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Predicting bioconcentration factors of highly hydrophobic chemicals. Effects of molecular size*

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Abstract: The bioconcentration factor (BCF) is a parameter that describes the ability of chemicals to concentrate in aquatic organisms. Traditionally, it is modeled by the log–log quantitative structure–activity relationship (QSAR) between the BCF and the octanol–water partition coefficient (K_{ow}). A significant scatter in the parabolic log(BCF)/log(K_{ow}) curve has been observed for narcotics with log(K_{ow}) greater than 5.5. This study shows that the scatter in the log(BCF)/log(K_{ow}) relationship for highly hydrophobic chemicals can be explained by the molecular size. The significance of the maximal cross-sectional diameter on bioconcentration was compared with the traditionally accepted effective diameter. A threshold value of about 1.5 nm for this parameter has been found to discriminate chemicals with log(BCF) > 3.3 from those with log(BCF) < 3.3. This critical value for the maximum diameter is comparable with the architecture of the cell membrane. This threshold is half thickness of leaflet constituting the lipid bilayer. The existence of a size threshold governing bioconcentration is an indication of a possible switch in the uptake mechanism from passive diffusion to facilitated diffusion or active transport. The value of the transition point can be used as an additional parameter to hydrophobicity for predicting BCF variation. The effect of molecular size on bioconcentration has been studied by accounting for conformational flexibility of molecules.

INTRODUCTION

Halogenated organic chemicals are routinely detected in a wide number of terrestrial and aquatic species [1–10]. The studies showed a dramatic rise in the concentration of organochlorinated and brominated compounds in sediment, biota, and breast milk. This has led to an increasing international concern to identify chemicals that have persistence, bioconcentration/bioaccumulation, and toxicity potential [11–15]. The tendency of chemicals to bioconcentrate in biota generally is expressed as a bio-

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concentration factor (BCF), defined as the ratio of the chemical concentration in biota to that in its environment at steady state [16]. Typically, fish are used for BCF assessments. The bioconcentration process is generally considered as a partitioning between the water and the lipid phase of the animal. This fundamental assumption is based on the work of Hamelink et al. [17] and established linear correlations between BCF log transformations and the chemical's partitioning between octanol and water, i.e., the log octanol/water partition coefficient log(K_{ow}) [18–20].

Several authors have shown that the linear relationships between log(BCF) and log(K_{ow}) are not adequate for chemicals with log(K_{ow})values greater than 6. The critical reexamination of the accepted correlation between octanol/water partitioning and BCF resulted in complex models varying from the high-order polynomial model of Connell and Hawker to the four-linear BCF-Syracuse model [21–30]. Although the best models provide average goodness of fit within one-half log unit for the compounds in the training sets, there is great scatter of the data in the range of the maximum of the log(BCF)/log(K_{ow}) relationship [29,30]. In order to improve the accuracy of BCF predictions, a set of correction factors and rules based on molecular geometry and weight were introduced.

The lack of bioaccumulation of congeners that have a cross-section >0.95 nm has been explained by limited membrane permeability in the guppy [31]. However, hexabromobenzene was taken up by rainbow trout after aqueous exposure [31]. Other polyhalogenated pollutants with large cross-sections, such as polychlorinated dibenzo-*p*-dioxins and polybrominated biphenyls and diphenylethers, have been also found to be taken up by fish and other species [1,9,10,32–38]. The usefulness of another commonly suggested criterion, molecular weight, for discrimination between bioaccumulative and nonbioaccumulative compounds, is questionable, and there are no experimental data to support a specific threshold for this parameter.

The main goal of this study is to describe experimentally supported criteria that discriminate between bioaccumulative and nonbioaccumulative compounds.

MATERIALS AND METHODS

In order to reveal the relation between the molecular geometry and bioconcentration, we have used 694 experimental data for BCF and K_{ow} values for fish [29]. The BCF values for 463 polar and nonpolar narcotics were used to derive a nonlinear log(BCF)/log(K_{ow}) model [30]. Such a restriction of the analysis for narcotics only is motivated by the fact that for reactive chemicals the concept of "steady-state concentration" in the test organism is in conflict with their reactive mode of action. Generally, the compounds were categorized according to the response-surface approach as noncovalent-acting toxicants, including narcotics, esters, amines, polar narcotic phenols and anilines, and chemicals possessing well-defined reactive groups such as aldehydes, acrylates, α,β-unsaturated halides, etc. [39]. Figure 1 illustrates the relationships between measured 694 log(BCF), calculated log(BCF) [30], and log(K_{ow}) values.

