Chapter 7

Membrane-Azaarene Interaction: A Preliminary Molecular Mechanics Approach

Abstract

Narcotic effects are the result of toxicant-induced membrane disturbances. Relationships between narcosis and hydrophobicity (log $K_{ow}$) of toxicants are well established. However, especially when isomers are involved, not all toxicant-specific differences may be explained. We argue, therefore, that more detailed, mechanism-based investigations of toxicant-membrane interactions may be necessary. The present study investigates the interactions between a computationally constructed model membrane and a selection of azaarenes (as model substances). The membrane model was constructed from 16 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine molecules forming a bilayer. Membrane-azaarene interaction energies were then calculated from solvation energies to evaluate the importance of three different regions in the membrane: in the polar headgroup, between the alkyl chains, and between the two layers. Finally, a QSAR approach was used to compare the resulting energies with previously measured toxicity data. Significant energy differences between the compounds were only found in the headgroup and between the layers. Of these two regions, the headgroup seems to be the most important for narcosis, resulting in significant relationships between interaction energies and narcosis. Although log $K_{ow}$ shows still higher correlation coefficients, it is less successful in predicting narcosis for isomers. Furthermore, log $K_{ow}$ values are less informative in terms of mode of action than solvation energies are. In addition, the membrane model used is a simplified model. A further improvement of the membrane model may, therefore, perform better than the log $K_{ow}$ model. Thus, based on the preliminary results presented here, the approach used is a promising tool in predicting narcosis.
Introduction

In an organism chemicals partition between hydrophilic compartments like cytosol, hydrophilic proteins and extracellular fluid, and hydrophobic compartments like organellar and plasma membranes, hydrophobic proteins and storage fat. Hydrophobic compounds will accumulate mostly in the hydrophobic parts, including the cell membrane, the first hydrophobic part that an extracellular compound encounters (Van Wezel et al., 1996). Compounds entering the membrane can partition through the bilayer directly (Jin and Hopfinger, 1996) or bind to some kind of receptor that either facilitates or hampers membrane partitioning (Franks and Lieb, 1990). This may either result in or oppositely prevent bioaccumulation and toxicity. The receptors can occur in five different regions: in the extracellular fluid, attached to the outer membrane surface, embedded within the bilayer, attached to the inner membrane surface, or in the cell lumen (Jin and Hopfinger, 1996).

For narcosis, a type of toxicity believed to result from membrane disturbance, relationships with hydrophobicity of the compounds are well known. The hydrophobicity is often expressed by the n-octanol-water partition coefficient ($K_{ow}$) (Könemann, 1981; Veith and Broderius, 1990). Such relationships, however, may not explain all toxicant specific differences in narcotic effects, especially when isomers are involved (Bleeker et al., 1998). We argue, therefore, that more detailed, mechanism-based investigations of toxicant-membrane interactions may be necessary.

In this study azaarenes were chosen as model toxicants. Azaarenes are a group of polycyclic aromatic hydrocarbons in which one in-ring carbon atom is substituted by a nitrogen atom. As a result azaarenes are more water soluble than their homocyclic analogues (Pearlman et al., 1984). This may increase their ecotoxicological significance, especially in aquatic environments. In a series of recent studies (Bleeker et al., 1998; Bleeker et al., 1999ab) the toxicity and genotoxicity of members of this group of compounds have been reported.

For the azaarenes a first attempt to explain toxicity using toxicant-membrane interactions was conducted by (Kraak et al., 1997), who suggested that isomer differences in toxicity towards zebra mussels were caused by differences in the way the compounds fitted between the alkyl-chains of the phospholipids. These chains, however, are only part of the membrane lipids. Since azaarenes are
reasonably hydrophilic, the polar headgroup of the phospholipid may be a much stronger barrier for these compounds than the alkyl chains, because the azaarenes will dissolve better in the polar region, which may hamper further diffusion. Furthermore, a third region can be distinguished within a biological membrane, i.e. between the two bilayers (Jin and Hopfinger, 1996). The importance of each of these regions depends on the location of the toxicant receptor (e.g. a membrane protein). For instance, if the receptor is located between the bilayers, the toxicant has to pass through both the head group and the alkyl chains in order to reach the receptor, so the more difficult this is, the less toxic the compound will be.

