Bioavailability and detoxification of cationics: I. Algal toxicity of alkyltrimethyl ammonium salts in the presence of suspended sediment and humic acid

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A B S T R A C T

We investigated the role of sorption in reducing bioavailability and consequently the measured toxicity of monoalkyl trimethyl ammonium salts (MAQ) under environmental conditions. The observed toxicity of MAQ in the presence of various types of sorbents was tested on algae. Decreased toxicity was found in the presence of all particulate sorbents tested, i.e. montmorillonite, silica, different sediments and for dissolved humic acid. Silica had a minor effect on the observed toxicity, whereas the clay mineral montmorillonite detoxified very effectively. The detoxification by several real-world sediments being mixtures of sand and clay was in-between silica and montmorillonite. Humic acid detoxified most effectively on weight basis. Polynomial relationships ('QSARs') were established for algal toxicity of MAQ in the presence of suspended matter and humic acid with carbon chain length as descriptor. Freundlich sorption isotherms for MAQ C16 for a clay sediment, montmorillonite, silica and for algae confirmed their relative detoxification in toxicity tests.

Humic acid significantly reduced bioavailability and observed toxicity of quaternary ammonium salts. Sorption of positively charged amines to suspended sediment is effectively controlled by its silt/clay fraction, not by the organic fraction. Purchased defined quality montmorillonite can serve as model sorbent in standard tests mimicking the clay fraction of natural suspended matter. It enables to study the effect of bioavailability on toxicity in a standardised way.

The QSARs developed for ecotoxicity of MAQ in the presence of suspended matter and humic acid enable quantitative incorporation of bioavailability in effect assessment. This increases realism in risk assessments of these fatty amine derivatives.

1. Introduction

In many cases ecotoxicological information is based on laboratory toxicity tests with pure liquid media containing the test chemical. In such tests the exposure is optimised to approach the intrinsic ecotoxicological properties of chemical products to indicate potential hazard. Risk assessments are mostly based on these hazard properties. At the same time it is broadly recognized that the fate of many chemicals in aquatic systems is influenced by various factors which can strongly reduce the toxicity observed in laboratory tests (Carey et al., 1987; Suffet et al., 1994; Alexander, 2000). Sorption to suspended matter or dissolved organic carbon such as humic acid, is particularly effective in mitigating toxic effects (Freidig et al., 1998; Urrestarazu Ramos et al., 1998). This has also been demonstrated for fatty amine derivatives such as quaternary ammonium salts (Giolando et al., 1995; Woltering and Bishop, 1989). Fatty amine derivatives with long alkyl chains strongly bind to sorbents in the aquatic environment such as suspended sediment and humic acid due to their multiple sorptive interactions. The carbon chains are usually assumed to sorb to the organic fraction of suspended sediment and of humic acid through van der Waals forces. The nitrogen group, which is positively charged at environmental pH, strongly binds electrostatically to the negative binding sites of these sorbents. A good understanding of sorption in relation to toxicity is needed to understand the relevant mitigating effects for chemicals. To incorporate such effects in risk assessment procedures the detoxification in the presence of different sorbents also needs to be quantified. In current risk assessment practice the inclusion of the effect of sorption of apolar chemicals to the organic matter fraction of suspended sediment is often taken into account. The relationships applied were found to be valid for a large group of
hydrophobic nonpolar chemicals and could conveniently be quantified in general terms as a function of the organic matter content (e.g. Di Toro et al., 1991).

Quaternary ammonium salts however, behave as cationic surfactants under environmental conditions and cannot be described by sorption to organic matter only. For these chemicals log Kow or Koc values are normally not meaningful. Quaternary ammonium salts and other fatty amine derivatives with even numbered chain lengths from 8 to 18 carbon atoms are used in various industrial applications such as asphalt, mining, emulsifiers and biocides. The current study was undertaken to establish the effects of bioavailability on the toxicity of monoalkyl quaternary ammonium salts (MAQ) to pelagic species. The standard species Pseudokirchneriella subcapitata, a green alga, was used in the tests. Algae are considerably more sensitive to MAQ than fish and crustaceans. In a related paper the toxicity of cationics to sediment-dwelling organisms is reported, more specifically on e Caenorhabditis elegans in artificial and natural substrates (Thomas et al., 2009).

