Photoenhanced toxicity of azaarenes to marine phytoplankton.

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Many natural and man-made compounds in water are subject to photochemical processes that alter the molecular structures and, consequently, the effects of these compounds on aquatic organisms. The exposure of compounds to sunlight depends on the wavelength of incident radiation that varies with season, cloud coverage, and latitude and is modified much by attenuation in water. Sunlight, a natural source of radiation, is composed of radiation in the wavelength region of 290 to 800 nm. The range of 400 to 800 nm is visible light, whereas the lower wavelength range (290–400 nm) is non-visible ultraviolet radiation (UV). This UV radiation is vital in photochemical processes (Leifer, 1988).

Currently, changes in our climate (global warming), such as an observed increase of UV radiation (Slaper et al., 1996), have drawn the attention to changes in photochemical reactions in the atmosphere. At the same time an increased pollution of the aquatic environment by compounds derived from the use of fossil fuels, such as polycyclic aromatic hydrocarbons (PAHs) is observed (Mastral and Callen, 2000). Polycyclic aromatic compounds are widespread contaminants and consequently present in elevated levels in sediments, surface water and aquatic organisms. Among the PAHs azaarenes are relatively water-soluble (Pearlman et al., 1984) and serve as model compounds in this thesis. Also these azaarenes are subject to photo-

chemical transformation in surface waters. Yet, the composition of water determines to a large extent the exposure of azaarenes to radiation: in ocean waters sunlight penetrates to greater depths than in inland waters, where high concentrations of natural organic compounds occur and phytoplankton is abundant (Leifer, 1988; Maclntyre et al., 2000). Phytoplankton development and light-dependent transformation of compounds can both be considered as photochemical processes in water. The similarity of both processes and their concurrence in the surface waters of ocean-, coastal areas and inland waters triggered this study on the interaction of the biological and chemical processes involved.

Azaarenes

Nitrogen containing polycyclic aromatic hydrocarbons (NPAHs), PAHs in which one or more carbon atoms of the aromatic ring have been replaced by nitrogen atoms (see Figure 1.2), are found in biological systems as mycotoxins, defence toxins of plants and sponges, electron carriers, alkaloids and nucleotides (Tomkins and Ho, 1982; Kuhn and Suflita, 1989; Bodzek et al., 1996; Kaiser et al., 1996). Apart from their natural origin, NPAHs enter the environment as spills or waste materials generated by mining industry, coal tar- and oil shale processing operations, wood preserving facilities and chemical manufacturing plants (Tomkins and Ho, 1982; Kaiser et al., 1996). NPAHs are present in the environment in amounts up to 1–10% of those of their homocyclic analogue PAHs (Wild and Jones, 1995).

Azaarenes, a group of NPAHs containing one nitrogen atom, are present in detectable concentrations in fresh water and in the sea (van Genderen et al., 1994), sediments (Fernández et al., 1992; Swartz et al., 1995; Kozin et al., 1997) and in groundwater (Pereira et al., 1983). Azaarenes accumulate in sediments (Furlong and Carpenter, 1982) and organisms (Duxbury et al., 1997) and are widely distributed in aquatic ecosystems (Stuermer et al., 1982). A large fraction of azaarenes found in the environment is from anthropogenic origin, since almost no azaarenes have been found in older sediments (Wakeham, 1979). In Lake Zurich sediments concentrations of 27 and 10 μg/kg dw of respectively acridine and phenanthridine have been found (Wakeham, 1979). In Lake Ketelmeer concentrations were 45, 95 and 0.76 μg/kg dw for benz[α]acridine, benz[c]acridine and dibenz[c,h]acridine,
respectively (Kozin et al., 1997). In groundwater under a coal tar distillation site (Pereira et al., 1983) and in aquifer contaminated with wood-treatment chemicals (Pereira et al., 1987) the following concentrations were detected: quinoline 288 μg/L; isoquinoline 29 μg/L; acridine 106 μg/L; 9(10H)-acridone 119 μg/L. Concentrations of the azaarenes quinoline, isoquinoline and acridine varied between 0.1–1 μg/L in Dutch surface waters (van Genderen et al., 1994).

