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Bioconcentration of Chlordane by the Green Alga *Scenedesmus quadricauda*

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Concentration of DDT and other organochlorine insecticides by biota in terrestrial and aquatic ecosystems is well documented. Several studies have demonstrated bioconcentration by phytoplankton (GREGORY et al 1969, VANCE and DRUMMOND 1967, COX 1970, RICE and SIKKA 1973).

Technical chlordane is a mixture of 26 organochlorine compounds whose H_2O solubility has been reported as 9 µg/L (NRC 1975). Chlordane in the low ppb range has been found to be acutely toxic to freshwater and estuarine fish and invertebrates (HENDERSON et al 1969, SANDERS and COPE 1966, PARRISH et al 1976). Toxic effects of chlordane upon the photosynthesis of estuarine phytoplankton was reported at 1.0 ppm (Anon. 1965). However, such a high pesticide concentration is unrealistic in nature.

Recent experimental results indicate that chlordane concentrations ranging from 0.1 to 100 µg/L significantly stimulate growth of S. quadricauda, a common planktonic green algal (GLOOSCHENKO and LOTT 1977). Chlordane, in concentrations from 0.1 to 50 µg/L, is likewise stimulatory to the growth of Chlamydomonas sp., a soil alga. However in Chlamydomonas chlordane at 100 µg/L was inhibitory to cell division (GLOOSCHENKO and LOTT 1977).

Although mammals apparently do not accumulate chlordane (FAO/WHO 1968) bioconcentrations of chlordane and its major component heptachlor, have been noted in fish (GODSIL and JOHNSON 1968, SCHIMMEL 1975) and in clams and oysters (WILSON 1965). SANBORN et al (1976) using ^{14}C-cis:trans (75:25) chlordane (water solubility 0.056 ppm) reported a bioconcentration factor of 98,000 with cultures of the filamentous green alga Oedogonium.

The present experiment used Scenedesmus quadricauda, a common test organism in freshwater algal toxicology for several decades (PALMER and

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MALONEY 1955, VANCE and DRUMMOND 1967, LUARD 1973, LARSSON and TILLBERG 1975). The experiment described here was designed to examine technical chlordane bioconcentration factors in S. quadricauda at the same low levels which stimulated growth of this phytoplankton species. The bioconcentration factor is defined as the ratio of measured residue compared to residue of the pesticide in the ambient air, water, or soil environment of the organism (KENAGA 1972).

METHODS

S. quadricauda, obtained from the Canada Center for Inland Waters, Burlington, Ontario, was cultured in Bristol's solution with added trace elements. Equal volumes of cells from a single culture were used to innoculate 2 liters of culture solution in each of six 3-liter Erlenmeyer flasks. High cell numbers were obtained in the cultures by constant aeration of the cultures and a constant light regime of 0.3 ly/min.

When a final cell number of approximately $2x10^6$ cells/ml was attained, contents of all six flasks were thoroughly mixed so that the cells were evenly distributed when returned to the six flasks. One flask was designated as control and one flask was designated for each of the five chlordane treatment levels of 0.1, 1.0, 10, 50 and 100 µg/L. Addition of chlordane was made, and the cultures were again aerated under lights for 24 hr. One liter of culture was then taken from each of the flasks and filtered through wet preweighed 0.45 µm membrane filters. The filters containing the cells were then placed in preweighed 25-ml vials, and the weight of the cells determined. Acetone was then added to dissolve the membrane filter and to make the solution up to a standard volume. The combined weight of the vial, membrane filters, cells and acetone was determined for each treatment level. A similar procedure was followed for day five of the experiment.

In a separate experiment four liters of culture containing approximately $2x10^6$ cells/ml, were divided into two equal portions. After heating the culture to 60°C to kill the cells one of the two flasks was injected with chlordane at 10 µg/L. Three hr later the cells were filtered out and handled as previously described for the cultures containing living cells.

Vials containing cells, and bottles containing measured volumes of supernatant from the controls and each chlordane treatment were taken to the

Provincial Pesticide Residue Testing Laboratory in Guelph for analysis. The supernatant was extracted with dichloromethane, partitioned into hexane and followed by a Langlois column clean-up (LANGLOIS et al 1964). This was injected into a gas chromatograph equipped with a Ni^{63} electron capture detector. A 1.8 m coiled glass column containing a mixed phase solid support of 2.5% OV 210-2.5% OV 101 was used.