The algal toxicity of MAQ with different chain lengths was investigated in the presence of silica, montmorillonite and sampled sediments. The observed toxicity was related to the sorption characteristics of MAQ to these particulate sorbents and to algae by establishing Freundlich sorption isotherms. The effects of the dissolved sorbent humic acid on MAQ was tested in a similar way. Several quantitative relationships (QSARs) were established to enable the impact of sorption to be taken into account in environmental risk assessments of MAQ.

2. Material and methods

2.1. Chemicals

The chemicals used in this study were obtained from Akcros Organics (decyltrimethylammonium bromide), Fluka Chemie (dodecyltrimethylammonium chloride, tetradecyltrimethylammonium bromide, octadecyltrimethylammonium chloride and montmorillonite, K10 surface 200 ± 20 m² g⁻¹ and CEC 30 meq 100 g⁻¹), JT Baker Chemicals bv (hexadecyltrimethylammonium bromide), Aldrich (huminic acid) and Merck (Silia gel 100, 0.2–0.5 mm, surface 300–400 m² g⁻¹). Natural sediment of Oostvaardersplassen was obtained from RIZA, homogenised and stored at 20 °C. The abbreviations purity and molecular weight of the quaternary ammonium substances used in this study are as follows, ‘MAQ C10’: decyltrimethylammonium bromide, 99%, mw 280.28; ‘MAQ C12’: dodecyltrimethylammonium chloride, 98%, mw 263.89; ‘MAQ C14’: tetradecyltrimethylammonium bromide, 98%, mw 336.41; ‘MAQ C16’: hexadecyltrimethylammonium bromide, 99%, mw 364.46 and ‘MAQ C18’: octadecyltrimethylammonium chloride, 98%, mw 348.05.

2.2. Algal toxicity tests

The algal tests were carried out with the freshwater green alga P. subcapitata (formerly Selenastrum capricornutum), CCAP 278/4 obtained from Collection of Algae and Protozoa, The Ferry House, Cumbria, Ambleside, United Kingdom (ISBN 1 871105056). The tests were done according to the European Economic Community/Organisation for Economic Cooperation and Development guidelines (OECD, 1984; EEC, 1992), with some minor deviations concerning the composition of the medium. The culture medium was modified by increasing the KH₂PO₄ concentration from 1.6 to 160 mg L⁻¹ and the NaHCO₃ concentration from 50 to 100 mg L⁻¹, to improve the growth of algae and the buffer capacity of the medium. Growth was determined spectrophotometrically, measuring at 436 nm each 24 h. The inhibition of growth at different concentrations after 96 h was calculated on the basis of the area under the growth curve. The algae were cultured in an illuminated orbital incubator at 22 °C under constant light conditions between 6000 and 10,000 lux.

2.3. Test strategy

For each toxicity test five concentrations were tested in triplicate with six control replicates containing only algal medium. All tests were carried out twice. The difference between EC50 values was less than a factor 2 in all cases. Adequate amounts of stock solution were added with a dispenser to 40 ml algal medium in 100 ml Erlenmeyer flasks. Before adding the algae, the vessels were rotated in an illuminated orbital incubator at 100 rpm for one and a half hour to obtain an equilibrium of MAQ between the liquid and solid-phases. Preliminary tests had shown that longer periods (up to a few days) did not significantly alter the toxicity results. This concurs with findings by other authors for cationic surfactants where fast sorption was reported (Hayworth and Burns, 1996). For benzyltrimethyl ammonium a time to equilibrium of less than a day has been reported (Polubesova et al., 1997). The vessels containing silica or sediment were shaken manually just before the absorbance was measured after a short period of sedimentation. The vessels containing montmorillonite were shaken and measured immediately. The inhibition of growth after 72 h was calculated for each concentration on the basis of the area under the growth curve.