As a consequence of their chemical structure, azaarenes are more soluble in water than non-substituted PAHs (Pearlman et al., 1984). This often implies a higher availability to aquatic organisms (expressed as n-octanol/-water partition coefficient, Figure 1.1). Water quality criteria, such as maximum permissible concentrations (MPCs) and negligible concentrations, which are available for a small group of homocyclic PAHs, are lacking for azaarenes (Kalf et al., 1995). Toxic effects of azaarenes have been studied in several fish species, but only in a few aquatic invertebrates and algal species and even less is known about the effects of azaarenes on marine organisms (AQUIRE, www.epa.gov/ecotox/, see Figure 1.1).

![Figure 1.1. The effect concentrations of azaarenes (italic) and homocyclic PAHs on different groups of organisms (AQUIRE 2000) plotted against the octanol/-water partition coefficients (log K_{ow}). The water solubilities of these compounds lie within the gray area.](image)
Figure 1.2. A selection of homocyclic PAHs (left) and the selected azarenes (right).
Photochemical reactions of azaarenes in the aquatic environment

**Mechanisms of photochemical reactions**

![Diagram](image)

**Photosensitization**

\[ \text{PAH} \xrightarrow{hv} \text{PAH}^* \rightarrow \text{energy transfer} \rightarrow \text{biological effects} \]

**Photomodification**

\[ \text{PAH} \rightarrow \text{oxygenation products} \rightarrow \text{biological effects} \]

**Figure 1.3.** Schematic view of photochemical reactions of PAHs and related biological effects in the water column. Modified after Foote (1991).

UV radiation can alter the structures of PAHs by essentially two routes (Figure 1.3): photomodification and photosensitization (Larson and Berenbaum, 1988). Photomodification (photooxidation and/or photolysis) structurally alters PAHs to a variety of oxygenation products, via unstable endoperoxide and/or peroxide intermediates, thereby altering the toxicity to aquatic organisms (Zepp and Schlotzhauer, 1979; David and Boule, 1993; 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 (Larson and Berenbaum, 1988). In the presence of oxygen, PAH-oxygen complexes will be formed (also called charge transfer or CT complexes), which can be excited by photons. Upon excitation, the CT complex can dissociate along several pathways (Onodera et al., 1985; Kochany and Maguire, 1994a). The excited singlet-state PAH can undergo intersystem crossing to the excited triplet state, after which it can react with ground triplet-state oxygen \((^{3}\text{O}_2)\) to form \(^1\text{O}_2\) (Onodera et al., 1985; Larson and Berenbaum, 1988). In contrast to the lifetimes of singlet-state molecules, triplet-state lifetimes of PAHs are sufficiently long for diffusion-limited reactions with \(^3\text{O}_2\) and are therefore effective photosensitizers (Krylov et al., 1997). It should be noted, however, that triplet state PAH can excite ground state oxygen (22 kcal/mol required) only if the triplet-singlet conversion of the PAH leads to an energy gain (> 38 kcal/mol). Excited oxygen, although having an extremely short lifespan, will attack those carbon atoms in the benzene ring opposite (i.e. para
oriented) to those having the highest electron density of the HOMO (Highest Occupied Molecular Orbital) resulting in 1,4-adducts, also called endoperoxides (Yamaguchi et al., 1985). Endo-compounds, on their turn, can dissociate into the parent PAH and $^{1}O_2$. Formed within an organism $^{1}O_2$ is capable of oxygenating or oxidizing many different biomolecules, altering their chemical structure, and consequently inhibiting or inactivating them (Larson and Berenbaum, 1988).

**Kinetics of photochemical reactions**

The kinetics and products of photochemical reactions of homocyclic PAHs and azaarenes in aquatic environments are reviewed by Kochany and Maguire (1994a). Compared to homocyclic PAHs little work has been done on photochemical reactions of azaarenes in water. Because solubilities of azaarenes are 1000–10000 times higher than those for PAHs, photochemical reactions are thought to be more important for azaarenes than for PAHs (Pearlman et al., 1984; Kochany and Maguire, 1994a).

The exposure of PAHs to UV radiation depends on the season, latitude, depth and composition of the water (Leifer, 1988). Dissolved compounds, pigments, and suspended material absorb and scatter UV radiation. Especially the humic acids, which form a part of the dissolved organic matter (DOM), attenuate UV radiation in the water. So, high concentrations of organic matter lead to a strong decrease of UV with increasing depth, and consequently to a decrease of photochemical reactions of PAHs. Nevertheless, in the presence of humic acids increasing rates of photolysis of azaarenes were also found, suggesting that humic acids can act as sensitizers (Kochany and Maguire, 1994a).

