Photoenhanced toxicity of azaarenes to marine phytoplankton.

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Photoenhanced toxicity of azaarenes to marine phytoplankton

UV absorbance dependent toxicity of acridine to the marine diatom *Phaeodactylum tricornutum*

Abstract

The present study seeks quantitative measures for photoenhanced toxicity under natural light regimes, by comparing the effects of an aromatic compound under natural and laboratory light. To this purpose, the influence of light irradiance and spectral composition on the extent of photoenhanced toxicity of acridine, a three-ringed azaarene, to the marine diatom *Phaeodactylum tricornutum* was analyzed. Under laboratory light containing ultraviolet radiation (UV), the 72 h EC50 growth value for acridine was 1.55 μM. Under natural light, a 72 h EC50 value for acridine below the lowest test concentration (0.44 μM) was observed. Under both laboratory and natural light, the toxicity of acridine was equally enhanced by total UV-(UV-A and UV-B) and UV-A-radiation, while in the absence of UV no enhancement of toxicity was observed. Hence, the UV-A region of light was dominant in the photoenhanced toxicity of acridine to *P. tricornutum*, in accordance with its absorption spectrum in the UV-A region. Therefore, the total amount of UV radiation absorbed by aqueous acridine was calculated,

for each separate treatment. The amount of UV absorbed by acridine effectively described the effect of acridine on the growth of *P. tricornutum* in a dose-response dependent manner. It is concluded that photoenhanced toxicity of aromatic compounds expressed as a function of the actually absorbed UV may circumvent some of the variability between studies using different concentrations of the phototoxic compounds and light sources. The UV quantity absorbed by these compounds allows a comparison with the absorption characteristics of natural waters and, thus, is a key parameter to determine the role of photoenhanced toxicity in water.

**Introduction**

Several polycyclic aromatic compounds strongly absorb light in the UV region, which can alter their structure and consequently their toxicity by two photochemical pathways: photosensitization and photomodification (Larson and Berenbaum, 1988; Leifer, 1988; Veith *et al.*, 1995; Huang *et al.*, 1997a). In photomodification reactions the aromatic compounds are structurally altered to a variety of oxygenation products, via unstable endoperoxide and/or peroxide intermediates (Zepp and Schlotzhauer, 1979; Onodera *et al.*, 1985; Huang *et al.*, 1993; Huang *et al.*, 1995). In photosensitization reactions the influence of UV on the fate of PAHs in water is to a large extent determined by the presence or absence of reactive species such as singlet-state oxygen (Larson and Berenbaum, 1988). This activation of aromatic compounds by UV radiation, via photosensitization and/or photomodification reactions, into reactive and toxic products is defined as photoenhanced toxicity. For azaarenes, heterocyclic aromatic compounds containing one in-ring nitrogen atom, both modes of photochemical reactions were found to contribute to an increase in toxicity to marine algae (Wiegman *et al.*, 1999; Wiegman *et al.*, 2001).

Different models have been developed to define the increased toxicity of aromatic compounds by UV radiation, incorporating photochemistry laws in which light intensity at wavelength $\lambda$ ($I_\lambda$) and compound specific characteristics such as molar absorptivity at wavelength $\lambda$ ($\varepsilon_\lambda$) and quantum yield ($\phi$) are used (Morgan and Warshawsky, 1977; Oris and Giesy, 1987; Diamond *et al.*, 2000). In addition, phosphorescence lifetimes, triplet state energies and HOMO-LUMO gap values, characteristics related to the stability of
compounds under UV radiation, have been used to predict the photo-
enhanced toxicity of aromatic compounds (Morgan et al., 1976; Newsted
and Giesy, 1987; Mekenyan et al., 1994b; Veith et al., 1995; Sinks et al.,
1997). Particularly, the HOMO-LUMO gap values, i.e. the energy differ-
ences between the highest occupied and lowest unoccupied molecular orbi-
tals, of these molecules accurately predicted the light absorption and conse-
quently photoenhanced toxicity of aromatic compounds (Mekenyan et al.,
1994b; Veith et al., 1995). Although these relationships may have been
clarified, they remain difficult to apply to the complex light conditions as
prevailing in natural waters.

