



Application of an algal growth inhibition assay to determine distribution coefficients of benzalkonium ions between kaolinite and water

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ABSTRACT

Benzalkonium compounds are widely used and found in environmental samples. Due to their amphiphilic nature, it is important to know sorption coefficients to account their bioavailability. However, currently available models describing their partitioning were developed using low molecular weight homologues and it cannot be ascertained whether they are applicable to their higher molecular weight homologues. Reasons for the scarcity of data on highly sorptive compounds include the lack of reliable quantification techniques for analyzing these chemicals at environmentally relevant levels. This study, therefore, reports on an algal growth inhibition assay-based method for the determination of kaolinite/water distribution coefficients for benzalkonium compounds at their environmentally relevant concentration range. Sorption to clay was computed using the difference between median effective concentration determined in a culture with kaolinite and that derived from a culture grown in standard medium. A kinetic model was used to account for uptake into algal cells and to calculate free concentrations. Due to the sensitivity of the algal species, *Pseudokirchneriella subcapitata*, it was possible to determine distribution coefficients below micromole per liter concentrations. The computed distribution coefficients showed a linear increase with number of carbon atoms in the alkyl chain up to 14. The proposed bioassay-based method should be applicable to determine distribution coefficients for highly hydrophobic chemicals and ionic liquids at a concentration range lower than typical analytical limits.

1. Introduction

Benzalkonium compounds are a group of surfactants with a permanently charged central nitrogen atom that is bonded to a benzyl group and three other alkyl groups. Due to their amphiphilic properties they find use in both industrial and domestic detergents (Martínez-Carballo et al., 2007; Ruan et al., 2014; Zhang et al., 2015). They are also potent antimicrobials and therefore they are often used for disinfection in various applications such as food processing (Xian et al., 2016) and medical uses (Choi et al., 2018; Lukáč et al., 2013). It is, therefore, not surprising that they have been detected in the environment at concentrations of up to $65 \mu\text{g L}^{-1}$ in receiving surface water and up to $3610 \mu\text{g kg}^{-1}$ and $1530 \mu\text{g kg}^{-1}$ in sewage sludge and sediment, respectively (Zhang et al., 2015). Since they are designed to be bioactive, benzalkonium compounds might present a risk to both aquatic and terrestrial ecosystems.

The presence of hydrophobic groups (alkyl and benzyl) and the positively charged nitrogen atom enables benzalkoniums to sorb to various matrices through both hydrophobic and ionic interactions. For over two decades, several studies have focused on sorption of

benzalkoniums to clay surfaces with the aim of producing organoclays, and these have been comprehensively reviewed (de Paiva et al., 2008; He et al., 2014). Consequently, the loading rates in those studies are several orders of magnitude above environmentally relevant concentrations. Studies that have examined sorption with the purpose of understanding bioavailability and toxicity have also often used loading rates much higher than the environmental levels (Ismail et al., 2010; Khan et al., 2017; Xiang et al., 2016). This is mainly due to unavailability of suitable analytical methods for reliably quantifying benzalkonium compounds at levels below micro-mole per liter in the aqueous medium. For non-polar organic compounds, distribution coefficients derived at environmentally relevant concentrations have been shown to differ substantially from those calculated at higher concentrations (Endo et al., 2009). For ionic compounds, it can also be expected that distribution coefficients measured at higher concentrations are inadequate to describe partitioning in environmental matrices at lower, and more environmentally relevant concentration levels.

By far, the work of Droge and Goss (2012, 2013a, 2013b, 2013c) has been the most comprehensive attempt at describing the sorption behavior of cationic organic compounds including benzalkonium ions.

