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Effects of Chlorinated Benzenes on Diatom Fatty Acid Composition and Quantitative Morphology. II. 1,3,5-Trichlorobenzene

L. Sicko-Goad, J. Hall, D. Lazinsky, and M. S. Simmons

Great Lakes Research Division, The Institute of Science and Technology, The University of Michigan, Ann Arbor, Michigan 48109, USA

Abstract. Cells of the diatom *Cyclotella meneghiniana* were exposed in a closed system to 0.245 ppm 1,3,5-trichlorobenzene. Response of the diatom was measured by quantitative ultrastructure and fatty acid percent composition over a 5-day period. During that time, 28 significant morphological and 13 significant fatty acid percent composition changes occurred. Autophagic-like vacuoles were observed consistently through the sampling periods. In comparison with exposure to 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene exerts fewer effects in the parameters studied, and these effects were observed most frequently in membranous components during the initial eight hours of exposure. It is suggested that the amount of cellular lipid and the relative reactivity of the isomer are responsible for the observed effects.

While lipid content is an important factor in bioconcentration of lipophilic toxicants in fish (Earnest and Benville 1971; Ernst 1979; Goerke 1984; Geyer *et al.* 1985), little attention has been paid to the role of lipids as a contributing factor in toxicity studies with phytoplankton. It has been suggested that increased toxicity of more octanol-soluble compounds to unicellular green algae may be related either to the greater ability of these compounds to penetrate and damage the lipoprotein cell membrane or to overall lipid content of the algae (Hutchinson *et al.* 1980).

Increases in phytoplankton storage product accumulation are usually associated with onset of adverse environmental parameters such as nutrient or toxicant stress (Davis 1972; Sicko-Goad 1982) or senescence and/or spore formation (McLean 1968; Schlichting 1974; Anderson 1975; von Stosch and Fecher 1979; French and Hargraves 1980; Sicko-Goad 1986; Sicko-Goad *et al.* 1986). Bisalputra and Antia (1980) demonstrated that reillumination of dark cultures of *Porphyridium* resulted in the deposition of significant numbers of starch grains within three hours and they attributed this to resumption of active growth by those cells surviving prolonged darkness. Similarly, rejuvenating resting cells of *Melosira granulata* produce copious amounts of lipid when first exposed to light and presumably these storage products are used to sustain the rapid burst of growth upon resumption of vegetative growth (Sicko-Goad *et al.* 1986).

However, relatively little is known about photoperiodic changes in lipid content in photosynthetic organisms. Shifrin and Chisholm (1981) demonstrated that changes in total lipids over a daily cycle are associated directly with cell growth and reproduction. Similarly, Wada *et al.* (1987) found that fatty particles appeared in a Raphidophycean alga in the dark. Rivkin (1985) reported that diatoms utilize lipids and low molecular weight polymers for respiration and/or protein synthesis at night. Recent work in our lab has demonstrated that lipid volume increases in the late part of the light period and reaches a maximum in the dark (Sicko-Goad *et al.* 1988). When cells of *Cyclotella meneghiniana* were exposed to the lipophilic toxicant 1,2,4-trichlorobenzene, more significant changes in morphological components and fatty acid composition were observed after the cells had gone through the dark period of the daily photocycle, suggesting that lipid status of cultures may ameliorate or delay effects of lipophilic toxicants (Sicko-Goad *et al.* 1989).

