Arch. Environ. Contam. Toxicol. 24, 243–248 (1993)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Effect of Diatom Lipid Composition on the Toxicity of Trichlorobenzene. II. Long-Term Effects of 1,2,3-Trichlorobenzene

Linda Sicko-Goad and Norman A. Andresen

Center for Great Lakes and Aquatic Sciences, The University of Michigan, 2200 Bonisteel Blvd, Ann Arbor, Michigan 48109-2099, USA

Abstract. Exposures of four diatoms, *Cyclotella meneghiniana, Melosira italica, Melosira varians,* and *Synedra filiformis* to 0.3 ppm 1,2,3-trichlorobenzene were initiated at the 8th and 11th hours of the light period on a 16:8 h L/D cycle at 20°C. Cell counts, lipid content, and lipid class composition were monitored for 10 days. *Melosira italica* demonstrated the most long-term effects. Cell counts and chlorophyll *a* were greatly reduced on the 10th day when exposure was initiated in the 11th hour of the light period in *S. filiformis* and in the 8th hour of the light period in *M. varians*. The data demonstrate that more immediate effects occur when exposures are initiated during periods of high polar lipid content or when polar lipids are being synthesized. Long-term effects are observed when exposure initiation occurs during periods of high neutral lipid content and higher total extractable lipid. The results suggest that the response of diatoms to low levels of chlorinated benzenes is related to normal variation in diel lipid composition. These results are repeatable, they vary with species, and exposures initiated at different times of the day may produce quite different results.

Although lipophilic compounds such as organochlorines have been demonstrated to affect phytoplankton populations adversely (*e.g.*, Galassi and Vighi 1981; Mahanty *et al.* 1983; Geyer *et al.* 1985; Wong *et al.* 1984; Halfon and Reggiani 1986), lower and/or chronic exposure of natural assemblages to a wide variety of toxicants often leads to increased phytoplankton abundance (North *et al.* 1964; Federle *et al.* 1979; Vargo *et al.* 1982). This is not to suggest that organic toxicants do not affect phytoplankton growth and survival. What has often been demonstrated is that chlorophyll synthesis (Conner and Mahanty 1979) and photosynthesis are reduced in these natural assemblages, often in combination with reduced predation rates (Vargo *et al.* 1982). What often occurs in impacted areas is the replacement of oligotrophic and mesotrophic algae with less desirable species of algae. In many areas of concern in the Laurentian Great Lakes phytoplankton standing stocks are high, but consist of blue-green and green algae, organisms which are low in food quality and inhibit zooplankton grazing (Arnold 1971; Richmond and Dodson 1983).

Diatoms appear to be particularly good food sources for many animals since they often have a high lipid content and high concentrations of eicosapentaenoic acid (Sicko-Goad *et al.* 1988; Volkman *et al.* 1989; Ahlgren *et al.* 1990). It is for this reason that we have continued toxicity studies with diatoms.

Our earlier studies (Sicko-Goad *et al.* 1989a–1989d; Sicko-Goad and Andresen 1993) demonstrated that exposures of diatoms to chlorinated benzenes for up to 5 days resulted in some detrimental changes in the cultures. However, it was not possible to predict from these studies whether or not the populations would recover from exposures. Some evidence of recovery was suggested. Therefore, we designed exposure experiments in which four diatom taxa were exposed to 1,2,3-trichlorobenzene for 10 days, with exposures initiated at 2 different times of days. The results of these experiments are presented here.

Materials and Methods

Most materials and methods have been described in the preceding paper (Sicko-Goad and Andresen 1993). For this experiment, however, two additional diatoms, *Melosira italica* (Ehrenb.) Kütz., and *Synedra filiformis* Grun. in Cl. & Grun., were used as experimental organisms. Culture conditions were identical to those previously described. The experiments were conducted in May and June, 1990 and consisted of an exposure of all four diatom taxa (individually) to 0.3 ppm 1,2,3-trichlorobenzene at 20°C. Several parameters were changed between this experiment and the preceding paper. First, no methanol was used as a carrier. Second, although the 16:8 h L/D cycle was maintained, the on-off timing was changed so that the lights came on three hours later in the day. Exposures were initiated at 2 pm and 5 pm in the afternoon, corresponding to the 8th and 11th hours of the light period. These changes were made in an attempt to ascertain if the periodicities observed in lipid composition were a function of entrainment with the light cycle, or if they were intrinsic with time of day. All samples were taken at the time of day the exposure was initiated (i.e., the 8th or 11th hour of the light period) and cultures were sampled on days 0, 1, 3, 5, 7 and 10.

