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Effects of Chlorinated Benzenes on Diatom Fatty Acid Composition and Quantitative Morphology. III. 1,2,3-Trichlorobenzene

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Abstract. Cells of the diatom *Cyclotella meneghiniana* were exposed in a closed system to 0.245 ppm 1,2,3-trichlorobenzene. Response of the diatom was measured by quantitative ultrastructure and fatty acid percent composition over a 5-day period. During that time, 35 significant morphological and 12 fatty acid percent composition changes occurred. The most pronounced morphological change that occurred was a significant increase in lipid volume. In addition, changes were observed in vacuolar relative volume, suggesting that the tonoplast became more permeable. Fewer significant changes were observed in fatty acid percent composition upon exposure to this isomer. However, there was a consistent increase in oleic acid (C18:1). The observed changes in morphological and fatty acid percent composition were uniformly distributed with time after the first hour of exposure. Results support the hypothesis that increased lipid stores may alter the timing of response to lipophilic toxicants.

In previous papers (Sicko-Goad *et al.* 1989a, 1989b, 1988), we suggested that while lipophilicity and octanol/water partition coefficients may be indicative of bioaccumulation potential, both the physical-chemical properties of toxicants and the physiological state of cells exposed to a lipophilic toxicant may play an important role in ameliorating or amplifying effects of toxicants. We have shown in these papers that lipid content of the diatom *Cyclotella meneghiniana* fluctuates with the daily photocycle and that two trichlorobenzene isomers having very similar octanol/water partition coefficients produce different patterns of effect with respect to the timing of the observed effect. For example, exposure to 1,2,4-trichlorobenzene produces the greatest number of alterations in morphological and fatty acid composition after longer exposure periods (Sicko-Goad *et al.* 1989b) whereas exposure to 1,3,5-trichlorobenzene results in large numbers of alterations in these components within 24 hr (Sicko-Goad *et al.* 1989a) We speculated that either lipid content of the diatoms which fluctuates with the daily photocycle or chemical reactivity of the two isomers could explain the pattern of change we observed.

This paper is the third in a series reporting results of exposures to trichlorobenzene isomers and discusses results obtained with exposure to 1,2,3-trichlorobenzene. By virtue of its structure, this isomer has three vicinal carbon atoms (Williams *et al.* 1975) and would be expected to be the most reactive in terms of epoxide formation and extent of metabolism of the three trichlorobenzene isomers.

Materials and Methods

All methods have been described in detail (Sicko-Goad *et al.* 1989b, 1988). The cultures of *Cyclotella* used in this experiment differed from cultures previously described in that sampling was begun much earlier in the day. Experiments with this isomer were initiated at 8 am, during the 6th hour of a 16/8 hr light/dark cycle. Consequently, 10 min and 1 hr samples were taken in what we defined as the early light period (Sicko-Goad *et al.* 1988), and the 8-hr sample was withdrawn 2 hr before dark.

Results

The most pronounced morphological changes that occur on exposure to 1,2,3-trichlorobenzene are the consistently elevated levels of lipid and autophagic-

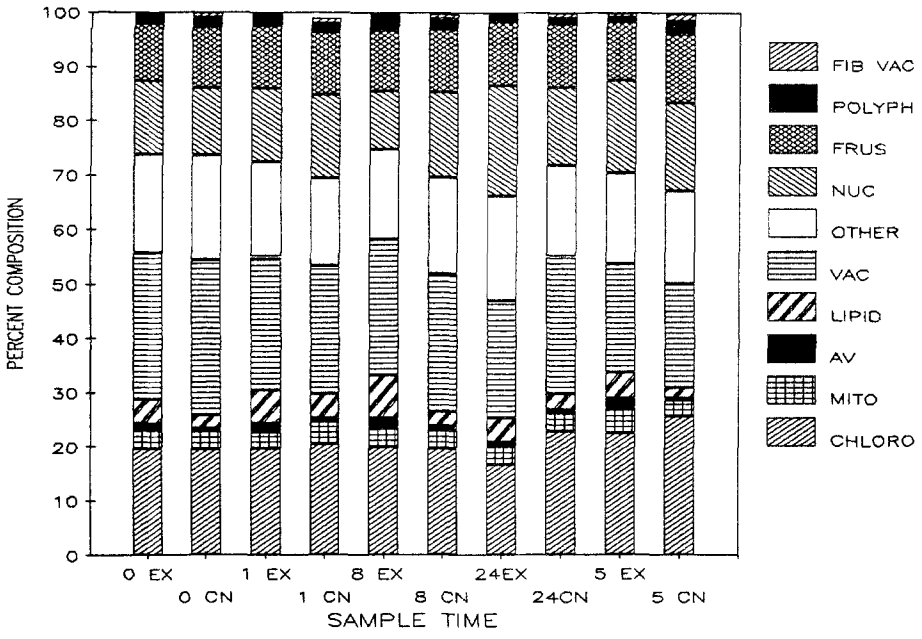


Fig. 1. Percent composition (relative volume) of all cytoplasmic volume components in *Cyclotella meneghiniana* control (CN) and experimental (EX) samples

like vacuole volume in exposed cells relative to control cells throughout the entire experiment (Figure 1 and Table 1). There also appears to be a small increase in chloroplast volume as a result of culture age in control cells. However, exposure to 1,2,3-trichlorobenzene reverses this trend in the 24-hr and 5-day samples. Fibrous vacuole is also consistently lower in exposed cells. Nuclear volume decreases in control cells throughout the first 8 hr of the experiment. However, exposed cells exhibit a decrease in volume in the same time period. Nuclear volume is also unusually large in cells exposed to 1,2,3-TCB at 24 hr and 5 days.