Toxicity was calculated using the measurements of absorbance, which were transferred to cell density with a linear regression curve (N = 2.648·10⁻⁶E + 0.0793·10⁻⁶, R² = 0.993). The mean value of the measured extinctions for each test substance concentration was used to determine the area under the growth curve from which the inhibition was calculated. The percentage inhibition was written as

\[ I_a = \frac{100}{1 + 10x + \beta \log(\text{conc})} \]

where \( I_a \) is the percentage inhibition and \( x \) and \( \beta \) are parameters. After rewriting the equation as

\[ \log \left( \frac{100 - I_a}{I_a} \right) = x + \beta \log(\text{conc}) \]

the parameters \( x \) and \( \beta \) were estimated for each MAC by linear regression. When the percentage inhibition is 50, \( x + \beta \log(\text{conc}) = 0 \) and \( \text{EC}_{50} = 10 - x/\beta \), from which the \( \text{EC}_{50} \) (Effect concentration based on biomass) is calculated.

2.4. Sorption

The actual sorption of MAQ C16 was studied to better understand the reduction of toxicity through bioavailability. For many organic chemicals the relationship at equilibrium between adsorbed and dissolved concentrations can be described by a Freundlich isotherm (De Haan and Zwerman, 1978 and Harmens, 1982). Sorption was determined for MAQ C16 based on OECD test guideline 106 (OECD, 2000). At least five concentrations of sorbent (i.e. algae, silica, montmorillonite and OVP sediment) were prepared in algal medium in centrifuge tubes. To allow the test substance to equilibrate, the tubes were shaken for one and a half hour on a shaker with 50 motions per minute. Preliminary tests demonstrated that equilibrium was reached almost instantaneously for all sorbents (results not presented). The slowest was silica, but also for this sorbent a sorption of more than 95% was reached in 1.5 h. Supernatants were separated by centrifugation at 5000g. To remove the remaining algae and sorbent, the supernatants were filtered over a 0.22 μm filter. Four control replicates without sorbent were included. Two controls were also centrifuged and
filtered, the other two were immediately decanted to correct for sorption to the tubes and filters. The MAQ C16 concentration was quantified according to the methylene blue method which forms a stable coloured complex with fatty ammonium compounds, Chatten and Okamura, 1973; Standard Method of Analysis, 1995. Adsorption isotherms were described by the Freundlich equation: 

\[
\frac{X}{m} = K_d \cdot C_e^{1/n}
\]

according to Hamaker and Thompson (1972) where \(X/m\) is the amount of substance sorbed per unit weight of sorbent, \(C_e\) is the amount of substance in solution per unit of volume of liquid phase, \(K_d\) is the Freundlich constant, reflecting a quantitative estimate of the relative affinity of a substance for a sorbent.

All tests were checked for their validity. The increase factor of biomass is a validity check required by the EEC/OECD test guidelines to demonstrate sufficient growth. This factor was >16 for all tests and in most cases >50. No contamination by other organisms was observed in the test vessels at the end of the tests.

2.5. Sampling and characterisation of sediments

A sediment sample from Oostvaardersplassen (OVP), Flevopolder, The Netherlands was available from RIZA (Lelystad, The Netherlands). Other sediments were sampled in the region of Arnhem, The Netherlands. Five centimeter of the upper layer of sediment was collected at each site, up to a volume of about 10 L. In the laboratory a subsample was air-dried and subsequently sieved through a 2 mm sieve and stored in a refrigerator till further analysis. The remaining parts of the samples were also stored in a refrigerator.

The amount of organic carbon (OC) was determined by quantifying the chemical oxygen demand (COD). To this end, about 10 mg of homogenised dried soil was oxidised at 148 °C for 2 h in the presence of sulfuric acid and silver sulphate as catalyst. The Cr(III) formed is analysed spectrophotometrically at 605 nm. The test was performed in triplicate. The organic matter and organic carbon contents were calculated from the COD of the samples according to Locher and Bakker (1987).

The particle size distribution was determined from a sample from which the OM was removed (oxidation with 30% hydrogen peroxide). The particle size of sediments was analysed through subsequent sedimentation tests for the fractions 0–2, 0–50 and 50–2000 μm, according to Locher and Bakker (1987). Two sediments from IJssel (IJ) and Breuly (Br) consisted mainly of sand (>97%), whereas the Oostvaardersplassen (OVP) and ditch (SL) sediments only contained about 25% sand. The combined silt and clay fraction of OVP and SL represented about 75% of the total.