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Another research group has also tried to build on the work of Droge and Goss and improve the predictive power by incorporating the effect of competing inorganic ions using probe molecules (Jolin et al., 2017). However, both research groups only dealt with low-molecular weight compounds. They did not study the longer chain homologues like benzyldimethyldodecyl ammonium and benzyldimethyltetradecyl ammonium which constitute the main components of the commercial detergent and biocide formulations (Van De Voorde et al., 2012) and are also more commonly detected in environmental samples (Li et al., 2014; Li and Brownawell, 2010; Martínez-Carballo et al., 2007; Ruan et al., 2014). One of the reasons they might have preferred not to study these compounds is that they were not compatible with their chromatographic elution methods. The retention times for more hydrophobic compounds were too long (Droge and Goss, 2013a; Jolin et al., 2016). Whilst the two research groups developed models that are meant to be applicable to a broad spectrum of cationic organic pollutants, it cannot be ascertained whether the linear range of these models extends to the more hydrophobic compounds. In fact, in the Droge and Goss (2013c) study, benzytrialkyl ammonium compounds clearly deviated from the proposed model. A need, therefore, exists for methods that can yield better estimates of sorption coefficients for the more hydrophobic quaternary ammonium compounds.

It is well-acknowledged that toxicity by organic chemicals in water is determined by their freely dissolved concentration (Escher and Hermens, 2004; Heringa et al., 2004; Roh et al., 2014). Because sorption to particulate or dissolved organic matter or to mineral surfaces lowers the bioavailable concentration, it would be possible to apply a sensitive aquatic toxicity test to observe the changes in bioavailable fraction, leading to the determination of distribution coefficients.

The aim of the study was to develop a method that can be applied to determine soil/water distribution coefficients of benzalkoniums and similar cationic organics at environmentally relevant levels. A standardized algal growth inhibition test (OECD, 2011), using *Pseudokirchneriella subcapitata*, was adapted to measure the bioavailability of benzalkoniums sorbed to kaolinite, as a model inorganic material. The test was conducted in media supplemented with kaolinite to estimate the sorption capacity of the clay. A toxicokinetic model was then employed to compute the free concentration by accounting for benzalkoniums sorbed to algal lipids, assuming that body-burden causing baseline toxicity is independent of the length of hydrophobic chains of benzalkonium ions. Values of distribution coefficients obtained using algal growth inhibition test were also compared to those obtained using conventional batch sorption test at higher concentration range in order to evaluate the concentration dependence of the distribution coefficient.

2. Materials and methods

2.1. Materials

The kaolin clay used in this study was purchased from Fischer Scientific (Loughborough, United Kingdom). The test chemicals were benzyldimethyloctyl ammonium chloride (BAC8), benzyldimethyldecyl ammonium chloride (BAC10), benzyldimethyldodecyl ammonium chloride (BAC12), benzyldimethyltetradecyl ammonium chloride (BAC14), benzyldimethylhexadecyl ammonium chloride (BAC16) and benzyldimethyloctadecyl ammonium chloride (BAC18). Their molecular structures are shown in Table S1 (Supplementary material). All the benzalkonium compounds were acquired from Sigma-Aldrich (St. Louis, MO, USA) with the exception of benzyldimethyloctadecyl ammonium chloride which was obtained from Tokyo Chemical Industries (Tokyo, Japan). Salts that were used in the determination of cation exchange capacity were also purchased from Sigma-Aldrich. The algal species, *Pseudokirchneriella subcapitata* (strain number 278/4), was obtained from the Culture Collection of Algae and Protozoa in Oban, Scotland.

2.2. Methods

2.2.1. Determination of cation exchange capacity

The cation exchange capacity of kaolinite was determined using the ammonium acetate method with some modifications (Tan, 2005). Since cation exchange capacity of kaolinite is pH-dependent (Ma and Eggleton, 1999), the pH of the displacing solution (ammonium acetate) was adjusted to 8.00 to reflect the experimental conditions in the algal test. Soil samples were mixed with the displacing solution, shaken for 1 h and incubated overnight at 25 °C. The solution was then separated by centrifuging at 5000 × g for 10 min. To remove excess ammonium ions, the residue was washed 3 times with 95% ethanol. Potassium chloride was used to leach the sorbed ammonium ions, which were then quantified using the salicylate-hypochlorite method on a DR/4000U UV/vis spectrophotometer (Hach Co., Loveland, CO, USA) at 650 nm (Tan, 2005).