The third change in experimental design was the addition of cell counts for all samples. Since the diatoms were growing rapidly and the experiment was 10 days in duration, changes in cell counts were expected. Concurrent with dry weight analyses, smaller volumes of culture medium (9mL) were withdrawn and placed in a tube containing paraformaldehyde and glutaraldehyde at final concentrations of 1% in

0.05M sodium cacodylate buffer at pH 7.2. Cell counts were performed with either a hemocytometer or plankton counting chamber, depending on cell size. The counts used for determining percent change in this parameter were averages of four replicates.

Results

Cyclotella meneghiniana

Percent change in lipid class composition of *C. meneghiniana* occurring in exposed cultures (compared with controls) for four parameters, cell count, chlororphyll *a*, chlorophyll *a*/neutral lipid ratio, and the neutral/polar lipid ratio are presented in Figure 1. At the time both exposures were initiated, (t = 0), chlorophyll/neutral lipid ratios were relatively high and the neutral to polar lipid ratios were low. On day 1, exposure to this isomer resulted in a reduction in chlorophyll in the 11th hour exposure and an increase in the neutral/polar lipid ratio. Increases in chlorophyll and the chlorophyll *a*/neutral lipid ratio were observed starting on day 3 and no further reductions were observed. Although cell counts were slightly diminished with exposure, by the 10th day, counts were virtually identical with control cultures. After the initial increase in neutral lipids, exposed cultures demonstrated a consistent decline in the neutral/polar lipid ratio, which appeared to result from increases in all polar lipid classes uniformly.

Melosira italica

The overall neutral/polar lipid ratios of *M. italica* were the lowest of the four diatoms. Values in the 8th hour of the light cycle are consistently lower than in the 11th hour. In general, *M. italica* appears to be more sensitive to this isomer than *C. meneghiniana* (Figure 2). Cell counts in cultures exposed in the 8th hour of the light cycle were reduced approximately 20% whereas those exposed in the 11th hour of the light period were reduced by ca. 50%. With the exception of chlorophyll measurements on days 1 and 10 in the 11th hour exposure (which demonstrated high standard errors), a general pattern of decreased chlorophyll *a*, decreased chlorophyll *a*/neutral lipid ratio, and increased neutral/polar lipid ratio was observed in both exposures. However, it appears that effects in lipid class composition were more pronounced in the 8th hour exposure whereas cell counts effects were more pronounced in the 11th hour exposure.

Melosira varians

In general, neutral/polar lipid ratios of *M. varians* were consistent in the 8th and 11th hours of the light cycle in control cultures and these ratios declined with growth. Figure 3 demonstrates that cell counts were rather erratic for this taxon. However, reductions were observed in cell counts by day 10. Chlorophyll *a* as well as the chlorophyll *a*/neutral lipid ratios were consistently reduced in both exposures. The greatest effect observed was the long term increase in the neutral/polar lipid ratio in the 8th hour exposure.