When data are regrouped into four major cellular compartments (frustule, nucleus, cytoplasm, and vacuole), apparent vacuole volume decreases (Figure 2) in spite of the increase in lipid volume which is located in the vacuole and cytoplasmic components increase proportionately. There appears to be no significant change in chloroplast numbers with exposure, although there is a trend of slight reduction in exposed cells through 24 hr (Figure 3). In general, polyphosphate bodies increase in numbers in exposed cells during the early sampling period while this trend is reversed at 8 hr (Figure 4). Mitochondrial and chloroplast lipid droplet numbers remain relatively constant and patterns of increase or decrease appear insignificant and within the realm of sampling error. Summaries of significant changes in morphological components (i.e., >20%) for all time periods examined are as follows (Table 1 and Figure 1):

10 min—Increases in autophagic vacuole and lipid; decrease in fibrous vacuole.
1 hr—Increases in autophagic-like vacuole and lipid; decreases in mitochondria and fibrous vacuole.
8 hr—Increases in autophagic-like vacuole, lipid, chloroplast lipid and polyphosphate; decreases in nucleus and fibrous vacuole.
24 hr—Increases in autophagic-like vacuole, lipid, polyphosphate, and nucleus; decreases in chloroplast, chloroplast lipid and fibrous vacuole.
5 day—Increases in mitochondria, autophagic-like vacuole, and lipid; decreases in chloroplast lipid, polyphosphate, and fibrous vacuole.

Fatty Acid Composition

The pattern of fatty acid composition is somewhat altered in this experiment (Figure 5) in comparison with the previously described chlorinated benzene exposure experiments (Sicko-Goad *et al.* 1989a, 1989b). With this experiment, the distribution of C16 fatty acids varied, with the relative percent composition of both fatty acids in the 30–40% range although the sum of these two acids is still approximately 70%. Minor fatty acids (designated as others) constitute a greater, although still small percentage of the total fatty acids. The C18:0 fatty acid appears to be present in higher percentages in control cells. Conversely, the C18:1 fatty acid is

Table 1. Relative volumes (% composition) and numbers per volume of cellular components of *Cyclotella menighiniana* control cells and cells exposed to 0.245 ppm 1,2,3 trichlorobenzene. Values reported are the mean (±1 S.E.) of a sample size of 25

