

Effects of Chlorinated Benzenes on Diatom Fatty Acid Composition and Quantitative Morphology. IV. Pentachlorobenzene and Comparison with Trichlorobenzene Isomers

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Abstract. Cells of the diatom *Cyclotella meneghiniana* were exposed in a closed system to 0.095 ppm pentachlorobenzene over a period of 5 days. Changes in fatty acid and morphological percent composition were monitored to determine the effect of the toxicant. The greatest morphological change observed was an increase in lipid volume. Most morphological changes occurred in the 1 hour and 5 day sampling periods. Few changes in morphological characteristics or fatty acid percent composition were observed at eight hours, when the cells were in the dark. The C18:1 and C20:5 fatty acids were most variable with exposure to pentachlorobenzene. Results suggest that at sub-lethal doses, lipophilic toxicants exert effects that are biphasic. That is, immediately measurable effects are observed in the cells that include increases in storage products and changes in membranous organelles. Long-term effects are postulated to be the result of mobilization of lipophilic toxicants that have partitioned into lipid stores and are more available when lipids are metabolized. Although pentachlorobenzene has a higher octanol/water partition coefficient, it appears to exert fewer cellular changes than any trichlorobenzene isomer.

Previous work in our laboratory (Sicko-Goad *et al.* 1989a, 1989b, 1989c, 1988) has suggested that toxicity of chlorinated benzenes to diatoms is dependent on a variety of parameters including the physiological state of the cell, environmental parameters, and structure-activity relationships of the toxicant. In addition, we have shown that exposure to sub-lethal doses of trichlorinated benzenes having similar octanol-water partition coefficients

produces changes in fatty acid composition and quantitative morphology that vary in number, timing, and magnitude. Although the number of detrimental effects relates to relative reactivity, it is apparent that the physiological state of the cell, particularly lipid content, plays an important role in determining response to lipophilic toxicants.

Pentachlorobenzene has been reported to be more toxic to and have a greater bioaccumulation factor in freshwater algae and a variety of other organisms than the trichlorobenzene isomers (Wong *et al.* 1984; Geyer *et al.* 1984; Halfon and Reggiani 1986). Toxicity and lipophilicity of chlorinated benzenes are believed to increase with increasing chlorination. The following report is the fourth paper in a series reporting changes in fatty acid composition and quantitative morphology in the diatom, *Cyclotella meneghiniana*, on exposure to chlorinated benzenes and reports the results of exposure to pentachlorobenzene.

Materials and Methods

For the pentachlorobenzene exposure experiment, 3 L of 7-day-old exponential phase cells of the diatom *Cyclotella meneghiniana* at a density of 102,000 cells/ml were exposed to the chemical at a concentration of 0.095 ppm, since the solubility of pentachlorobenzene in water is considerably less than trichlorobenzene. However, in this experiment, as with the trichlorobenzene isomers, concentration was well below maximum water solubility and no carrier such as acetone was used to facilitate dissolution. Cells were withdrawn by aspiration at 10 min, 1 hr, 2 hr, 8 hr, 24 hr, and 5 days for fatty acid analysis. For electron microscopy, only 3 time samples were analyzed: 1 hr, 8 hr, and 5 days. In terms of chronological sequence, this was the first of the four experiments run, and in subsequent experiments with exposure to the trichlorobenzene isomers, more detailed time analyses of morphological components were carried out.

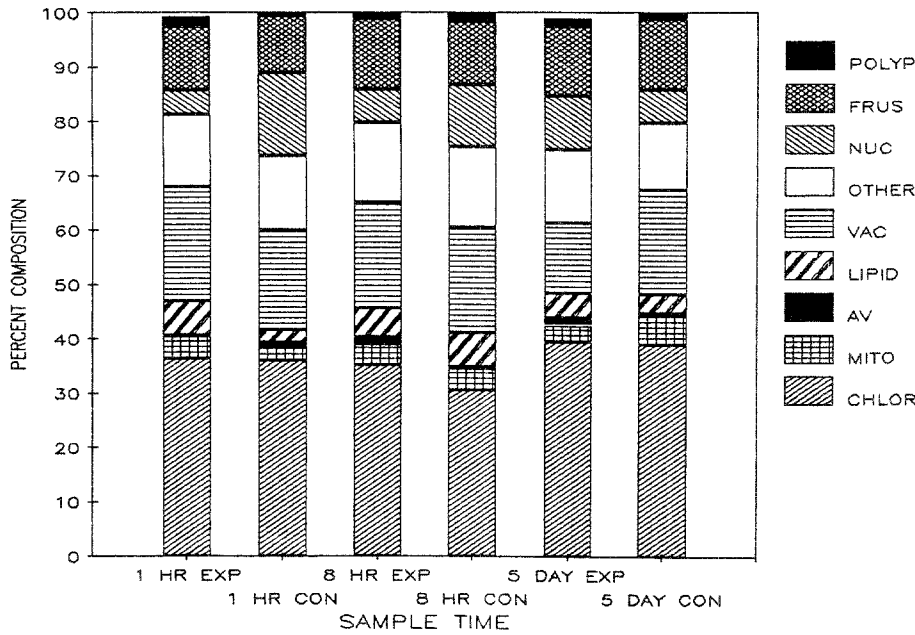


Fig. 1. Morphological percent composition of experimental (EXP) and control (CON) cells of *Cyclotella meneghiniana*.

Table 1. Relative volumes (% composition) and numbers per volume of cellular components of *Cyclotella meneghiniana* control cells and cells exposed to 0.245 ppm pentachlorobenzene (with standard errors). Values reported are the mean (± 1 S.E.) of a sample size of 25

