Toxicity of azaarenes: mechanisms and metabolism.
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Chapter 4

Comparative Ecotoxicity of NPAHs to Larvae of the Midge Chironomus riparius

Abstract

Acute toxicity of seven azaarenes (NPAHs) has been determined using larvae of the midge Chironomus riparius. Clear dose-response relationships for mortality of first instar larvae were observed, but surviving larvae grew equally well as the controls. LC$_{50}$ values (96 h) for a two-ringed structure (quinoline), four three-ringed structures (acridine, phenanthridine, benzo[f]quinoline and benzo[h]quinoline) and two four-ringed structures (benz[a]acridine and benz[c]acridine) were generally one order lower than the few values reported for other aquatic species in the literature. Dibenz[a,i]acridine, a five-ringed structure, was not acute toxic at its maximum dissolved concentration. Acute toxicity increased with increasing number of aromatic rings of the compound; accordingly correlations between the LC$_{50}$ values and size, shape and topology-related molecular properties were strong. However, between the four benzoquinoline isomers tested also differences in toxicity were observed: acridine was significantly more toxic than the other benzoquinoline isomers tested. This is most likely caused by transformation of acridine under UV light, indicated by a HOMO-LUMO gap in the highly phototoxic region, a high correlation between isomer toxicity and heat of formation and a low recovery of acridine in the water during the experiment.
Introduction

Nitrogen in-ring substituted polycyclic aromatic hydrocarbons (NPAHs), analogues of parent polycyclic aromatic hydrocarbons (PAHs), contain one or more nitrogen atom(s) in place of a carbon atom. Apart from their natural origin (e.g. as alkaloids), NPAHs are formed and released into the environment by incomplete combustion of fossil fuels, in spills or effluents of several industrial activities, oil drilling, refining and storage (Kochany and Maguire, 1994) and coal tar distillation (Pereira et al., 1983). N-heterocycles are also associated with wood preservation (Pereira et al., 1983) and pesticide use (Kuhn and Suflita, 1989).

Van Genderen et al. (1994) demonstrated the occurrence of some NPAHs in freshwaters in the Netherlands and Blumer et al. (1977), Wakeham (1979), Furlong and Carpenter (1982) and Bleeker et al. (1996) detected NPAHs in marine and freshwater sediments. Despite the potentially carcinogenic, mutagenic, teratogenic and (geno)toxic activity of certain chemicals within the group of NPAHs (Davis et al., 1981; Santodonato and Howard, 1981; Walton et al., 1983), acute toxic effects have been studied in only a few aquatic macroinvertebrate species, among which daphnids (Southworth et al., 1978; Newsted and Giesy, 1987; Johnson et al., 1990), the midge Chironomus tentans (Cushman and McKamey, 1981), the calanoid copepod Diaptomus clavipes (Cooney and Gehrs, 1984) and the zebra mussel Dreissena polymorpha (Kraak et al., 1997ab). For all these species, acridine and quinoline are often the only compounds tested. Thus, a reliable hazard assessment for this family of compounds is hampered by a lack of toxicity data, while also differences in toxicity are hardly understood. The aim of this study was therefore to determine the acute toxicity of seven different NPAHs, ranging from a two-ringed structure to a five-ringed structure. It was attempted to relate differences in toxicity to molecular properties, such as molecular surface area or volume, connectivities, charge distribution or dipole moment and also correlations with physico-chemical properties like partition coefficients (e.g., n-octanol/water or Henry’s law constant) and chromatographic retention were analysed (De Voogt et al., 1988; De Voogt et al., 1990; De Voogt et al., 1991).

The midge Chironomus riparius was used as a test species, representing a most abundant group of insects in freshwater ecosystems. They are a major
food source for fish and other vertebrate and invertebrate predators and thus occupy an important position in aquatic food webs (Pinder, 1986). Because chironomid larvae live in close contact with the sediment, which shows accumulation of NPAHs, they may be exposed to these toxicants via water and via ingestion of sediment.