	Experimental Treatment									
Cell Component	10 min Control	10 min Exposed	1 hr Control	1 hr Exposed	8 hr Control	8 hr Exposed	24 hr Control	24 hr Exposed	5 day Control	5 day Exposed
Chloroplast V_v	19.0	19.0	20.5	18.9	19.0	19.0	21.8	15.9	24.0	21.4
	(1.1)	(1.5)	(1.5)	(1.2)	(1.7)	(1.5)	(1.4)	(1.5)	(1.3)	(1.5)
Chloroplast lipid V_v	0.6	0.7	1.0	0.9	0.8	1.0	1.1	0.9	1.6	1.2
	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)	(0.3)	(0.2)	(0.2)	(0.2)	(0.2)
Mitochondria V_v	3.4	3.4	4.2	2.9	3.5	3.7	3.4	3.3	3.0	4.6
	(0.4)	(0.5)	(0.5)	(0.4)	(0.4)	(0.6)	(0.5)	(0.5)	(0.5)	(0.6)
Autophagic-like vacuole V_v	0.4	1.3	0.6	1.7	0.5	1.6	0.6	0.8	0.4	1.7
	(0.2)	(0.4)	(0.7)	(0.3)	(0.3)	(0.3)	(0.2)	(0.2)	(0.2)	(0.5)
Lipid V_v	2.5	4.4	4.5	6.1	2.7	8.0	2.9	4.5	1.9	4.8
	(0.7)	(1.1)	(1.3)	(1.3)	(0.7)	(2.0)	(0.7)	(1.1)	(0.6)	(1.4)
Vacuole V_v	28.6	26.9	23.6	24.5	25.4	25.0	25.2	21.6	19.2	20.0
	(2.3)	(2.4)	(1.9)	(2.3)	(2.1)	(2.4)	(2.4)	(2.0)	(2.2)	(2.0)
Other	19.1	18.1	16.1	17.5	17.7	16.4	16.8	19.2	17.0	16.8
	(1.3)	(1.1)	(1.2)	(1.4)	(1.1)	(0.9)	(1.1)	(1.3)	(0.8)	(1.2)
Nucleus V_v	12.3	13.5	15.4	13.5	15.7	10.8	14.3	20.3	16.3	17.0
	(1.7)	(1.9)	(1.9)	(1.9)	(2.0)	(1.8)	(1.5)	(1.9)	(2.0)	(1.4)
Frustule V_v	11.4	10.6	11.5	11.5	11.6	11.2	11.9	11.8	12.7	10.8
	(0.4)	(0.7)	(0.6)	(0.8)	(0.8)	(0.6)	(0.6)	(0.6)	(0.8)	(0.4)
Polyphosphate V_v	1.7	1.5	1.7	2.0	1.9	2.9	1.1	1.4	2.4	0.9
	(0.4)	(0.3)	(0.5)	(0.4)	(0.4)	(0.6)	(0.4)	(0.3)	(0.6)	(0.3)
Fibrous vacuole V_v	1.0	0.5	0.9	0.4	1.0	0.4	0.9	0.4	1.4	0.9
	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.1)	(0.3)	(0.2)
Chloroplast N_V	0.13	0.11	0.12	0.11	0.18	0.10	0.12	0.10	0.11	0.11
($/\mu m^3$ cell)	(0.02)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.02)
Chloroplast lipid N_V	19.86	18.19	15.37	18.42	20.00	19.70	20.42	23.25	23.76	14.57
($/\mu m^3$ chloroplast)	(2.90)	(4.30)	(2.40)	(4.60)	(3.90)	(3.60)	(5.40)	(3.70)	(2.10)	(2.70)
Mitochondria N_V	0.06	0.10	0.09	0.07	0.10	0.064	0.06	0.07	0.04	0.05
($/\mu m^3$ cell)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Polyphosphate N_V	0.79	1.19	0.50	1.39	0.74	0.33	0.50	0.51	0.95	0.81
($/\mu m^3$ cell)	(0.19)	(0.34)	(0.19)	(0.53)	(0.34)	(0.12)	(0.19)	(0.12)	(0.42)	(0.37)

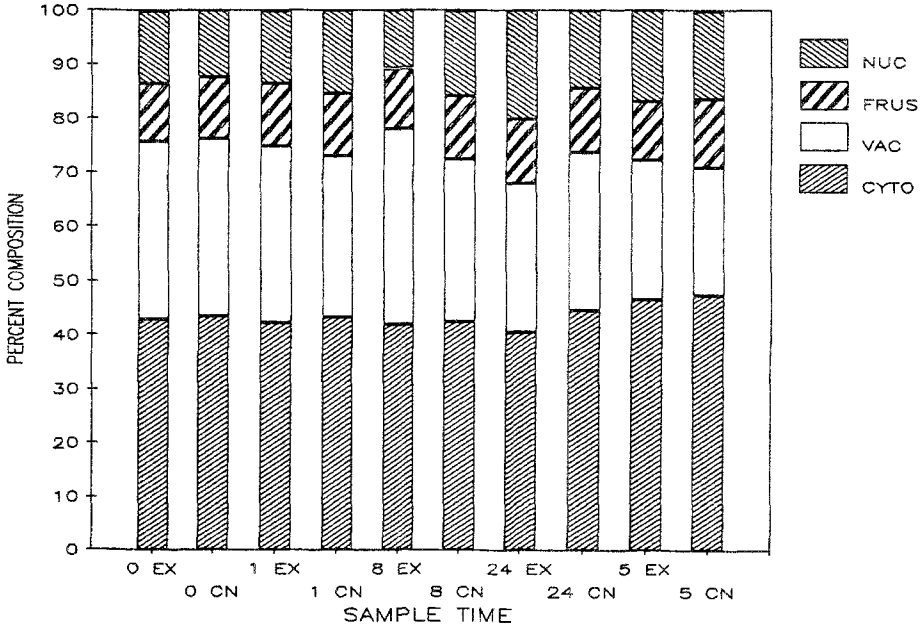


Fig. 2. Percent composition of regrouped major cellular compartments in exposed and control cells