The aim of the present study was to evaluate the role of diffusion in narcosis, together with other types of interactions between membranes and azaarene substrates. To that end a computational approach was used. First, a membrane model was constructed from available crystallographic data, based on 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC) (Pearson and Pascher, 1979; Hauser et al., 1981), one of the main phospholipids in biological membranes (Downer, 1985). Next, interaction energies were calculated to evaluate the importance of three different regions that can be distinguished in the DMPC bilayer model for membrane-substrate interaction. Finally, the resulting energies were compared in a QSAR approach with previously measured toxicity data.

**Methods**

**Azaarene optimisation**

The following azaarenes were selected for the study: quinoline (QUI), acridine (ACR), phenanthridine (PHE), benzo[f]quinoline (BFQ), benzo[h]-quinoline (BHQ), benz[a]acridine (BAA), benz[c]acridine (BCA), benz[g]isoquinoline-5,10-dione (BID), benzo[g]quinoline-5,10-dione (BQD), acridone, both in enol (ACOe) and keto form (ACOk), and phenanthridinone, also in both tautomeric forms (PHOe and PHOk, respectively). Although the keto form is more stable and, therefore, more favourable than the enol form (Roberts and Caserio, 1967), the enol form can be favourably influenced by a number of causes, summarised by Elguero et al. (1976). Partial bond fixation e.g. as in
3-hydroxyisoquinoline favours this tautomer over the 3-isoquinolone form. More importantly perhaps are the effects of phase and temperature. The enol form is favoured in nonpolar solvents and in the gas phase, and at high temperature in polar or low temperature in nonpolar solvents. For example, in water the conversion of the hydroxy into oxo form is exothermic ($\Delta H < 0$), in less polar solvents it becomes less exothermic, and in dioxan it is endothermic. Furthermore, the annelation of two benzene rings in acridone, compared to 4-pyridone, considerably increases the relative stability of the oxo form. For these reasons, in this study, both mesomeric forms of acridone and phenanthridinone were incorporated.

![Figure 7.1. Atom charges for all optimised azaarenes, calculated by the AM1 and PM3 methods. The different symbols refer to the different atom types as defined by HyperChem. The arrows point at specific atoms (see text for details).](image)

To calculate membrane-azaarene interaction energies molecular mechanics were used (see below), but to this end atom charges had to be calculated for each azaarene. All azaarene molecules have been drawn in HyperChem (Hypercube Inc., Version 5.1), followed by the option Model Build to get the 3D-structure. The geometry of this 3D-structure was then further optimised,
using either AM1 or PM3 with the Polak-Ribiere optimiser. The convergence limit was set to 0.05 (kcal/mol), accelerate convergence was turned on and the criterion of the RMS gradient was set to 0.05 (kcal/Å-mol).

Comparison of calculated atom charges from AM1 and PM3 shows that in general there is a strong linear correlation between the two methods ($r^2 = 0.88$). However, differences occur when atom charges for the keto forms of acridone and phenanthridinone are calculated, especially when the nitrogen atom is considered (Fig. 7.1, open triangles). With PM3 a positive charge is calculated for this atom, while AM1 results in a negative charge. In addition, other atoms from or directly connected to the N-containing ring also showed deviations from the linear correlation (Fig. 7.1, arrows), indicating that especially in this case PM3 and AM1 use different parameterisation for calculating charges.

Therefore, a third calculation method was used for both tautomers of acridone and phenanthridinone: the minimal (STO-3G) ab initio method. This method was chosen, because ab initio methods are generally considered less simplified and, therefore, more accurate than semi-empirical methods (Doucet and Weber, 1996). Ab initio computational time, however, is much longer than that of semi-empirical methods. In addition, in the literature more results are available for semi-empirical than for ab initio methods. Therefore, in this study the ab initio method was only used to enable a choice between the AM1 and PM3 methods. The ab initio calculations resulted in a negative charge for the nitrogen atom in the keto form, and consequently in a better correlation between STO-3G and AM1 calculated atom charges ($r^2 = 0.83$) than between STO-3G and PM3 ($r^2 = 0.48$). Consequently, in this study, AM1 calculated atom charges were used.