Recently, Diamond et al. (2000) argued that by determining concentrations
of polycyclic aromatic compounds and quantifying spectral characteristics
of sites contaminated with polycyclic aromatic compounds, the risk of these
compounds to invertebrates in aqueous environments can be predicted more
accurately. Most algae and plants, however, are less sensitive to the photo-
enhanced toxicity of aromatic compounds than invertebrates and fish,
although responses vary between tests species (Arfsten et al., 1996). This
lower sensitivity of algae to photoenhanced toxicity of aromatic compounds
is probably due to a rapid attenuation of UV radiation. Especially in water
UV radiation is absorbed by dissolved compounds, humic acids, suspended
matter and pigments, subsequently decreasing the rate of photochemical
reactions (Leifer, 1988; Kochany and Maguire, 1994a) and, hence, the
extent of photoenhanced toxicity of aromatic compounds (Gensemer et al.,
1998; Weinstein and Oris, 1999). However, research in environmental
photochemistry has focused mainly on attenuation of UV by humic mate-
rials (Oris et al., 1990; Wang et al., 1995; Gensemer et al., 1998; Weinstein
and Oris, 1999), whereas the influence of attenuation of UV by pigments is
hardly included in studies investigating the photoenhanced toxicity of aro-
matic compounds. In the present study, therefore, the roles of attenuation of
UV by cells of Phaeodactyllum tricornutum and UV absorbance by acridine
were distinguished. These attenuation characteristics were incorporated in a
quantitative measure describing the photoenhanced toxicity by relating the
actual amount of UV absorbed by acridine to the observed effects.
**Materials and methods**

*Culturing of Phaeodactylum tricornutum*

*Phaeodactylum tricornutum* (Bohlin) was cultured in batches artificial seawater medium (Admiraal and Werner, 1983) under cool-white fluorescent light (90 μmol m\(^{-2}\) s\(^{-1}\), Philips TLD 36W/33) at 20 °C. The light-dark regime was 16:8 h. Tris-buffer (3.7 mM, Merck) was added to the artificial seawater medium to maintain the pH at 8.

*Acridine toxicity under laboratory and natural light*

For the determination of the EC50 value for acridine (97% purity; Aldrich) under laboratory light, 180 ml glass jars were placed in an incubator equipped with a mercury light source that contains a more natural spectral composition of UV (80 μmol m\(^{-2}\) s\(^{-1}\), Philips HPI-T 400 W). The light-dark regime was 16:8 h. The water was kept at a constant temperature of 20 °C. The jars were filled with 130 ml algae suspension of *P. tricornutum* and each jar was aerated with a glass Pasteur pipette to provide a water circulation.

The algae were added to the jars 24 h before adding acridine, to avoid a lag phase in algal growth and to allow the algae to acclimatize. At the start of the toxicity experiment (t\(_0\)), 24 h after the acclimatization period, the algal concentration was approximately 10\(^6\) cells/ml. At time t\(_0\), a concentration series of acridine using the carrier solvent dimethyl sulphoxide (DMSO, > 99.5%, Merck) was added. To each experimental treatment 50 μl of the corresponding stock solution was added to ensure equal DMSO concentrations in all acridine treatments (0.04% v/v). Besides controls (algae without acridine and without DMSO), DMSO controls (algae with DMSO but without acridine) were incorporated to check for effects of the carrier solvent on growth. The series of concentrations (including all controls) was tested in duplicate. A chemical control (0.66 μM acridine in 0.04% v/v DMSO in sterilized medium without algae) was added to determine the loss of acridine due to sorption, evaporation or photochemical transformation. The average actual acridine concentrations in the water were 0.41, 0.67, 0.79, 3.48 and 8.00 μM.
Of each jar, a 1 ml water sample was measured daily with a Coulter counter (Coulter Multisizer) with a counting tube aperture of 70 μm to determine cell numbers and volumes (in triplicate). For each treatment, the growth rate \( \mu \) (day\(^{-1} \)) of the algal population was calculated over 4 days of exponential growth during the exposure to acridine. For the highest tested acridine concentrations sometimes negative growth rate values were calculated, indicating that the algae died. Therefore, negative growth rate values were set to zero in curve fitting calculations. The growth rate values were plotted against average actual acridine concentrations in the water. The acridine concentrations that reduced growth rates of \( P.\ tricornutum \) to 50% of the (DMSO) controls (EC50 values) were obtained by fitting the following equation (Haanstra et al., 1985) through the dose-response plots with Kaleidagraph:

\[
y = \frac{c}{1 + \exp^{b(X - a)}}
\]  

(4.1)

where \( Y \) is the effect on growth rate, \( X \) is the 10\(^{th}\) log acridine concentration (μM), \( a \) is the 10\(^{th}\) log EC50, \( b \) is the slope of the logistic curve and \( c \) is the average growth rate of (DMSO) controls. The corresponding 95% confidence intervals were calculated according to Miller and Miller (1984).