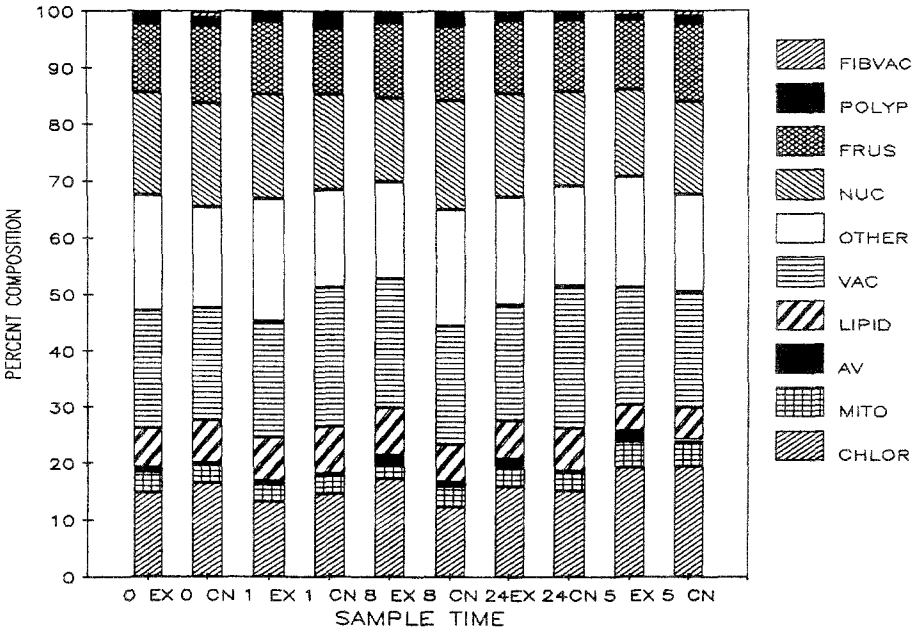

Fig. 1. Relative volumes (percent composition) of cellular compartments in *Cyclotella meneghiniana* during 1,3,5-trichlorobenzene exposure experiments (EX - experimental; CN - control)

Environmental chemistry of the three trichlorobenzene isomers would predict that the 1,3,5-isomer would be least reactive (Sicko-Goad *et al.* 1989). Wong *et al.* (1984) demonstrated that the EC_{50} is slightly higher for this isomer than the other two isomers of trichlorobenzene. This is the second paper in a series that reports changes in quantitative ultrastructure and fatty acid composition as a result of exposure to trichlorobenzenes and presents results of exposure to 1,3,5-trichlorobenzene.

Materials and Methods

All materials and methods have been described in detail in the first paper in this series (Sicko-Goad *et al.* 1989). The only deviation from the experiment with 1,2,4-trichlorobenzene was the timing of withdrawal of samples for analysis. With 1,2,4-trichlorobenzene, the 8 hr sample was taken in the second hr of the dark period. With 1,3,5-trichlorobenzene all samples were withdrawn in the early and late light periods, the times corresponding to low lipid content (Sicko-Goad *et. al.* 1988).

Results

Cytological Changes

The most pronounced morphological change that occurs on exposure to 1,3,5-trichlorobenzene is the increase in autophagic-like vacuole with time (Figure 1 and Table 1). Lipid volume decreases in later sampling periods and the reduction is more pronounced in exposed cells. During short-term sampling periods (10 min and 1 hr), chloroplast relative volume is reduced in experimental cells although this trend is reversed in the later sampling periods. Five day control and exposed cells are remarkably similar (Table 1 and Figure 2). Greatest variability in all morphological parameters occurs at 8 hr of exposure. Polyphosphate and lipid relative volume are generally lower in exposed cells (Table 1).

When data are regrouped into major cellular compartments (Figure 3) nuclear volume appears to be reduced in the 8 h experimental sample whereas vacuole volume is largest in the 1 h control sample. There appears to be no significant change in chloroplast numbers with exposure (Figure 4, Table 1). In general, polyphosphate body numbers per volume increase somewhat in exposed cells while mitochondrial numbers show a reduction with time. This effect is enhanced by exposure to 1,3,5-trichlorobenzene. Chloroplast lipid droplets (plastoglobuli) are more numerous in exposed cells. The increase in chloroplast lipid droplet number is also accompanied by an increase in relative volume. Summaries of significant changes in morphological components (>20%) for all time periods examined are as follows (Table 1, Figure 1):

10 min—Increases in autophagic vacuole and polyphosphate; decrease in fibrous vacuole.