**Materials and Methods**

All experiments lasted for 96 hours and were carried out under static conditions in an incubator at 20 ± 2 °C. A 16/8 h light/dark regime was provided. An experimental treatment consisted of fifty newly hatched first instar larvae, originating from a laboratory culture, placed in a glass vessel (180 ml) containing 100 ml Dutch Standard Water (DSW) (Nederlands Normalisatie Instituut, 1980). They were fed 1 ml of a solution of grounded Trouvit and Tetraphyl® in DSW (10 g and 0.5 g, respectively, in 200 ml). During the experiments the vessels were covered with transparent plastic foil. The average actual concentrations of the toxicants tested were for quinoline 0.92, 1.86, 4.27, 8.51 and 18.94 mg/L, for acridine 0.013, 0.032, 0.076, 0.152 and 0.301 mg/L, for phenanthridine 0.030, 0.069, 0.166, 0.287, 0.476, 0.970 and 2.119 mg/L, for benzo[f]quinoline 0.042, 0.101, 0.233, 0.496 and 0.922 mg/L, for benzo[h]quinoline 0.332, 0.644, 1.311 and 2.602 mg/L, for benz[a]acridine 0.0007, 0.0017, 0.0057, 0.0123 and 0.0237 mg/L, for benz[c]acridine 0.0037, 0.0073 and 0.0130 mg/L, and for dibenz[a,i]acridine 0.012 mg/L.

For each compound, the series of concentrations (including two controls) was tested in triplicate, except for quinoline, which was tested in duplo and dibenz[a,i]acridine, which was tested once at maximum dissolved aqueous concentration (0.012 mg/L).

At the start of each experiment the length of at least 20 newly hatched larvae was measured and after 96 h surviving larvae were counted and their length measured. Survival was expressed as percentage of the mean of the two corresponding controls. For each compound, LC$_{50}$ values based on average actual azaarene concentrations in the water (see below) with 95 % confidence limits were estimated. Survival values were plotted against corresponding actual toxicant concentrations in the water. With the computer program
KaleidaGraph® (Abelbeck Software 1993) the following equation (Haanstra et al., 1985) was fitted through the dose-response plots:

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

in which \(Y\) = survival (%), \(c\) = survival in control (set to 100 %), \(a\) = log LC\(_{50}\) (mg/L), \(b\) = slope and \(X\) = log concentration (mg/L).

EC\(_{50}\) values for growth were not calculated, because exposed larvae that survived, tended to grow the same as under control conditions (average 1.1 vs. 1.2 mm in 96 h, respectively).

All compounds were added to the water once at the start of the experiment, using stock solutions. A quinoline stock solution was made by dissolving 40 mg quinoline in 1 L DSW, resulting in a stock solution concentration of 40 mg/L. For all other compounds tested, stock solutions were made using the following generator column technique: each compound was dissolved in methanol, to which chromosorb GAW (60-80 mesh; Chrompack) was added, after which the methanol was evaporated using nitrogen gas flow in an incubator at 50 °C. The toxicant-loaded chromosorb was placed in a 30 cm high glass column. One litre DSW was pumped into the bottom side of the column by a peristaltic pump (Gilson minipuls 2) and left the column at the top where it was stored in a flask. To prevent photodegradation, the column and flask were wrapped in aluminium foil. This method resulted in the following stock solution concentrations: 34 mg/L for acridine, 32 mg/L for phenanthridine, 26 mg/L for benzo[f]quinoline, 36 mg/L for benzo[h]quinoline, 0.30 mg/L for benz[a]acridine, 0.05 mg/L for benz[c]acridine, and 0.012 mg/L for dibenz[a,i]acridine. The purity of the compounds was 97 % for quinoline and acridine (Aldrich), 98 % for phenanthridine (Aldrich), 99+ % for benzo[f]quinoline (Janssen), 97 % for benzo[h]quinoline (Janssen) and 99+ % for benz[a]acridine, benz[c]acridine and dibenz[a,i]acridine (European Community Bureau of Reference).