2.2.2. Algal growth inhibition test

The algal cultures were grown in OECD media following a standard protocol (OECD, 2011). The media is composed of several mineral salts—with sodium (0.60 mM), ammonium (0.27 mM), calcium (0.12 mM) and magnesium (0.12 mM) as the major cations. Culture media was prepared in deionized water, sterilized by autoclaving at 121 °C for 15 min or filtration (0.2 µm cellulose filter). The algae were grown in glass flasks kept in a shaking incubator (120 rpm) at 25 °C. Continuous lighting was provided by an LED lamp with a yield of 3000 lx. Growth inhibition experiments were conducted under the same conditions for a period of 48 h. Inhibition experiments were done in OECD media with or without kaolin clay at 0.4 g L⁻¹. The media was then spiked with benzalkonium chloride salt solutions. The initial concentrations of benzalkonium ions in the test media ranged from 0.002 to 40 µmol L⁻¹. Spiked media was incubated for 12 h to allow for equilibration of the organic cations between clay and water. Preliminary experiments had demonstrated that equilibrium is attainable within five hours. Growth inhibition tests were started by inoculating the media to a density of 5000 cells/mL. At the end of the test algal biomass was quantified using a calibrated AquaFluor™ hand-held fluorometer (Turner Designs, Sunnyvale, CA, USA). The fluorometer is designed for chlorophyll quantification at an excitation wavelength of 395 ± 65 nm and an emission wavelength of ≥660 nm. The calibration was done by manually counting cells using a hemocytometer. The r² values of the linear regression were greater than 0.99.

Algal growth inhibition was calculated using growth rate as specified in the OECD guideline (OECD, 2011). Inhibition data were modeled following the log-logistic model (Eq. (1)) using the *drc* package in R software (Ritz et al., 2015).

$$\% \text{Inhibition} = \frac{100}{1 + \left(\frac{x}{e}\right)^b} \quad (1)$$

where x is concentration of the test substance, e is the median inhibitory concentration (EC₅₀) and b is the gradient of the curve when x is equal to EC₅₀. Presence of a sorbent in the medium reduces the bioavailable concentration thereby increasing the nominal EC₅₀ (Chen et al., 2014; van Wijk et al., 2009). Therefore, the amount of benzalkonium sorbed to kaolinite was calculated by subtracting the nominal EC₅₀ of the culture without clay from that of the kaolinite containing culture as shown in Eq. (2) and illustrated on Fig. 1.

$$A_{\text{sorbed}} = V(\text{EC}_{50(\text{kaolinite})} - \text{EC}_{50(\text{ref})}) \quad (2)$$

where A_{sorbed} is the amount of ion sorbed to clay in micromoles, V is the volume (in liters) of the culture media in each flask whilst EC_{50(kaolinite)} and EC_{50(ref)} are the median inhibitory concentrations (in micromoles per liter) observed in the media with or without kaolinite respectively.

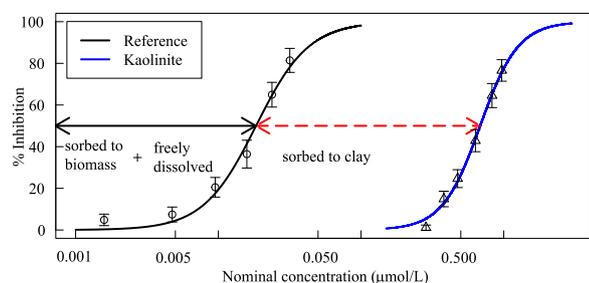


Fig. 1. Representative dose-response curves for OECD media with kaolinite (blue) and without kaolinite (Reference, black). Nominal EC_{50} value for the reference is shown by the black arrow whilst that for the kaolinite containing culture is shown by the black and red arrows. The red arrow shows difference between the two values and represents the amount sorbed to kaolin clay. Horizontal error bars denote standard deviation of triplicate analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2.3. Batch sorption tests