Table 1. Relative volumes (% composition) and numbers per volume of cellular components of *Cyclotella meneghiniana* control cells and cells exposed to 0.245 ppm 1,3,5 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	15.7	14.2	14.1	12.6	11.9	16.3	14.6	15.1	18.4	18.4
	(1.2)	(1.3)	(1.6)	(1.3)	(1.2)	(1.1)	(2.0)	(1.5)	(2.0)	(1.7)
Chloroplast lipid V_v	0.8	0.6	0.5	0.6	0.4	1.0	0.6	0.8	1.0	0.9
	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)	(0.2)
Mitochondria V_v	3.5	3.6	3.6	3.1	3.6	2.6	3.4	3.5	4.3	4.5
	(0.5)	(0.4)	(0.5)	(0.5)	(0.5)	(0.2)	(0.5)	(0.5)	(0.8)	(0.6)
Autophagic-like vacuole V_v	0.0	0.8	0.0	0.6	0.9	1.6	0.1	1.4	0.4	2.0
	(0.0)	(0.4)	(0.0)	(0.3)	(0.4)	(0.3)	(0.06)	(0.38)	(0.14)	(0.56)
Lipid V_v	7.6	7.1	8.3	7.7	6.6	8.3	7.6	6.8	5.8	4.6
	(1.7)	(1.8)	(1.7)	(1.6)	(1.7)	(1.8)	(1.5)	(1.7)	(2.2)	(1.5)
Vacuole V_v	20.0	20.9	24.7	20.7	21.1	23.1	25.3	20.6	20.5	20.8
	(1.8)	(1.7)	(2.3)	(1.8)	(1.4)	(2.0)	(2.1)	(1.8)	(2.1)	(1.9)
Other	17.9	20.4	17.3	21.6	20.6	17.1	17.6	19.1	17.2	19.5
	(1.3)	(1.2)	(1.3)	(1.2)	(1.5)	(1.0)	(1.2)	(1.4)	(1.1)	(1.0)
Nucleus V_v	18.4	18.1	16.9	18.6	19.3	14.8	16.8	18.3	16.5	15.6
	(2.3)	(1.9)	(1.7)	(1.9)	(2.3)	(1.6)	(2.3)	(2.2)	(1.9)	(2.4)
Frustule V_v	13.6	12.1	11.6	12.8	12.9	13.3	12.5	12.8	13.7	12.1
	(0.7)	(0.5)	(0.6)	(0.5)	(0.9)	(0.62)	(0.76)	(0.92)	(0.74)	(0.62)
Polyphosphate V_v	1.4	2.1	2.4	1.1	2.0	1.3	1.0	1.1	1.2	0.90
	(0.3)	(0.6)	(0.4)	(0.3)	(0.5)	(0.26)	(0.28)	(0.30)	(0.68)	(0.24)
Fibrous vacuole V_v	1.0	0.2	0.5	0.5	0.5	0.50	0.50	0.50	1.0	0.70
	(0.4)	(0.1)	(0.2)	(0.1)	(0.2)	(0.16)	(0.20)	(0.18)	(0.26)	(0.03)
Chloroplast N_V	0.08	0.09	0.10	0.08	0.12	0.08	0.09	0.08	0.09	0.10
(/μm^3 cell)	(0.02)	(0.03)	(0.02)	(0.01)	(0.03)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)
Chloroplast lipid N_V	28.44	31.86	22.67	23.39	26.55	29.31	39.19	41.19	34.59	30.07
(/μm^3 cell)	(2.11)	(2.02)	(1.38)	(4.1)	(1.21)	(11.20)	(3.83)	(12.10)	(3.14)	(10.8)
Mitochondria N_V	0.08	0.10	0.08	0.10	0.13	0.05	0.09	0.06	0.06	0.06
(/μm^3 cell)	(0.01)	(0.02)	(0.02)	(0.03)	(0.04)	(0.01)	(0.02)	(0.01)	(0.02)	(0.01)
Polyphosphate N_V	0.403	0.58	0.22	0.34	0.34	0.35	0.34	0.43	0.36	0.35
(/μm^3 cell)	(0.13)	(0.17)	(0.09)	(0.17)	(0.11)	(0.15)	(0.11)	(0.13)	(0.16)	(0.12)

1 hr—Increases in chloroplast lipid droplets, autophagic-like vacuole and "other"; decrease in polyphosphate.
8 hr—Increases in chloroplast, chloroplast lipid, and autophagic-like vacuole; decreases in mitrochondria, nucleus and polyphosphate.
24 hr—Increases in chloroplast lipid and autophagic-like vacuole
5 day—Increase in autophagic-like vacuole; decreases in lipid, polyphosphate, and fibrous vacuole.

A total of 19 significant morphological changes in relative volume occurred during the five sampling periods. In addition, 9 significant changes were observed in numbers per volume of chloroplast, chloroplast lipid, and mitochondria. The number of significant morphological changes reached a maximum at 8 hr and then declined. Autophagic-like vacuoles appeared immediately upon exposure and were consistently present throughout the 5-day sampling period.