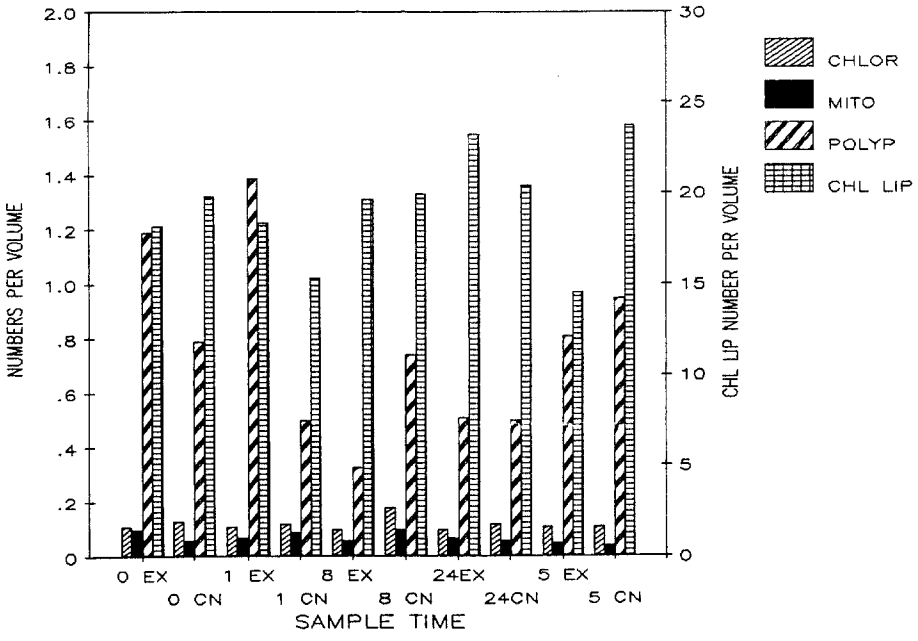


Fig. 3. Numbers per volume of selected inclusions and organelles in control and exposed cells. Chloroplast lipid droplets (plastoglobuli) are reported as numbers per m^3 chloroplast. All other values are reported per μm^3 cell

generally greater in experimental cells. Summaries of significant changes (>20%) in fatty acid composition are as follows:

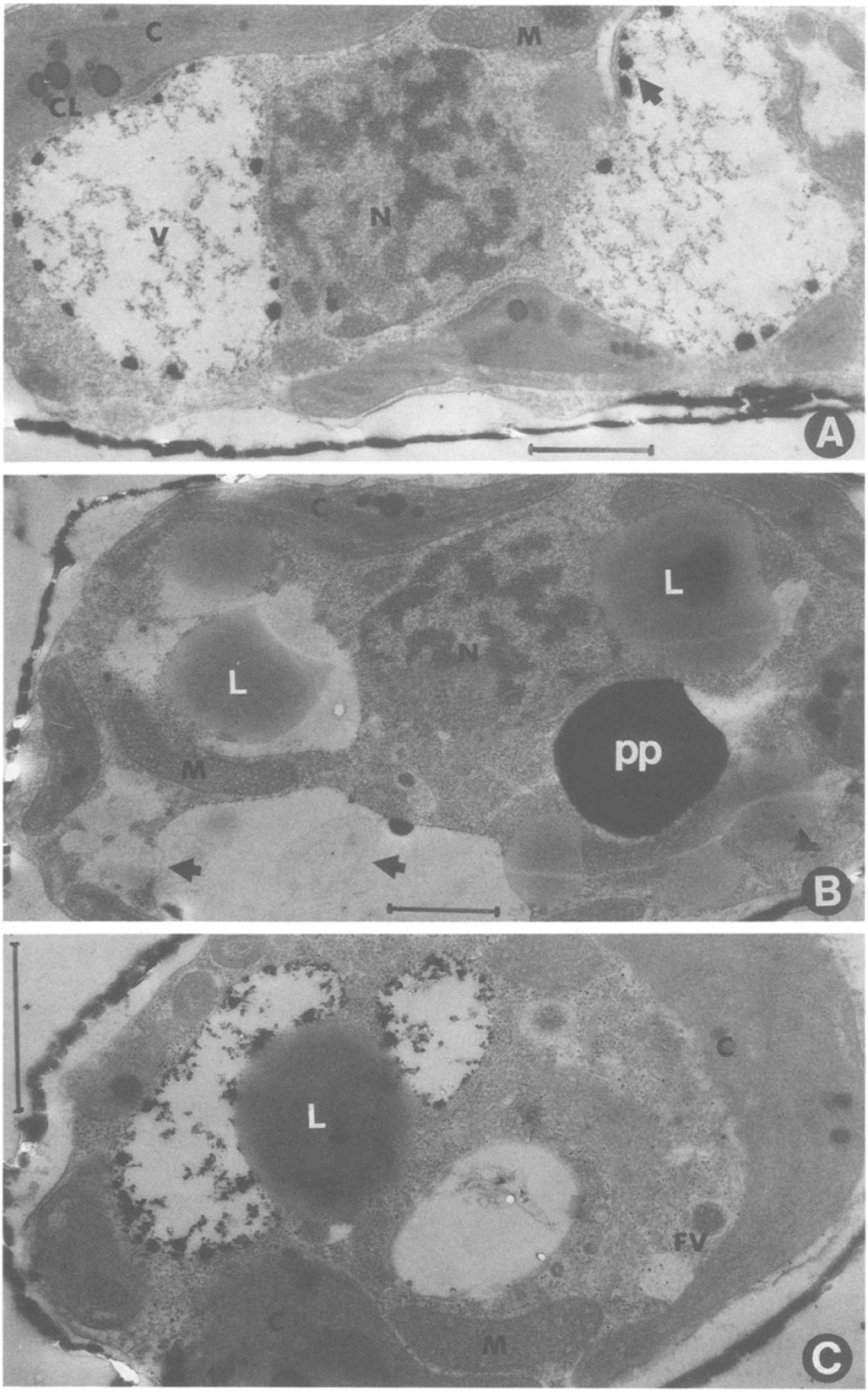
10 min—Increases in C18:1; decrease in C20:5 (Figure 6a).
1 hr—Increases in C16:1 and C20:5; decreases in C18:1 and C18:0 (Figure 6b).
8 hr—Increases in C18:1 and C20:5 (Figure 6c).
24 hr—Increase in C18:1; decrease in C20:5 (Figure 7a).
5 day—Increase in C18:1; decrease in C18:0 (Figure 7b).