To further optimise the structures and the membrane-azaarene complexes the molecular mechanics method MM+ was used. This MM+ method can be applied in two different modes for the calculation of electrostatic interactions: based on either bond dipoles or atomic charges. To evaluate which mode gave better results, three structures were selected for which experimental dipole moments were available: phenol, pyridine, and quinoline. For these molecules atom charges were calculated as before with AM1, starting with geometry optimisation, followed by a single point calculation for the resulting geometries.
The resulting structures were then further optimised twice using MM+, either with atomic charges or with bond dipoles. The dipole moments calculated with bond dipoles appeared much higher than those based on atomic charges, which is in agreement with the literature (Stewart, 1990). Experimental data showed still higher values. We concluded that for MM+ calculations the bond dipole method seems more appropriate than the atomic charges method. In addition, the membrane model used (see below) had also been optimised with the bond dipole method.

**Construction of the phospholipid bilayer membrane model**

The membrane used in this study is constructed from 16 molecules of 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC) and 2 water molecules per DMPC molecule (Govers, in prep.). This resulted in a phospholipid bilayer of 8 against 8 DMPC molecules with water molecules within the polar head groups (Fig. 7.2). Atomic charges of all DMPC atoms were set at zero, except for nitrogen (+1) and the two equivalent oxygen atoms bonded to the phosphorous atom (each -0.5). Atom charges of H₂O atoms were obtained from an AM1 optimisation (H: +0.191; O: -0.383).

To decrease calculation times some restrictions were incorporated into the membrane model. Inner and outer radii for atom-atom parameter summation were set at 10 and 14 Å, respectively, and restraints (128 for the full membrane) were applied to distances (corresponding to z = 9.077 and x = 9.285 Å) of nitrogen and phosphorous atoms in the polar heads and carbon atoms at the end of the alkyl chains, and to angles (90°) between x and z edges (further details for the bilayer construction can be found in Govers (in prep.). After optimisation, the restraints were removed and a single point MM+ calculation was performed to calculate the unrestrained energy of the membrane.

**Incorporating azaarenes into the membrane**

Optimised azaarene structures were placed into the membrane at positions along the central channel of the membrane model (Ch in Fig. 7.2C). This channel was chosen to minimise effects of the vacuum surrounding the model. For ACR and PHE and both tautomers of their respective metabolites acridone (ACOe and ACOk) and phenanthridinone (PHOe and PHOk) a total of nine positions was tested, denoted by H1a, H1b, H2, H3, C1, C2, M1, M2 and M3.
(see below). The 6 structures were selected to look for differences between isomers and between tautomers. From the calculations with these six structures and nine positions, two positions emerged that showed the most distinct differences between the six compounds. Only these two positions were then used to calculate membrane-azaarene interaction energies for the remaining seven azaarenes.

![Figure 7.2](image_url) The DMPC bilayer membrane model seen from three different sides (A,B,C). The Ch in C indicates the central channel through which the azaarenes were placed in the membrane. H1, H2 and H3 are the sites where azaarenes were placed in the headgroup, C1 and C2 those between the alkyl chains and M1-3 indicates the locations where azaarenes were placed between both layers (see text for details).

The H1 position (Fig. 7.2) was optimised twice for each molecule, i.e. with different starting positions (a and b), rotated 180° between each other in such a way that the N-atom is either pointing away from the phosphorous bound oxygen (H1a) or pointing towards it (H1b). Position H2 is situated more inside the membrane, while position H3 is at a similar position, but between different chains (Fig. 7.2BC). C1 and C2 positions are situated between the alkyl chains, where C1 is situated closer to the polar region of the membrane. M1 and M2 are
positioned between the layers (Fig. 7.2, M1-3), M1 with the length and width of
the molecule along the $y$ and $x$ axis respectively, and M2 with those along the
$x$ and $y$ axis, i.e. rotated 90°. The M3 position is somewhat tilted between the
layers in the cavity formed by two opposing 'short' chains.