Fatty Acid Composition

Few striking changes are observed in fatty acid composition (Figure 5). The C14:0 fatty acid is consistently higher in exposed cells. The C16:0 fatty acid appears to decrease with exposure in the 10 min and 1 hr samples, and appears to decline somewhat with culture age. A slight increase in the C18:1 fatty acid is also observed in the early sampling periods, with this trend reversing itself during the later sampling periods. Summaries of significant changes (i.e., > 20%) in fatty acid composition are as follows:

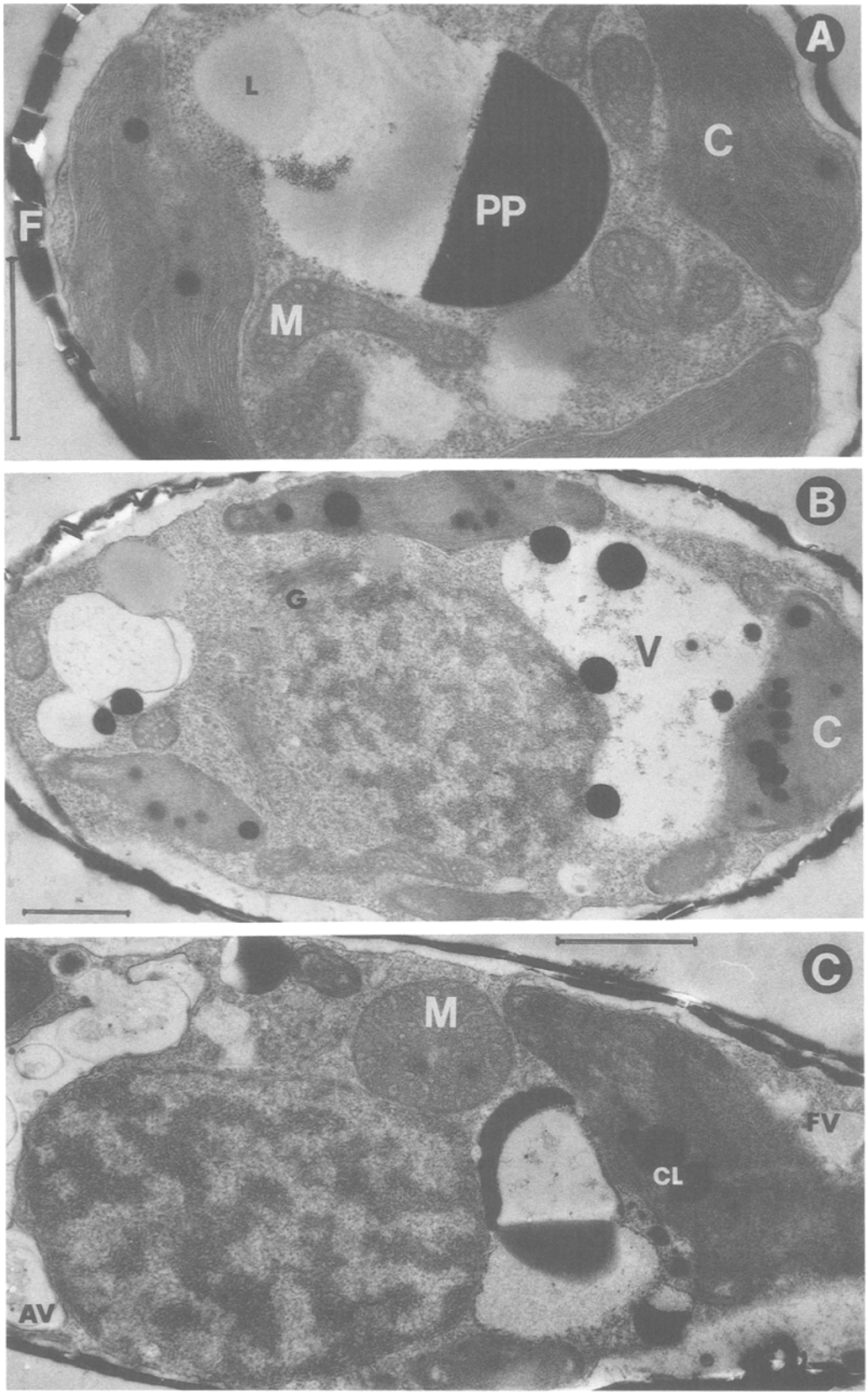

Fig. 2. Electron micrographs of control and experimental cells. Key to figure legends: Autophagic-like Vacuole (AV), Chloroplast (C), Plastoglobuli (CL), Fibrous Vacuole (FV), Frustule (F), Golgi (G), Lipid (L), Mitochondria (M), Polyphosphate (PP), Vacuole (V). Marker bars = 1 μm. **A** One hr control cell. A large polyphosphate body and lipid are present in central vacuole. **B** One hr exposed cell. Numerous small polyphosphate bodies are present in the vacuole. Chloroplast lipid droplets appear numerous and enlarged. **C** Five day exposed cell. Vesiculation, membranous aggregates (AV), and a partially degraded polyphosphate body are present in the vacuole