Cell component	Experimental treatment					
	1 hr Control	1 hr Exposed	8 hr Control	8 hr Exposed	5 day Control	5 day Exposed
Chloroplast V_v	34.1 (1.9)	35.5 (1.7)	30.0 (2.4)	34.3 (1.9)	37.8 (2.0)	37.4 (2.1)
Chloroplast lipid V_v	0.9 (0.2)	0.9 (0.2)	0.7 (0.1)	0.8 (0.1)	1.3 (0.2)	2.1 (0.3)
Mitochondria V_v	2.7 (0.5)	4.1 (0.6)	3.9 (0.4)	3.8 (0.5)	5.5 (1.0)	3.1 (0.6)
Autophagic-like vacuole V_v	0.6 (0.2)	0.2 (0.1)	0.3 (0.1)	1.2 (0.3)	0.2 (0.2)	1.4 (0.3)
Lipid V_v	2.4 (0.7)	6.3 (1.7)	6.3 (2.1)	5.2 (1.1)	3.5 (1.5)	4.5 (1.3)
Vacuole V_v	18.3 (2.0)	21.0 (1.9)	19.4 (2.3)	19.6 (1.9)	19.2 (2.5)	12.9 (1.9)
Other	13.7 (1.1)	13.4 (1.3)	14.8 (1.3)	14.7 (0.9)	12.4 (1.3)	13.5 (1.1)
Nucleus V_v	15.2 (2.1)	4.4 (1.2)	11.4 (2.3)	6.0 (1.9)	6.1 (2.0)	10.8 (2.2)
Frustule V_v	10.5 (0.5)	11.5 (0.6)	11.6 (0.6)	12.9 (0.9)	12.8 (0.9)	12.5 (0.8)
Polyphosphate V_v	1.6 (0.3)	2.0 (0.4)	1.6 (0.4)	1.4 (0.3)	1.2 (0.5)	1.7 (0.3)
Chloroplast N_v (/ μm^3 cell)	0.12 (0.02)	0.10 (0.02)	0.11 (0.01)	0.02 (0.02)	0.10 (0.01)	0.12 (0.01)
Chloroplast lipid N_v (/ μm^3 chloroplast)	8.10 (0.22)	7.32 (0.26)	13.80 (0.39)	14.30 (0.44)	7.87 (0.29)	13.47 (0.44)
Mitochondria N_v (/ μm^3 cell)	0.04 (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	0.05 (0.01)	0.04 (0.01)
Polyphosphate N_v (/ μm^3 cell)	0.70 (0.41)	0.31 (0.08)	0.36 (0.21)	0.38 (0.33)	0.30 (0.15)	0.50 (0.20)

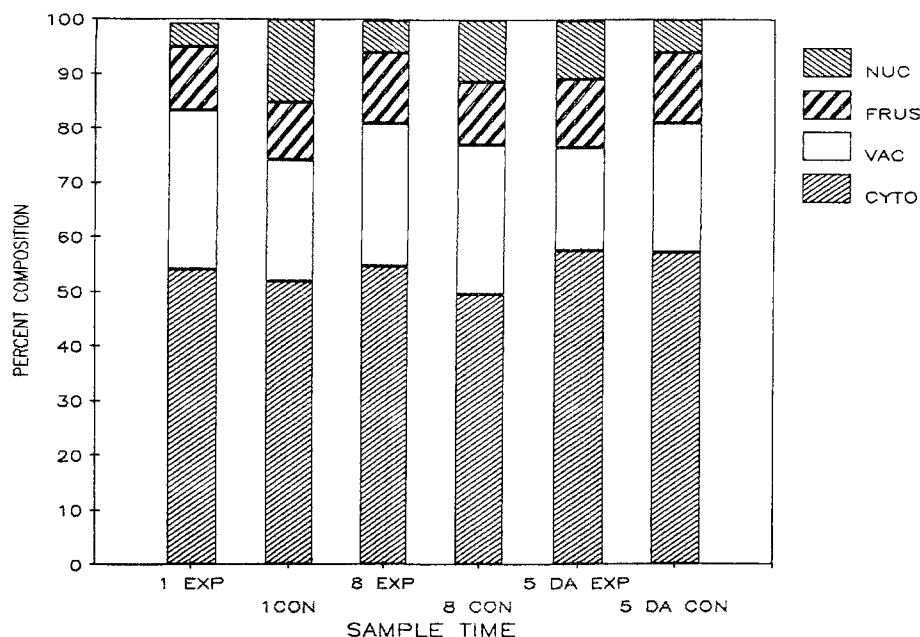


Fig. 2. Comparison of major cellular compartments (regrouped data) on exposure to pentachlorobenzene

In addition to the changes in chemical concentration and cell densities, this experiment, in terms of sampling in the daily photocyclus, was most similar to the 1,2,4-trichlorobenzene experiment. The experiment was initiated at 1230 hr, with the 10-min, 1-hr, 2-hr, 24-hr, and 5-day samples being withdrawn in the latter part of the light period. The 8-hr sample was withdrawn 1 and 1/2 hr into the dark period of the photocyclus.

Results

Figure 1 and Table 1 demonstrate that the most pronounced cellular changes that occur on exposure to pentachlorobenzene are the increase in lipid volume for 1-hr and 5-day samples and the large reduction in nuclear volume for the 1- and 8-hr exposure samples. This difference in nuclear volume is accentuated when morphological data are regrouped into four major cellular compartments (Figure 2) as previously described (Sicko-Goad *et al.* 1989c). In addition, Figure 2 also demonstrates that vacuole volume is variable, and the variability cannot be fully explained by the changes in lipid volume, although the lipid is found in the vacuole.

Exposure to pentachlorobenzene results in a short-term increase in mitochondrial volume. However, at 5 days, mitochondrial volume is greater in control cells. Mitochondria are also aberrant in 5-day exposed cells (Figure 3). Cristae are less numerous and inclusions are often observed in the mitochondria. Increases in mitochondrial relative volume are accompanied by an increase in numbers per volume. Polyphosphate volume is slightly greater in exposed cells at 1 hr and 5 days. These

numbers, however, are not significant. Although fibrous vacuole was not enumerated as a separate category in this experiment, it was observed in the cells adjacent to the chloroplasts (Figure 3).

Although there are some significant changes in numbers per volume of chloroplasts, chloroplast lipid droplets, mitochondria, and polyphosphate, there appears to be no consistent pattern of change (Figure 4). However, it should be noted that significant changes that do occur are found in the 1-hr and 5-day sampling periods, with variations of only up to 6% over controls in the 8-hr sampling period.

Few significant trends in fatty acid composition are apparent from Figure 5. The most significant fluctuations in percent composition are found in the C18:1 fatty acid, although this fatty acid accounts for only 1–2% of the total fatty acid percent composition in *Cyclotella*. It also appears that the levels of the C16 fatty acids are more evenly distributed in percent composition of total fatty acids, although they still comprise approximately 70% of all fatty acids.

To further discriminate changes occurring as a result of exposure to pentachlorobenzene, increases or decreases for all sampling categories were examined for each time period. Those changes greater than 20% occurring in morphological components may be summarized as follows:

1 hr—Increases in mitochondria, lipid, and polyphosphate; decreases in autophagic-like vacuole and nucleus.

8 hr—Increase in autophagic-like vacuole; decrease in nucleus.