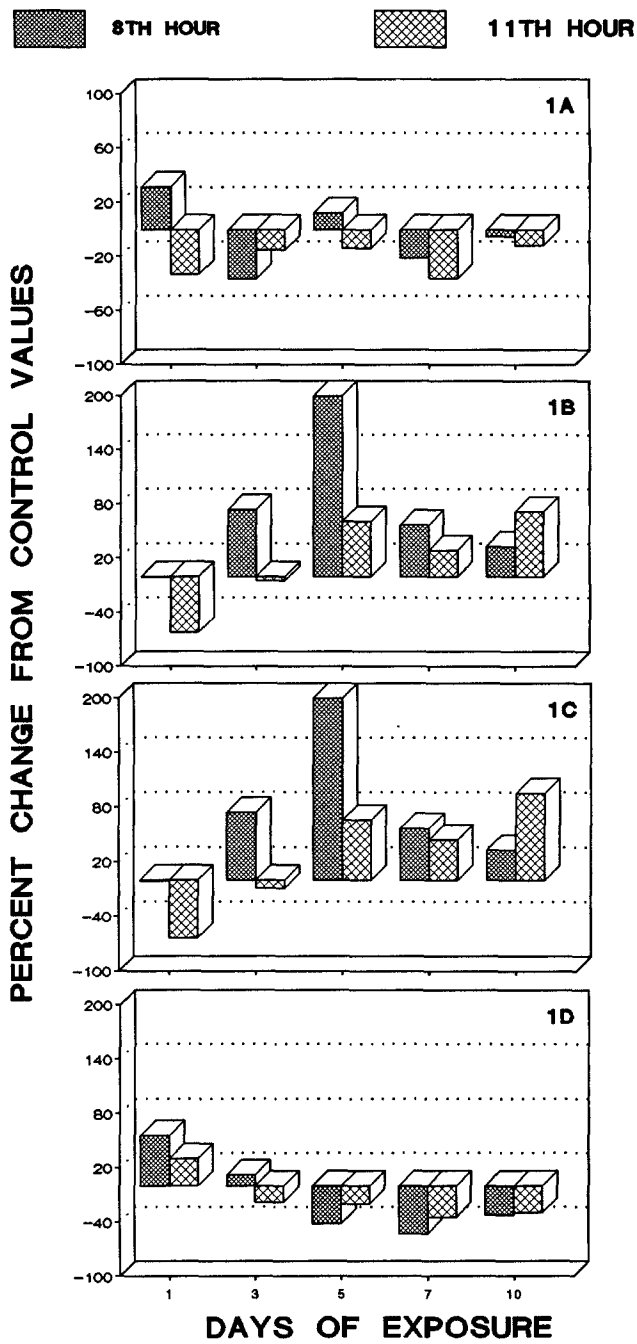

Fig. 1. Change in selected parameters and ratios in *Cyclotella meneghiniana* as a function of hour of exposure and time: **A** cell count; **B** chlorophyll *a*; **C** chlorophyll *a*/neutral lipid ratio; **D** neutral/polar lipid ratio

Synedra filiformis

Unlike the other three diatoms, *S. filiformis* demonstrated no short-term (*i.e.*, 1 day) effect with exposure at either time (Figure 4). However, at the time exposures were initiated, the neutral/polar lipid ratio was lower at the 11th hour of the light period. Cell counts were largely unaffected until the 10th day when an approximately 80% reduction occurred in the 11th hour exposure. Chlorophyll *a* and chlorophyll *a*/neutral lipid ratios increased in the 11th hour exposure between days 3

Table 1. Total extractable lipid (TEL) of cultures at onset of exposure. Values reported are percent of dry weight ± (standard error)

Organism	8th h	11th h
Cyclotella meneghiniana	5.4 (0.6)	5.7 (1.5)
Melosira italica	5.3 (0.8)	5.1 (1.0)
Melosira varians	9.4 (1.4)	6.9 (0.7)
Synedra filiformis	6.1 (1.0)	8.0 (1.3)

and 7. However, this ratio is reduced in both exposures by the 10th day. Although chlorophyll *a* appeared to be reduced with exposure to the chlorinated benzene, the neutral/polar lipid ratio decreased (Figure 4), largely as a result of increases in both AMPL and PL at 10 days. The magnitude of reduction was most similar to the data presented for *C. meneghiniana* (Figure 1).

Total Extractable Lipid (TEL) at the Time of Exposure

TEL as a percent of dry weight for cultures at the onset of exposure are presented in Table 1. Values reported are considerably lower than those presented in our previous paper. However, trends are similar for *C. meneghiniana* and *M. varians*. For *Cyclotella*, TEL appears to be constant, although perhaps slightly lower in the 8th hour of the light period. Significant increases in TEL are observed in the 8th hour of light in *M. varians* and in the 11th hour of light in *S. filiformis*.