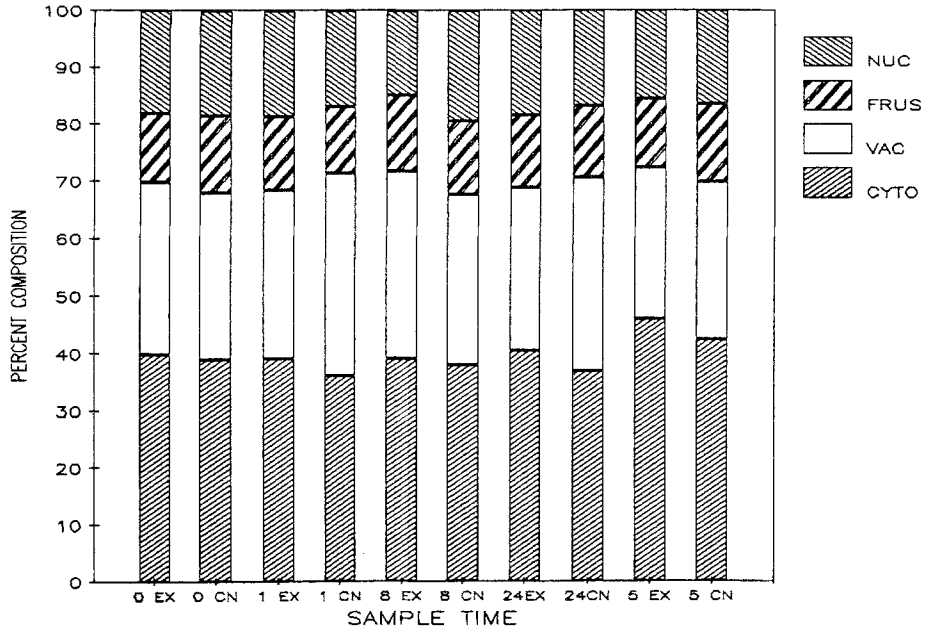

Fig. 3. Percent composition of total cell volume for four aggregate cellular compartments

10 min—Increases in C14:0, C18:1, C20:5; decrease in C16:0 (Figure 6a).
1 hr—Increases in C14:0 and C20:5; decreases in C16:0 and C18:0 (Figure 6b).
8 hr—Decreases in C18:0 and C18:1 (Figure 6c).
24 hr—Decrease in C20:5 (Figure 7a).
5 day—Increases in C18:0 and C20:5 (Figure 7b).

Significant changes were observed in the shorter chain fatty acids during the first hour of exposure. The C20:5 fatty acid showed a uniform decrease. The C18 fatty acids were variable with exposure to this isomer. Overall, 13 significant changes were observed in fatty acid percent composition with exposure, with the greatest numbers of changes occurring within the first 8 hr.

Discussion

The data presented here suggest that 1,3,5-trichlorobenzene exerts fewer detrimental effects on *Cyclotella meneghiniana* than 1,2,4-trichlorobenzene in agreement with the results of Wong *et al.* (1984). We observed a total of 41 significant changes in both morphological and fatty acid composition during 5 time periods examined, in contrast with the 49 observed on exposure to 1,2,4-trichlorobenzene. However, these effects, while significant, were not of the magnitude observed with 1,2,4-trichlorobenzene exposure. The greatest difference observed in the distribution of effects related to the timing of the exposure. That is, most effects from 1,3,5-trichlorobenzene were observed in the initial eight hours of exposure whereas most changes produced by exposure to the 1,2,4- isomer were observed in the 24 hr and 5-day exposures. In addition, most of the changes observed on exposure to the 1,3,5- isomer during the early sampling times were in membranous components.