Conventional batch sorption tests were also carried out using OECD media as the liquid phase. This was done to allow for comparison with the bioassay-based method since, sorption of cationic compounds to kaolinite is affected by both pH and the ionic strength of the liquid medium (Ishiguro and Koopal, 2016). Approximately 25 mg of kaolinite were weighed into a 20 mL glass vial and then 10 mL of benzalkonium solution in OECD media were added. A total of six different initial concentrations were run for each benzalkonium homologue and they ranged from 40 to 600 $\mu\text{mol L}^{-1}$. The range was much greater than those for algal growth inhibition tests due to high detection limits in the medium and low EC_{50} values in growth inhibition. Each concentration was replicated thrice. For each initial nominal concentration, a parallel set of control vials without clay were run to account for losses other than sorption to clay. The mixture was incubated for 24 h at 25 °C with shaking at 200 rpm. Preliminary tests using BAC12 had shown that equilibrium is attained within 1–5 h. At the end of incubation, the concentration of benzalkonium in the liquid phase was measured. Samples were filtered through a 0.2 μm syringe filter and then injected directly, without further purification, into the chromatograph for analysis. The amount of benzalkonium sorbed to the clay was calculated by mass balance—comparing each test vial with its respective control. Sorption accounted for 50–90% decrease in the free concentration. Recovery rates based on the initial spiked concentration were above 70% for all analytes.

2.2.4. Analysis of benzalkonium compounds

The concentration of benzalkonium ions was determined using a Waters Acquity™ Ultra pressure liquid chromatograph (UPLC) equipped with a Waters sample manager and a photo diode array detector (Waters Co., Milford, MA, USA) set at 209 nm. Separation was achieved using an Acquity UPLC BEH C18 column (2.1 × 50 mm). Column temperature was set at 35 °C. The mobile phase was made up of methanol (90%) and 100 mmol L^{-1} ammonium acetate (10%) run in isocratic mode at a flow rate of 0.35 mL min^{-1} . The detection limits ranged from 1.3 $\mu\text{mol L}^{-1}$, for BAC8 to 3.5 $\mu\text{mol L}^{-1}$ for the BAC18. The quantification limit was set at 3 times the detection limit.

3. Theory

The distribution coefficient of a compound is defined as the ratio of the sorbed concentration to that in the aqueous phase (Eq. (3)).

$$K_d = \frac{C_s}{C_w} \quad (3)$$

where K_d is the distribution coefficient (L kg^{-1}), C_s and C_w are the sorbed ($\mu\text{mol kg}^{-1}$) and freely dissolved ($\mu\text{mol L}^{-1}$) concentrations,

respectively. Whereas the sorbed concentration can be easily derived from the EC_{50} values of the reference and kaolinite cultures using Eq. (2), the free concentrations can only be estimated after considering the amount sorbed to algal biomass, mainly membrane lipids. Since conditions of the test require that algae be in the exponential growth phase, the concentration of benzalkoniums in biomass is best modeled considering uptake and depuration kinetics (Kwon et al., 2016):

$$C_o(t) = \frac{k_u}{k_g + k_d} C_w [1 - \exp(-(k_g + k_d)t)] \quad (4)$$

where C_o is the lipid-normalized concentration of the chemical in the test organism ($\mu\text{mol/kg}_{\text{lipid}}$), k_u , and k_d are the uptake ($\text{L kg}_{\text{lipid}}^{-1} \text{d}^{-1}$) and depuration (d^{-1}) rate constants, respectively, k_g is the algal growth rate constant (d^{-1}) and t is the time in days. Substituting C_w in Eq. (4) and isolating K_d gives Eq. (5)—which makes it possible to calculate the kaolin/water distribution coefficient.