3. Results

3.1. Toxicity of MAQ in the presence of particulate standard sorbent

The effect of montmorillonite and silica on the measured algal toxicity of five MAQ was studied. The concentration of montmorillonite and silica during these tests was 0.125 g L\(^{-1}\) to Locher and Bakker (1987).

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- Pure: log EbC50 = 14.23–2.13 · CL + 0.071 · CL\(^{-2}\) (R\(^2\) = 0.957).
- Silica: log EbC50 = 8.40–1.16 · CL + 0.036 · CL\(^{-2}\) (R\(^2\) = 0.967).
- Montmorillonite: log EbC50 = 3.68–0.34 · CL + 0.0083 · CL\(^{-2}\) (R\(^2\) = 0.928).

in which ‘CL’ means chain length.

Montmorillonite is very effective in detoxifying MAQ of all tested chain lengths (C10–C18). Montmorillonite is a clay mineral and can be considered representative of the clay fraction of suspended matter. The above results therefore underscore that the silt/clay fraction representing the smallest particles of natural suspended matter and sediment, are particularly effective in reducing the bioavailability and subsequently the toxicity of MAQ. Silica, representative for the sand fraction of sediments, was much less effective. Since MAQs with longer carbon chains have a larger hydrophobic fraction within the molecule, an increasing toxicity for higher chain lengths would be expected based on experience with hydrophobic narcotic chemicals (e.g. Königemann, 1981). An increased toxicity was observed for chain lengths from C10 to C14, but MAQ with higher chain lengths were less toxic. The toxicity maximum was observed at MAQ C14. The trend of a toxicity maximum at the middle chain length was most obvious for pure MAQ (without sorbents). For the tests with montmorillonite longer carbon chains were more toxic, but this trend was not significant. For pure MAQ and for montmorillonite the observed increase of toxicity of C12 as compared to C10 was significant, but not for silica (Student’s t-test at 95% confidence level, Krachten, 1997).

The algal toxicity in the presence of different amounts of sorbent was determined for MAQ C16- testing montmorillonite in a range up to 0.19 g L\(^{-1}\) and silica up to 8.0 g L\(^{-1}\). The EbC50 increases linear with increasing sorbent concentration (in g L\(^{-1}\)). For montmorillonite the relationship was EbC50 = 17.377 · conc + 0.1279 (R\(^2\) = 0.927) and for silica EbC50 = 0.0125 · conc + 0.0341 (R\(^2\) = 0.810). The slope for montmorillonite is much steeper than for silica, which corresponds well with the higher detoxification capacity.

Table 1 Sediment characteristics representing average values of triplicates.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OVP</th>
<th>SL</th>
<th>Br</th>
<th>IJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>26.2</td>
<td>25.9</td>
<td>97.3</td>
<td>98.5</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>57.4</td>
<td>54.5</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>19.0</td>
<td>21.8</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>OC (%)</td>
<td>4.0</td>
<td>3.5</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Fig. 1. The toxicity of MAQ with different chain lengths in the presence and absence of sorbents, expressed as log EbC50 values, expressed as values corrected to 100% active ingredient.
3.2. Toxicity of MAQ C16 in the presence of natural sediment

The toxicity of MAQ C16 in the presence of natural sediment with different characteristics was tested with two sandy and two clay sediments. Details on sediment characteristics are given in Table 1. The results of the toxicity tests with these sediments are presented in Table 2. The results for silica and montmorillonite with MAQ C16 from experiments described earlier are also included in the table for comparison. The EbC50 values for these sorbents are also included in Table 2. The EbC50 values represent the concentration of MAQ C16 that reduces the toxic effect by 50%.