These results suggest several possible explanations. First, the increased lipophilicity of the 1,3,5-isomer, as predicted by the octanol/water partition coefficent (Wong *et al.* 1984), facilitates partitioning of the chemical into the cell. After exposure, the cells accommodate and recover, in a fashion similar to that reported for short-term exposures to heavy metals at sub-lethal doses (Sicko-Goad 1982). However, the lipophilicity and EC_{50} of these two compounds are not substantially different and we consider it unlikely that these properties contribute to the differences in reactivities.

A second possible explanation for the differences observed between the two isomers is the chemical reactivity. Galassi and Vighi (1981) found that 1,2,3-trichlorobenzene was more toxic than the 1,2,4- isomer in *Selanastrum*. Williams *et al.* (1975) found that the extent of metabolism of chlorinated benzenes and potential epoxide formation depended on the number of unsubstituted vicinal carbons. 1,3,5-trichlorobenzene has no free vicinal carbon positions and thus cannot form epoxides

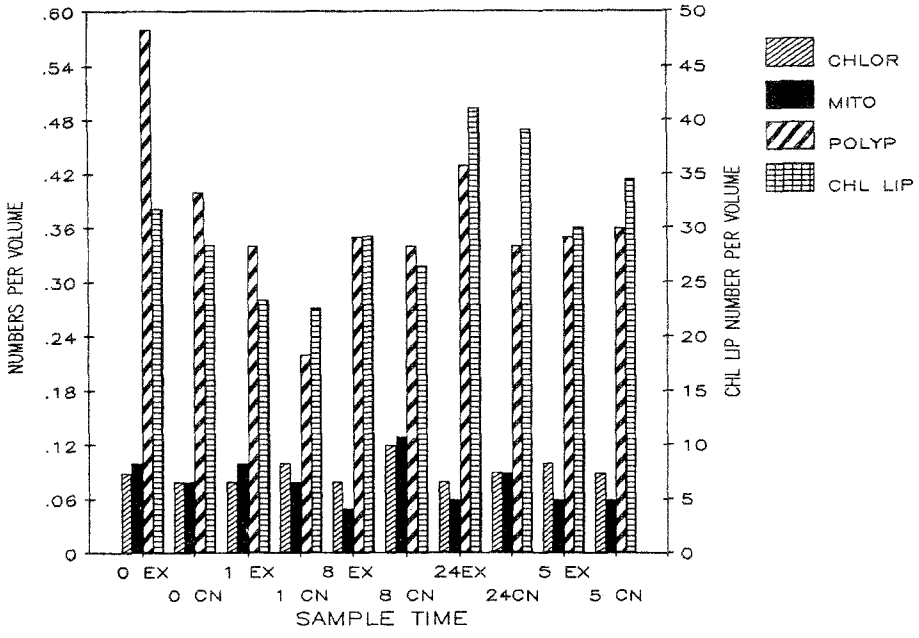

Fig. 4. Numbers per volume of chloroplast, mitochondria, polyphosphate, and chloroplast lipid. N_v for chloroplast is number/μm^3 chloroplast. All other inclusions and organelles are reported per μm^3 cell

whereas the 1,2,4- isomer has 2 vicinal positions and the 1,2,3- isomer has 3. In animals, the metabolism of chlorobenzenes includes hydroxylation to mono- and dihydric phenols followed by the conjugation of these with glucuronic acid and sulfate, and the formation of mercapturic acid (Williams *et al.* 1975). Metabolic pathways of most chlorinated aromatic hydrocarbons are initiated by microbial mono- and dioxygenases resulting in: (1) incorporation of oxygen into the C-H bond, (2) oxidation of the halogen substituents, and (3) oxidation of the C-C bond via epoxidation leading to subsequent cleavage of the benzene ring (Dagley 1975; Gibson *et al.* 1968). Consequently, we may be observing two phenomena: the first reaction is partitioning of the toxicant into lipid components of the cell re-