The pH of the water also determines the photolysis rates of azaarenes are. In the aromatic system of azaarenes the electron density is higher than for PAHs, because of the non-bonded electron pair on the nitrogen atom. In acid water the nitrogen atom will be protonated (for example, 70% of quinoline is protonated at a pH of 4.4), lowering the electron density within the molecule and thereby decreasing photolysis rates (Mill et al., 1981; Kochany and Maguire, 1994a).
Photoenhanced toxicity of azaarenes to marine phytoplankton

Figure 1.3. Decrease of acridine concentration in milli-Q water (%; \(C_0 = 10 \, \mu M\)) by sunlight (1% v/v solvent acetonitrile or DMSO) and by UV-A radiation (0.033% v/v solvent DMSO) plotted against exposure time (h) (unpublished data; Wiegman et al., 1999).

In laboratory studies azaarenes dissolved in pure water degraded rapidly under short-waved radiation, UV-B radiation being more effective than UV-A (Mill et al., 1981; Kochany and Maguire, 1994b). In outdoor experiments however, the photochemical reactions proceeded slower (Mill et al., 1981). This is shown in Figure 1.3 in which photolysis rates of acridine under different light sources and in different seasons are compared. Acridine degraded faster when exposed to laboratory UV radiation than to sunlight (52°21’ N, 4°57’ E, The Netherlands) in September and May, respectively (unpublished data; Wiegman et al., 1999). Compared to sunlight with a broad spectral composition (290–800 nm), laboratory UV radiation contains a small range of wavelengths with relatively high intensities.

Besides the composition of the light or the availability of light \((I_\lambda)\), the absorption spectrum of a compound \((\varepsilon_\lambda)\) and the efficiency of direct photochemical reaction or quantum yield \((\phi)\) determines the photolysis rates of compounds dissolved in water. Photolysis rates are proportional to the overlap between absorption spectrum of the compound and the incident light \((\Sigma(I_\lambda\varepsilon_\lambda))\) and to the quantum yield \((\phi)\). The quantum yield of PAHs – the ratio
between the number of molecules undergoing photochemical reaction and the number of photons absorbed – is assumed to be independent of wavelength (Zepp, 1978; Leifer, 1988). However, the quantum yield of quinoline was lower in photolysis experiments conducted under natural light than under lamps with a smaller region of UV (Mill et al., 1981). Such contrasting findings show that especially for azaarenes, the kinetics of photolysis are still unclear.

Products of photochemical reactions

Compared to PAHs, such as anthracene, phenanthrene and benzo[a]pyrene (Mill et al., 1981; McConkey et al., 1997; Mallakin et al., 1999), minor progress has been made in identifying the products of photochemical reactions of azaarenes. Photoproducts determined for indoles were 0-acylaminoketones (Picel et al., 1987). For quinoline some photoproducts were tentatively determined, mainly hydroxyquinolines (Mill et al., 1981). Parallel to the present study de Voogt et al. (1999) found that 9(10H)-acridone is the main photoproduc t of acridine. For acridine and quinoline similar oxygenated products are formed by biological transformation involving mono-oxygenases (Kaiser et al., 1996; de Voogt et al., 1999). The scarcity of knowledge on intermediates or final photoproducts is an obstacle in determining the adverse biological effects of irradiated azaarenes.

Photoenhanced toxicity of PAHs

Photoenhanced toxicity of homocyclic PAHs

Under standard laboratory light conditions, most of the four- and five-ringed PAHs are not acutely toxic at or below their aqueous solubilities (see Figure 1.1). However, a number of PAHs have been found to be acutely toxic to aquatic organisms at concentrations well below their solubilities in the presence of environmentally relevant UV radiation (Kagan et al., 1985; Holst and Giesy, 1989; Gala and Giesy, 1993; Gala and Giesy, 1994; Pelletier et al., 1997; Wernersson and Dave, 1997). This increase of toxicity of PAHs by UV radiation, via photosensitization and/or photomodification, into reactive and toxic products is defined as photoenhanced toxicity.
Table 1.1. Log $K_{ow}$ and HOMO-LUMO gap energies of PAHs and azaarenes (italic) (compounds with HOMO-LUMO gap energies within the phototoxic window of 6.6–7.6 eV are bold)