Algae were extracted with acetonitrile in H_2O (85:15), partitioned into hexane and followed by a Langlois column clean-up. Determinations were made in the Hewlett-Packard 5830A gas chromatograph equipped with the same column as described previously. Cis (α) and trans (γ) chlordane, two major components of technical chlordane, were analyzed in this experiment. They are readily identifiable as separate peaks when using gas-liquid chromatographic techniques and have been found to represent approximately 40% by weight of technical chlordane (VELSICOL 1971). Peaks of other components of technical chlordane, because of algae-substrate interference require tedious separation procedures and were not determined in this experiment.

RESULTS

Bioconcentration factors for cis (α) and trans (γ) chlordane by S. quadricauda after 24 hr and five days are shown in Table 1. Based on the bioconcentration definition, the factors were obtained by dividing the total cis (α) and trans (γ) chlordane residues found in the algae at each concentration level by the total cis (α) and trans (γ) residues left in the the culture media.

Results indicate that S. quadricauda concentrated these chlordane components to levels 6,000 to 15,000 times the original concentration in the medium within 24 hr of chlordane addition. No linear increase of concentration factors was observed with the increasing amounts of chlordane used. It was concluded by SODERGREN (1968) and RICE and SIKKA (1973) that algal uptake of DDT was passive, since absorption of the pesticide by killed and living cells was similar. In the present experiment, heat-killed cells (60°C) exposed for three hr to chlordane at 10 µg/L also show apparent passive uptake of this pesticide. The bioconcentration factor for heat-killed cells was 7,300. Uptake of cis (α) and trans (γ) chlordane on day five (see Table 1) was depressed under day one levels by 19 to 37% with exception of the 1.0 µg/L treatment which showed an apparent increase of 40%. No evidence of cis (α) or trans

TABLE 1

BIOCONCENTRATION OF Cis (α) PLUS Trans (γ) CHLORDANE

Technical Chlordane added to Culture Media (µg/L)	Days of Exposure	Amount of Cis(α) plus Trans (γ) Chlordane added to Culture Media (µg/L)	Components of Technical Chlordane in Culture Media (µg/L)			Components of Technical Chlordane in Algae (µg/g, wet weight)			Bioconcentration factor
			Cis (α) Chlordane	Trans (γ) Chlordane	Total	Cis (α) Chlordane	Trans (γ) Chlordane	Total	
0.1	1	0.04	0.002	0.002	0.004	0.02	0.02	0.04	10,000
1.0	1	0.40	0.03	0.02	0.05	0.16	0.14	0.3	6,000
10	1	4.0	0.15	0.09	0.24	2.0	1.6	3.6	15,000
50	1	20.0	1.2	0.7	1.9	9.7	7.1	16.8	8,800
100	1	40.0	2.0	1.0	3.0	19.8	14.2	34.0	11,300
0.1	5	0.04	0.003	0.003	0.006	0.02	0.02	0.04	6,700
1.0	5	0.40	0.02	0.02	0.04	0.18	0.16	0.34	8,500
10	5	4.0	0.20	0.16	0.36	2.3	1.4	3.7	10,300
50	5	20.0	1.5	0.8	2.3	10.5	6.1	16.5	7,200
100	5	40.0	2.6	1.3	3.9	20.5	12.5	33.0	8,500

(γ) chlordane peaks in control samples was observed.

DISCUSSION

Results of the present experiments show a bioconcentration factor of 6,000 to 15,000 for cis (α) and trans (γ) chlordane at all treatment levels after 24 hr. This is in contrast to a bioconcentration factor of 98,000 observed in the unbranched filamentous algal Oedogonium (SANBORN 1976). The greater algal biomass represented by Oedogonium may have contributed to increased pesticide uptake. Our work shows the major isomers of technical chlordane are concentrated by Scenedesmus from initial environmental levels as low as 0.1 µg/L. The 0.1 µg/L level is approximately 1/100 of the water solubility of technical chlordane. The data indicate that bioconcentration was rapid, occurring within the first 24 hr. It has been noted that DDT uptake in Chlorella after one min was equivalent to the amount retained after 24 hr (SODERGREN 1968).

Determination of cis (α) and trans (γ) chlordane residues in S. quadricauda after five days showed a 19 to 37% decrease in bioconcentration from day one with the exception of the 1 µg/L treatment level. Therefore some metabolism of the chlordane residues or their biodegradation to other unidentified components may also occur in this species.

Bioconcentration of chlordane apparently affects algal physiology. During time of the maximum pesticide uptake within 24 hr of addition, a significant stimulation of respiration occurred in S. quadricauda with all the chlordane concentrations used in these bioconcentration experiments (GLOOSCHENKO and LOTT 1977). Technical chlordane, at concentrations of 1 to 100 µg/L produced a significant ($P < .05$ to $P < .001$) stimulation of cell numbers over the control levels during the course of a 12 day experiment (GLOOSCHENKO and LOTT 1977).

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