Since culturing of algae was performed under fluorescent light (standard culturing light in toxicity tests; NEN 6506; EPA/600/4-91/002), whereas the toxicity experiments were performed under mercury light, light containing UV radiation, the algae may have suffered from a change of light source at the beginning of the toxicity experiment. Therefore, the effect of test conditions on the toxicity of acridine to algae under mercury light was investigated by using two different cultures of \( P.\ tricornutum \); one cultured under fluorescent light as described above and the other one cultured under mercury light simultaneously. For the algae cultured under mercury light, the controls, DMSO controls (0.04% v/v DMSO) and a series of acridine concentrations was tested in duplicate. The average actual acridine concentrations in the water were 0.35, 0.66, 0.72, 3.65 and 8.47 μM. No significant difference was found (\( p > 0.05 \)) between the EC50 values of acridine for \( P.\ tricornutum \) cultured under fluorescent light (1.55 μM, 95% confidence limits 1.37–1.75 μM) and mercury light (1.53 μM, 95% confidence limits 1.27–1.83 μM), demonstrating that the change of light source did not affect the determination of the EC50 value of acridine.
Hence, the results were grouped and one EC50 value of acridine for *P. tricornutum* under mercury light was calculated (1.55 μM, 95% confidence limits 1.35–1.79 μM).

These experiments were performed with algal densities of 10^6 cells/ml to reach a high level of UV attenuation by algal biomass, but in standard toxicological test 10^4 cells/ml are used. During the exposure time, the pH of the medium increased in the control treatments concurrently with an increase of algal biomass (in the treatment with the highest biomass a pH of 10 was measured). For this reason, growth rates of *P. tricornutum* (as described in the first section) were also determined under mercury light using lower initial algal densities (10^4 cells/ml). The average growth rate was 0.88 ± 0.09 day^−1 (n = 4) and an increase of 8.1 to 8.5 was measured for the pH of the medium. The average growth rate of *P. tricornutum* under mercury light, using cell densities of 10^6 cells/ml, was 0.85 ± 0.07 and 0.81 ± 0.01 day^−1 for the control and DMSO control treatments, respectively (n = 4), and equalled the average growth rate of *P. tricornutum* in the tests using lower algal density (10^4 cells/ml). Therefore no adverse effect of the increase of pH or of the higher algal densities on growth rates of *P. tricornutum* was expected in the following experiments.

The range of effect concentrations of acridine was also determined under natural light. To this purpose, 10 glass aquaria of 1.5 L were placed in a tray (1 × 1.5 m) filled with water on the roof of the Biological Centre in Amsterdam (52°21' N, 4°57' E), the Netherlands, on May 11–15, 1999. The aquaria were filled with 1 L of algae suspension and all aquaria were aerated with glass Pasteur pipettes. The algae suspension was added to the glass aquaria, 24 h before adding acridine. At the start of the toxicity experiment, a concentration series of acridine (0.01% v/v DMSO) was added. The controls and acridine concentrations were tested in duplicate. One DMSO control (algae with DMSO but without acridine) and one chemical control (6.53 μM acridine in 0.01% v/v DMSO) were added. The average actual acridine concentrations in the medium were 0.44, 1.42 and 4.83 μM. All aquaria were sampled daily for algal density (5 ml). The growth rates and EC50 values were calculated as described above.
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*Effects of UV radiation*

To modify the UV spectral characteristics, a polycarbonate filter (Lexan 80330, 1 mm), absorbing all UV radiation (transmitting visible radiation), and a cellulose diacetate filter (Melinex AS 539, 0.175 mm), absorbing UV-B radiation, while transmitting UV-A and visible radiation, were used (see Figure 4.1). For each experiment a new set of filters was used (see also http://cc.joensuu.fi/~aphalo/plastics.html) to prevent effects of aging of the filters.