Figure 7.3. Schematic overview of the calculations performed in this study (see text for details).
For each position 6 energy terms were calculated by HyperChem as a measure of the way the membrane and the azaarene change due to their mutual interactions. These energy terms consist of the interaction energies of the atoms, both within and between molecules, and they include: the energy of the membrane before insertion of an azaarene ($E_{Mo}$), the energy of the azaarene before insertion into the membrane ($E_{Ao}$), the energy of the total azaarene-membrane complex ($E_{MA}$), the energy of the azaarene-adapted membrane ($E_{M}$), and the energy of the membrane-adapted azaarene ($E_{A}$) (Fig. 7.3). From these energies a solvation energy ($\Delta E_{MA} = E_{MA} - [E_{Mo} + E_{Ao}]$), a membrane cavity formation energy ($\Delta E_{M} = E_{M} - E_{Mo}$), and an azaarene adaptation energy ($\Delta E_{A} = E_{A} - E_{Ao}$) can be calculated.

In previous studies significant relationships were found between toxicity and log $K_{ow}$ of azaarenes (Bleeker et al., 1998; Bleeker et al., 1999). Although not all toxicant-specific effects could be explained by these relationships, they indicate that the role of the surrounding water in toxicity mechanisms could not be neglected. Therefore, in the present study a model of pure water was used in which the same azaarenes were placed to calculate hydration energies. This model consisted of a periodic box (HyperChem option; dimensions: 18.12 x 18.53 x 18.43 Å) with 208 water molecules (= 624 atoms), which results in dimensions comparable to one third of the membrane model. Furthermore, this resulted in a the natural density of 1.000 g water/cm$^3$. Atom charges of the water molecules were again obtained from an AM1 optimisation (H: +0.191; O: -0.383) and geometry of the model was again optimised with MM+ (bond dipoles).

This model was then used to calculate the hydration energy for each azaarene. The azaarene was placed in the middle of the periodic box with water, after which the water-azaarene complex was optimised with the MM+ bond dipole method. Inner and outer radii for atom-atom parameter summation were set at 5.06 and 9.06 Å, respectively, and convergence limit was set at 0.20 for a Steepest descent pre-optimisation. The complex was then further optimised in Newton-Raphson mode with 0.05 convergence limit. Finally, and analogously to the DMPC model, the periodic box was removed to calculate the actual energy of the system.
The hydration energy ($\Delta E_{WA}$) can then be calculated with the following formula

$$\Delta E_{WA} = E_{WA} - (E_{Ao} + E_{Wo})$$

in which $E_{WA}$ is the energy of the optimised water-azaarene complex (without the box), $E_{Ao}$ is the energy of the isolated azaarene, and $E_{Wo}$ is the energy of the water model (without the azaarene and the box).

An overview of all calculations is given in Fig. 7.3.

Finally, relationships were sought between (geno)toxicity data and calculated solvation and hydration energies, to gain insight in the role of membrane-azaarene and water-azaarene interactions in (geno)toxicity. In addition, relationships were sought between (geno)toxicity and energy differences, i.e. between hydration energies and either H1a or M1 solvation energies as a measure for diffusion from the water into the membrane, and between H1a and M1 solvation energies as a measure for diffusion within the membrane.

### Results

Fig. 7.4 presents the solvation energies for each starting position of acridine, phenanthridine and both tautomers of both acridone and phenanthridinone. This figure shows that the differences between solvation energies of various azaarenes amount to only a few kcal/mol at positions H2, H3, C1, C2, M1 and M2. Positions H1 (both a and b) and M3 yield larger differences, which can be as high as several tens of kcal/mol.

Considering this, H-positions and M-positions seem most suitable for determining differences in azaarene-membrane interactions between closely related compounds, i.e. isomers on the one hand and parent vs. metabolite on the other. For the other seven azaarenes, therefore, one H-position and one M-position were selected for energy calculations.