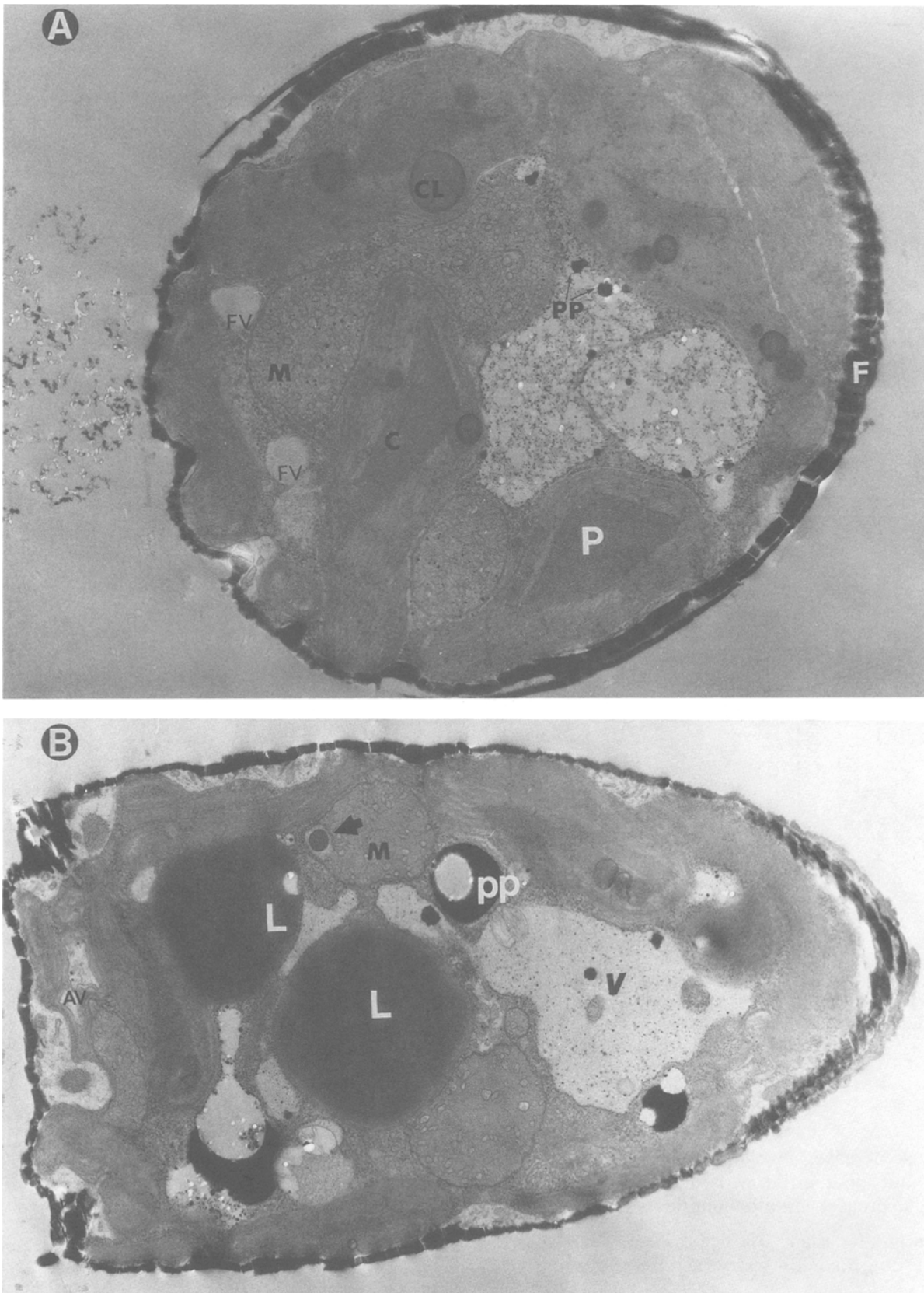


Fig. 3. Electron micrographs of *Cyclotella meneghiniana*. Key to figure legends: Autophagic-like Vacuole (AV), Chloroplast (C), Chloroplast Lipid (CL), Frustule (F), Fibrous Vacuole (FV), Lipid (L), Mitochondria (M), Pyrenoid (P), Polyphosphate (pp), Vacuole (V). Marker bars = 1 μm . **A** Five day control cell. Mitochondria, chloroplast lipid, and pyrenoids are prominent. Small polyphosphate bodies are seen at the periphery of the vacuole. **B** Five day exposed cell. Lipid and polyphosphate are prominent in the vacuolar region. Mitochondrial cristae appear to be reduced in numbers and mitochondria have inclusions (arrow). Membranous aggregates (AV) are located adjacent to chloroplast and mitochondrion.

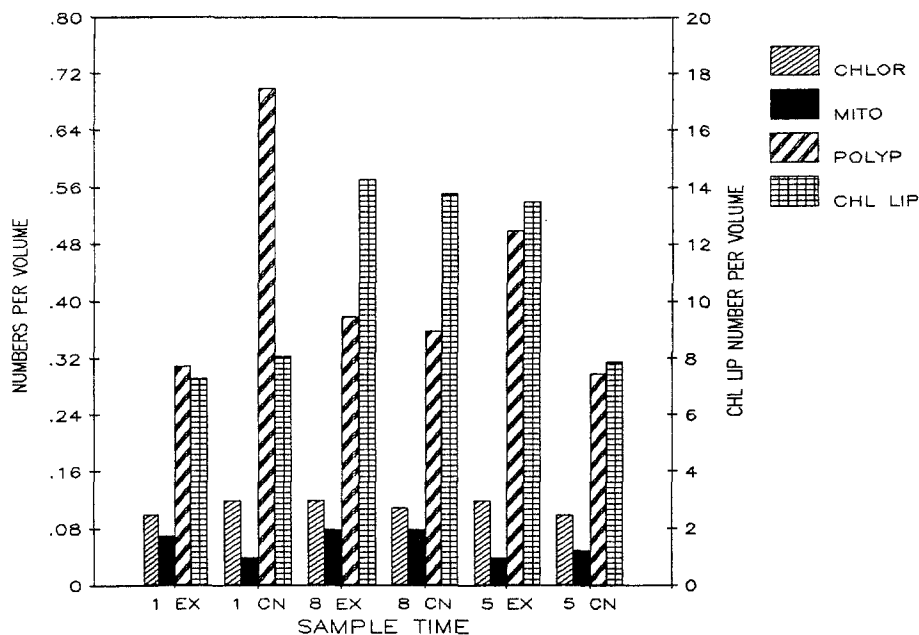


Fig. 4. Numbers per volume of selected organelles. Numbers of chloroplast lipid droplets are reported per μm^3 chloroplast; all other numbers are reported per μm^3 cell