![Graph showing transmittance of natural light through filters](image)

**Figure 4.1.** Relative transmittance of natural light (%) through a cellulose diacetate filter (that absorbs all UV-B) and a polycarbonate filter (that absorbs all UV) and the absorbance spectrum of acridine.

The effects of the composition of the UV on toxicity of acridine were tested under natural light and a 16:8 h light-dark regime of mercury light. For each light source and spectral treatment combination, the EC50 value for acridine, obtained from the experiments described above, was tested in duplicate and compared to two corresponding DMSO controls. For each light source and spectral treatment combination, one chemical control (sterilized medium with DMSO and acridine) was included.
The toxicity tests under laboratory light were performed as described in the first section. Under natural light, the effects of the UV irradiance on acridine (0.6 μM acridine in 0.01% v/v DMSO) toxicity were tested on the roof of the Biological Centre in Amsterdam, on September 21–25, 1999 as described in the previous section.

The UV irradiance of the used mercury light (280–400 nm) was 161 ± 6 μW/cm² without UV-filters, 128 ± 5 μW/cm² applying the UV-B-filter and 13.4 ± 1.5 μW/cm² applying the total UV-filter. In the toxicity test under natural light at midday, average UV irradiances (280–400 nm) of 660 ± 173, 494 ± 55 and 92 ± 25 μW/cm² applying no filter, the UV-B-filter and the total UV-filter, respectively, were measured (52°21' N, 4°57' E, Amsterdam, The Netherlands).

**Calculation of UV absorbance by acridine**

Using measurements of UV irradiance, attenuation of UV by algal biomass and the UV absorption spectrum of acridine, the UV irradiance absorbed by acridine was calculated as described in Leifer (1988). To this purpose the spectral intensities of UV irradiance were measured in all treatments at the surface of the medium using an UV-Visible spectroradiometer (OL 752-O-PMT, Optronic Laboratories, Orlando, Florida, USA). The spectroradiometer was calibrated with NIST-traceable lamps (1000 W, S-794 and F-314, Optronic Laboratories). Under mercury light conditions, the UV spectra were scanned from 280 to 500 nm at 1-nm intervals. Under natural light conditions (at midday), the UV spectra were scanned from 280 to 500 nm at 2-nm intervals. For each scan, the total UV irradiance was calculated by integrating spectral irradiance values from 280 to 400 nm.

The attenuation of UV radiation by artificial seawater and different algal densities was measured using quartz test tubes with a path length of 1 cm. Quartz tubes were filled with 100 ml medium, and with 100 ml algae suspension of *P. tricornutum* with concentrations of respectively 2.1 and 7.0 × 10⁶ cells/ml. The corresponding UV spectra were measured by placing the tubes on the sphere of the UV-visible spectroradiometer. Total UV irradiance was calculated by integrating spectral irradiance values from 280 to 400 nm (see Figure 4.2). The attenuation of UV, Kd (cm⁻¹), was calculated with equation 4.2:
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Figure 4.2. Spectral distribution of laboratory light, measured through quartz cuvets (with a path length of 1 cm) filled with artificial seawater medium and different concentrations of *Phaeodactylum tricornutum*.

Figure 4.3. The attenuation coefficient $K_d$ ($\text{cm}^{-1}$) for the wavelength range of 280 to 400 nm plotted against algal densities ($10^6$ cells ml$^{-1}$) of *Phaeodactylum tricornutum*. 
**Chapter 4 — UV absorbance dependent toxicity of acridine**

\[ K_d = \ln \left( \frac{I_{x1}}{I_{x2}} \right) / \Delta x \]  

(4.2)

in which \( I_x \) is the intensity of irradiance measured through the quartz tubes with algal density \( x \) and \( \Delta x \) the difference between the algal densities of the corresponding tubes. Subsequently, a linear relation between the attenuation of UV and algal biomass could be determined and is illustrated in Figure 4.3.

The UV absorption spectrum of acridine was measured using a Perkin Elmer Lambda 2 UV-visible spectrophotometer as described in Wiegman et al. (1999), which is also illustrated in Figure 4.1. From the UV absorption spectrum, the molar absorptivity \( \varepsilon_{\lambda} (\text{M}^{-1} \text{cm}^{-1}) \) of acridine was calculated with the Lambert-Beer law.

