Bull. Environ. Contam. Toxicol. (2002) 68:600–605

DOI: 10.1007/s00128-001-0296-3

Bulletin of Environmental Contamination and Toxicology

Acute and Chronic Toxicity of Pyrene to the Unicellular Marine Alga *Phaeodactylum tricornutum*

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Received: 12 June 2001/Accepted: 15 November 2001

Polyaromatic hydocarbons (PAHs) are common contaminants in the coastal waters throughout the world and are known to be bioaccumulated in the environment by unicellular marine algae (Gossiaux et al, 1998; Okay et al, 2000). Previous studies have shown that unicellular algae play an important role in the fate of these pollutants in marine and freshwaters through such processes as biodegradation, absorption and adsorption, though the quantitative importance of these processes depends on light conditions, the concentration of PAHs to which the algae are exposed and the phototoxicity of PAHs and their metabolites (Warshawsky et al, 1995). The influence of unicellular algae on the fate of PAH is especially important in nutrient-enriched coastal areas where frequent algal blooms take place, but few data are available in the literature which define the toxicity of PAHs to these organisms (Gala and Giesy, 1992; Hamada et al, 1996; Soto et al, 1977).

The aim of the present study was to determine the toxic effects of pyrene, an environmentally abundant combustion-derived PAH, on the marine microalga *Phaeodactylum tricornutum* using short and medium-term laboratory-based toxicity tests. Pyrene has been shown to be phototoxic to some plant species (Ren et al., 1994,). As this phenomenon is of potentially great importance in natural waters, levels of pyrene were measured during the medium-term batch exposure experiments to establish whether and by how much the pyrene degraded and/or was removed by the algae under different conditions of temperature and illumination.

MATERIALS AND METHODS

Toxicity tests were performed in a temperature controlled room (T = 20 ± 1°C) continuously illuminated by cool-white fluorescent lamps (3500-4000 lux). Species were cultured in f/2 medium (Guillard and Ryther, 1962). Pyrene was dissolved in acetone before adding the test cultures. The acetone concentrations in test solutions were adjusted to be 0.1 %. The same amount of acetone was also added to the Control (termed the Control-Vehicle). Filtered (GF/C) and sterilised seawater (salinity 20 ppt) was used as dilution water. The solubility of pyrene in water is 135 ppb, thus the exposure concentrations used both for short-

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term and medium-term batch experiments were no higher than the solubility limits.
For short term toxicity test, stock pyrene solution was prepared in acetone and then a series of (0-100 ppb) pyrene solutions (in seawater algal medium) and *Phaeodactlylum tricornutum* (10000 cells/ml) were pre-incubated for 4 hours before adding $NaH^{14}CO_3$ solution into each bottle. The samples were then incubated for 2 more hours and filtered through 0.2 μm membrane filter. The radioactivities on the filter papers were counted in a liquid scintillation counter (Packard 1550 Tri-Carb). The results were expressed as % inhibition relative to the control versus concentration of pyrene.

In batch bioassays, *Phaeodactylum tricornutum* cells were exposed to 20, 40 and 80 ppb pyrene solutions in 500 mL erlenmeyer flasks. The growth of algae was monitored for 17 days by measuring the fluorescence intensity at chlorophyll wavelengths ($\lambda_{Ex} = 430$, $\lambda_{Em} = 663$).

During the algal batch bioassays, a portion of each solution (20, 40 and 80 ppb pyrene) was filtered through GF/C filters every two days and the pyrene concentrations in the filtrate were measured at excitation = 240 nm and emission = 383 nm wavelengths with a Perkin Elmer Model LS 50 B spectrofluorometer to determine the changes in concentration. Additionally, the same concentrations of pyrene solutions were prepared in algal medium (without algae), the solutions being maintained for 17 days in the dark (both at 4 and 20°C) and in light (at 20°C); the pyrene concentrations were determined throughout the period to check whether there was a decrease in pyrene concentration in the absence of algae. A linear calibration was obtained up to 80 ppb for pyrene in filtered, sterilised seawater; pyrene was added to the seawater in acetone giving a maximum acetone concentration of 0.1 %. Pyrene concentrations were evaluated for two replicates.

RESULTS AND DISCUSSION

Figure 1 shows the results of short term algal toxicity tests. This type of acute toxicity testing is mainly used for screening of chemicals and wastewaters (Kusk and Nyholm, 1991). The results were expressed as % inhibition of algal ^{14}C assimilation rate versus pyrene concentration. From Figure 1, the EC_{50} value was found to be 68-70 ppb. Pyrene affects the CO_2 assimilation capacity of *Phaeodactylum tricornutum* in short-term exposures and, can therefore be concluded to be acutely toxic to phytoplankton. This is especially important for phytoplankton communities in coastal waters affected directly by the entrance of wastewaters.