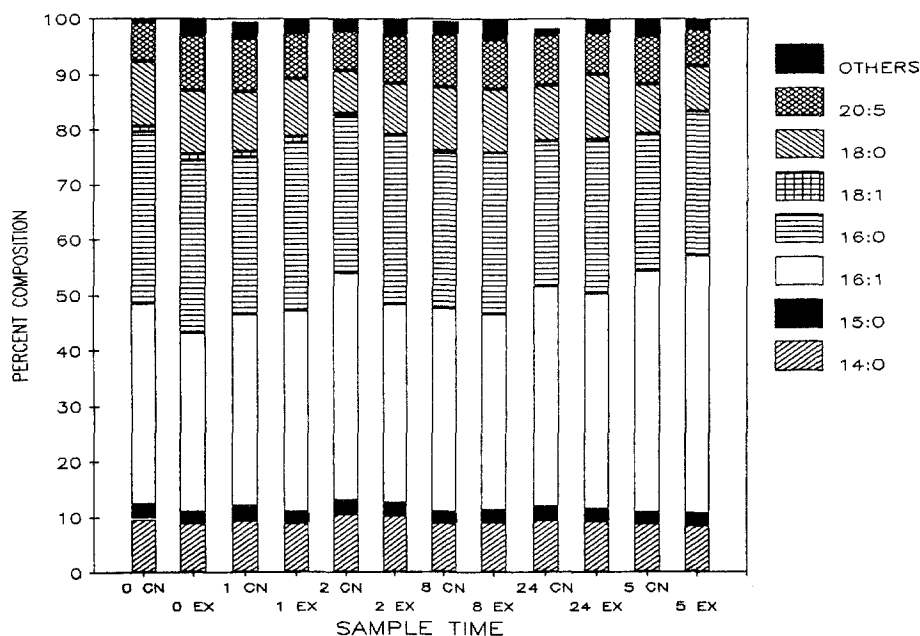


Fig. 5. Change in fatty acid percent composition for all control (CN) and experimental samples. Others represent a composite category of confirmed fatty acids individually present in amounts less than 1%

5 day—Increases in chloroplast lipid, nucleus, lipid, autophagic-like vacuole and polyphosphate; decreases in mitochondria and vacuole.

Changes greater than 20% occurring in fatty acid percent composition may be summarized as follows:

- 10 min—Increase in C20:5; decrease in C15:0. (Figure 6a)
- 1 hr—Decrease in C15:0. (Figure 6b)
- 2 hr—Increase in C18:0; decrease in C18:1. (Figure 7a)

8 hr—Decrease in C18:1. (Figure 7b)

24 hr—Increase in C18:1 (Figure 8a)

5 day—Decreases in C18:1 and C20:5 (Figure 8b)

A total number of 19 significant changes occurred in relative volume and numbers per volume categories examined during the three sampling periods. In addition, 9 significant changes occurred in fatty acid percent composition during 6 sampling periods examined. Changes in fatty acid percent composition were evenly distributed with time whereas the morphological changes occurred in the

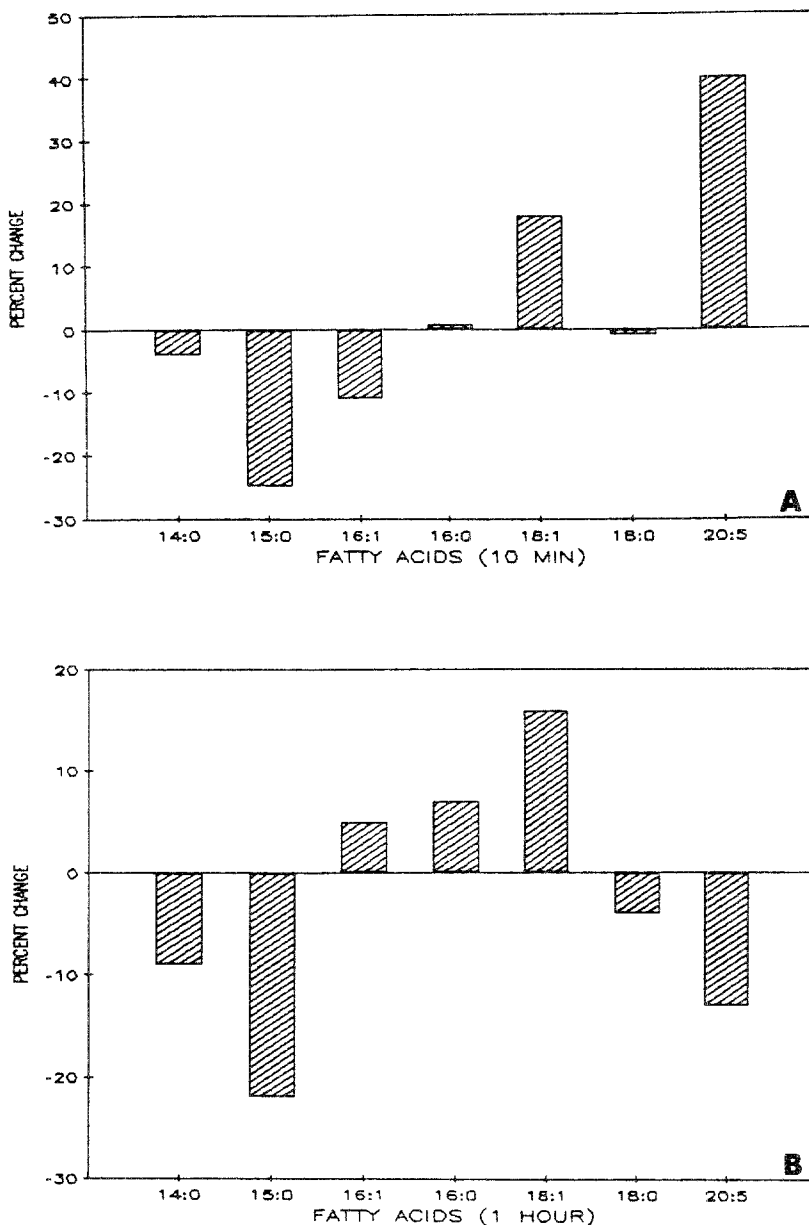


Fig. 6. Changes in fatty acid percent composition with exposure. A 10 min exposure. B 1 hr exposure

1-hr and 5-day samples. Autophagic-like vacuole increased by 300 and 600% in the 8-hr and 5-day samples, respectively. Overall, lipid and polyphosphate, two storage products, increased with toxicant exposure. The percentage of morphological changes that were significant was similar to the three trichlorobenzene isomers, although there were fewer changes in fatty acids. The C18 fatty acids were most variable with exposure to pentachlorobenzene.

Discussion

Pentachlorobenzene has an octanol-water partition coefficient (log P) of 4.88 whereas values for tri-

chlorobenzene isomers range from 3.9–4.1 (Wong *et al.* 1984). Based on this coefficient, one would expect greater partitioning of pentachlorobenzene into lipoprotein membranes (Hutchinson *et al.* 1980) and greater potential for bioaccumulation. Wong *et al.* (1984) have demonstrated that, based on the EC50, pentachlorobenzene is much more toxic to *Ankistrodesmus falcatus* than any of the trichlorinated benzene isomers. Similarly, Geyer *et al.* (1984) demonstrated that both experimentally determined and calculated bioaccumulation factors for pentachlorobenzene were higher than for trichlorobenzene in *Chlorella*. These data would suggest that pentachlorobenzene has a greater toxicity potential than any of the less chlorinated benzene derivatives.