The daily amount of UV absorbed (280–400 nm) by the system with algae and acridine could be determined from the experiments conducted under mercury light, using the attenuation coefficient \( K_d (\text{cm}^{-1}) \) calculated for cell numbers of \( P. \) tricornutum and the actual acridine concentration in the water (M). For each wavelength, the UV irradiance absorbed by the aqueous system \( (I_{s\lambda}) \) with a depth of 1 cm is calculated using equation 4.3 (for example, Leifer (1988)):

\[ I_{s\lambda} = (I_{0\lambda} - (I_{0\lambda} \times \exp^{(Kd + \varepsilon[M]))}) \]  

(4.3)

in which \( I_{\lambda} \) is the incident irradiance (in \( \mu \text{mol cm}^{-2} \text{s}^{-1} \)) and \( I_{s\lambda} \) the irradiance of light absorbed by the aqueous system with a depth of 1 cm (in \( \mu \text{mol cm}^{-3} \text{s}^{-1} \)). The UV radiation absorbed by algae only \( I_{s\lambda}' \), is calculated according to equation 4.4:

\[ I_{0\lambda}' = (I_{0\lambda} - (I_{0\lambda} \times \exp^{Kd})) \]  

(4.4)

The UV quantity absorbed during the exposure time was calculated by integrating daily UV absorbance (in \( \mu \text{mol cm}^{-3} \text{day}^{-1} \)) over 72 h, for \( I_{s\lambda} \) as well as \( I_{0\lambda}' \). The amount of UV irradiance absorbed by acridine during the exposure time (\( \mu \text{mol cm}^{-3} = \text{mmol L}^{-1} \text{ per 72 h} \)) is defined as the difference between these daily absorbencies \( (\Delta I_{s\lambda}' = I_{s\lambda} - I_{0\lambda}') \). In this manner, the amount of UV absorbed by acridine was corrected for both the decrease in acridine concentration and the change in algal biomass over time. This
approach enabled us to relate the actual amount of UV absorbed by acridine to the effect on growth rates of *P. tricornutum*. All our toxicity experiments were conducted at the same distance from the mercury light. Therefore, processes such as reflection and/or refraction of light were not included in our calculations. Daily UV irradiance, obtained from the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands) and UV compared with irradiance measured at our test location, was used to validate the laboratory results with the results from the experiment conducted under natural light.

**HPLC analysis**

Water samples (2 ml) were taken directly after addition of acridine \( (t_0) \) and after 24-, 48- and 72-h, in order to determine the actual acridine concentrations in the test medium. After centrifugation (3000 rpm, 10 min) 1 ml of the supernatant was taken for analysis by High-Performance Liquid Chromatography (HPLC), using UV detection (Applied Biosystems model 785A) for acridine and fluorescence detection (Kratos Spectroflow 980) for the transformation products. A 150 × 4.6 mm Lichrosorb 5 μm RP-18 analytical column was used with a 4 × 4 mm Lichrosorb 5 μm RP-18 guard column. The column temperature was kept at room temperature (20 °C). The flow of the mobile phase, an isocratic mixture of 65% acetonitrile (J.T. Baker Analyzed HPLC Reagent, > 99.9%) and 35% water (J.T. Baker Analyzed HPLC Reagent), was 1 ml/min. Of each sample 20 μl was automatically injected. Acridine was detected at a wavelength of 249 nm and the transformation products at emission wavelengths of > 370 nm. Acridine concentrations were calibrated with standards of acridine in methanol (J.T. Baker Analyzed HPLC Reagent, > 99.8%). From the measured acridine concentrations in the water an actual average exposure concentration was calculated assuming exponential decrease over time. Recovery of the acridine concentration during the experiments was defined as the quotient of the actual concentration at 72 h and the actual start concentration expressed as percentage \( (C_{72}/C_0 \times 100) \).