$$K_d = \frac{k_u}{k_g + k_d} \frac{C_{\text{clay}}}{C_o} [1 - \exp(-(k_g + k_d)t)] \quad (5)$$

When the free concentration is equal to EC_{50} , C_o is defined as the critical body burden. For ionic organic compounds, the critical body burden in *P. subcapitata* is given by Bittermann and Goss (2017) as 1250 $\mu\text{mol kg}_{\text{lipid}}^{-1}$. The size-dependent uptake rate constant for algae ($10^7 \text{ L kg}_{\text{lipid}}^{-1} \text{d}^{-1}$) was adopted from Kwon et al. (2016), whilst the depuration rate constant was calculated as the ratio of the uptake rate constant to the compound specific lipid/water partition coefficient assuming that biotransformation is negligible. The lipid/water partition coefficient for benzalkonium compounds was calculated using a poly parameter model developed for cationic surfactants (Timmer and Droge, 2017). Lipid content in *P. subcapitata* is reported to be 2% of the cell dry weight (Gonçalves et al., 2013; Patil et al., 2007).

4. Results and discussion

4.1. Experimental determination of EC_{50}

Compared to cultures grown in standard OECD media, algae grown in kaolinite containing media showed approximately 10% reduced growth rates. However, this was considered acceptable since the cultures met the specified minimum of a 16-fold increase over the duration of the experiments (OECD, 2011). The observed reduction in growth rate was likely due to scattering of light by the clay suspension, effectively slowing down photosynthesis. Sorption of mineral ions in the media to clay surfaces might also lead to a reduction in growth rate due to a decrease in the bioavailability of nutrients. Growth rate can be slightly improved by reducing the clay content below 0.4 g L^{-1} , but this would reduce the difference between the kaolinite and reference EC_{50} values. The calculated sorbed amount would be low and the lower clay content would increase the margin of error in estimating sorbed concentrations.

Whereas kaolinite has been shown to bind to algal cells—inhibiting cell multiplication (Louzao et al., 2015; Sengco and Anderson, 2004), the marginal reduction in growth rate observed in this study implies that such binding was not significant at the kaolinite dose studied. Notably, the final algal biomass at EC_{50} is about 3 mg L^{-1} . This is at least two orders of magnitude below the mass of clay particles. If there was significant interaction between clay and algal cells, then algal growth would have been completely inhibited. Therefore, it is reasonable to assume that the distribution of benzalkonium ions between kaolinite and water was not affected by introducing algal cells in the test system.

All the benzalkonium compounds in the current study were highly toxic to *P. subcapitata* with nominal EC_{50} values in the sub-micromole per liter range (Table 1.). All dose-response relationships for benzalkonium homologues are shown in Fig. S1 (Supplementary material).

Table 1

Benzalkonium toxicity to *Pseudokirchneriella subcapitata* in OECD media (reference) and kaolinite-supplemented media (kaolinite). The numbers in parentheses are the 95% confidence intervals.

Test compound	EC ₅₀ (μmol L ⁻¹)	
	Reference	Kaolinite
BAC8	4.57 (3.64–5.50)	10.1 (8.23–12.1)
BAC10	0.612 (0.513–0.711)	2.02 (1.80–2.24)
BAC12	0.158 (0.141–0.176)	0.868 (0.748–0.988)
BAC14	0.0184 (0.0162–0.0207)	0.684 (0.602–0.766)
BAC16	0.0171 (0.0153–0.0195)	1.25 (1.05–1.46)
BAC18	0.0165 (0.0137–0.0192)	1.58 (1.41–1.75)