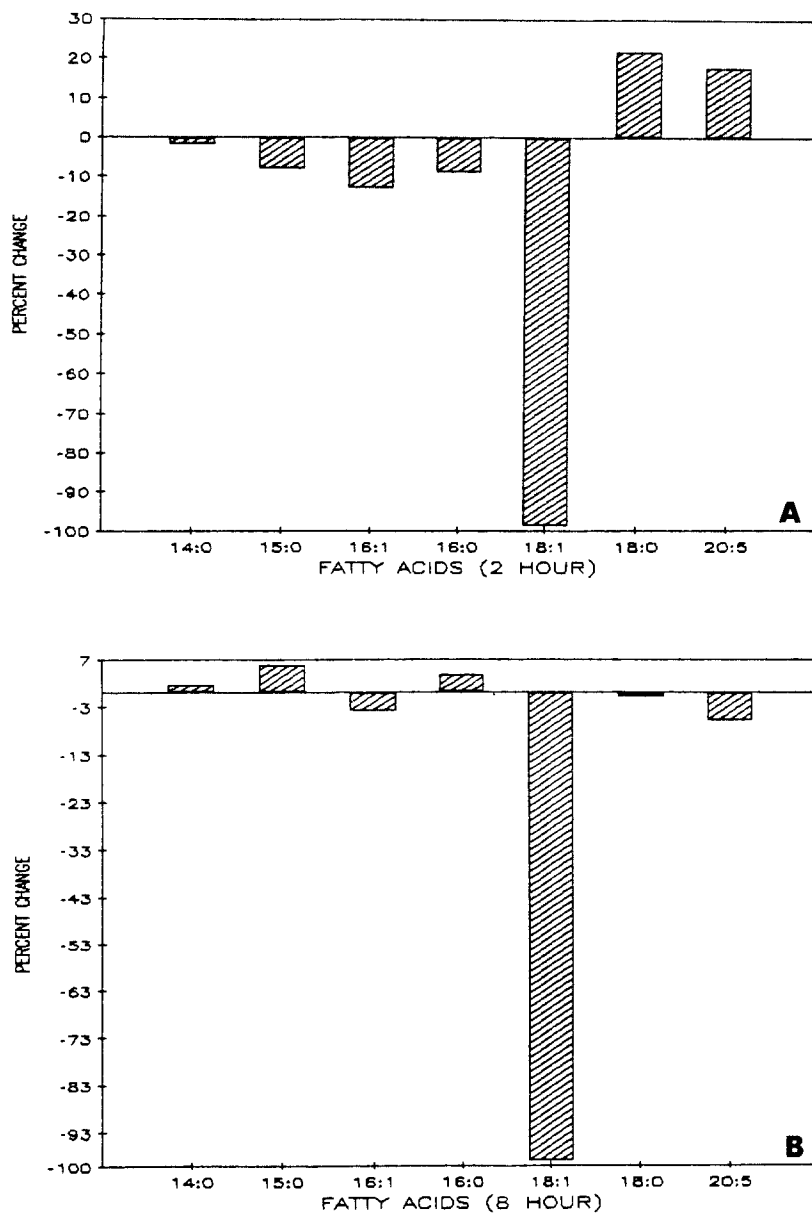


Fig. 7. Changes in fatty acid percent composition with exposure. A 2 hr exposure. B 8 hr exposure

Our data suggest that, at concentrations well below maximum water solubility, partition coefficients cannot be used reliably to predict toxicity. We have suggested (Sicko-Goad *et al.* 1989a, 1989b, 1989c) that for trichlorobenzene isomers, toxicity was related to a combination of factors including chemical reactivity and physiological state of the cell, particularly lipid content. The data presented in this paper support this hypothesis.

For example, numbers of significant deviations from control cells were plotted for changes greater than 20% (Figure 9a) and greater than 50% (Figure 9b). Both figures demonstrate that the numbers of significant changes for the three time periods sampled that were common to all experiments follow a distinct pattern: the results of exposure to 1,2,3-

and 1,3,5-trichlorobenzene are similar while pentachlorobenzene and 1,2,4-trichlorobenzene are similar. Using a criterion of 50% change in any category as being significant (Figure 9b), it appears that pentachlorobenzene exposure results in more changes in the first hr and at 5 days of exposure and this in fact may be related to the greater lipophilicity of the compound. However, pentachlorobenzene, which would not be expected to form epoxides or diols, produces similar numbers of effects at 5 days in comparison with 1,2,4-trichlorobenzene, a less lipophilic but potentially more reactive compound. The only explanation that we can offer is that the cells of *Cyclotella meneghiniana* that were exposed to pentachlorobenzene had a greater lipid content, since these cells were taken