**Statistical analyses**

*P. tricornutum* cultured under fluorescence light and *P. tricornutum* cultured under mercury light were concurrently exposed to acridine in the presence
of mercury light. To test for differences in toxicity of acridine between the two different cultures, these toxicity data were fit simultaneously to logistic models that differed in their slope parameters but had the same EC50 parameter, using SPSS 9.0 (SPSS Inc.) for windows (van Gestel and Hensbergen, 1997). The student's t test (one way) (Microsoft Excel 98) was used to test for significant differences between the average growth rates of acridine treatments and their corresponding DMSO controls in the UV radiation tests. The growth values of *P. tricornutum* were fit against the corresponding fraction of UV absorbed by acridine to logistic models (SPSS 9.0) as described in the first paragraph (van Gestel and Hensbergen, 1997) to test for differences between used set-ups.

**Results and discussion**

**Acridine toxicity under laboratory and natural light**

Under laboratory light a clear dose-response curve for the effects of acridine on growth of the marine diatom *Phaeodactylum tricornutum* was observed. A 72 h EC50 value of 1.55 (95% confidence limits of 1.35–1.79) μM was found (Table 4.1). The EC50 value for acridine under laboratory light for *P. tricornutum* is within the range of EC50 values (1.23 to 4.35 μM) reported for the freshwater algal species *Scenedesmus acuminatus, Selenastrum capricornutum, Chlamydomonas eugametos, Staurastrum* spec. (Dijkman et al., 1997) and the marine species *Dunaliella tertiolecta* (Wiegman et al., 2001), all determined using the same mercury light source.

Under natural light, however, the growth rates of *P. tricornutum* exposed for 72 h to all tested acridine concentrations equalled zero or were negative, implying that the actual EC50 value in these conditions is below the lowest concentration tested (< 0.44 μM, Table 4.1). The higher toxicity of acridine under natural light compared to laboratory light observed in the present study is most likely due to the higher UV irradiance. Sink's et al. (1997) observed for example that the photoenhanced toxicity of acridine was higher in the presence of 8 than in the presence of 2 UV bulbs, suggesting that the photoenhanced toxicity of acridine is indeed related to UV irradiance. Also for the PAHs fluoranthene, pyrene, and anthracene, the photoenhanced toxicity increased with increasing UV irradiance (Holst and Giesy, 1989;
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Huang et al., 1993; Gala and Giesy, 1994; Ankley et al., 1995; Ankley et al., 1997). In the present study, the tested UV irradiance of mercury light is moderate (161 µW/cm²), when compared to that of natural light measured at noon and a clear sky in May in the Netherlands (3972 µW/cm²; de Lange, 1999). These observations clearly demonstrate the importance of quantifying the influence of UV irradiance in determining the effects of phototoxic aromatic compounds.

Table 4.1. Effect concentrations and 95% confidence limits of acridine (µM) under laboratory and natural light

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 value</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory light</td>
<td>1.55</td>
<td>1.35–1.79</td>
</tr>
<tr>
<td>Natural light (May '99)</td>
<td>&lt;0.44</td>
<td>nc</td>
</tr>
</tbody>
</table>

<sup>a</sup>72 h exposure; <sup>b</sup>48 h exposure

Effects of UV radiation on acridine toxicity

The spectral overlap between the light absorption of the PAH and the emitted irradiance of the light source (Σ(IE)λ) determines the extent of photoenhanced toxicity of PAHs (Arfsten et al., 1996; Diamond et al., 2000). Acridine absorbs especially UV radiation in the wavelength region of 320 to 410 nm (see Figure 4.1) with a maximum absorption peak at 355 nm. Therefore, UV-A is likely to enhance the toxicity of acridine.

Figure 4.4 shows the effect of acridine on the growth rates of *P. tricornutum* exposed to laboratory or natural light in the presence of total UV- (no filter), in the presence of UV-A- (UV-B-filter) and in the absence of UV-radiation (total UV-filter). Under non-filtered laboratory light, acridine reduced the growth rates of *P. tricornutum* to 41% of the corresponding DMSO controls. In the presence of UV-A-radiation, the growth rates were comparably reduced to 53% of the DMSO controls. In the absence of UV, growth rates were reduced to 86% of the DMSO controls (Figure 4.4). Under natural light, the same pattern was observed as under laboratory light: total UV- and UV-A-radiation enhanced the toxicity of acridine to the marine algae. In the absence of UV, however, acridine did not reduce the growth rates. Hence, the UV-A region of light was dominant in the photoenhanced toxicity of acridine to *P. tricornutum*, in accordance with its absorption spectrum in the UV-A region.