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clay content (%)</th>
<th>EbC50 (µmol 100% A.I/l)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure</td>
<td>0</td>
<td>0.030</td>
<td>0.016</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>0.125</td>
<td>2.617</td>
<td>0.690</td>
</tr>
<tr>
<td>OVP</td>
<td>0.125</td>
<td>0.513</td>
<td>0.220</td>
</tr>
<tr>
<td>SL</td>
<td>0.125</td>
<td>0.382</td>
<td>0.095</td>
</tr>
<tr>
<td>Br</td>
<td>2</td>
<td>0.224</td>
<td>0.046</td>
</tr>
<tr>
<td>IJ</td>
<td>2</td>
<td>0.092</td>
<td>0.042</td>
</tr>
<tr>
<td>Silica</td>
<td>2</td>
<td>0.072</td>
<td>0.024</td>
</tr>
</tbody>
</table>

The EbC50 values are averages of triplicates except for pure, which is an average of duplicates. For sediment characteristics see Table 1, for abbreviations see text, EbC50 values expressed as values corrected to 100% active ingredient.

3.3. Toxicity of MAQ C16 and sorption isotherms

Freundlich sorption isotherms were determined for MAQ C16 with silica, montmorillonite, algae and OVP sediment to characterise the effect of sorption on algal toxicity. The Freundlich plots presented in Fig. 3 showed linear relationships for the three sorbents and for algae. Table 3 summarizes the Freundlich sorption characteristics for these sorbents. The Kd values illustrate that the relative affinity of the sorbents for MAQ C16 decreases in the order montmorillonite > algae > OVP sediment > silica. This is consistent with the results obtained in the detoxification experiments, illustrating that MAQ C16 very effectively sorbs to montmorillonite, strongly reducing its bioavailability and toxicity. The affinity of MAQ C16 to OVP sediment consisting of both clay and sand was in-between that of montmorillonite and silica, as is expected based on its composition.

3.4. Toxicity of MAQ in the presence of humic acid

The effect of dissolved organic carbon on the toxicity of MAQ with different chain lengths was studied in the presence of 10 and 20 mg/L humic acid (HA). These values represent realistic environmental concentrations and are also used in EPA toxicity test guidelines designed to include the effect of reduced bioavailability (US EPA, 1993) and in EU guidelines for testing difficult substances. Since the toxicity results of MAQ with different chain lengths in the presence of 10 and 20 mg/L were very similar, only the 10 mg/L results are shown in Fig. 4, together with toxicity results without humic acid (“pure MAQ”). The highest toxicity in the presence of 10 mg/L humic acid was found with MAQ chain lengths of C12 and C14. The toxicity of MAQ with different chain lengths in the
presence of 10 mg/L humic acid fitted a second order polynomial relationship with a correlation coefficient of 0.97 using the carbon chain length (CL) as descriptor: \( \log E_{bC50} = 0.0555CL^2 - 1.5184CL + 9.6351 \) \( (R^2 = 0.9684) \).

To study the effect of different humic acid concentrations on the toxicity of MAQ C16 tests were done in the presence of 10, 12, 14, 16, 18 and 20 mg/L humic acid. A decrease in toxicity was observed with increasing humic acid concentrations. This relationship was linear for the log transformed parameters (\( \log E_{bC50} = 0.43log(C(HA) - 1.34) R^2 = 0.994 \)). On a weight basis humic acid detoxifies approximately 2.5 times more effectively than montmorillonite. Probably the positively charged hydrophilic part of MAQ molecules will interact with the negatively charged groups of humic acid (mainly carboxylic acid groups). Simultaneously, the hydrophobic carbon chain of MAQ molecules probably interacts with the apolar organic fraction of humic acid. Apparently, the combination of ionic and hydrophobic sorption sites of humic acid constitutes a strong sorption mechanism.

### 4. Discussion

#### 4.1. Sorption characteristics and mechanisms

Sorption is an important mechanism determining the environmental fate of many chemicals. The reduction of bioavailability through sorption has been studied extensively. For non-polar hydrophobic chemicals with a \( \log K_{ow} \) between approximately 3 and 6 sorption behaviour is understood relatively well and can be largely explained through sorption to the organic carbon fraction of suspended matter and sediment (Di Toro et al., 1991). The reduced bioavailability due to this equilibrium partitioning is regularly incorporated in risk assessments and is included in current European risk assessment guidelines (TGD, 2003; Versteeg and Shorter, 1991). Although electrostatic sorption mechanisms are also well known in scientific literature, for example for metals and amines (Davis, 1993), their importance is not always recognized in regulatory approaches for risk assessment (TGD, 2003).