The growth curves of *Phaeodactylum* cells incubated in 20, 40 and 80 ppb pyrene solutions are presented in Figure 2. Growth was monitored by measuring the fluorescence intensities of the cultures. The growth curves representing

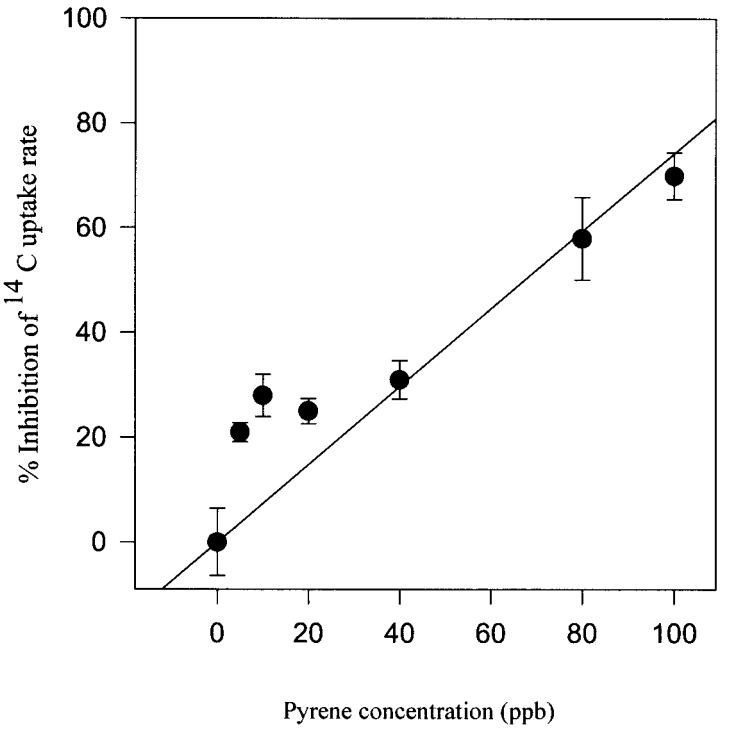

Figure 1. Short-term algal toxicity test results

cultures incubated in three different pyrene solutions show nearly the same behaviour. Of the cultures incubated, the lag phases of the cells incubated in 40 and 80 ppb were slightly longer than that of the Control-V and 20 μg/L solutions, but beyond the 7th day, the difference disappeared although the 40 and 80 ppb data points were still lower than the others at day 11. Thus in the log phase of growth, the medium-term batch growth experiments gave results broadly consistent with those of the short term photosynthesis test, confirming the toxicity of pyrene at higher exposure levels. However, the dose-response relationship was much less clear in batch growth experiments than in the short term photosynthesis test. The dose-response relationship in the batch experiments disappeared completely when the cultures reached the stationary phase; they showed nearly the same growth rate and chlorophyll-a value. Previous exposure experiments with this alga but with different chemicals and a batch culture design in which the photosynthetic rate was measured in aliquots of algae taken at different parts of the growth curve, indicated that once the log phase is reached, photosynthetic activity almost ceases (Okay et al, 1996). Pyrene only appears to have an impact on actively growing cells. In another