<table>
<thead>
<tr>
<th>Compound</th>
<th>rings</th>
<th>$log K_{ow}$</th>
<th>HOMO-LUMO gap (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinoline</td>
<td>2</td>
<td>2.03$^a$</td>
<td>8.72$^c$</td>
</tr>
<tr>
<td>Isoquinoline</td>
<td>2</td>
<td>2.08$^a$</td>
<td>8.46$^c$</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2</td>
<td>3.32$^b$</td>
<td>10.06$^d$/10.16$^g$</td>
</tr>
<tr>
<td><strong>Acridine</strong></td>
<td>3</td>
<td>3.20/3.41$^b$</td>
<td>7.53$^c$</td>
</tr>
<tr>
<td>Benzo[f]quinoline</td>
<td>3</td>
<td>3.43$^c$</td>
<td>8.28$^c$</td>
</tr>
<tr>
<td>Benzo[h]quinoline</td>
<td>3</td>
<td>3.43$^c$</td>
<td>8.18$^c$</td>
</tr>
<tr>
<td>Phenanthridine</td>
<td>3</td>
<td>3.48$^c$</td>
<td>8.37$^c$</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>3</td>
<td>3.90$^d$</td>
<td>8.24$^d$</td>
</tr>
<tr>
<td>Fluorene</td>
<td>3</td>
<td>4.32$^a$</td>
<td>8.48$^g$/8.51$^h$</td>
</tr>
<tr>
<td>Anthracene</td>
<td>3</td>
<td>4.57$^b$</td>
<td>7.28$^g$,$^h$</td>
</tr>
<tr>
<td>Phenanthenene</td>
<td>4</td>
<td>4.67$^b$</td>
<td>8.20$^g$/8.21$^g$</td>
</tr>
<tr>
<td><strong>Benz[a]acridine</strong></td>
<td>4</td>
<td>4.49$^c$</td>
<td>7.63$^c$</td>
</tr>
<tr>
<td><strong>Benz[c]acridine</strong></td>
<td>4</td>
<td>4.49$^c$</td>
<td>7.56$^c$</td>
</tr>
<tr>
<td><strong>Pyrene</strong></td>
<td>4</td>
<td>4.90$^d$</td>
<td>7.24$^d$</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>4</td>
<td>5.22$^b$</td>
<td>7.68$^g$/7.70$^g$</td>
</tr>
<tr>
<td>Chrysene</td>
<td>4</td>
<td>5.79$^b$</td>
<td>7.69$^g$</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>5</td>
<td>5.80$^d$</td>
<td>7.59$^d$</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>4</td>
<td>5.90$^d$</td>
<td>7.39$^g$</td>
</tr>
<tr>
<td>Benzo[b]anthracene</td>
<td>4</td>
<td>5.90$^g$</td>
<td>6.52$^g$</td>
</tr>
<tr>
<td><strong>Dibenz[a,ij]acridine</strong></td>
<td>5</td>
<td>5.67$^d$</td>
<td>6.81$^k$</td>
</tr>
<tr>
<td><strong>Dibenz[c,h]acridine</strong></td>
<td>5</td>
<td>6.27$^e$</td>
<td>7.64$^k$</td>
</tr>
<tr>
<td><strong>Dibenzo[a,h]anthracene</strong></td>
<td>5</td>
<td>6.40$^e$</td>
<td>7.45$^g$</td>
</tr>
<tr>
<td><strong>Benzo[a]pyrene</strong></td>
<td>5</td>
<td>6.10$^b$</td>
<td>6.81$^g$</td>
</tr>
<tr>
<td><strong>Benzo[e]pyrene</strong></td>
<td>5</td>
<td>6.44$^d$</td>
<td>7.36$^g$</td>
</tr>
<tr>
<td><strong>Perylene</strong></td>
<td>5</td>
<td>6.44$^d$</td>
<td>6.70g/6.71$^n$</td>
</tr>
</tbody>
</table>

$^a$(Hansch and Leo, 1995), $^b$(Kalf et al., 1995), $^c$(Bleeker et al., 1998), $^d$(Boese et al., 1998), $^e$(Helweg et al., 1997), $^f$(Baumard et al., 1998), $^g$(Mekenyan et al., 1994b), $^h$(Veith et al., 1995), $^i$(Wiegman et al., 2001), calculated with Hyperchem, calculated with MOPAC package.