Discussion

Our previous short-term experiments with *Cyclotella* (Sicko-Goad *et al.* 1989a–1989d; Sicko-Goad and Andresen 1993) and *M. varians* (Sicko-Goad and Andresen 1993) indicated that *M. varians* was more sensitive to exposure to 1,3,5-trichlorobenzene. The data presented here suggest both species of *Melosira* are more sensitive to 1,2,3-trichlorobenzene than *Cyclotella*. Furthermore, in the experiment conducted longer than 5 days, no reductions in cell count are observed in *Cyclotella*, and chlorophyll *a* content is actually higher in cells exposed to this isomer. In contrast, both *M. varians* and *M. italica* are sensitive to long-term exposure to this isomer. Reductions in cell counts as well as reductions in the chlorophyll *a*/neutral lipid ratio are observed. The neutral/polar lipid ratio increases in both species, with a more pronounced time effect when the exposure is initiated in the 8th hour of the light period.

Synedra filiformis is largely unaffected by the isomer from the lipid perspective. Cell counts vary little with exposure and the neutral/polar lipid ratio actually declines. However, a decline in cell count is observed at 10 days, when exposure is initiated in the 11th hour of the light period.

The short-term effects (i.e. 1-3 day) observed with both 1,3,5,- and 1,2,3-trichlorobenzene are similar and appear to be related to the low triacylglycerol content and high polar lipid content in both the 8th and 11th hour exposures in *Cyclotella*. Of the two *Melosira* species, *M. italica* experienced more overall effects than *M. varians*. Of the four organisms studied, *M. italica* had the lowest neutral/polar lipid ratios, suggesting that toxicity effects are much more pronounced in rapidly growing

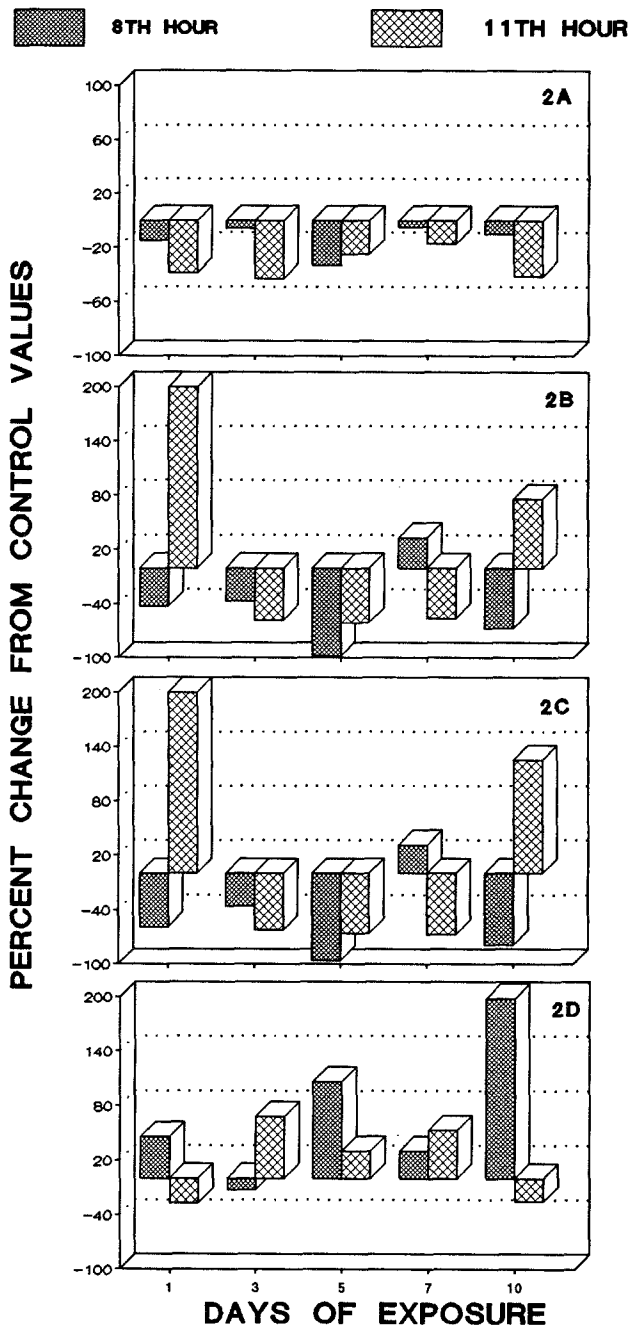

Fig. 2. Change in selected parameters and ratios in *Melosira italica* as a function of hour of exposure and time: **A** cell count; **B** chlorophyll *a*; **C** chlorophyll *a*/neutral lipid ratio; **D** neutral/polar lipid ratio

cells, which is consistent with our previous observations. Where small declines were observed in cell numbers (11th hour), the neutral/polar lipid ratio was lower when the exposure was initiated.