Water samples (2.5 ml) were taken after 1 h and after 96 h to determine actual NPAH concentrations in the water. After centrifugation (3000 rpm, 10 min.) 1 ml of the supernatant was taken for analysis by High-Performance Liquid Chromatography (HPLC), using fluorescence detection (Kratos Spectroflow 980) for the (di)benzacridines and UV detection (Applied Biosystems model 785) for the other compounds. A 150*4.6 mm LiChrosorb
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5 µm RP-18 analytical column was used with a 4 × 4 mm LiChrosphere 5 µm RP-18 guard-column. The column temperature was maintained at 30 ± 1 °C and 20 µl of each sample was automatically analysed. The flow of the mobile phase was 1 ml/min. Quinoline was detected at a wavelength of 220 nm and was eluted with a mixture of 65 % methanol (J.T. Baker Analyzed HPLC Reagent, min. 99.8 %) and 35 % 13.4 mM phosphate buffer (Merck, 1.0998 Titrisol®, pH 7.00 ± 0.02). Acridine, phenanthridine, benzo[j]quinoline and benzo[h]quinoline were detected at a wavelength of 254 nm after elution with a mixture of 80 % methanol and 20 % phosphate buffer (acridine and benzo[j]quinoline) or a mixture of 80 % acetonitrile (J.T. Baker Analyzed HPLC Reagent, min. 99.9 %) and 20 % water (J.T. Baker Analyzed HPLC Reagent) (phenanthridine, benzo[h]quinoline). The benzacridines were detected at an excitation wavelength of 285 nm and an emission wavelength of > 354 nm and eluted with a mixture of 80 % acetonitrile and 20 % water. Dibenzo[a,i]acridine was detected at an excitation wavelength of 299 nm and an emission wavelength of > 354 nm and eluted with a mixture of 90 % acetonitrile and 10 % water.

From the toxicant concentrations in the water samples, the average actual concentrations and start concentrations (at t = 0 h) were calculated using integral calculus. An exponential decrease of toxicant concentrations in the water was therefore assumed. Recovery was defined as the quotient of the average actual concentration during the 96 h exposure period and the actual concentration at the start of the experiment.

Based on the concept that to elicit a biological effect, a chemical has to cross membranes and interact with one or more receptors and that, therefore, both hydrophobic and electronic forces may play a role, hydrophobicity-related and electronic molecular parameters were selected which may describe the transport and partition process(es) involved. Since photo-induced toxicity and/or transformation may also be involved when considering NPAHs (see below), parameters related to the reactivity of the compound were also included.

Correlation coefficients were calculated between the estimated log based LC₅₀ values and the following molecular descriptors: molecular volumes (Vol) and surface areas (SA), valence-connectivity-indices for path orders 3, 4 and 5 (χ3p, χ4p, χ5p), dipole moments (D) and energies of the lowest unoccupied and
highest occupied molecular orbits (LUMO and HOMO), which reflect the ionisation potential (IP) and electron affinity (EA) respectively. In several studies, good correlations were found between acute toxicity of organic pollutants and octanol-water partition coefficients, $K_{ow}$ (Könemann, 1981; Veith et al., 1983). Therefore, these coefficients were also incorporated in this study, together with molecular connectivities, volumes and surface areas, which highly correlate with $K_{ow}$ values (De Voogt et al., 1988).

**Figure 4.1.** Survival (%) during the 96 h experiments of first instar larvae of *Chironomus riparius* at different concentrations of quinoline (Qui), acridine (Acr), phenanthridine (Phe), benzo[f]-quinoline (BfQ), benzo[h]quinoline (BhQ), benz[a]acridine (BaA) and benz[c]acridine (BcA) in the water, plotted as percentage of the corresponding controls. The lines represents the curve-fit after Haanstra et al. (1985).
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Log $K_{ow}$ values are taken from Med Chem's ClogP model. Vol and SA were calculated by the SAVOL programme using cartesian co-ordinates obtained after optimisation of the molecular geometry by a molecular mechanics modelling method (MM1 option in MOPAC). Van der Waals radii used for hydrogen, aromatic carbon and nitrogen were 1.01, 1.77 and 1.50 ($10^{-10}$ m), respectively. Atom-atom distances used were: C-H, 1.084; C-C, 1.395; C-N, 1.338 ($10^{-10}$ m). A solvent radius of zero was assumed. D, IP and EA were calculated by the semi-empirical quantum mechanical AM1 method using the same optimised molecular conformations (MM1 option in MOPAC). This program also generates heats of formation. Valence connectivity indices for path orders 3, 4 and 5 were calculated by the CFUNC-programme. Regression was calculated with the computer program KaleidaGraph® (Abelbeck Software, 1993).