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A comparison of the outdoor experiments conducted in May and September 1999, confirms that the toxicity of acridine was stronger during the spring period when UV irradiance was higher (Table 4.1 and Figure 4.4). Bowling et al. (1983) found that the short-term effects of anthracene, a phototoxic PAH, corresponded with daily peak hours of sunlight (10:00–14:00 h). Therefore, strong daily and seasonal UV related variation in photoenhanced toxicity of PAHs must be accounted for.

![Figure 4.4. Average growth rates \((n = 2)\) of *Phaeodactylum tricornutum* in DMSO controls (white bars) and in acridine treatments (gray bars) under laboratory (mercury light) or natural light for 72 h. Significant differences between the DMSO controls and acridine treatments are indicated by **\((p < 0.05)\) and *(\(p < 0.10)\).](image)

**Transformation of acridine**

Based on the actual acridine concentrations at the start and the end of the different experiments, it was also demonstrated that the spectral composition of light influences the transformation rate of acridine: in the absence of UV for both laboratory and natural light, the recovery of acridine was higher (≥53%) than in the presence of total UV- or UV-A-radiation (approximately 30%, Table 4.2), suggesting that UV radiation contributes to the decrease in acridine concentrations. Besides these photochemical reactions, we also
suspect that *P. tricornutum* actively contributed to the transformation of acridine, as found for the freshwater alga *S. capricornutum* (Dijkman et al., 1997; de Voogt et al., 1999), because in the treatments with algae recoveries of acridine were low under both laboratory and natural light (59% and 37%, respectively) when compared to the chemical controls (> 95%, Table 4.1). The main transformation product of acridine (resulting from both biotransformation and photochemical reactions) is 9(10H)acridone (Kraak et al., 1997a; de Voogt et al., 1999; Wiegman et al., 1999). In the different treatments in the present study, however, only a low percentage (±10%) of the loss off acridine was regained as the main transformation product (9(10H)-acridone). Therefore, besides 9(10H)acridone, unidentified products may have been formed, but the exact nature for the acridine loss remains elusive.

**Table 4.2.** The recovery of acridine concentrations (C72/C0 in %) in the different treatments under laboratory and natural light (no algae = chemical controls; standard deviations between parentheses)

<table>
<thead>
<tr>
<th>Recovery (%)</th>
<th>laboratory light</th>
<th>natural light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>algae</td>
<td>no algae</td>
</tr>
<tr>
<td>Concentration series</td>
<td>59 (22)</td>
<td>116</td>
</tr>
<tr>
<td>Total UV</td>
<td>31 (14)</td>
<td>100</td>
</tr>
<tr>
<td>UV-A</td>
<td>29 (1)</td>
<td>107</td>
</tr>
<tr>
<td>No UV</td>
<td>53 (0.1)</td>
<td>110</td>
</tr>
</tbody>
</table>

\(^a\)n = 20, \(^b\)n = 6 (May 99), \(^c\)n = 2 (natural light; Sept. 99).

*Absorbance of UV by acridine*

The rate of photochemical reactions of PAHs in aqueous solutions is determined by the wavelength specific molar absorptivity of the compound (\(\varepsilon_\lambda\)), the concentration of the compound in the medium (M), the attenuation of UV (Kd) and the irradiance at each wavelength (\(I_\lambda\)). These photochemical parameters have been used to predict the phototoxic effects of PAHs to aquatic invertebrates (Morgan and Warshawsky, 1977; Oris and Giesy, 1987; Holst and Giesy, 1989; Ankley et al., 1995; Ankley et al., 1997; Diamond et al., 2000), but the effects varied with test organism and light conditions (Arfsten et al., 1996).

In the present study, we observed that the transmittance of UV radiation in the water column strongly depended on the algal densities. Therefore, we incorporated the attenuation of UV radiation (Kd) by the densities of *P.*
tricornutum (Figure 4.3) in the calculation of the amount of UV absorbed by acridine. Using the actual aqueous acridine concentrations, the UV absorption by acridine is corrected for, besides changes in algal biomass, acridine concentrations in the medium, since acridine concentrations in the water were influenced by algal biomass as well. For aromatic compounds, including azaarenes, it is observed that different excitation pathways can contribute to photoenhanced toxicity (Larson and Berenbaum, 1988; Leifer, 1988; Wiegman et al., 1999). In our study, the toxicity of acridine was enhanced by UV radiation within the specific molar absorptivity wavelength of acridine. Assuming, that only aqueous acridine was activated by UV, allowed us to calculate the amount of UV absorbed by acridine.