Considering the head-group, a selection had to be made between H2, H3, H1a and H1b. The H3 position is situated outside of the central channel, so the vacuum around the model might influence the calculations. As for the H2 position, it does not show significant differences between the compounds. Therefore, an H1 position seems more appropriate for describing differences in azaarene-membrane interactions. This leaves us with the question whether to choose H1a or H1b. In this respect, the relatively large difference between the
two ACOe orientations (cf. Fig. 7.4) is striking, but during the optimisation of the H1b position, this particular azaarene is moved to the outside of the membrane, partly into the vacuum, logically resulting in a very low solvation energy. In reality, however, the membrane is surrounded by water molecules, which are likely to make it more difficult to move the azaarene out of the membrane. Since for the other molecules the H1a position results in solvation energies lower or equivalent to that of the H1b position, the H1a position is selected for calculations with the other seven azaarenes.

![Figure 7.4. Solvation energies for the optimised membrane-azaarene complexes, calculated by MM+ for 9 possible locations in the membrane. See text for details.](image)

Considering the M-positions, M2 shows little distinction between the compounds, narrowing the choice for an M-position to either M1 or M3. The M3 starting position, however, seems unfavourable, because the molecule, when put in this position, starts to move through the membrane, resulting in an optimised position near or partly in the vacuum around the model. The vacuum is of course absent in reality and, therefore, calculated energies for the optimised M3-position are probably less reliable. Hence, the H1a and M1 position were used for calculations of the other seven azaarenes.
Table 7.1 shows the calculated (differences between) solvation and hydration energies for each of the thirteen compounds, together with LC \textsubscript{50} values for the midge *Chironomus riparius* and LOEC values of the Mutatox\textsuperscript{TM} genotoxicity tests (both taken from Bleeker et al., 1999ab). Since accurate LC \textsubscript{50} values for the metabolites and LOEC values for BFQ and BCA are lacking, these compounds were not included in the corresponding correlations. Furthermore, during optimisation of BHQ in the H1a position, this molecule moves to a H2 like position, resulting in a much lower solvation energy in comparison with the other benzoquinolines. Therefore, relationships with H1a solvation energies were also calculated without considering BHQ.

**Table 7.1.** Calculated energy values (kcal/mol), log \( \text{K}_{\text{ow}} \) values and (geno)toxicity data (µM) for the azaarenes tested. H1a: solvation energy in position H1a; M1: solvation energy in position M1; H1a-M1: difference between solvation energies in position H1a and M1; hydration: hydration energy; M1-Hydr.: difference between hydration energy and solvation energy in M1 position; H1a-Hydr.: difference between hydration energy and solvation energy in H1a position; log LC \textsubscript{50}: logarithm of 96 h LC \textsubscript{50} value for *Chironomus riparius*; log LOEC: logarithm of LOEC\textsubscript{genotoxicity} tested with the Mutatox\textsuperscript{TM} test; see text for compound abbreviations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvation energy</th>
<th>log ( \text{K}_{\text{ow}} )</th>
<th>log LC \textsubscript{50}</th>
<th>log LOEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1a</td>
<td>M1</td>
<td>H1a-M1</td>
<td>Hydration</td>
<td>H1a-Hydr.</td>
</tr>
<tr>
<td>QUI</td>
<td>10.48</td>
<td>-7.17</td>
<td>17.65</td>
<td>-30.93</td>
</tr>
<tr>
<td>ACR</td>
<td>9.40</td>
<td>-10.57</td>
<td>19.97</td>
<td>-26.14</td>
</tr>
<tr>
<td>PHE</td>
<td>19.72</td>
<td>-2.35</td>
<td>22.06</td>
<td>-24.09</td>
</tr>
<tr>
<td>BFQ</td>
<td>9.97</td>
<td>4.25</td>
<td>5.72</td>
<td>-23.58</td>
</tr>
<tr>
<td>HQ</td>
<td>-13.56\textsuperscript{e}</td>
<td>-2.24</td>
<td>-11.27</td>
<td>-21.48</td>
</tr>
<tr>
<td>BAA</td>
<td>0.29</td>
<td>5.41</td>
<td>-5.13</td>
<td>-29.55</td>
</tr>
<tr>
<td>BCA</td>
<td>-2.67</td>
<td>4.02</td>
<td>-6.69</td>
<td>-31.46</td>
</tr>
<tr>
<td>BID</td>
<td>10.76</td>
<td>-0.42</td>
<td>11.18</td>
<td>-41.85</td>
</tr>
<tr>
<td>BGD</td>
<td>10.55</td>
<td>-3.14</td>
<td>13.69</td>
<td>-36.63</td>
</tr>
<tr>
<td>ACOe</td>
<td>24.32</td>
<td>-7.83</td>
<td>32.15</td>
<td>-27.82</td>
</tr>
<tr>
<td>ACOk</td>
<td>4.56</td>
<td>-7.57</td>
<td>12.13</td>
<td>-36.88</td>
</tr>
<tr>
<td>PHOe</td>
<td>19.78</td>
<td>-2.43</td>
<td>22.22</td>
<td>-22.99</td>
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<tr>
<td>PHOk</td>
<td>17.48</td>
<td>-1.28</td>
<td>19.12</td>
<td>-29.28</td>
</tr>
</tbody>
</table>