Kagan et al. (1987) noted that the degree of photoenhanced toxicity of PAHs under laboratory UV radiation is related to UV absorption characteristics for each compound. The absorption peak of the 'phototoxic' PAHs is found to be in the UV-A range of laboratory light (> 320 nm) and of the non-phototoxic PAHs near the UV-B range (< 320 nm). Holst and Giesy (1989) and Ankley et al. (1995) observed that increasing UV intensities coincide with increasing toxicity of PAHs to aquatic organisms. The energy difference between the Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital (HOMO-LUMO gap) of PAH molecules is
an effective parameter to explain the light absorption and photoenhanced toxicity of PAHs. Photoenhanced toxicity can occur for PAHs with ground-state HOMO-LUMO gap energies in the range of 6.5 to 8.0 eV with maximum effect near 7.2 ± 0.4 eV (Mekenyan et al., 1994b). PAHs falling within this highly 'phototoxic window' are anthracene, pyrene, benzo[a]anthracene, benzo[e]pyrene, benzo[a]pyrene, dibenz[a,h]anthracene and perylene and are listed in Table 1.1 (Mekenyan et al., 1994b; Veith et al., 1995; Boese et al., 1998). The HOMO-LUMO gap of PAHs defines the energy that is necessary to elevate an electron from the HOMO to the LUMO state. PAHs with small HOMO-LUMO gaps absorb energy at higher wavelengths (more visible range) of light and are photochemical less stable, whereas PAHs with larger HOMO-LUMO gaps absorb at smaller wavelengths (UV; greater energy) and are photochemical reactive (Mekenyan et al., 1994b; Veith et al., 1995).

Most research concerning photoenhanced toxicity in the aquatic environment has focused on acute (photoenhanced) toxicity of PAHs to freshwater species, e.g. the water flea, *Daphnia magna* (Kagan et al., 1985; Wernersson and Dave, 1997), the mosquito, *Aedes aegypti*, (Kagan et al., 1985) and *Chironomus tentans* (Ankley et al., 1994), the oligochaete, *Lumbriculus variegatus* (Ankley et al., 1995), the fish *Pimephales promelas* (Kagan et al., 1987) and *Lepomis macrochirus* (Oris and Giesy, 1985; Oris and Giesy, 1986), the alga *Selenastrum capricornutum* (Gala and Giesy, 1993; Gala and Giesy, 1994) and the macrophyte *Lemna gibba* (Huang et al., 1993) and is reviewed by Arfsten et al. (1996). Photoenhanced toxicity of PAHs varies between test species and compounds. Compared to invertebrates and fish, algae and plants are expected to be less sensitive to these effects of PAHs. In algae, highly coloured pigments can protect against photoenhanced toxicity of PAHs (Gala and Giesy, 1993). Indeed, a higher resistance to phototoxic PAHs has been observed (Arfsten et al., 1996). Holst and Giesy (1989) reported the chronic effects of anthracene under UV-A radiation on *D. magna*. Exposure to anthracene in the presence of ecologically relevant UV-A intensities decreased the survival and fecundity of *D. magna* at concentrations well below their aqueous solubility limits, indicating that low PAH concentrations can adversely affect population dynamics in the environment.

A few experiments have been conducted with field-collected sediments
containing a combination of different PAHs (Arfsten et al., 1996). Especially the benthic organisms *Hyalella azteca* and *L. variegatus* accumulated PAHs from the test sediments and, subsequently, UV enhanced the toxicity of the PAHs to these organisms in both laboratory and field experiments (Ankley et al., 1994; Monson et al., 1995).

**Photoenhanced toxicity of azaarenes**

Photoenhanced toxicity of azaarenes in aqueous environments has been scarcely investigated, while toxicity of azaarenes to aquatic organisms has only been reported for two-ringed, three-ringed and four-ringed structures; quinoline (two-ringed) and acridine (three-ringed) are the most investigated compounds (see Figure 1.1). Acridine and the four-ringed structures benz [a]acridine and benz[c]acridine are the only azaarenes that can display photoenhanced toxicity in water (Table 1.1). Acridine has been predicted to be the most pronounced phototoxic compound, because the HOMO-LUMO gap energy of acridine falls in the highly phototoxic region of 7.2 ± 0.4 eV (Mekenyan et al., 1994b).