A total number of 26 significant morphological volume changes occurred during the five sampling periods examined. In addition, there were 9 significant changes in numbers per volumes of organelles, and 12 changes in fatty acid percent composition. The fewest significant changes were observed in the first 10 minutes of exposure. The number of significant changes observed during the other time periods examined was constant.

Discussion

The most pronounced morphological alteration found in *Cyclotella meneghiniana* exposed to 1,2,3-trichlorobenzene is the consistent increase in lipid volume, accompanied by a decrease in the structure we refer to as "fibrous" vacuole. The relationship between these two components is not clear. Fibrillar-like vesicles have been described in several diatoms (Drum and Pankratz 1964; Stoermer *et al.* 1965; Taylor 1972; Walker *et al.* 1979; Edgar 1980; Sicko-Goad 1986). In *Caloneis amphisbaena*, they appear in close association with the nuclear envelope (Walker *et al.* 1979). However, in *Melosira granulata*, they are located near the nucleus and Golgi (Sicko-Goad 1986). In *Cyclotella*, they are usually located in close proximity to the storage products, and it has been suggested that this type of vesicle stores and exports extracellular products (Gibson 1979; Daniel *et al.* 1980; Edgar and Pickett-Heaps 1984; Gordon 1987). Although this inverse relationship was not evident with exposure

Fig. 4. Electron micrographs of control and experimental cells of *Cyclotella*. Key to figure legends: Chloroplast (C), Chloroplast Lipid (CL), Fibrous Vacuole (FV), Lipid (L), Mitochondria (M), Nucleus (N), Polyphosphate (PP), Vacuole (V). Marker bars = 1 μm. **A** Eight hr control cell. The central cytoplasmic bridge show the nucleus and portions of chloroplasts and mitochondria. Small polyphosphate bodies (arrows) are located at the vacuole periphery of the vacuole. **B** Eight hr exposed cell. Large polyphosphate and lipid inclusions fill portions of the vacuole. Membranous remnants are observed in other portions of the vacuole (arrows). **C** Five day exposed cell. Fibrous vacuoles can be observed adjacent to the chloroplast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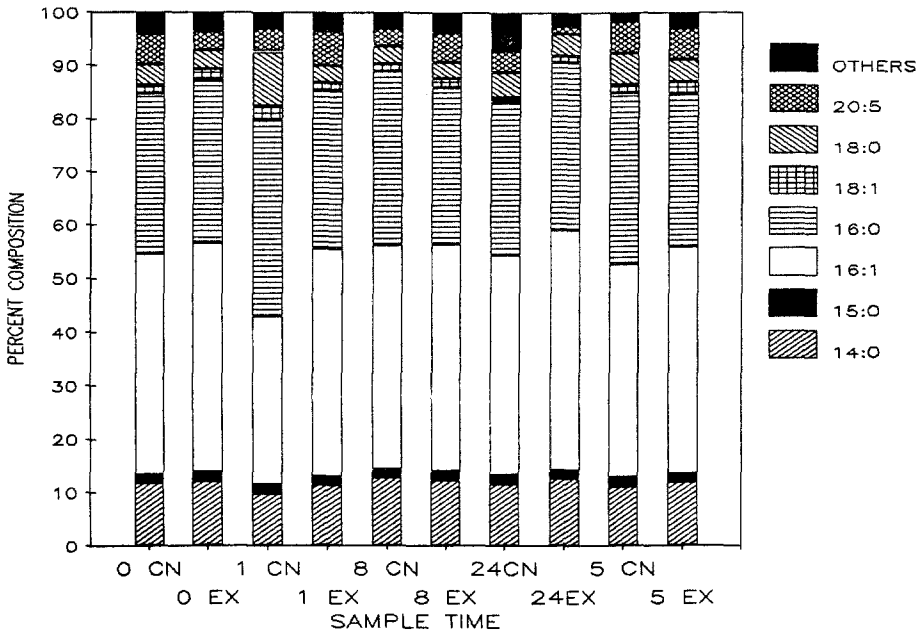

Fig. 5. Relative fatty acid percent composition in control and experimental cells. "Others" represent minor confirmed fatty acids present in indivdual amounts less than 1%

to 1,2,4- or 1,3,5-trichlorobenzene, it is possible that the toxicological mode of action of the isomers varies in its effect on carbon recycling. In any case, this morphological component seems to respond quickly to exposure and its biochemical properties deserve further investigation. Increases in storage product accumulation have also been noted for other organic toxicants (Soto *et al.* 1977; Abarzua *et al.* 1985).