\textsuperscript{a} this molecule is moved during optimisation to a more H2-like position; \textsuperscript{b} these log \( \text{K}_{\text{ow}} \) values are experimental data, taken from literature (De Voogt et al., 1988); \textsuperscript{c} these log \( \text{K}_{\text{ow}} \) values are estimated with the ClogP model provided by the Environmental Science Center of Syracuse Research Corporation (internet: http://esc.syrres.com/~ESC/kowexpdb.htm).
The first part of Table 7.2 shows regression statistics for (linear) relationships between experimental LC$_{50}$ values and either log K$_{ow}$ or energy descriptors. It can be seen that for LC$_{50}$ values vs. energy related descriptors no significant (i.e. p > 0.05) correlation coefficients could be found, unless BHQ was left out of the data set. In the latter case LC$_{50}$ values showed significant relationships with solvation energies in the H1a position and with the differences between solvation energies in the H1a and the M1 positions. Furthermore, correlation coefficients of LC$_{50}$ with log K$_{ow}$ and with the difference between H1a solvation energies and hydration energies improve when the group of compounds is made more homogeneous by leaving out BID and BQD (Table 7.2; no BHQ/BID/BQD).

Table 7.2. Regression statistics for correlations of selected energy parameters with log LC$_{50}$ and log LOEC$_{genotoxicity}$, respectively. N: number of azaarenes used in the regression; r$^2$: the proportion of the variance that is described by the regression line; slope: the slope of the regression line; p: the probability of the regression line; see text for compound abbreviations.

<table>
<thead>
<tr>
<th>LC$_{50}$ regression statistics (all without ACOe, ACOk, PHOe and PHOk)</th>
<th>All compounds</th>
<th>no BID/BQD</th>
<th>no BHQ</th>
<th>no BHQ/BID/BQD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 9)</td>
<td>(N = 7)</td>
<td>(N = 8)</td>
<td>(N = 6)</td>
</tr>
<tr>
<td>Log K$_{ow}$</td>
<td>0.55 -0.69 0.02</td>
<td>0.77 -0.66 0.11</td>
<td>0.61 -0.73 0.02</td>
<td>0.82 -0.70 0.01</td>
</tr>
<tr>
<td>H1a</td>
<td>0.13 3.49 0.34</td>
<td>0.12 3.47 0.44</td>
<td>0.54 4.73 0.04</td>
<td>0.57 5.12 0.08</td>
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<tr>
<td>H1a-M1</td>
<td>0.24 6.03 0.18</td>
<td>0.23 6.05 0.28</td>
<td>0.50 7.27 0.05</td>
<td>0.51 7.72 0.11</td>
</tr>
<tr>
<td>H1a-Hydr.</td>
<td>0.05 2.77 0.58</td>
<td>0.04 2.12 0.67</td>
<td>0.31 4.46 0.15</td>
<td>0.66 4.15 0.05</td>
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</table>