For each narcotic with log(K_{ow}) greater than 5.5, conformers were generated and their geometric characteristics were calculated. The threshold of log(K_{ow}) = 5.5, was selected in order to eliminate the influence of reduced accumulation owing to low lipophilicity of chemicals under these values. In other words, the present analysis was performed in the K_{ow} range, where factors other than lipophilicity control the bioconcentration of chemicals in the cells.

In the present work, we have decided to use a 4:1 ratio between initial population and new individuals (population size 20 and 5 new individuals), which provides relatively high reproducibility and robustness of GA runs, and subsequent high coverage of conformational space. Each of the generated conformations was submitted to a strain-minimization technique (pseudo-molecular mechanics, PMM), which is based on a simple energy-like function, where only the electrostatic terms are omitted [41]. Subsequently, conformational degeneracy, due to molecular symmetry and geometry convergence, was detected within a 60° range of torsion angle differences. The geometry optimization was next completed by employing MOPAC 93 [42], using the AM1 Hamiltonian with the key words >PRECISE= and

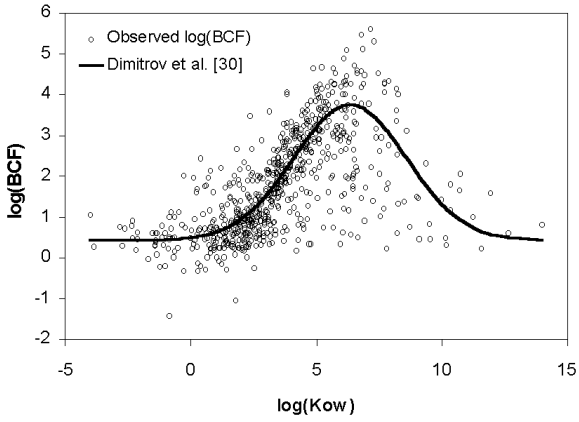

Fig. 1 Measured log(BCF) values vs. log(K_{ow}) for the 694 compounds in the BCF database [29].

>NOMM=. Finally, the energetically reasonable conformers were screened, having heat of formation ΔH^o no greater than 20 kcal/mol from the ΔH^o associated with the conformer with absolute energy minimum [43,44]. The compounds were divided into rigid and flexible chemicals depending on the number of their energetically reasonable conformers. All chemicals with more than two conformers were denoted as flexible.

RESULTS AND DISCUSSIONS

The complexity of the effect of molecular geometry and flexibility on membrane permeation and subsequently on bioconcentration can be illustrated with *n*-pentadecane. The conformer with lowest heat of formation (ΔH^o = –84.5 kcal/mol) is shown in Fig. 2a.

The length of the *n*-pentadecane molecule, known also as the maximum diameter, is D_{max} = 2.14 nm. The other two related dimensions, the effective and minimum cross-sections for this conformer, are D_{eff} = 0.499 nm and D_{min} = 0.492 nm, respectively. Due to the high hydrophobicity of *n*-pentadecane [log(K_{ow}) = 7.71] and small effective cross-section of its lowest energy conformer, which is far below the critical 0.95 nm [27], one anticipates high bioconcentration for this chemical. The bioconcentration predicted by the models of Connell and Hawker [21], Meylan et al. [29], and Dimirov et al. [30] is in the range of 3.2 to 4.3 log units. Two other energetically reasonable conformers of *n*-pentadecane (ΔH^o = –71.8 kcal/mol and ΔH^o = –68.5 kcal/mol) are present in Figs. 2b and 2c, respectively. Based on the effective cross-section of the third conformer, D_{eff} = 1.15 nm, a loss of membrane permeation may be expected, resulting in low bioconcentration. The experimentally measured BCF of *n*-pentadecane in carp, expressed as log(BCF), is in the range of 1.12 to 1.29 [45]. Two conclusions could be drawn from this simple example. First, molecular cross-sectional diameters appear to be strongly dependent on molecular flexibility. Secondly, it is not clear which of the molecular dimensions controls the membrane permeation of chemicals.

The complex effect of molecular geometry and flexibility on the chemicals' permeability is confirmed by superposition of the molecular effective cross-sectional diameters of the studied lipophilic chemicals with their experimentally measured BCFs (Fig. 3).