Longer term reductions in cell counts were observed in *M. varians* when exposed in the 8th hour of the light cycle and in *S. filiformis* when exposed in the 11th hour of the light period. Since TEL was higher in these cultures at the time of exposure, we consider this further evidence that increased lipid content at the time of exposure may lead to longer term effects.

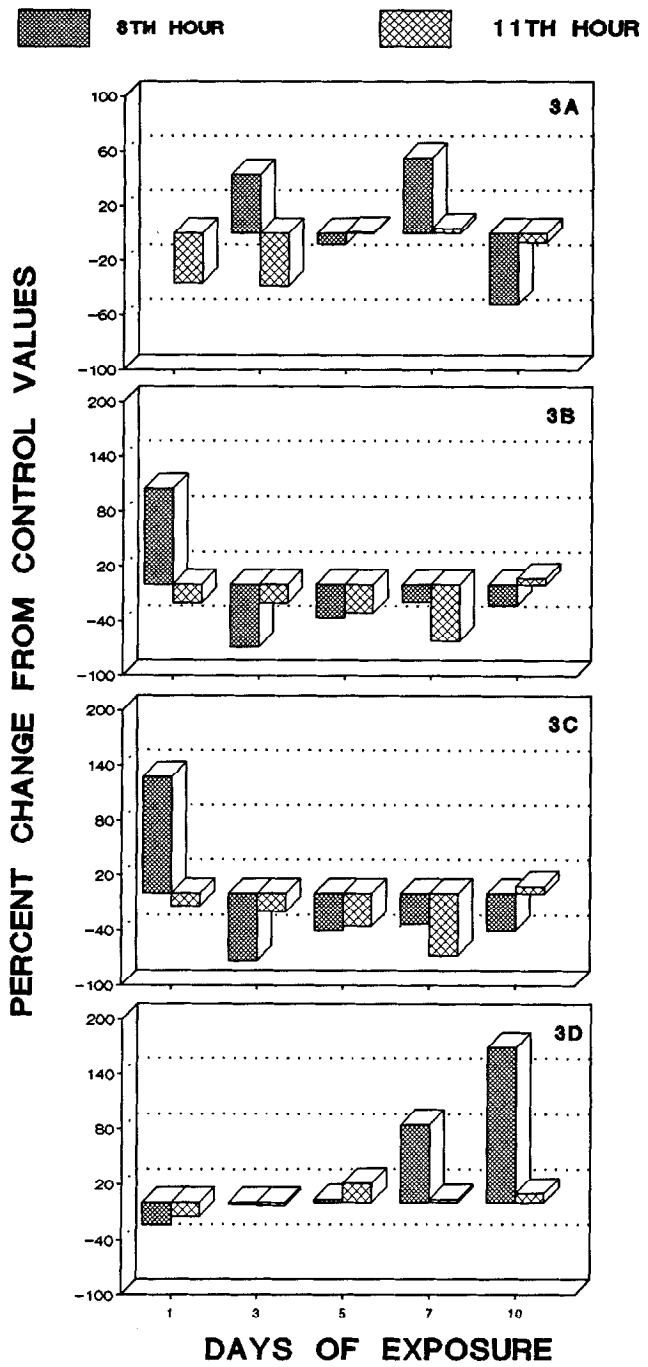

Fig. 3. Change in selected parameters and ratios in *Melosira varians* as a function of hour of exposure and time: **A** cell count; **B** chlorophyll *a*; **C** chlorophyll *a*/neutral lipid ratio; **D** neutral/polar lipid ratio