The structure of quaternary ammonium salts and other fatty amine derivative molecules facilitates sorption via two binding mechanisms. The carbon chains are capable of apolar sorption to organic matter through van der Waals forces, such as the organic fraction of sediment, humic acid and suspended matter. The positively charged nitrogen group binds electrostatically via ion-exchange to the negatively charged sites of clay or humic acid. Due to the combined mechanisms, quaternary ammonium salts sorb very strongly to various sorbents ubiquitously present in the environment. These properties are useful in various practical applications. For example hexadecyltrimethylammonium with montmorillonite is applied as a commercial product as carrier in wastewater biotreatment, combining sedimentation capabilities and sorption to organic substances (Yang et al., 2002). Brownawell et al. (1990) reported that sorption of organic cations to soils depends more on the cation exchange capacity (CEC) of the sorbent than on the organic matter content. It supports the observation in this study that the organic matter content of suspended sediment was not correlated with the observed detoxification. The additional electrostatic sorption by the clay fraction of suspended sediment explains the strong detoxification.

#### 4.2. Sorption mechanism of protonated amines to plant cells

Crist et al. (1992) found that sorption of ethylenediamine to the freshwater algae Vaucheria was essentially an ion-exchange process at pH 5. At this pH ethylenediamine is protonated and behaves as a cation that displaces an equivalent amount of Ca or Mg, associated with the anions of the cell wall. Positively charged MAQ are likely to behave in a similar way. Because of the additional van der Waals interaction of the long alky chain with organic matter, amines are expected to have a stronger binding capacity than ethylenediamine. This supports the observation that MAQs can adsorb in quantities significantly above the CEC of sorbents such as montmorillonite. Zhang et al. (1993) investigated the different types of sorption of MAQ C16 on montmorillonite up to its CEC of 30 meq/100 g and found that adsorption on non-exchangeable sites may exceed that on cation exchangeable sites. Polubesova et al. (1997) studied the sorption of benzytrimethylammonium and benzyltri-ethylammonium salts to montmorillonite and found a relation between the sorption of these substances and the CEC. Montmorillonite is a clay mineral in which the Si and Al layers are assembled in a 2:1 ratio. These clays can expand and may adsorb organic compounds on both external and internal surfaces. Montmorillonite has a high CEC. Because of the expanding structure and high CEC it will adsorb MAQ very well (Bolt and Bruggenwert, 1978; Alexander, 1994).

Multiple mechanisms of binding interaction with algal cell wall constituents have also been demonstrated for heavy metal chelation on brown algae (Davis et al., 2003). Cell walls of brown algae act as sorptive surfaces via different mechanisms, including van der Waals binding and ion-exchange. The review by Davis et al. (2003) provides many details of interactions between metals and the key functional groups of algal cell walls, recognizing the complexity behind the observed sorption characteristics. Isotherms represent a suitable means to quantify sorption mechanisms allowing their application in practice, without the need to fully understand all details. Freundlich isotherms have been employed to quantify the equilibrium of biosorption systems, but was later interpreted as sorption to heterogeneous surfaces supporting different affinities (Davis et al., 2003). Specifically, the Freundlich isotherm is obtained with a log-normal affinity distribution. The stronger binding sites are occupied first and the binding strength

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**Table 3**

Freundlich parameters (for explanation see text) for sorption of MAQ C16.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>log_{Kd}</th>
<th>1/n</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>4.222</td>
<td>0.39</td>
<td>0.951</td>
</tr>
<tr>
<td>Silica</td>
<td>2.830</td>
<td>0.56</td>
<td>0.929</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>5.280</td>
<td>0.41</td>
<td>0.996</td>
</tr>
<tr>
<td>OVP</td>
<td>4.003</td>
<td>0.38</td>
<td>0.944</td>
</tr>
</tbody>
</table>

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![Fig. 4](image.png)

**Fig. 4.** Influence of humic acid on the toxicity of MAQ to algae, expressed as log EbC50 values corrected to 100% active ingredient.
decreases with increasing degree of site application (Smith, 1981; Stumm and Morgan, 1996). Freundlich exponents less than one, as found in this study, indicate a non-linear sorption which can be expected, because of the complex heterogeneous binding mechanisms of MAQ. Crum et al. (1999) also found non-linear sorption of an amine-based pesticide to macrophyte tissue, in contrast to other types of pesticides showing linear sorption.