The absence of any correlation indicates that this geometric characteristic is not related to the variation of BCF for highly hydrophobic chemicals. The hypothesis that the effective diameter controls permeability of chemicals assumes a strict spatial orientation of the molecules toward the cell membrane surface in a way that the molecular projection over the membrane does not exceed a certain threshold (anticipated to be around 0.95 nm) (Fig. 4a). The appropriate orientation, however, is pre-

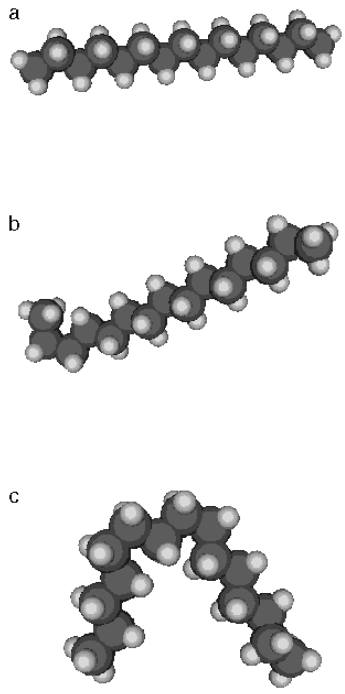

Fig. 2 Cross-sectional diameters for energetically reasonable conformers of *n*-pentadecane. (a) Conformer 1 (lowest-energy conformer; ΔH^{o} = –84.5 kcal/mol), D_{max} = 2.14 nm; D_{eff} = 0.499 nm; D_{min} = 0.492 nm. (b) Conformer 2 (ΔH^{o} = –71.8 kcal/mol), D_{max} = 1.98 nm; D_{eff} = 0.663 nm; D_{min} = 0.512 nm. (c) Conformer 3 (ΔH^{o} = –68.5 kcal/mol), D_{max} = 1.40 nm; D_{eff} = 1.15 nm; D_{min} = 0.552 nm.

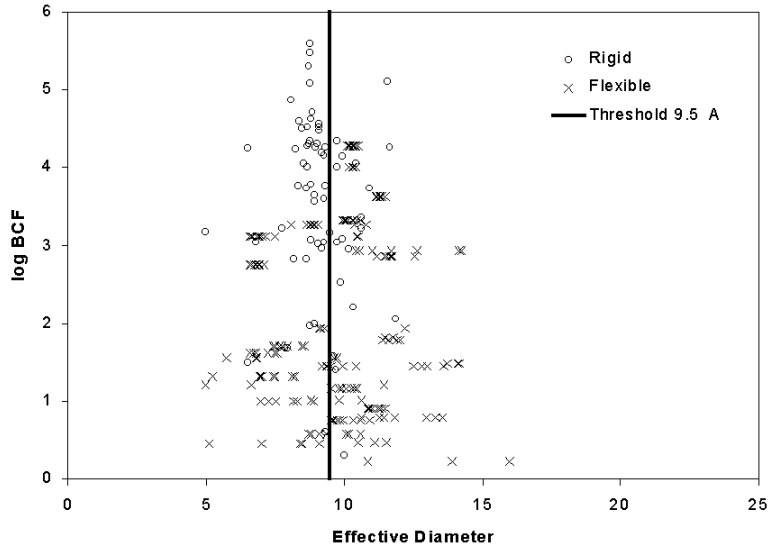

Fig. 3 Log(BCF) vs. effective cross-sectional diameter. (a) Appropriate orientation; (b) Inappropriate orientation.

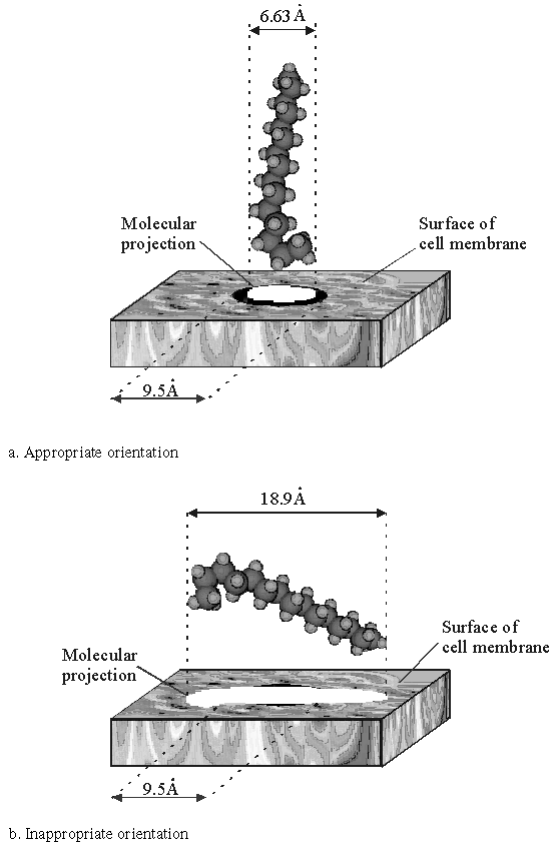

Fig. 4 The chaotic movement and molecular projection over the membrane.

vented by entropy (i.e., by chaotic movement of the molecules), which can explain the insufficiency of the effective cross-section of molecules to explain their permeability (Fig. 4b).

