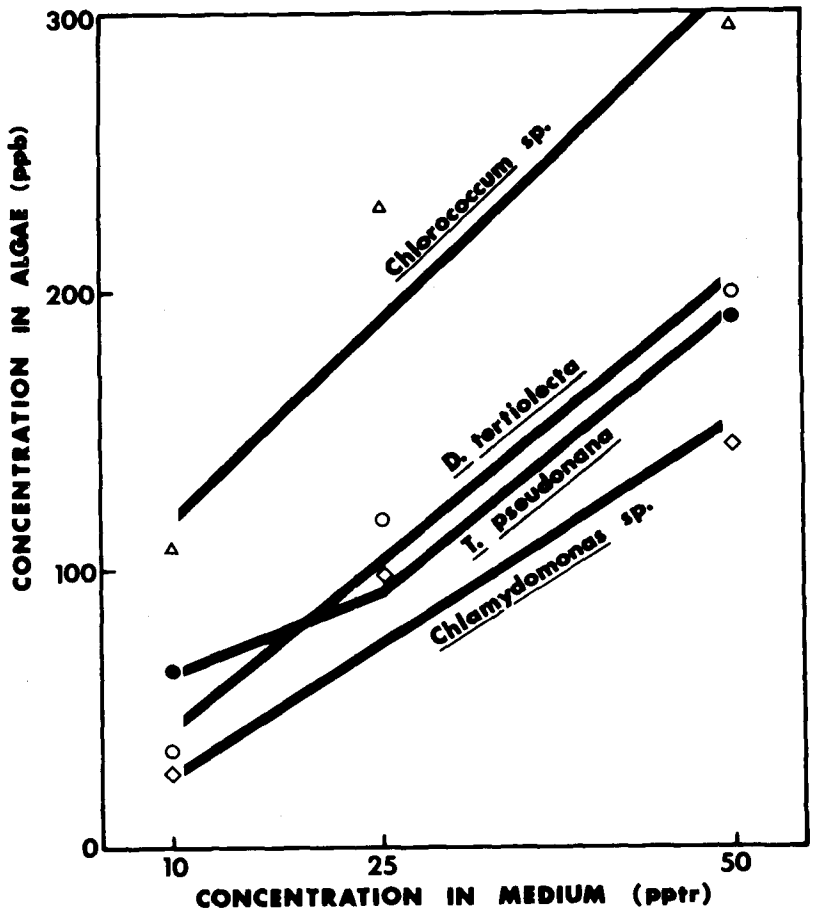

Figure 2. Uptake of mirex by algal populations after seven days exposure.

D. tertiolecta 4,100 x, Chlamydomonas sp. 3,200 x and T. pseudonana 5,000 x the concentration in the medium.

DISCUSSION

Accumulation of mirex by the algae was evident. Within seven days, for example, Chlorococcum sp. accumulated mirex from the nearly non-detectable concentration of 10 pptr in the medium and concentrated it to more than 100 ppb on cells, a concentration factor of 10,000 x. If a similar condition existed in nature, marine unicellular algae could accumulate mirex and, when grazed upon, act as passive transporters of the toxicant to consumers in the food chain.

In summary, these studies show that under our laboratory conditions, mirex had no significant effect on either population growth or oxygen evolution of selected species of marine algae. It was however, accumulated from the water by the algae.

REFERENCES

BORTHWICK, P.W., T.W. DUKE, A.J. WILSON, JR., J.I. LOWE, J.M. PATRICK, JR. and J.C. OBERHEY. Pestic. Monit. J. 7, 6 (1973).

de la CRUZ, A.A. and S.M. NAQVI. Arch. Environ. Contam. Toxicol. 1, 255 (1973).

RICE, C.P. and H.C. SIKKA. Bull. Environ. Contam. Toxicol. 9, 116 (1973).

SODERGREN, A. Oikos 19, 126 (1967).

UMBREIT, W.W., R.H. BURRIS and J.F. STAUFFER. Manometric Techniques, 4th ed., Burgess Publ. Co., Minneapolis, Minn., 305 pp. (1964).

VANCE, B.D. and W. DRUMMOND. J. Am. Water Works Ass. 61, 360 (1969).

WALSH, G.E. In Progress Report, Bureau of Commercial Fisheries Center for Estuarine and Menhaden Research, U.S. Dept. Interior Circular (Unpubl.).