When cellular components are regrouped to consider changes in two major compartments, vacuole and cytoplasm, it appears that the containing volume of vacuole is higher in exposed cells than in control cells. This volume increase cannot be accounted for solely by the increase in lipid volume. This suggests that the tonoplast or vacuolar membrane became more permeable so that solute moved into the vacuole, resulting in an apparent increase in vacuole volume in exposed cells. Lipophilic membranes are particularly sensitive to the effects of hydrocarbons and in view of the fact that toxicants can either partition into membranes or absorb on them, it is not surprising to find changes that suggest alterations in membrane permeability. Such changes in permeability have been demonstrated for a variety of toxicants (Boyles 1980; Gotham and Rhee 1982; Sicko-Goad 1982; Smith 1983; Moore 1985; Sicko-Goad and Lazinsky 1986).

Fewer significant changes were observed in fatty acid percent composition as a result of exposure to 1,2,3-trichlorobenzene than were observed for the other two isomers (Sicko-Goad *et al.* 1989a, 1989b). There was a consistent increase in the 18:1 fatty acid (oleic acid) upon exposure to this isomer. Similar increases in oleic acid in copper exposed *Fucus* have been demonstrated by Smith and Harwood (1984) and Smith *et al.* (1985), who postulated that copper lowered the activity of acyl-ACP transacylases.

Fisher and Schwarzenbach (1978) demonstrated that C16:0 and C16:1 fatty acids in *Thalassiosira pseudonana* were stored in triglycerides and were partially or completely oxidized when cells were in the dark for prolonged periods of time or were exposed to PCB. We demonstrated that there was an increase in lipid volume in *Cyclotella* as darkness approached that paralleled the increase in the C16:1 fatty acid (Sicko-Goad *et al.* 1988) Although the numbers are not significant, this increase in the C16:1 fatty acid may also be observed in the present data set.

We had anticipated that 1,2,3-trichlorobenzene would produce the greatest effects in the components evaluated during the course of these experiments. However, it is apparent from the data that the isomers differ in the pattern of changes produced as well as in the absolute numbers of change (Figures 8a-b). For example, the pattern of change is most similar for 1,3,5- and 1,2,3-trichlorobenzene (Figures 8a-b). Assuming that our criterion of 20% change (Figure 8a) was not a stringent enough requirement to determine significance, we reexamined our data and plotted the total number of changes that were greater than 50% for all categories (relative volumes, numbers per volume, and fatty acid percent composition) against sample time for all three isomers. The pattern of change remains the same (Figure 8b), with 1,2,3- and 1,3,5-trich-