Results and Discussion

Fig. 4.1 shows the survival of first instar larvae of *Chironomus riparius* at different concentrations of the NPAHs in the water as percentages of the corresponding controls. Survival in the controls always exceeded 80 %. Clear dose-response relationships were observed for the effects of all toxicants, except for dibenz[a,z']acridine. From these results, LC$_{50}$ values and their 95 % confidence limits were calculated (Table 4.1). EC$_{50}$ values for growth could not be calculated because surviving exposed larvae tended to grow the same as under control conditions (average 1.1 vs. 1.2 mm in 96 h, respectively).

All LC$_{50}$ values in this study are based on actual toxicant concentrations in the water, but big differences were observed between the recoveries of the different compounds (Table 4.1). Recoveries of more than 80 % were found for quinoline, phenanthridine, benzo[f]quinoline and benzo[k]quinoline, while for acridine and the two benzacridines recoveries reached only 48 % and about 25 %, respectively. The recovery percentages clearly reflect the increasing lipophilicity in the tested series of nitrogen heterocyclic compounds. Especially in the case of the relatively lipophilic benzacridines, concentrations in the water dropped rapidly, probably caused by sorption to the glass, food (present as dissolved and particular organic matter) and midge larvae. When dealing with
such poorly water-soluble compounds, the uptake of toxicants via food is likely to become a relevant accumulation pathway (Carvalho et al., 1995). LC\textsubscript{50} values based on actual toxicant concentrations in the water therefore underestimate the total exposure. Measuring concentrations of toxicants in the midge larvae, and expressing effect-concentrations as lethal body burdens (LBB) seems a promising alternative.

Table 4.1. Recovery (%) ± standard deviations and calculated effect concentrations (96 h LC\textsubscript{50}; mg/L). 95 % confidence limits are given between brackets.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery (%)</th>
<th>96 h LC\textsubscript{50} (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinoline</td>
<td>90.4 ± 4.2</td>
<td>4.8967 (3.0800 - 7.7849)</td>
</tr>
<tr>
<td>Benzo[f]quinoline</td>
<td>96.3 ± 10.2</td>
<td>0.8312 (0.3815 - 1.8108)</td>
</tr>
<tr>
<td>Phenanthridine</td>
<td>85.7 ± 9.2</td>
<td>0.6131 (0.5172 - 0.7267)</td>
</tr>
<tr>
<td>Benzo[n]quinoline</td>
<td>93.2 ± 2.8</td>
<td>0.6059 (0.4988 - 0.7360)</td>
</tr>
<tr>
<td>Acridine</td>
<td>48.1 ± 8.2</td>
<td>0.0714 (0.0612 - 0.0833)</td>
</tr>
<tr>
<td>Benz[a]acridine</td>
<td>25.2 ± 8.6</td>
<td>0.0153 (0.0138 - 0.0169)</td>
</tr>
<tr>
<td>Benz[c]acridine</td>
<td>22.3 ± 6.2</td>
<td>0.0069 (0.0067 - 0.0072)</td>
</tr>
</tbody>
</table>

Table 4.2 presents the calculated molecular descriptors for the seven NPAHs tested. Except for the dipole moment, all descriptors are highly correlated (\(r > 0.9\)) with the observed toxicity (log based values) (e.g. Fig. 4.2). All correlation coefficients presented in this study are calculated using mean LC\textsubscript{50} values and the values of the molecular descriptors. Because the standard errors of the LC\textsubscript{50} values could not be incorporated in the calculation of the correlation coefficients, no unequivocal significance of the regression lines could be assessed, and is therefore not presented. However, taking the low standard errors of LC\textsubscript{50} values for most compounds tested into account, these correlation coefficients are sufficiently precise for the purpose of this study.

For dibenz[a,i]acridine, a five-ringed structure, no effect was observed at its maximum dissolved concentration. The actual average exposure concentration of dibenz[a,i]acridine could not be calculated because the concentration after 96 h in the water was below detection limits of the HPLC system.