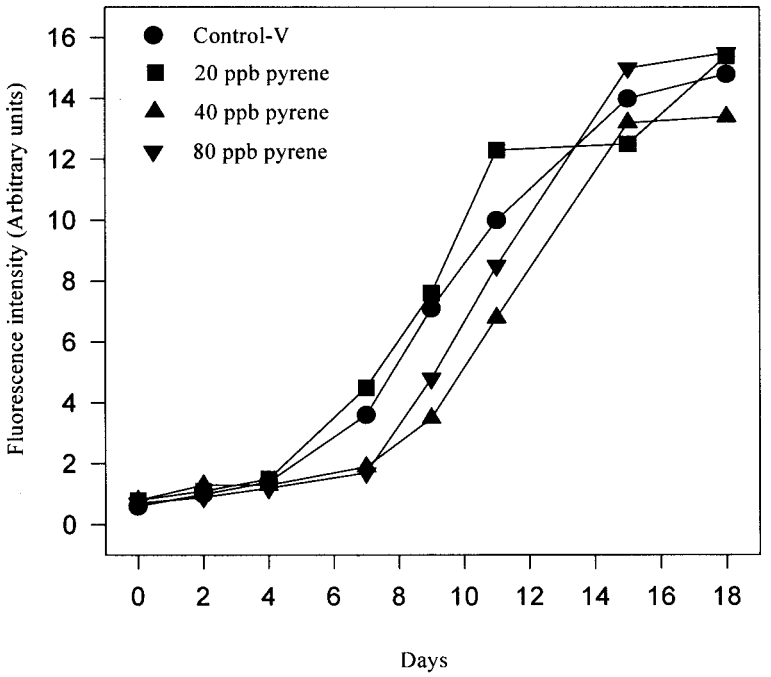

Figure 2. Batch algal toxicity test results

study with PAHs, pyrene was found not to be phototoxic to the freshwater unicellular alga, *Selenastrum capricornutum* (Warshawsky et al, 1995), though it was phototoxic to some marine organisms such as the bacteria *Vibrio fischeri* (Steevens et al, 1999), and the duckweed *Lemna gibba* L. (Huang et al, 1995), to marine invertebrate larvae and juveniles (Pelletier et al, 1997) and to fish (Schirmer at al, 1998).

Figure 3 shows that the batch growth experiment has significant limitations as a means of testing the medium-term toxicity of pyrene. The Figure shows the pyrene concentrations in the filtrate and also in the pyrene solutions left under the same experimental conditions as the batch cultures. The curves denoted "with algae" and "without algae" on the Figures correspond to the pyrene concentrations in the filtrates in the presence of algae and in the pyrene solutions in the absence of algae respectively. Whereas there was no difference in the pyrene concentrations measured in both cases at 20 ppb there were significant differences at 40 and 80 ppb. The half life of 40 ppb pyrene incubated together with algae was shorter (app 7 days) than the pyrene in the solution without algae (16 days). A similar half life was observed in 80 ppb pyrene solution in the

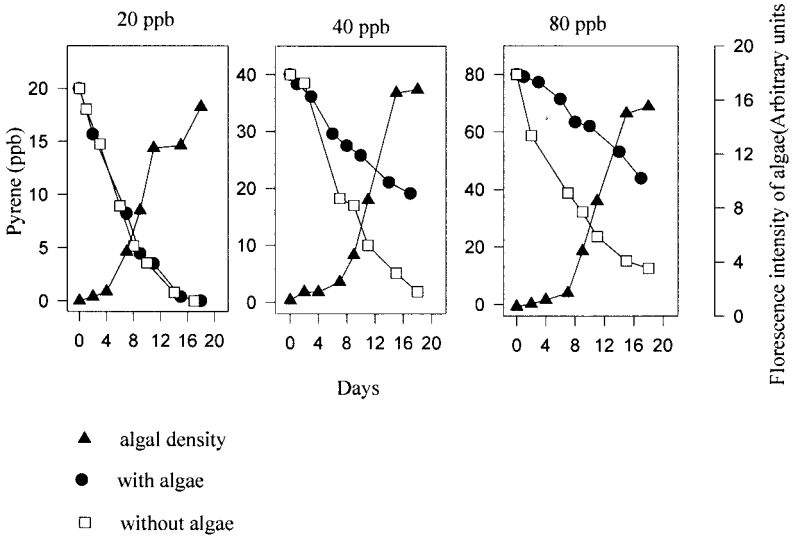

Figure 3. Changes in pyrene concentrations with and without algae

absence of algae. Moreover no change in the pyrene concentrations kept in the fridge at 4°C and in the dark (by wrapping the flasks with Al-foil to prevent the light penetration) was observed at 20°C for 17 days. These results indicate that, under the physical conditions of the batch experiment, pyrene is lost from solution. The most likely mechanism is photodegradation. Though we did not measure any degradation product, the disappearance of pyrene in the solution logically indicates that degradation products must have been present.

In the presence of light and algae, losses from solution are even greater. Several mechanisms are possible, including adsorption, absorption, metabolic degradation and algae-enhanced photodegradation (Warshawsky et al, 1995). The role of the algae in influencing pyrene behaviour was greater at higher pyrene concentrations. However, these mechanisms had the effect of substantially reducing the pyrene concentration in solution throughout the course of the experiment, so as the culture increased in density, the pyrene dose per cell declined severely, resulting in much less clarity in the toxicity dose response data than in the short-term radioactive bicarbonate fixation experiment.

Acknowledgments. The authors wish to thank Mehtap Çelemen and Gülümser Yakupoğlu for laboratory work. This study was performed within the framework of a collaborative research project financially supported by British Council.

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