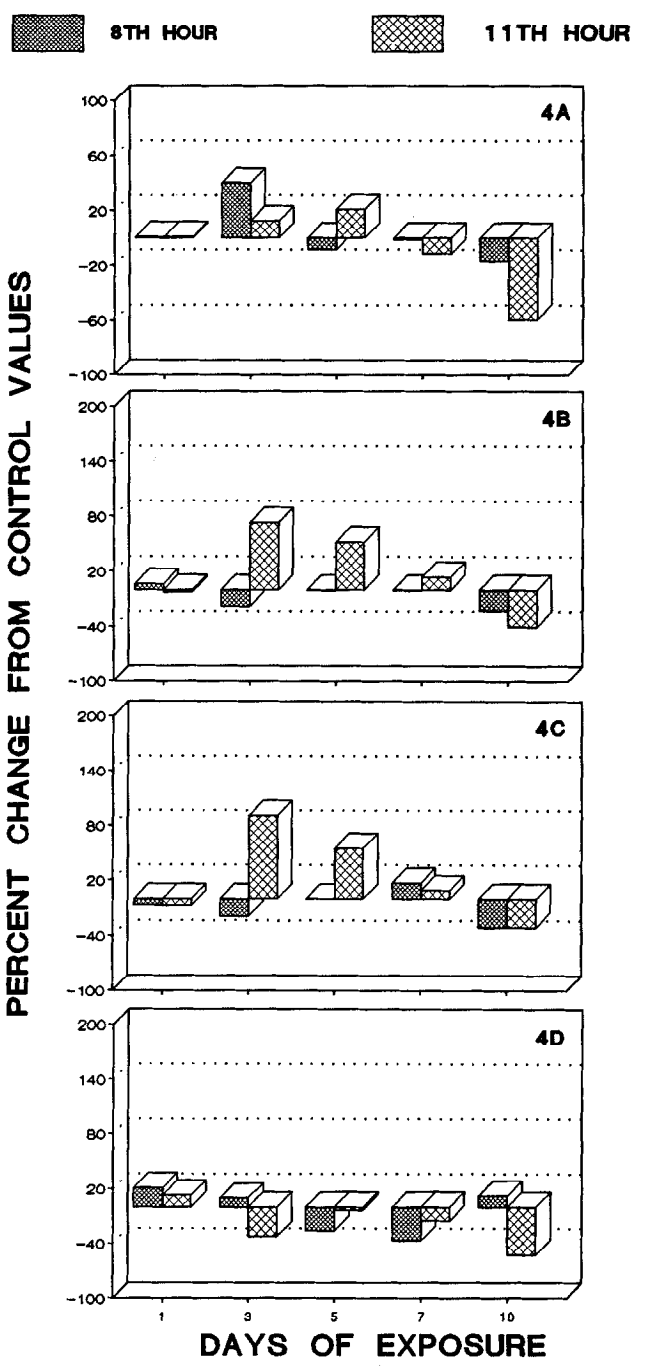

Fig. 4. Change in selected parameters and ratios in *Synedra filiformis* as a function of hour of exposure and time: **A** cell count; **B** chlorophyll *a*; **C** chlorophyll *a*/neutral lipid ratio; **D** neutral/polar lipid ratio

During the course of the experiments some macroscopic changes were observed in two taxa. For *M. varians* cultures, the normal growth condition is for filaments to form loosely adherent, hairlike clumps. In exposed flasks the hair-like masses began to break up or decrease in size by day 3, and by day 5, no clumps were observed. This effect was most pronounced in the eighth hour exposure. Mucilage secretion, which facilitates clumping, apparently had been affected by the toxicant. *Synedra filiformis* is normally planktonic. However in culture it adopts the growth habit of loosely attaching itself to a substrate by means of a mucilage pad, as a bushy, stellate colony in the manner of other members of the genus. Control cultures demonstrated this habit. Both exposed cultures did not exhibit the attaching growth habit but rather became single-celled. Thus in two unrelated taxa mucilage secretion was affected suggesting other metabolic systems are disrupted by exposure to chlorinated benzenes.