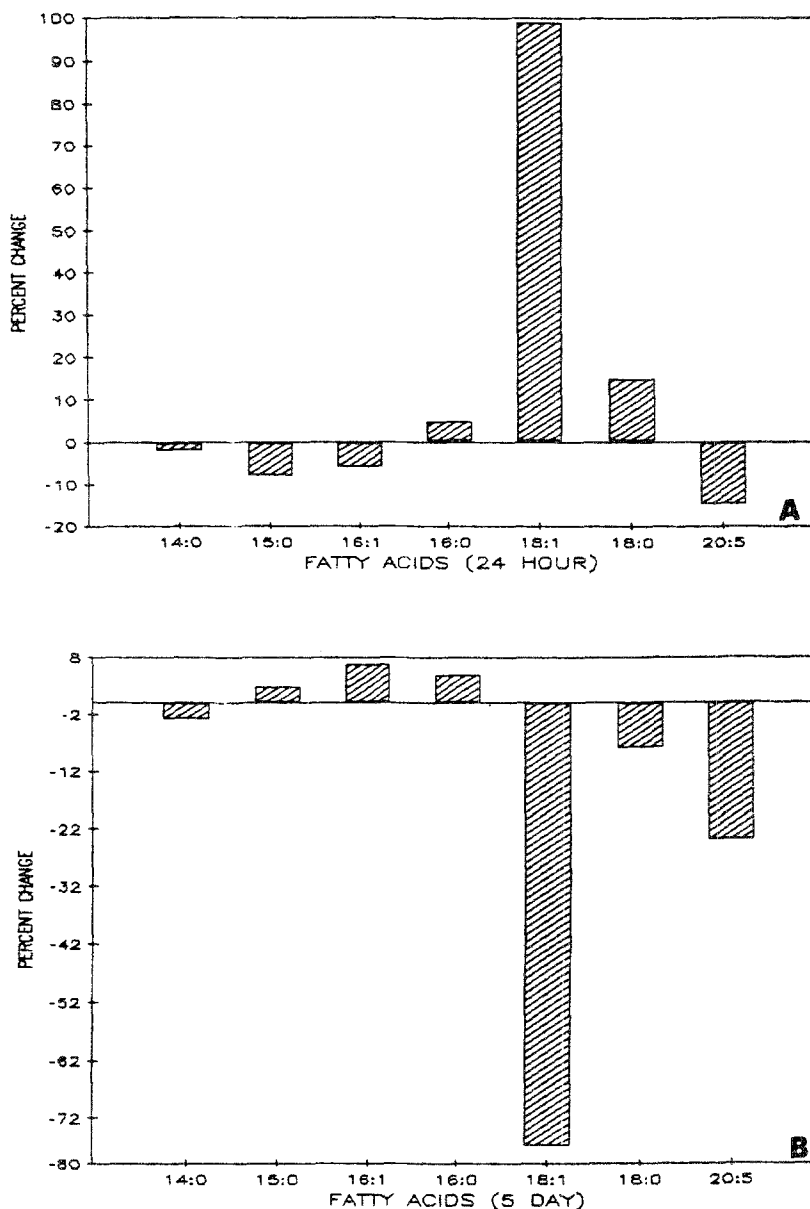


Fig. 8. Changes in fatty acid percent composition with exposure. A 24 hr exposure. B 5 day exposure

relatively late in the light period, and in fact the 8-hr sample was withdrawn in the first hr and a half of the dark period when lipid volume is at its maximum (Sicko-Goad *et al.* 1988). So while octanol-water partition coefficients may be useful predictors of acute toxicity and bioaccumulation at high doses (*i.e.*, greater than maximum solubility), they are not as useful in predicting effects at sub-lethal doses on primary producers.

A comparison of changes in morphological components within common sampling periods for all chlorinated benzene exposure experiments suggests that, in terms of relative volume, some organelles respond more quickly and with greater magnitude than others to chlorinated benzenes. For ex-

ample, Figure 10a demonstrates that chloroplast relative volume appears to be most sensitive to exposure to 1,3,5-trichlorobenzene, but on the whole, is rather insensitive to sub-lethal chlorinated benzene exposure. However, when chloroplast number per volume is plotted (Figure 10b), it is apparent that at eight hr of exposure there is a reduction in numbers per volume, coupled with an increase in relative volume. Since cell volumes from all treatments were not significantly different, it is apparent that the remaining chloroplasts increased in size to compensate for the reduction in numbers.

Plastoglobuli numbers per volume and relative volume follow the same general pattern for all experiments (Figures 11a, b) and increases and/or de-

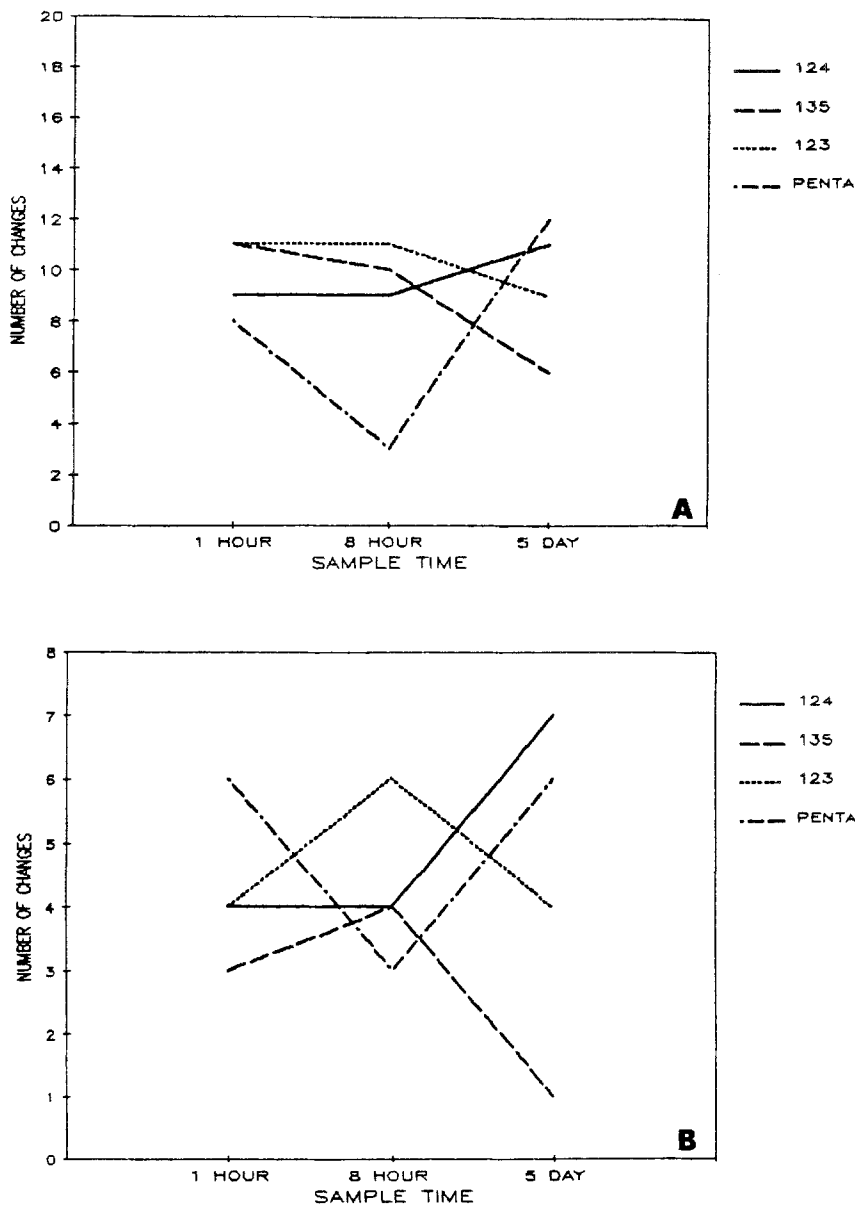


Fig. 9. Significant changes in morphological or fatty acid percent composition with exposure to chlorinated benzenes. **A** Changes greater than 20% of the control values. **B** Changes greater than 50% of the control values

creases in volume appear to be directly related to numbers. Autophagic-like vacuoles, which are indicative of lytic activity, increase uniformly for all chlorinated benzenes tested (Figure 12) and are still present at 5 days, suggesting continued response to the toxicant.

Based on our results, the toxicity of the chlorinated benzenes that we tested can be rated as follows: 1,2,4-TCB > 1,2,3-TCB > 1,3,5-TCB > Pentachlorobenzene. It is suggested that the physiological state of the cell, particularly lipid content as it relates to light cycle, is responsible for the increased toxicity of 1,2,4-trichlorobenzene. The data suggest that both cell cycle events and environmental parameters that favor lipid production in

phytoplankton (*e.g.*, dark, exposure to other toxicants, nitrate starvation) may alter both the timing and magnitude of response to exposure to lipophilic toxicants and may also alter bioaccumulation patterns in aquatic foodwebs.