It is generally assumed that surfactants show a non-specific polar or non-polar toxicity mechanism. Cationic surfactants have a relatively high toxicity to algae under standard laboratory conditions (e.g. Knezovich and Inouye, 1993). Under such conditions toxicity may be higher than would be expected based on the theoretical log $K_{ow}$, assuming a narcotic or polar-narcotic toxicity mechanism. However, the additional electrostatic sorption of MAQ is not taken into account in such considerations. The relative order of the Freundlich $K_F$ parameters of sorbents and algae found in this study support the view that also the toxicity of MAQ is mainly based on their sorption characteristics. The very high binding efficiency to negatively charged algal cell walls is likely causing the toxicity of MAQ in standard tests, rather than intracellular toxicity. Therefore, toxic effects as observed in standard tests are unlikely to occur under realistic environmental conditions, where the presence of other sorbents will compete with algae as substrate for sorption of MAQ and reduce toxicity.

### 4.3. Implications for risk assessment

As demonstrated and explained in this study, the strong combined interaction of fatty amines with negatively charged suspended solids, sediments and humic acid significantly influences their bioavailability and consequently their environmental risks. Knezovich and Inouye (1993) for example, demonstrated that the presence of sediment significantly reduces the bioaccumulation of hexadecylpyridinium bromide in fish due to reduced bioavailability. Another example of the influence of the bioavailability of quaternary ammonium salts has been provided by van Ginkel et al. (2003). Poorly water-soluble long-chain dialkyl(dimethyl)ammonium salts are resistant to biodegradation in standard ready biodegradability tests used to assess the environmental exposure (van Ginkel, 2004). The water-soluble didecyl(dimethyl)ammonium salt is readily biodegradable. This dialkyl(dimethyl)ammonium salt is metabolised through C–N alkyl cleavage following β-oxidation of the alkyl chains (Nissihara et al., 2000). Based on this biodegradation mechanism it would be expected that the potential of micro-organisms to degrade dialkyl(dimethyl)ammonium salts is independent of the alkyl chain lengths. Therefore reduced bioavailability of poorly water-soluble dialkyl(dimethyl)ammonium salts is expected to be responsible for the observed decline in the rate of degradation with increasing alkyl chain lengths. Indeed rapid degradation of the bioavailable desorbed fraction was demonstrated in column studies (van Ginkel et al., 2003). Because the effect of bioavailability on biodegradability is not reflected in current standard biodegradability test methods, the persistence of these strongly sorbing substances cannot be assessed in such tests.

For risk assessment purposes it is particularly important to quantify sorption of fatty amines to suspended matter and humic acid, which are the most relevant sorbents in the aqueous phase. Similar phenomena of reduced bioavailability and toxicity were observed in sediment and soil substrates (Thomas et al., 2009). Bioavailability of MAQ in presence of suspended matter and humic acid significantly impacts their toxicity. Due to the mechanisms discussed above, the sorption of MAQ is largely underestimated when considering only the ‘classic’ sorption based on organic carbon ($K_C$).

Since quaternary ammonium salts and other fatty amines are positively charged under environmental conditions, a realistic risk assessment should take into account the bioavailability in the presence of humic acid and suspended sediment. In laboratory testing this can be done by including characterised natural sorbents. To increase acceptance and comparability it is recommended to use standardised and commercially available sorbents. Humic acid as well as the clay mineral montmorillonite can be purchased with defined and consistent qualities. This study demonstrated that montmorillonite is a suitable model sorbent to mimic fine particles of natural suspended matter and sediment. The QSARs established in this study enable a more realistic assessment of toxicity of MAQ using laboratory test results.

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### References