Compared to other toxicity tests like luminescence inhibition of *Aliivibrio fischeri* and *Pseudomonas putida* growth inhibition that show toxicity in the milligram per liter range (Di Nica et al., 2017; Sütterlin et al., 2008), the algal growth inhibition assay is sufficiently sensitive for distribution coefficient determination at environmental levels. BAC8 was the least toxic, being at least one order of magnitude less toxic than the next homologue (BAC10). The toxicity increased from BAC8 through BAC14. The trend observed here agrees with previous reports on quaternary ammonium compounds where the length of the alkyl chain was found to be proportional to toxicity of the compound (Rosen et al., 2001; Zhu et al., 2010). Such a decrease in nominal EC₅₀ is predictable from the lipophilicity of the compounds which increases with alkyl chain length, as discussed in Section 4.2. However, there was significant overlap in the 95% confidence intervals for BAC14, BAC16 and BAC18. Unlike the current study, García et al. (1999) reported a decrease in methanogenesis-inhibition with increase in alkyl chain length. The apparent suppressed toxicity for longer alkyl chain compounds is less-likely due to differences in species sensitivity but rather reduced free concentrations as a result of sorption to solids in the medium. As described in Section 4.2, in the presence of sorbent particles, the longer alkyl chain homologues will show greater reductions in bioavailability as they have higher sorption coefficients.

4.2. Bioassay-based distribution coefficients

The lipid/water partition coefficients and sorption parameters are shown in Table 2. Amount of benzalkonium ions sorbed to kaolinite ranged from 10^{3.22} to 10^{4.15} μmol kg_{clay}⁻¹. Except for BAC8, the sorbed concentrations were all below 5% of kaolinite's cationic exchange capacity. At this loading rate, sorbate-sorbate interactions would be negligible; sorption is therefore expected to be dominated by ionic interactions (Droge and Goss, 2013a). Whereas Vasudevan et al. (2013) observed cooperative interactions at loading rates above 2.3% of the clay's cation exchange capacity, such interactions are highly unlikely in this study since the kaolinite used has a cation exchange capacity of 90 mmol kg⁻¹—one order of magnitude below the reported cut-off limit for cooperative interactions. Since all the benzalkoniums carry an

Table 2

Kaolinite/water distribution coefficients of benzalkonium compounds. K_{lipw} is the lipid/water partition coefficient (L kg_{lipid}⁻¹), C_{clay} and C_w are the sorbed (μmol kg_{clay}⁻¹) and the free (μmol L⁻¹) concentrations, respectively. K_d is kaolinite/water distribution coefficient.

Test compound	^a log K _{lipw}	log C _{clay}	log C _w	log K _d
BAC8	3.07	4.15	0.0270	4.12
BAC10	4.25	3.55	-1.15	4.70
BAC12	5.43	3.25	-2.32	5.47
BAC14	6.61	3.22	-3.39	6.61
BAC16	7.79	3.48	-3.85	7.33
BAC18	8.97	3.58	-3.90	7.48

^a From Timmer and Droge (2017).

Table 3

Kaolinite/water distribution coefficients of benzalkonium compounds determined using the conventional batch test method. K_d is kaolinite/water distribution coefficient whilst C_{clay} and C_w are the sorbed (μmol kg_{clay}⁻¹) and the free (μmol L⁻¹) concentrations, respectively.

	log K _d	log C _{clay}	log C _w
BAC8	2.47	3.40	0.92
BAC10	2.73	3.64	0.91
BAC12	2.90	4.47	1.57
BAC14	3.22	4.74	1.53
BAC16	3.21	5.08	1.87
BAC18	3.55	4.93	1.68

identical charge, it would be expected that the distribution coefficients will be comparable. However, the computed base-10 logarithmic distribution coefficients ranged from 4.12 for BAC8 to 7.48 for BAC18. An increase in lipophilicity/hydrophobicity was associated with increased sorption affinity. Although the contribution of hydrophobicity in sorption of cationic organics has not been adequately quantified, molecular dynamics simulations have suggested the spreading of the positive charge over a large area of the molecule is responsible for the increased sorption (Samaraweera et al., 2014). Other previous reports have attributed this to hydrophobic exclusion which makes it more energetically favorable for hydrophobic compounds to partition away from the aqueous phase (Jolin et al., 2017; Li and Gallus, 2007; MacKay and Vasudevan, 2012).