The sensitivity of algae to toxicants during different seasons of the year and under nutrient deprivation conditions has been suggested by other authors. For example, Hannan and Patouillet (1972) found that mercury toxicity to three algal species increased with decreasing nutrient concentrations. Similarly, Fisher *et al.* (1976) found that at reduced nitrate levels, the growth of a marine diatom was substantially reduced in the presence of pentachlorobenzene.

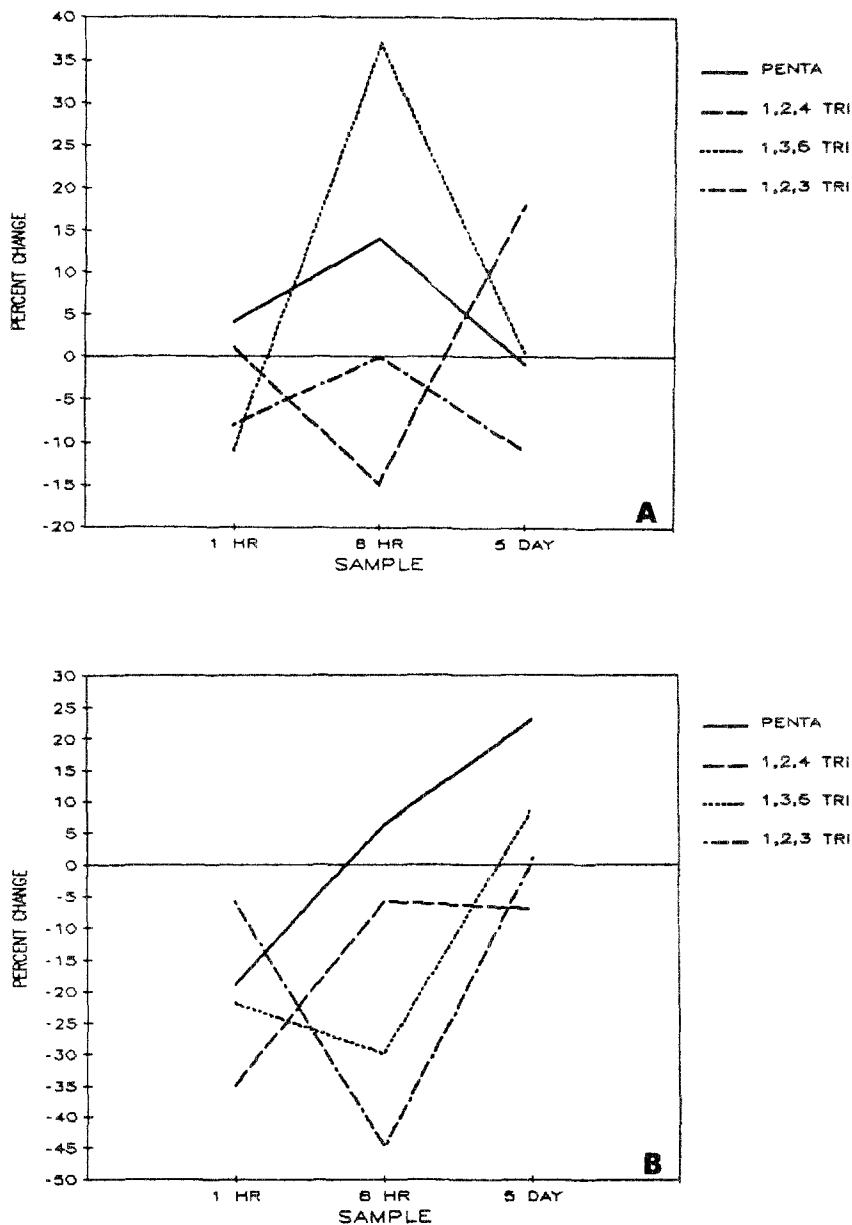


Fig. 10. Percent change in chloroplast relative volume and numbers per volume in sampling times common to all chlorinated benzene exposure experiments. A Chloroplast relative volume (V_v). B Chloroplast (N_v)

The partitioning of lipophilic toxicants into phytoplankton may result in four equally important ecological perturbations.

1. Lipid-rich metabolically inactive cells may accumulate lipophilic compounds and sink to the sediments resulting in increased sediment burdens of toxicants.
2. The lipid-rich cells may be entrained into the water column to more favorable growth conditions, resulting in the mobilization of lipid for metabolic purposes and subsequent release of the lipophilic toxicant into the cell.
3. The lipid-rich cells may be ingested by either planktonic or benthic herbivores, resulting in biomagnification of toxicants in the foodweb.

4. Planktonic and benthic herbivores may avoid ingesting lipid-rich cells containing lipophilic toxicants. This may result in reduced grazing on the phytoplankton community, resulting in higher standing stocks of phytoplankton and a reduction in herbivore standing stocks.

Such effects are likely to have a major role in the ecotoxicology of lipophilic contaminants in the Great Lakes ecosystem. For example, depositional areas in the offshore regions of the Great Lakes tend to be regions of relatively high concentrations of toxic contaminants (Frank *et al.* 1979, 1980, 1981). Sediments in such systems are fine-grained and relatively rich in living and dead diatoms (Stoermer *et al.* 1985). Thus, toxic substances in-