Quinoline, a two-ring structure, is significantly less toxic than acridine, phenanthridine, benzo[f]quinoline and benzo[k]quinoline, all three-ringed structures (\(p < 0.05\)). Benz[a]acridine and benz[c]acridine are significant more
toxic than each of the three-ringed compounds (p < 0.05). This increase in toxicity with an increase in number of rings, appears to be a general pattern for the acute toxicity of N-heterocycles. Acridine was more toxic than quinoline for *Chironomus tentans* (Cushman and McKamey, 1981; Millemann et al., 1984), *Daphnia magna* (Millemann et al., 1984), *Gammarus minus* and *Physa gyrina* (Millemann et al., 1984) and benz[a]acridine was more toxic than acridine for *Daphnia pulex* (Southworth et al., 1978). The increase in toxicity coincides with an increase in lipophilicity, facilitating membrane crossing and bioaccumulation and hindering excretion. These processes are affected by hydrophobic and electronic forces, as well as the size and shape of the molecular structure, indicated by high correlations between electronic-, size- and topology-related molecular properties (e.g. log $K_{ow}$, molecular volume, surface area and connectivities) and observed toxicities of the NPAHs tested.

Table 4.2. Values of molecular descriptors and their correlation with toxicity data. Qui: quinoline; BIQ: benzo[f]quinoline; Phe: phenanthridine; BhQ: benzo[h]quinoline; Acr: acridine; BaA: benz[a]acridine; BcA: benz[c]acridine; r: correlation coefficients with corresponding (log based) 96 h LC$_{50}$ values; Vol: Molecular volumes; SA: surface areas; HF: heats of formation; D: dipole moments; HOMO: highest occupied molecular orbitals; LUMO: lowest unoccupied molecular orbitals; $\chi^3p$, $\chi^4p$, $\chi^5p$: 3rd, 4th and 5th order path valence indices; $K_{ow}$: octanol-water partition coefficient.

<table>
<thead>
<tr>
<th></th>
<th>Vol</th>
<th>SA</th>
<th>HF</th>
<th>D</th>
<th>HOMO</th>
<th>LUMO</th>
<th>$\chi^3p$</th>
<th>$\chi^4p$</th>
<th>$\chi^5p$</th>
<th>log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qui</td>
<td>122.24</td>
<td>143.07</td>
<td>52.24</td>
<td>1.87</td>
<td>-9.18</td>
<td>-0.47</td>
<td>1.51</td>
<td>1.02</td>
<td>0.66</td>
<td>2.03</td>
</tr>
<tr>
<td>BIQ</td>
<td>165.97</td>
<td>185.98</td>
<td>69.28</td>
<td>1.96</td>
<td>-8.88</td>
<td>-0.60</td>
<td>2.51</td>
<td>1.83</td>
<td>1.30</td>
<td>3.20</td>
</tr>
<tr>
<td>Phe</td>
<td>165.99</td>
<td>186.17</td>
<td>68.07</td>
<td>2.06</td>
<td>-8.98</td>
<td>-0.60</td>
<td>2.50</td>
<td>1.81</td>
<td>1.28</td>
<td>3.20</td>
</tr>
<tr>
<td>BhQ</td>
<td>166.05</td>
<td>186.73</td>
<td>68.46</td>
<td>1.54</td>
<td>-8.75</td>
<td>-0.55</td>
<td>2.50</td>
<td>1.81</td>
<td>1.29</td>
<td>3.54</td>
</tr>
<tr>
<td>Acr</td>
<td>166.21</td>
<td>187.47</td>
<td>77.19</td>
<td>1.83</td>
<td>-8.57</td>
<td>-1.04</td>
<td>2.43</td>
<td>1.72</td>
<td>1.24</td>
<td>3.41</td>
</tr>
<tr>
<td>BaA</td>
<td>210.36</td>
<td>230.40</td>
<td>92.45</td>
<td>1.92</td>
<td>-8.62</td>
<td>-1.00</td>
<td>3.43</td>
<td>2.53</td>
<td>1.88</td>
<td>4.59</td>
</tr>
<tr>
<td>BcA</td>
<td>211.23</td>
<td>231.11</td>
<td>91.53</td>
<td>1.43</td>
<td>-8.54</td>
<td>-0.98</td>
<td>3.41</td>
<td>2.51</td>
<td>1.87</td>
<td>4.59</td>
</tr>
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</table>

$\chi^3p$, $\chi^4p$, $\chi^5p$ values for *Chironomus riparius* exposed to quinoline, acridine and benz[a]acridine are much lower than those found in the literature for other species (e.g. Southworth et al., 1978; Cushman and McKamey, 1981; Millemann et al., 1984). These differences may be caused by differences in test species, but also differences in exposure time and life-stage can play an important role.
Cushman and McKamey (1981), for example, used fourth instar *Chironomus tentans* larvae, while in the present study first instar larvae were used. Williams et al. (1986) demonstrated that first instar midge larvae are 1000x more sensitive to metals than fourth instar larvae and Walton (1980) determined that acridine was highly toxic to cricket (*Acheta domesticus*) eggs but not toxic to nymphs or adults. Therefore, differences in sensitivity between life-stages is the most likely explanation for the low LC$_{50}$ values for *C. riparius* compared to the strongly related *C. tentans*. In accordance we found that NPAH exposed larvae once surviving grew unimpaired later on.