It has been suggested that sensitivity of algae to toxicants varies with season of the year, nutrient conditions, and physiological state of the cell (Hannan and Patouillet 1972; Conter *et al.* 1987; Neumann *et al.* 1987; Winner and Owen 1991). Our results further suggest that even under conditions of rapid

growth, diel variations in physiological parameters are sufficient to result in different effects when a lipophilic toxicant is introduced at different times of the day. This is not surprising in view of the increasing reports on the variety of the physiological parameters which undergo diel fluctuations (Sournia 1974; Varnum *et al.* 1986; Villareal and Carpenter 1990; Sundberg and Nilshammer-Holmvall 1975; Harding *et al.* 1983; Erga and Skjolda 1990; Pettersson and Sahlsten 1990; Granata 1991; Sukenik and Carmeli 1990).

The diatoms utilized in this study were chosen to represent a variety of environmental trophic conditions. *Melosira varians* and *C. meneghiniana* have worldwide distributions and may be characterized as taxa of eutrophic waters (Hustedt 1938–1939; Stoermer and Ladewski 1976). *Melosira italica* and *S. filiformis* may be classified as oligotrophic to mesotrophic species (Stoermer and Ladewski 1976). The responses observed here do not appear to be correlated with observed distribution patterns in nature. That is, the cleaner water forms were not necessarily more susceptible to toxicant exposure. The most obvious correlations were with lipid content and composition.

In summary, it appears that lipid content and composition play an important role in predicting both short and long-term effects of lipophilic toxicants. Exposures initiated during periods of high polar lipid content, or when polar lipids are increasing usually result in immediate effects. Exposures commencing during periods of high neutral lipid content, high TEL content, or periods when neutral lipids are increasing most often result in delayed effects. Lipid content and composition vary through a diel cycle. Consequently, exposures initiated at different times of the day may produce quite different results.

Acknowledgments. Supported by grants 88-0315 from the Air Force Office of Scientific Research and R-814194 from the Office of Exploratory Research, U.S. Environmental Protection Agency. M. B. Edlund provided technical support. Contribution No. 553 of the Center for Great Lakes and Aquatic Sciences.

References

Ahlgren G, Lundstedt L, Brett M, Forsberg C (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J Plankton Res 12:809–818

Arnold DE (1971) Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of bluegreen algae. Limnol Oceanogr 16:906–919

Connor AJ, Mahanty HK (1979) Growth responses of unicellular algae to polychlorinated biphenyls: new evidence for photosynthetic inhibition. Maru Ora 7:3–17

Conter A, Dupouy D, Murat M, Planel H (1987) Influence of "age," physiological state, and light level on the sensitivity of *Synechococcus lividus* to hydrogen peroxide. Environ Exp Bot 27:21–28

Erga SR, Skjoldal HR (1990) Diel variations in photosynthetic activity of summer phytoplankton in Lindaspollene, Western Norway. Mar Ecol Prog Ser 65:73–85

Federle TW, Vestal JR, Hater GR, Miller MC (1979) Effects of Prudhoe Bay crude oil on primary production and zooplankton in arctic tundra thaw ponds. Mar Environ Res 2:3–18

Galassi S, Vighi M (1981) Testing of volatile substances with algae. Chemosphere 10:1123–1126

Geyer H, Scheunert I, Korte F (1985) The effects of organic environmental chemicals on the growth of the alga, *Scenedesmus subspicatus:* A contribution to Environmental Biology. Chemosphere 14:1355–1369

Granata TC (1991) Diel periodicity in growth and sinking rates of the centric diatom, *Coscinodiscus concinnus*. Limnol Oceanogr 36:132–139

Halfon E, Reggiani MG (1986) On ranking chemicals for environmental hazard. Environ Sci Technol 20:1173–1179

Hannon PJ, Patouillet C (1972) Effect of mercury on algal growth rates. Biotech Bioeng 14:93–97

Harding LW, Meeson BS, Tyler MA (1983) Photoadaptation and diel periodicity of photosynthesis in the dinoflagellate *Prorocentrum mariae-lebouriae*. Mar Ecol Prog Ser 13:73–85

Hustedt F (1938–1939). Systematische und ökologische Untersuchungen uber die Diatomeenflora von Java, Bali und Sumatra. Arch Hydrobiol Suppl-Band 15:131–177, 187–295, 393–506; 16:1–155, 274–394