<table>
<thead>
<tr>
<th>LOEC-regression statistics (all without BFQ, BCA, ACOe and PHOe)</th>
<th>All compounds</th>
<th>no BID/BQD/ACOe/PHOk</th>
<th>no BHQ</th>
<th>no BHQ/BID/BQD/ACOe/PHOk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 9)</td>
<td>(N = 5)</td>
<td>(N = 8)</td>
<td>(N = 4)</td>
</tr>
<tr>
<td>Log K$_{ow}$</td>
<td>0.46 -0.57 0.04</td>
<td>0.92 -0.60 0.01</td>
<td>0.52 -0.61 0.04</td>
<td>0.98 -0.62 0.01</td>
</tr>
<tr>
<td>H1a</td>
<td>0.04 1.59 0.57</td>
<td>0.04 1.88 0.73</td>
<td>0.39 2.83 0.10</td>
<td>0.30 2.69 0.45</td>
</tr>
<tr>
<td>H1a-M1</td>
<td>0.11 2.85 0.39</td>
<td>0.22 5.22 0.42</td>
<td>0.47 4.19 0.06</td>
<td>0.61 6.10 0.22</td>
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<tr>
<td>H1a-Hydr.</td>
<td>0.00 -0.61 0.87</td>
<td>0.04 1.91 0.76</td>
<td>0.04 1.06 0.62</td>
<td>0.57 2.92 0.25</td>
</tr>
</tbody>
</table>
Statistics for the linear relationships between energy related descriptors or log $K_{ow}$, and genotoxicity are given in the second part of Table 7.2. No significant correlation coefficients with membrane energy values could be found ($p > 0.05$). Log $K_{ow}$ values, however, do show significant correlations, and also H1a solvation energies and H1a-M1 solvation energy differences show significant ($p \leq 0.1$) correlation coefficients, if BHQ is left out. In these latter cases, however, the regression lines only describe small parts of the variation ($r^2 < 0.5$).

**Discussion and Conclusions**

Narcosis of azaarenes, as expressed by the LC$_{50}$ values, can be at least partly explained with solvation energies when positioned in the polar region of the membrane. The lower the solvation energy in the polar region, the higher the toxicity. This indicates that penetration of the membrane may be a driving force behind narcosis. For the diffusion parameter describing this process (the difference between H1a solvation energy and hydration energy), however, this only holds for a more homogenous group of azaarenes (Table 7.2; no BHQ/BID/BQD). Log $K_{ow}$ shows an even higher correlation coefficient with LC$_{50}$ values than membrane solvation energy values do. For isomers, however, log $K_{ow}$ is much less successful in predicting narcosis (Kraak et al., 1997). In addition, log $K_{ow}$ values are less informative in terms of mode of action than solvation energies are. Log $K_{ow}$ only describes the equilibrium between a hydrophobic solvent (octanol) and water, while the solvation energies used in the present study describe the interaction with the membrane itself. In particular, solvation energies for the H1a position show a correlation coefficient with LC$_{50}$ only slightly lower than that with log $K_{ow}$ ($r^2 = 0.54$ vs. 0.61). This is even more striking, when considering that the membrane model used is a strongly simplified model. The membrane in the present study was constructed from DMPC molecules, whereas in insect membranes it is known that DMPC is only one of the phospholipids involved (Downer, 1985). In addition, the model may be further improved by adding a water-layer to the outside of the membrane. For PCBs the importance of such a non-stirred water layer has been demonstrated (Dulfer et al., 1995; Dulfer and Govers, 1995).
Another point of inaccuracy is the fact that solvation energies only describe the enthalpy processes involved, i.e. entropy processes are ignored. It has been argued, however, that entropy might strongly influence water-membrane partitioning process (Dulfer, 1996; Vaes, 1998), especially when hydrogen bonding is involved.

For predicting the genotoxicity of azaarenes the membrane model calculation appears less appropriate than K_{ow}. This is of course not surprising, since genotoxicity implies additional interaction with the genome after initial passage of the membrane. Apparently, the type of interactions described by membrane solvation energies are not the limiting step(s) in the genotoxicity. Other processes, e.g. DNA-azaarene interaction or azaarene-transport protein binding are probably more important and seem to be better described by the K_{ow}.

In conclusion we can state that the preliminary calculations of solvation energies appear to be a promising tool for predicting narcosis of a compound. Especially when structurally related toxicants (e.g. isomers) are involved, an improved membrane-interaction model may perform better than the log K_{ow} model.

References