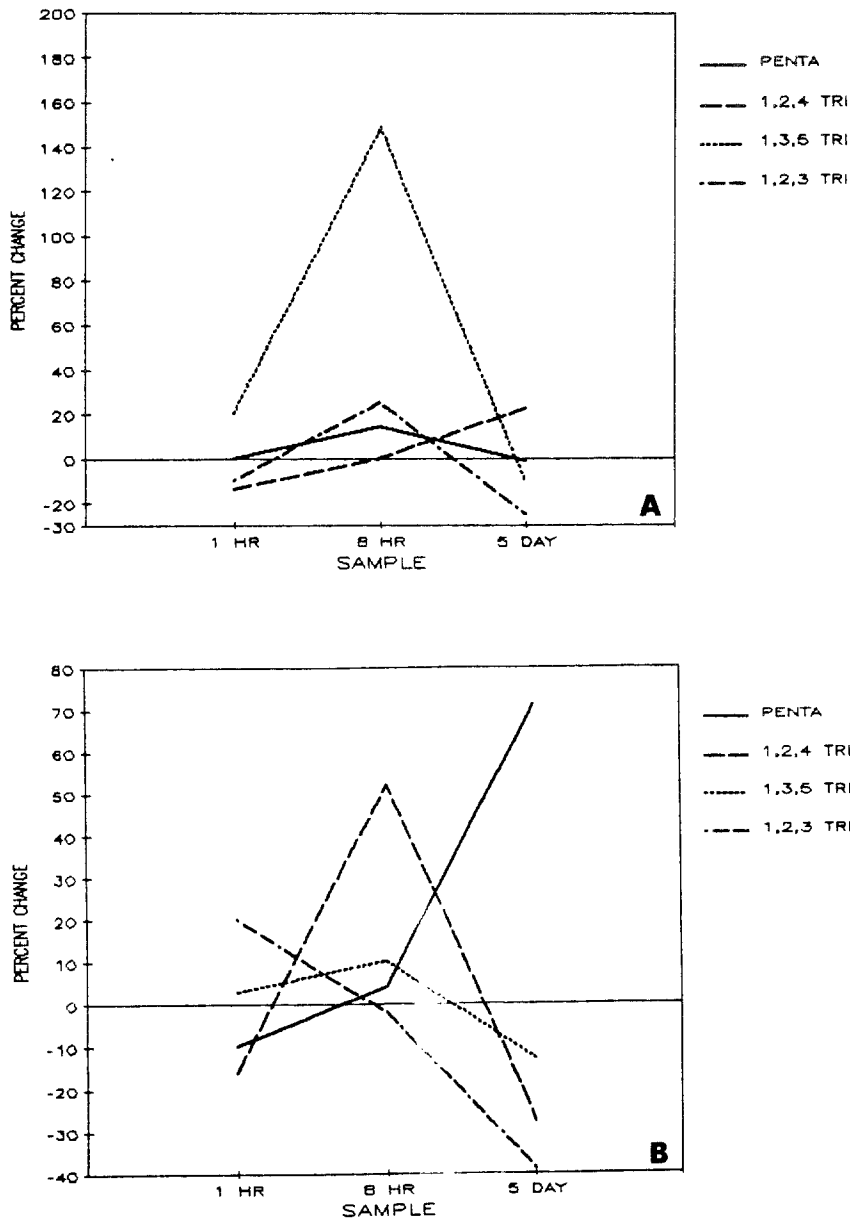


Fig. 11. Percent change in chloroplast lipid relative volume and numbers per volume in sampling times common to all chlorinated benzene exposure experiments. **A** Chloroplast relative volume (V_v). **B** Chloroplast (N_v)

incorporated into sedimenting phytoplankton may represent a major depositional pathway from the nearshore to the offshore and from the lake surface to the lake floor.

Sedimenting algae may be entrained into more shallow depths under a variety of physical conditions. The thermocline is eroded during spring and autumn, transporting nutrients and sedimented algae to shallower and more favorable depths (Brooks and Torke 1977). Vertical mixing is especially intense in winter and may reach the lake floor where minerals and sedimented organic matter (including phytoplankton) are resuspended (Eadie *et al.* 1984). Finally, summer upwelling events transport nutrients and sedimented algae to shallower

depths (Schelske *et al.* 1971), resulting in an increase in phytoplankton productivity.

Toxic substances may be biomagnified when planktonic and benthic herbivores consume contaminant-containing phytoplankton (Reinert 1972; Borgmann and Whittle 1983). This biomagnification appears to be especially significant for offshore populations of benthic invertebrates (Evans *et al.* 1982; Borgmann and Whittle 1983), possibly because of the interactive effects of relatively high concentrations of contaminants in the planktonic diet and in surficial sediments.

Several studies have shown that zooplankton grazing varies as a function of phytoplankton species composition. Certain species such as blue-

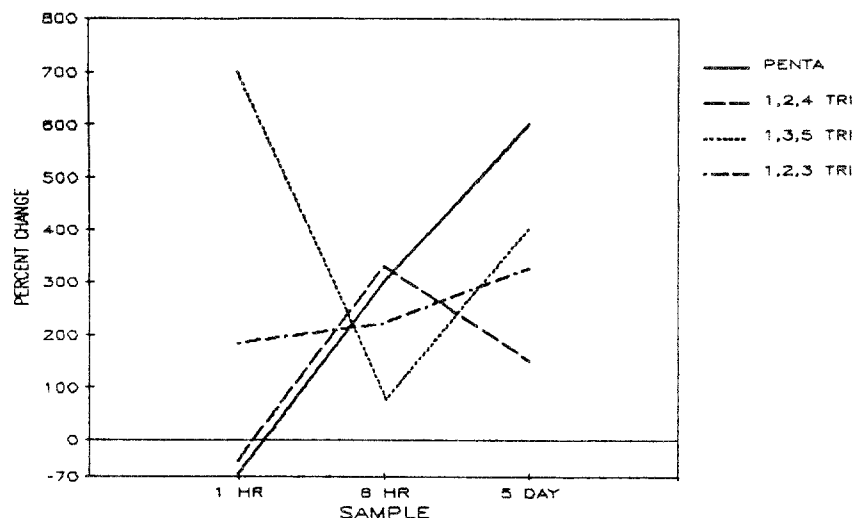


Fig. 12. Percent change in autophagic-like vacuole in sampling time common to all chlorinated benzene exposure experiments

green algae and greens are low in food quality and thus inhibit zooplankton grazing (Arnold 1971; Richman and Dodson 1983). More recent investigations have shown that zooplankton can discriminate between nutritionally-poor and nutritionally-rich phytoplankton of the same species (Butler *et al.* 1987), possibly suggesting that zooplankton may also be able to distinguish between contaminant-rich and contaminant-poor cells. Other studies have shown that zooplankton grazing may be inhibited by the presence of toxic substances (McNaught 1982; Gliwicz and Sieniawska 1986). Many of the areas of the Laurentian Great Lakes in which toxic substances are of concern also are areas in which phytoplankton standing stocks are high. Although such large phytoplankton standing stocks have been related to excessive nutrient loadings, reduced zooplankton grazing as a consequence of the presence of toxic substances cannot be discounted as a modifying factor (Evans and McNaught 1988).

Benthic organisms such as amphipods also have been shown to be capable of discriminating against sediments which contain toxic contaminants (Gannon and Beeton 1969). Thus, if sedimented phytoplankton contain high concentrations of toxic substances, this rich source of organic matter may not be consumed by the benthic community. This would result in a reduction in benthic productivity. Alternately, such contaminant-containing cells may be consumed but may adversely affect the benthos, *e.g.*, resulting in deformities in chironomid head capsules (Warwick 1988).

In summary, since numerous pathways for foodweb biomagnification exist, a basic understanding of the mechanisms by which toxicants can alter normal metabolic pathways and by which environmental and physiological parameters can ame-

liorate or amplify toxicant effects in phytoplankton is essential for predicting effects in the aquatic ecosystem.

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