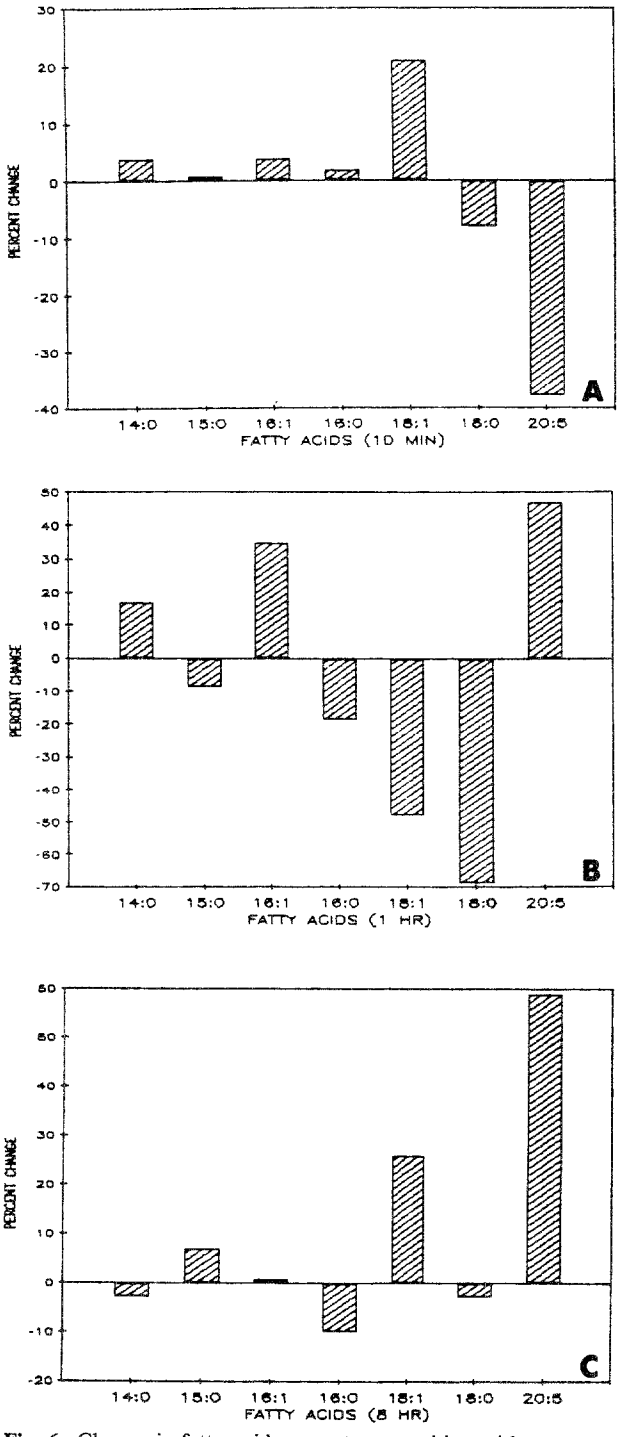

Fig. 6. Change in fatty acid percent composition with exposure to 1,2,3-trichlorobenzene. Early sampling periods. A 10 min exposure. B 1 hr exposure. C 8 hr exposure

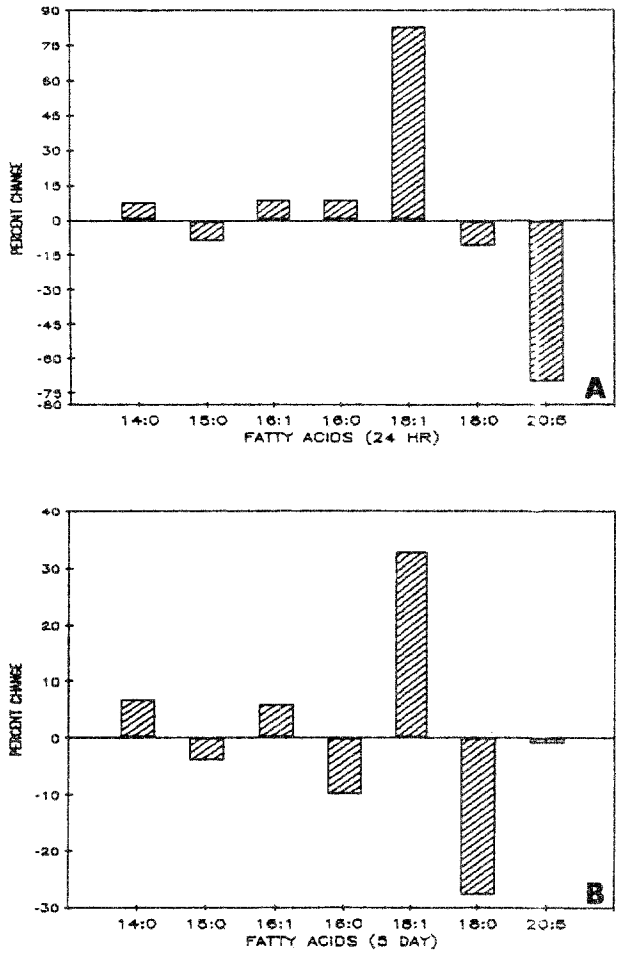

Fig. 7. Changes in fatty acid percent composition with exposure. Late sampling periods. A 24 hr exposure. B 5 day exposure

lorobenzene with respect to the timing of the alterations, although the absolute number of changes is the same over 5 sampling periods with either 1,2 4- or 1,2,3-trichlorobenzene.

We believe that the results presented here strongly suggest that the physiological state of the cell plays an important role in either ameliorating or amplifying toxicant effects. The greatest numbers of significant changes were observed in cells exposed to 1,2,4- and 1,2,3-trichlorobenzene. Of the three isomers, these two were predicted to be more reactive than 1,3,5- with 1,2,3- having the most potential for epoxide and metabolite production. The only difference between the cultures used in all three experiments was related to the timing of the experiments in the daily photocycle. The experiment with 1,2,4-trichlorobenzene was started late in the day, and we have shown previously that cells taken during the latter part of the light period have a greater lipid content (Sicko-Goad *et al.* 1988). In contrast, the cells that were exposed to 1,3,5- or 1,2,3-trichlorobenzene, presumably the compound having the greatest potential for toxicity, were exposed to these isomers very early in the light period when lipid reserves are lowest. Assuming that partitioning occurs into lipids, there was less potential for the isomers to partition into lipid reserves and