Photoenhanced toxicity of acridine is indeed reported for the freshwater ciliate *Tetrahymena pyriformis*, the water flea *D. magna*, the fathead minnow *P. promelas* and several freshwater algae by respectively Sinks et al. (1997), Newsted and Giesy (1987), Oris and Giesy (1987), Wernersson and Dave (1997), Bleeker et al. (1998) and Dijkman et al. (1997). In the presence of UV-A radiation (two UV-A-340-tubes, with a peak at 340 nm) toxicity of acridine increased with one order of magnitude to *D. magna* (Wernersson and Dave, 1997). Within 228 minutes of exposure to UV radiation, the mortality of *T. pyriformis* was 100% in the presence of acridine, whereas no mortality was observed for its isomer phenanthridine (Sinks et al., 1997). In the dark, the no observed effect concentration (NOEC) of acridine equalled the NOEC of phenanthridine to *T. pyriformis*. Also, an increase of UV intensity – an increase of 1 bulb to 8 bulbs of broadband black light (peak emission at 350 ± 50 nm) – increased the toxicity to *T. pyriformis* (Sinks et al., 1997). These studies demonstrated that especially UV radiation is responsible for photoenhanced toxicity of acridine. UV-A constitutes a larger fraction of sunlight at the earth surface than UV-B. UV-B is rapidly attenuated in the atmosphere and in water, while a large fraction of UV-A penetrates into the water column (Leifer, 1988; Nielsen and
Ekelund, 1995). Hence, especially the UV-A region of light will be of environmental importance to photoenhanced toxicity of PAHs and azaarenes in aquatic surroundings. Yet, the wavelength dependency of photoenhanced toxicity of azaarenes largely lacks experimental verification.

**Toxicity of photochemical products**

Although most studies demonstrated that electronic changes are responsible for photoenhanced toxicity of PAHs to aquatic organisms (Gala and Giesy, 1994; Mekenyan et al., 1994b; Veith et al., 1995; Ankley et al., 1997), oxygenated products have been proven to increase the environmental hazard of PAHs as well (Huang et al., 1993; Huang et al., 1995; Ren et al., 1996; Huang et al., 1997a; Huang et al., 1997b; McConkey et al., 1997; Mallakin et al., 1999). Whereas photosensitization of PAHs is restricted to PAHs exhibiting a HOMO-LUMO gap window of $7.2 \pm 0.4$ eV only, photomodification of PAHs is not. PAHs with HOMO-LUMO energy differences outside this HOMO-LUMO gap window can be transformed into more toxic photoproducts (Huang et al., 1993; Ren et al., 1994). Even though the toxicity of transformation products of azaarenes are barely investigated, photomodification reactions are likely to increase the environmental hazard of azaarenes, as observed for homocyclic PAHs.

**Photosynthesis of microalgae as an end-point for photoenhanced toxicity**

Growth rate of algal populations is a sensitive parameter, useful to measure chronic toxicity of toxic compounds, whereas photosynthesis has been proven to be a sensitive parameter to determine the acute effects of toxicants. Huang et al. (1997b) observed that some photomodified PAHs have a unique mechanism of intoxicating photosynthesis of macrophytes. Especially the oxygenation products seemed to block the electron transport between photosystem II and I, inhibiting the photosynthesis of the duckweed *Lemma gibba* and that of natural phytoplankton assemblages (Huang et al., 1997b; Marwood et al., 1999). Traditionally, photosynthetic activity of microalgae has been determined measuring the incorporation of radio-labelled carbon. Recently, the photosynthetic performance, and, in addition, the physiological state of microalgae can be determined, using a rapid technique, Pulse Amplitude-Modulated (PAM) fluorometry that measures chlorophyll a fluorescence *in vivo*. Because algae depend on light for photo-
synthesis and inhibition of photosynthesis has been reported as a key mechanism of toxicant action in plants and algae (Huang et al., 1997a; McConkey et al., 1997), photosynthesis will also be explored in the present thesis as an important end-point for determining the photoenhanced toxicity of azaarenes.