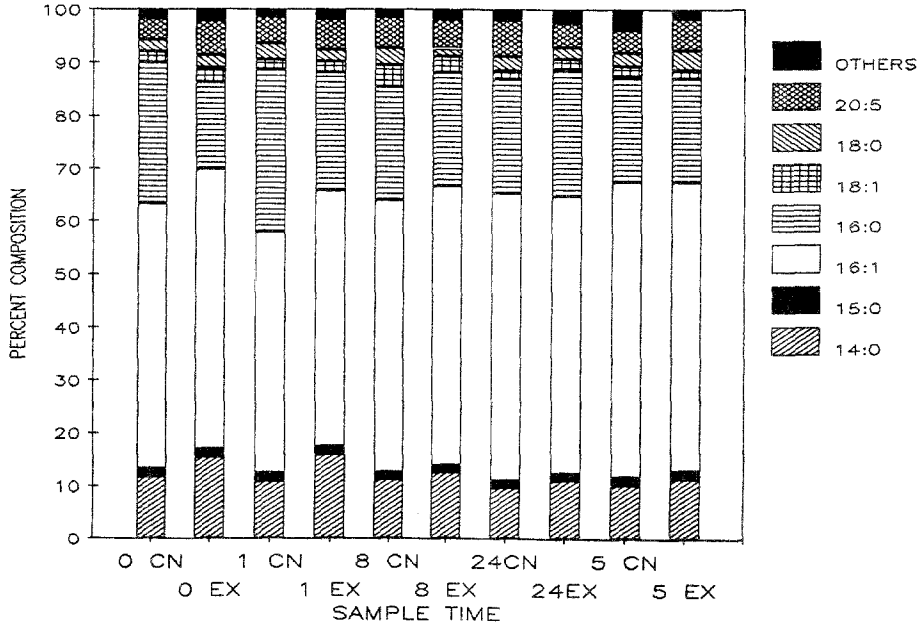

Fig. 5. Relative fatty acid percent composition for all sampling times and treatments

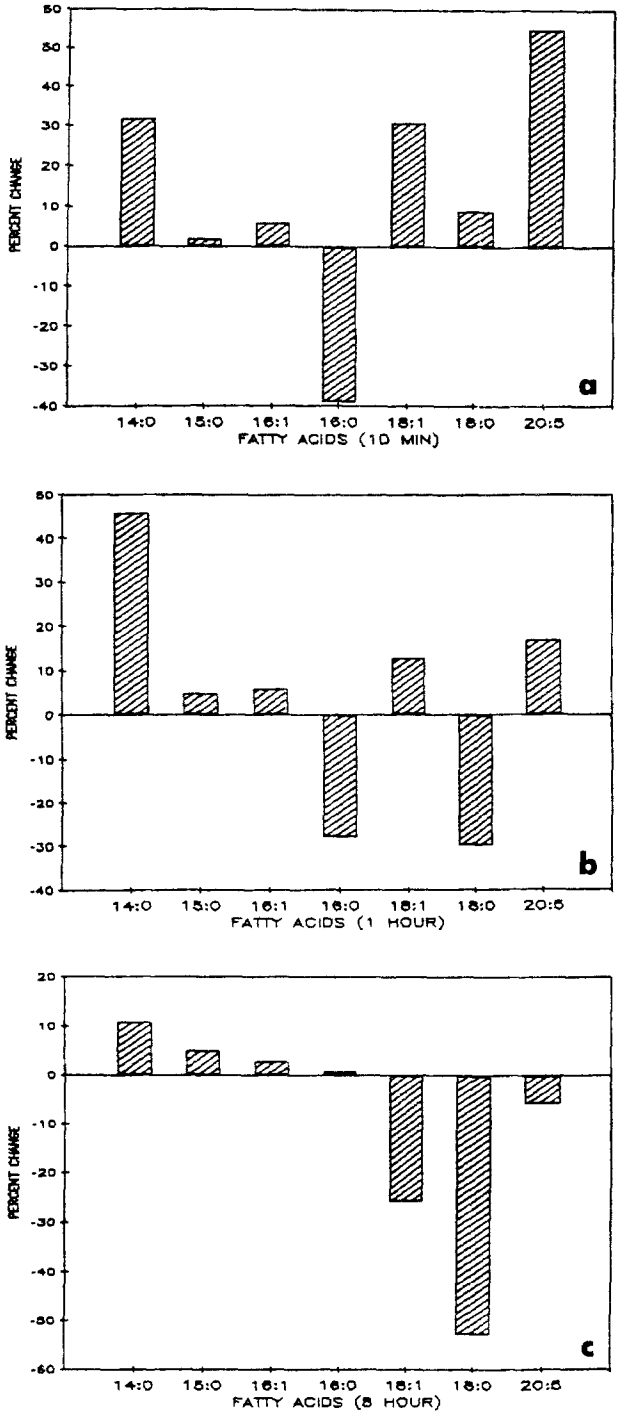

Fig. 6. Changes in fatty acid percent composition of cells exposed to 1,3,5-trichlorobenzene. A 10 min exposure. B 1 hr exposure. C 8 hr exposure