![Figure 4.2. Relationship between the 96 h LC$_{50}$ (mg/L) for *Chironomus riparius* midge larvae and the heat of formation values (kcal/mol) for seven NPAHs.](image)

Acridine was ten times more toxic than the three other benzoquinolines tested (p < 0.05), while toxicities of phenanthridine, benzo[hl]quinoline and benzo[f]quinoline did not differ significantly (p < 0.05). Benz[c]acridine is significantly more toxic than benz[a]acridine (p < 0.05). Explaining differences in toxicity between isomers by molecular descriptors requires that they are of sufficient statistical quality and distinctive enough to obtain significantly
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different values for isomers. Most descriptors, however, were insufficiently discriminating between isomers, although correlation with toxicity of the whole series of compounds (two to four ring) tested was high. Dipole moment and electron affinity discriminated most, but did not provide a good explanation for the big differences in the observed effects for the whole series of compounds tested (Table 4.2). Acute toxicity of NPAHs is well described by heat of formation values (high correlation and high discriminating power) (Fig. 4.2; Table 4.2). The difference in toxicity between acridine and the other benzoquinolines coincides with a significant difference in heat of formation. Combined with the low recovery of acridine, this may indicate that acridine is being transformed. Acridine is known to be metabolised by the zebra mussel into 9(10H)-acridone (Kraak et al., 1997a). However, because no acridone was found in the water samples at the end of the present experiments, transformation into another compound than acridone should be considered.

Photo-induced toxicity also plays an important role in eliciting toxic effects of NPAHs. Studies with a variety of aquatic species have shown that (N)PAHs can be orders of magnitude more toxic in the presence of UV light (Bowling et al., 1983; Newsted and Giesy, 1987). Photo-induced toxicity is the result of competing processes, such as stability and light absorbance, which interact to produce a complex relationship between toxicity and chemical structure. Mekenyan et al. (1994) described a relation between photo-induced toxicity and the HOMO-LUMO gap. Differences in linearity of aromatic polycycles have strong influence on the HOMO-LUMO gap. Mekenyan et al. (1994) demonstrated that PAHs exhibiting photo-induced toxicity were consistently within a HOMO-LUMO gap "window" of 7.2 ± 0.4 eV. The two benzacridines and acridine, being the most toxic NPAHs in the series of compounds tested in this study, with HOMO-LUMO gaps of 7.6 and 7.5 eV respectively, fall into this highly phototoxic region. All other compounds tested, with HOMO-LUMO gaps between 8.2 and 8.7 eV, are thus classified as chemicals for which phototoxicity is of no concern. Because the HOMO-LUMO gap of acridine falls in the highly phototoxic region and because a high correlation between formation heat values and toxicity was observed, degradation of acridine, under influence of UV light, may have taken place. This may explain why acridine was the most
toxic of all benzoquinolines and why recovery of acridine in the experiments was low.

In contrast quinoline is reported to be degraded easily by micro-organisms (Pereira et al., 1988; Aislabie et al., 1994; Sutherland et al., 1994) and fish (Bean et al., 1985), and also degrades rapidly in sunlight (Kochany and Maguire, 1994), but the recovery for quinoline in these experiments (Table 4.1) was high, showing that degradation does not occur under these conditions.

For the algae *Scenedesmus acuminatus* acridine was also significantly more toxic than the other three benzoquinolines (Van Vlaardingen et al., 1996), possibly also caused by photo-activation. In contrast to algae and midges, mussels are protected against UV light by their valves, preventing photo-induction. Consequently, for the zebra mussel *Dreissena polymorpha* a different order of toxicity for the four benzoquinolines was observed: phenanthridine was significantly more toxic than acridine (Kraak et al., 1997b), confirming the vital role of short-waved light in modifying the toxicity of polyaromatic compounds.

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**References**