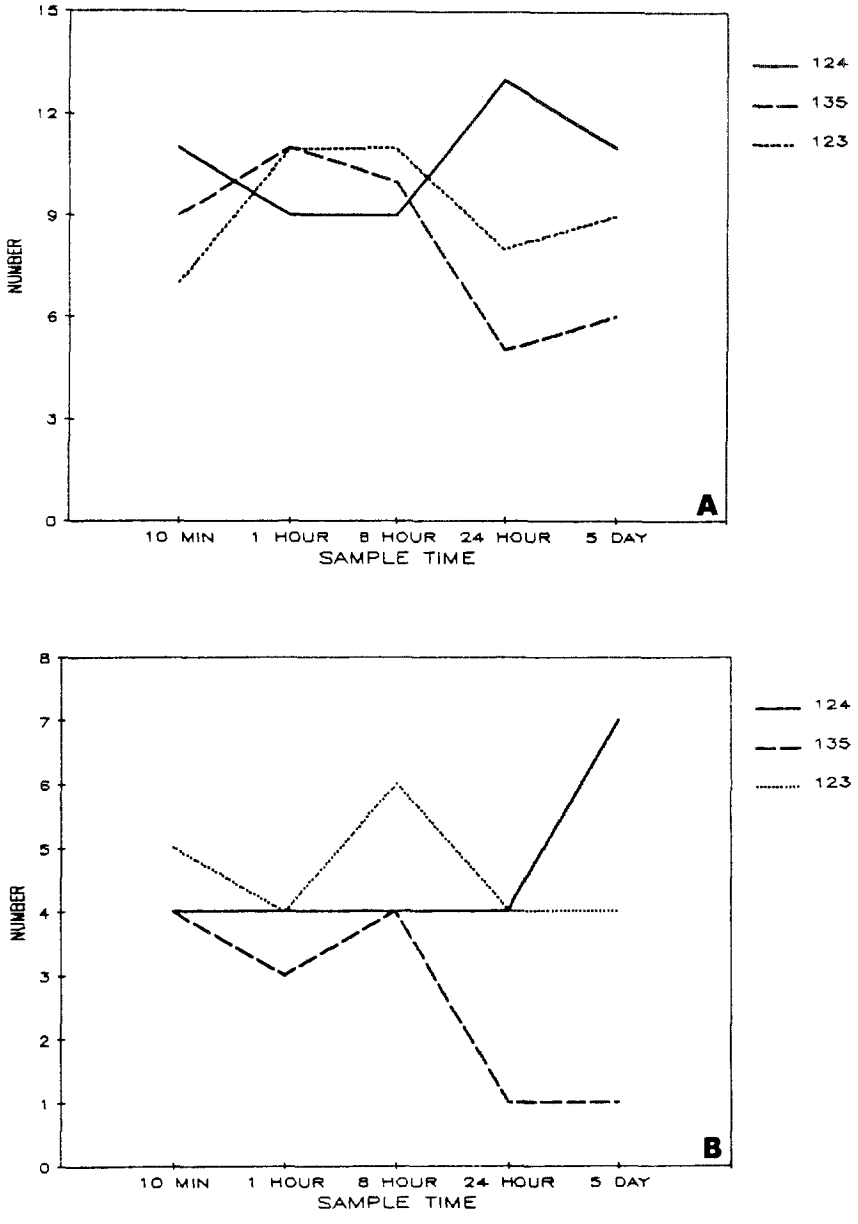

Fig. 8. Numbers of significant changes occurring in morphological and fatty acid percent composition by sample time and isomer. **A** Changes greater than 20%. **B** Changes greater than 50%

one would expect that more toxicant would be available to lipophilic membranous systems within the cell, resulting in greater numbers of detrimental changes during the early sampling periods and this, in fact, was observed (Figure 8b).

One would also expect that at sub-lethal doses lipophilic toxicants might exert effects that are somewhat biphasic. That is, effects would include those produced by the interaction of the toxicant with lipoproteins, resulting in immediately measurable effects such as changes in membrane permeability, photosynthesis, etc. Secondary measurable effects might include systems affected by the production of metabolites. Of the three tricholorobenzene isomers, the 1,3,5- isomer has the least capability of epoxide or metabolite formation and it is apparent that effects experienced in this experiment were short-term, *i.e.*, less than 8 hr (Figures 8a-b). Rapid recovery from either short-term or sublethal toxicant doses in not uncommon (Soto *et al.* 1977; Sandmann and Boger 1980; Sicko-Goad 1982; Hardy *et al.* 1985). In contrast, the more reactive isomers, 1,2,3- and 1,2,4-trichlorobenzene, exerted more long-term effects.

It has been suggested that environmental physical-chemical parameters and/or physiological state of the cell may play an important role in toxicity studies (Fisher *et al.* 1976; Conner and Mahanty 1979; Karydis and Fogg 1980; Sicko-Goad *et al.* 1986; Sicko-Goad and Lazinsky 1986; Neumann *et al.* 1987). We would further suggest that in phytoplankton, with their rapid generation times, even

daily photoperiodic events, which are not generally thought of in terms of being environmental perturbations, may alter the effects of toxicants. We also speculate that the increased numbers of effects observed with exposue to 1,2,4-trichlorobenzene were the result of greater partitioning of the lipophilic toxicant into cells that had a greater lipid content as a result of a natural photocycle. Consequently, naturally occurring, rather subtle changes in the physical environment may alter the physiological state of phytoplankton, so that cells become more susceptible to lipophilic toxicants.

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