Objectives of this thesis

The aim of this thesis is to analyse the influence of light, especially UV radiation, on the interaction between phototoxic compounds and phototrophic organisms. The objectives of the present thesis are:

- To quantify the reactivity of azaarenes in water in relation to different regions of the radiation spectrum.
- To discriminate the role of photomodification, photosensitization and the general toxicity of azaarenes, and to establish the relevant molecular descriptors.
- To determine the effect of intensity and wavelength of UV radiation on the extent of photoenhanced toxicity of azaarenes.
- To establish a key parameter for photoenhanced toxicity that is applicable for natural waters in which these complex light-dependent processes prevail.

In this thesis a series of azaarene isomers is selected as test compounds and marine microalgae are used as model organisms.

Selection of test compounds and test organisms

For several aquatic species, a linear relationship between the $n$-octanol/water partition coefficient ($\log K_{ow}$) and short-term toxicity of azaarenes has been demonstrated (de Voogt et al., 1988; Bleeker et al., 1999). However, deviations from these relationships have also been observed, even for closely related compounds such as isomers (Kraak et al., 1997b; Bleeker et al., 1999). Such deviations can be considered as indications for a specific mode of action. Hence, isomer-specific toxicity deserves further attention and therefore, four azaarene isomer pairs have been selected (see Figure
1.2); two two-ringed (quinoline and isoquinoline), two three-ringed (acridine and phenanthridine), two four-ringed (benz[a]acridine and benz-[c]acridine) and two five-ringed structures (dibenz[a,i]acridine and dibenz-c,h]acridine).

Both photoenhanced toxicity of aromatic compounds and algal photosynthesis are depending on light. The photoenhanced toxicity may be equally important in seawater and freshwater but information on photoenhanced toxicity of PAHs and azaarenes to marine algae is virtually absent. Therefore, two marine microalgae, the green flagellate *Dunaliella tertiolecta* (Chlorophyceae) and the diatom *Phaeodactylum tricornutum* (Bacillariophyceae), were selected. Both algal species are representative species for phytoplankton in coastal waters (Geel, 1997) and are commonly used test species in ecotoxicological research (Samson and Popovic, 1988; Visviki and Rachlin, 1994; Kudo et al., 1996; Muller and Wilhelm, 1997; Mooney and Patching, 1998).

**Outline of this thesis**

First, the toxicity of the four azaarene isomer pairs, ranging from two-ringed to five-ringed structures, was determined in 72 h growth experiments with the green flagellate *Dunaliella tertiolecta* (chapter 2). Molecular properties of the azaarenes were used to discriminate photoenhanced toxicity from a non-specific or general mode of action, and to gain insight into the mechanisms leading to photoenhanced toxicity.

Chapter 3 focuses on the photoreaction kinetics of the eight azaarenes and the toxic effects of their transformation products on the marine diatom *Phaeodactylum tricornutum*, formed under influence of UV radiation. First, photoreaction kinetic parameters were used to define the amount of energy necessary for photolysis, which provided insight in the stability or reactivity of azaarenes under UV radiation in the aquatic environment. Second, all azaarenes were irradiated with UV radiation prior to toxicity tests. The toxicity of non-irradiated azaarenes and mixtures with transformation products of azaarenes was tested using inhibition of photosynthetic activity (measuring $^{14}$C incorporation) as the end-point. The comparison between the toxicity of azaarenes and the toxicity of mixtures with transformation
products provided insight in the importance of photomodification as a pathway of toxic action.

The influence of spectral composition of irradiance on the extent of photoenhanced toxicity was analysed in chapter 4. This chapter reports on the development of quantitative measures for photoenhanced toxicity under natural light regimes. Therefore, the photoenhanced toxicity of acridine, a three-ringed azaarene, to growth of *P. tricornutum* was investigated under laboratory and natural light. By including spectral filters in the experimental set-ups, the wavelength ranges of UV radiation responsible for an enhancement of toxicity of acridine were determined.

Finally, chapter 5 investigates the possible role of adaptation of *P. tricornutum* to UV radiation as a protective mechanism against photoenhanced toxicity of acridine. Using the Pulse Amplitude-Modulated (PAM) fluorometry, physiological adaptations to UV and the effects of photoenhanced toxicity to photosynthesis of microalgae were studied.

The concluding remarks (chapter 6) discuss the main findings of this thesis and review the implications for risk assessment of phototoxic aromatic compounds.