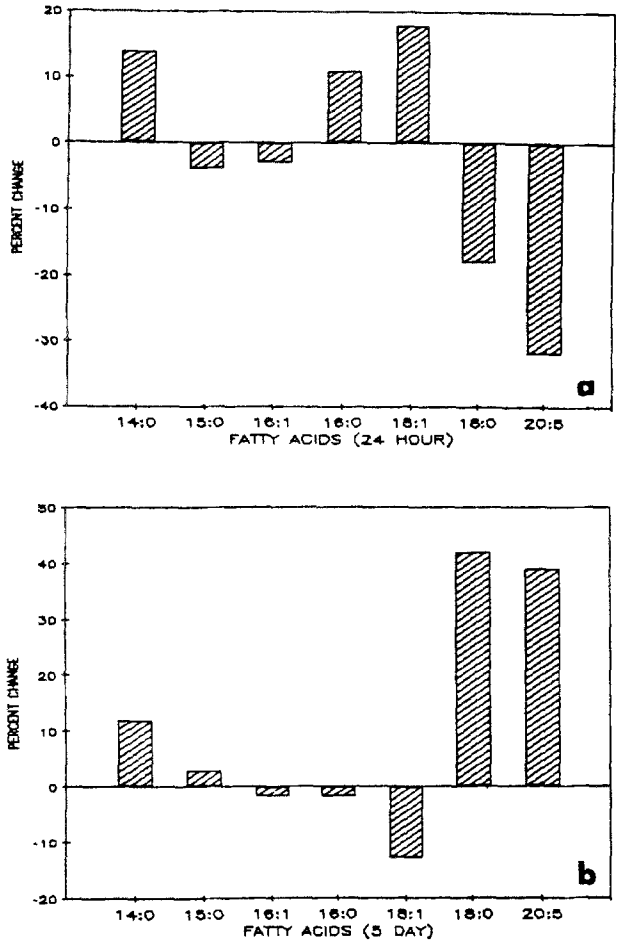

Fig. 7. Long term changes in fatty acid percent composition of cells exposed to 1,3,5-trichlorobenzene. A 24 hr exposure. B 5 day exposure

sulting in morphological alterations, while the second, longer-term effects produced by chlorinated benzenes may be the result of the production of toxic metabolites.

A third possible explanation lies in the timing of the dose of the toxicant. *Cyclotella* cells that were exposed to 1,3,5-trichlorobenzene were sampled two and a half hr earlier in the light period than the cultures exposed to the 1,2,4- isomer. Since we have demonstrated that lipid stores are accumulated throughout the light period, it is conceivable that there was reduced partitioning into cellular lipid reserves, and consequently either more toxicant was available to the cells immediately or less was released in the long term by utilization of lipid stores.

Chloroplast lipid relative volume in control cells showed a rather consistent decrease with time during the first 24 hr of exposure. Since chloroplast lipid droplet number and size are usually largest at the initiation of the light period (Puiseux-Dao 1981) or during the dark period and their numbers and volume are entrained with the photocycle (Sicko-Goad *et al.* 1988), it appears that chloroplast lipid

in exposed cells either was not being metabolized or its production was increased. Other indications of altered photosynthetic capacity were significant long-term increases in chloroplast V_v, decreases in cellular lipid reserves, indicating that lipid reserves were utilized to maintain metabolism, and changes in the C20:5 fatty acid. Except for the decrease in lipid reserves, all changes noted as a result of exposure to the isomer are similar to those observed in the dark (Sicko-Goad *et al.* 1988).

Photosynthetic inhibition of phytoplankton by toxicants is a common physiological effect (Sicko-Goad and Stoermer 1988). Conner and Mahanty (1979) and Conner (1980) found that phytoplankton were less sensitive to polychlorinated biphenyls when cultured heterotrophically than autotrophically, suggesting either or both of two possibilities. First, the phytoplankton were able to utilize supplied carbohydrate to bypass a photosynthetic block induced by PCBs, or the phytoplankton did not produce lipid reserves through normal photosynthetic pathways, consequently reducing the partitioning of the PCBs into the cells at lower exogenous concentrations. It seems apparent that while studies of bioconcentration based on octanol/water partition coefficients are useful as general predictors of toxicity, more studies need to be conducted that address other environmental factors and their relationship to toxicity, particularly physiological state of the cell and mode of action of the toxicant.

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