Mahanty HK, Fineran BA, Gresshoff PM (1983) Effects of chlorinated biphenyls (Aroclor 1242) on the ultrastructure of certain planktonic algae. Bot Gaz 144:56–61

Neumann W, Laasch H, Urbach W (1987) Mechanisms of herbicide sorption in microalgae and the influence of environmental factors. Pest Biochem Physiol 27:189–200

North WJ, Neushul M Jr, Clendenning KA (1964) Successive biological changes observed in a marine cove due to a large spillage of mineral oil. Comm Int Explor Mer Medit Symp Poll mar par Microorgan Prod Petrol, pp 335–354

Pettersson K, Sahlsten E (1990) Diel patterns of combined nitrogen uptake and intracellular storage of nitrate by phytoplankton in the open Skagerrak. J Exp Mar Biol Ecol 138:167–182

Richman S, Dodson SI (1983) The effect of food quality on feeding and respiration by *Daphnia* and *Diaptomus*. Limnol Oceanogr 28:948–956

Sicko-Goad L, Andresen NA (1993) Effect of lipid composition on the toxicity of trichlorobenzene isomers to diatoms. I. Short-term effects of 1,3,5-trichlorobenzene. Arch Environ Contam Toxicol 24:236–242

Sicko-Goad L, Evans MS, Lazinsky D, Hall J, Simmons MS (1989a) Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. IV. Pentachlorobenzene and comparison with trichlorobenzene isomers. Arch Environ Contam Toxicol 18:656–668

Sicko-Goad L, Hall J, Lazinsky D, Simmons MS (1989b) Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. II. 1,3,5-Trichlorobenzene. Arch Environ Contam Toxicol 18:638–646

———, ———, ———, ———(1989c) Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. III. 1,2,3-Trichlorobenzene. Arch Environ Contam Toxicol 18:647–655

Sicko-Goad L, Lazinsky D, Hall J, Simmons MS (1989d) Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. I. 1,2,4-Trichlorobenzene. Arch Environ Contam Toxicol 18:629–637

Sicko-Goad L, Simmons MS, Lazinsky D, Hall J (1988) Effect of light cycle on diatom fatty acid composition and quantitative morphology. J Phycol 24:1–7

Sournia A (1974) Circadian periodicities in natural populations of marine phytoplankton. Adv Mar Biol 12:325–389

Stoermer EF, Ladewski TB (1976) Apparent optimal temperature for the occurrence of some common phytoplankton species in southern Lake Michigan. Great Lakes Res. Div. Publ. 18, 49 pp

Sukenik A, Carmeli Y (1990) Lipid synthesis and fatty acid composition in *Nannochloropsis* sp. (Eustigmatophyceae) grown in a light-dark cycle. J Phycol 26:463–469

Sundberg I, Nilshammer-Holmvall M (1975) The diurnal variation in phosphate uptake and ATP level in relation to deposition of starch, lipid, and polyphosphate in synchronized cells of *Scenedesmus*. Z Pflanzenphysiol 76:270–279

Vargo GA, Hutchins M, Almquist G (1982) The effect of low, chronic levels of No. 2 fuel oil on natural phytoplankton assemblages in microcosms: 1. Species composition and seasonal succession. Mar Environ Res 6:245–264

Varum KM, Østgaard K, Grimsrud K (1986) Diurnal rhythms in carbohydrate metabolism of the marine diatom *Skeletonema costatum* (Grev.) Cleve. J Exp Mar Biol Ecol 102:249–256

Villareal TA, Carpenter EJ (1990) Diel buoyancy regulation in the marine diazotrophic cyanobacterium *Trichodesmium thiebautii*. Limnol Oceanogr 35:1832–1837

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219–240

Winner RW, Owen HA (1991) Seasonal variability in the sensitivity of freshwater phytoplankton communities to a chronic copper stress. Aquat Tox 19:73–88

Wong PTS, Chan YK, Rhamey JS, Docker M (1984) Relationship between water solubility of chlorobenzenes and their effect on a freshwater green algae. Chemosphere 13:990–996

Manuscript received May 19, 1992 and in revised form September 26, 1992.