![Laboratory light: 0.4-8.5µM acridine](Laboratory light: 2.8µM acridine (Fig. 4.4))

![Natural light: 0.6µM acridine (Fig. 4.4)](Dose-response curve; r = 0.95)

**Figure 4.5.** Growth rates (%) of *Phaeodactylum tricornutum* plotted against the absorbance of UV radiation (mmol L\(^{-1}\) per 72 h) by acridine. Growth rates are expressed as percentages of the corresponding (DMSO) controls. Wavelength region: 280–400 nm. For natural light the average day length was 12.2 h. UV irradiance of natural light of Sept. '99 was obtained from the RIVM, The Netherlands.

For each separate treatment of the experiments conducted under laboratory and natural light, the amount of UV irradiance absorbed by aqueous acridine
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over the 72 h exposure period was calculated for the wavelength region 280 to 400 nm and plotted against the corresponding growth rates of \textit{P. tricornutum} (Figure 4.5). Figure 4.5 shows a clear dose-response relationship between the amounts of UV absorbed by acridine and the growth rates of algae \((p < 0.001, r = 0.95)\). No significant differences were observed between the EC50 values calculated for the experiments conducted under laboratory and natural light \((p > 0.05)\). The amount of UV absorbed by acridine, reducing the growth rates of \textit{P. tricornutum} to 50\% of the DMSO controls, was 0.45 mmol L\textsuperscript{-1} 72 h\textsuperscript{-1} (95\% confidence limits; 0.38–0.53 mmol L\textsuperscript{-1} 72 h\textsuperscript{-1}). Our results suggest that the UV amount absorbed by a phototoxic aromatic compound in solution is a key parameter for determining the toxicity of this compound.

Membrane damage due to oxidation of lipids by singlet oxygen has frequently been proposed as the mechanism of photoenhanced toxicity (McCloskey and Oris, 1991; Arfsten \textit{et al.}, 1996). Most algae and plants, however, were found to be less sensitive to the photoenhanced toxicity of aromatic compounds than invertebrates and fish (Arfsten \textit{et al.}, 1996). It has been hypothesized previously that especially their coloured pigments protect them from the adverse effects of photoenhanced toxicity by acting as radical scavengers (Gala and Giesy, 1993; Arfsten \textit{et al.}, 1996). In addition, our results indicate that coloured pigments mitigate the photoenhanced toxicity of acridine by preventing acridine from being activated by UV radiation. Therefore we argue that the actual amount of UV absorbed by a phototoxic compound may help explaining the sensitivities between the different taxonomic groups of organisms.

The fraction of UV absorbed by an aqueous system \((1–10^{(Kd+e_i[M])})\) is determined by the UV attenuation coefficient (determined by e.g. algal densities and dissolved organic compounds) and the acridine concentration in the water (Figure 4.6). In oceanic waters attenuation of UV is mainly caused by phytoplankton, but their densities hardly attenuate UV (MacIntyre \textit{et al.}, 2000). Consequently, aromatic compounds may absorb UV and the toxicity may be photoenhanced. In estuarine and coastal zones, however, both phytoplankton and dissolved organic compounds (DOC) reduce the UV availability (MacIntyre \textit{et al.}, 2000) and, subsequently, the absorbed amount of UV by phototoxic compounds. Thus, incorporating the UV absorption characteristics for natural water in the calculation of UV radiation absorbed
by aqueous aromatic compounds allows quantifying the key parameter for adverse biological effects. This approach improves the basis for assessing the environmental effects of these compounds.

**Figure 4.6.** Fraction of UV radiation in the wavelength region of 280–400 nm absorbed by the aqueous system as a function of acridine concentrations (μM) and attenuation coefficient (K_{d_{UV}} in cm\(^{-1}\)). K_{d_{UV}} values used in our set-up varied between 0.06 and 1.7 cm\(^{-1}\) (see Figure 4.3), for coastal waters K_{d_{UV}} values between 0.01 and 0.16 cm\(^{-1}\) have been reported (Conde et al., 2000; Neale, 2001).