4.3. Batch sorption tests

The batch test sorption isotherms were found to be non-linear, even for an initial concentration range of only one order of magnitude (Fig. S2, Supplementary material). Therefore, the distribution coefficients near the quantification limits are shown in Table 3. The log K_d values ranged from 2.47 to 3.55. These values are approximately two to three orders of magnitude below those calculated using the bioassay-based method. This is due to higher concentrations used in the batch sorption tests—leading to sorbed concentrations that were between 50% and 150% of the clay's cation exchange capacity. At loading rates greater than 10% of cation exchange capacity, sorbate-sorbate interactions result in steric hindrances lowering the apparent K_d (Droge and Goss, 2013a). Sorption rates in excess of the cationic exchange capacity are due to the formation of a hydrophobic layer when the clay surface is saturated by organic cations. Zeta-potential measurements have shown a decrease in the net negative clay-surface charge, followed by a complete reversal of the charge as the sorbed organic cation concentration increased (Wang et al., 1999). The observed charge reversal is due to the formation of a secondary layer sorbed to the primary layer through hydrophobic interactions. Other studies have confirmed the formation of this double-layer through X-ray diffraction measurements (Ishiguro and Koopal, 2016; Tahani et al., 1999). The lower K_d values in the batch sorption test are also a result of the lower sorption affinity of the secondary layer bound through hydrophobic interactions (Zanini et al., 2013). The difference between batch test distribution coefficients and bioassay-based sorption data (Section 4.2) highlights the need for K_d values derived at environmentally relevant levels. Data derived at higher concentrations will lead to overestimation of bioavailability of benzalkoniums. This has implications in environmental risk assessment and biological remediation where desorption from soils might be the rate limiting step.

4.4. Equilibrium assumptions in growing algal cultures

A number of recent studies have argued that ionic organic compounds with high lipid/water partition coefficients can be defined as baseline toxicants (Bittermann and Goss, 2017; Di Nica et al., 2017;

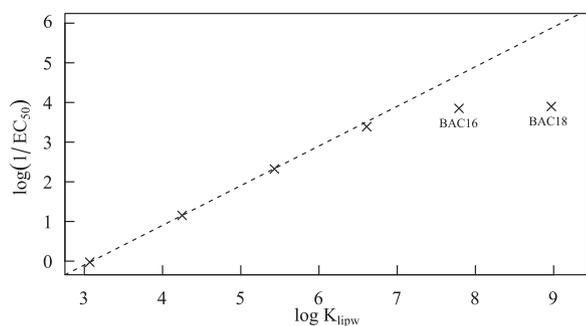


Fig. 2. A plot of Eq. (6) (dotted line) with benzalkonium toxicity data superimposed. Homologues with an alkyl chain of 14 carbon atoms and below conform to the model whilst the longer chains (BAC16 and BAC18) do not attain equilibrium under algal test conditions.

Escher et al., 2017; Fu et al., 2015; Qin et al., 2010). Baseline toxicity occurs as a result of intercalation of the toxicant in cellular membranes. Quaternary ammonium compounds are known to destabilize membrane structure by interacting with both the negatively charged membrane surface and the hydrophobic membrane interior (Lukáč et al., 2010). For a given median effective concentration, the concentration of toxicant in the membrane is defined as the critical body burden (Di Toro et al., 2000). The lipid-corrected critical body burden and the median effective concentration are connected by the relationship $C_o = K_{ipw}EC_{50}$. This relationship can be transformed into Eq. (6).

$$\log(1/EC_{50}) = \log K_{ipw} - \log C_o \quad (6)$$

The plot of the equation is a straight line where the slope is unity and the intercept is negative $\log C_o$. These conditions are only satisfied when the narcotic concentration in biomass is at equilibrium with the free aqueous concentration. However, in an algal growth inhibition test biomass increases at an exponential rate, thus equilibrium cannot be assumed. Therefore, the current study used a kinetic model (Eq. (4)) to describe benzalkonium ion partitioning into algal lipids. Fig. 2 shows the base-10 logarithm of the inverse of free concentrations computed using Eq. (4) plotted against the $\log K_{ipw}$ of the respective organic cations. Benzalkonium ions with alkyl chains of 14 carbon atoms and below conform to Eq. (6) whilst those with 16 and 18 carbon atoms deviated from the model (Fig. 2). This implies that an equilibrium model would sufficiently describe the distribution of compounds with a $\log K_{ipw}$ below 7 but would not be suitable for the relatively more lipophilic compounds. This phenomenon has been previously described for non-polar narcotic chemicals and has been attributed to the uptake rate being too slow for highly lipophilic compounds to attain equilibrium during the experimental period (Seto and Handoh, 2009). The same explanation can be given for reports from García et al. (2001) where BAC16 was less toxic than low molecular weight homologues in a 30-min bacterial luminescence inhibition assay. For such highly lipophilic compounds, experimental EC_{50} values are higher than what can be predicted from their partition coefficients. Thus, for risk assessment of these compounds, uptake rates should always be considered.

4.5. Potentials and limitations of the bioassay-based method

The method presented in this paper can be applied to other sorbate-sorbent combinations but might not be applicable to certain soil types. Especially, it would be difficult to interpret results from environmental soil samples already contaminated by other algal growth inhibitors. Interactions between contaminants in the soil and the spiked chemical will not be predictable. The method can, therefore, only be reliably applied to sorbents of a well-defined composition, or at the least where the components are known to have no effect on algal growth because association of clay particles with algae might affect chemical

distribution in the water-minerals-biota system. Also, the sorbates to be studied must be sufficiently toxic to cause significant inhibition at environmentally relevant concentrations. Sorption behavior of some high-molecular weight non-polar organic compounds might not be easily determined using the current method since their solubility limits are often too low to result in any noticeable toxicity (Kwon et al., 2016). However, the method must be suitable for high-molecular weight amphiphilic compounds like surfactants and ionic liquids.

The ionic composition of the media must meet the growth requirements of the chosen algal species. It cannot be altered without a detrimental effect on algal growth. This presents a challenge when effects of competing inorganic ions are to be investigated using the current method. The nutritional requirements of algal species imply that at least four major cations must be present in the test medium (OECD, 2011). It will therefore be impossible to study the effect of a single competing inorganic cation or to use homoionic clays as is often the case in many sorption studies (Droge and Goss, 2013a; Jolin et al., 2016; Samaraweera et al., 2014; Tahani et al., 1999; Wang et al., 1999). Additionally, the pH range at which the sorption experiments can be carried out is also limited by the pH-tolerance of the algal species. However, the method can still be used to differentiate between sorption behavior in marine and freshwater systems by employing either a marine or freshwater algal species respectively.

5. Conclusion

The method presented here is useful for the determination of soil/water distribution coefficients of trace pollutants that are difficult to analyze using conventional analytical methods. One major limitation of the method is that the estimates are less reliable for compounds with low sorption coefficients—as demonstrated for BAC8 in this study. However, for compounds with low partition ratios, sorption coefficients can be easily determined using conventional batch tests since the free concentrations are more likely to fall within quantification limits of instrumental methods. Also, the bioassay-determined distribution coefficients are not linearly related to the number of carbon atoms on the alkyl side chain. This implies that using models based on low molecular weight homologues to predict the sorption behavior of longer chain homologues is highly likely to yield misleading results. The current method is therefore relevant in assessing the bioavailability of high distribution coefficient chemicals at environmentally relevant concentrations. Because orders-of-magnitude greater distribution coefficients were obtained at lower concentration, further validation is required to explain non-linear behavior of benzalkonium ion sorption to mineral surfaces.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2019.03.011](https://doi.org/10.1016/j.ecoenv.2019.03.011).

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