Based on these results, we decided to relate BCF with the other molecular dimensions, and namely, the maximum and minimum diameters. The importance of the maximum diameter of molecules is illustrated in Fig. 5, where this parameter is related to bioconcentration.

This plot displays two interesting features. Firstly, there is a well-outlined tendency of decreasing bioconcentration with the increase of the maximum cross-sectional areas of molecular conformers. The higher the molecular length (i.e., its D_{max} value), the smaller are the chances of the molecule reaching the cell membrane at an appropriate angle. Secondly, as is evident from Fig. 5, most chemicals with D_{max} under ~1.5 nm achieve high log(BCF)—in the range of 3 to 6, while the chemicals with D_{max} greater than this threshold accumulate up to 3.3 units at most. The existence of such a transition point can be explained by a change in the mechanism of uptake of chemicals from passive diffusion through the phospholipid bilayer of the membrane to the more conservative passing of the membrane by the mechanism of exocytosis and endocytosis. Interestingly, the critical value of 1.5 nm for the threshold is comparable with the cell membrane architecture. The threshold of maximum diameter is comparable with the half thickness of leaflet constituting the lipid bilayer [46] of the cell membrane.

From the present results, one could conclude that diffusion through the cell membrane is limited to molecules having a length not exceeding the threshold of about 1.5 nm. The latter could be assumed as the maximum tolerance of the cell membrane.

Analysis of Fig. 5 reveals another tendency of decreasing log(BCF) with an increase in conformational flexibility of molecules. As seen, the rigid chemicals tend to populate the area associated with high bioaccumulation. This could be related to the enhancement of the entropy factor on membrane permeability of chemicals with increase of their conformational flexibility. The complexity of this relationship, however, requires more detailed analysis of conformational space of studied chemicals across the whole range of log(K_{ow}) values.

The significance of this result for explaining the scatter around the maximum of the log(BCF)/log(K_{ow}) curve for narcotics is visualized in Fig. 6.

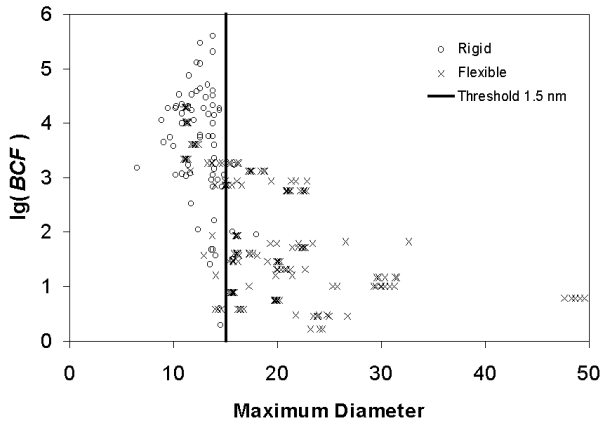

Fig. 5 Log(BCF) vs. maximum molecular diameter.

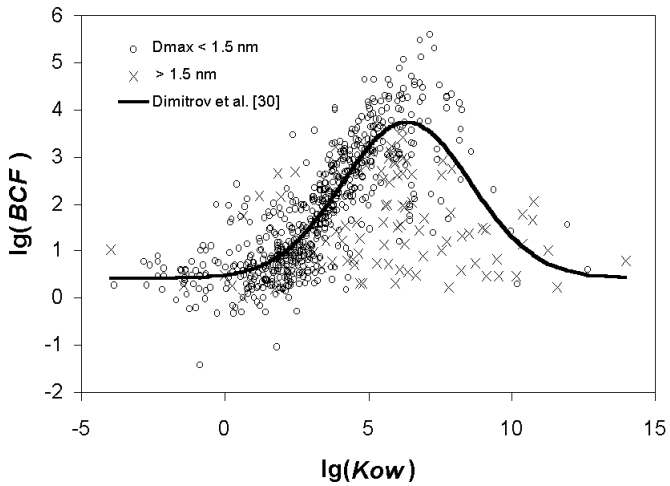

Fig. 6 Discrimination of chemicals by D_{max}.

CONCLUSIONS

Analysis of the BCF data for narcotics with $\log(K_{ow})$ greater than 5.5 revealed that the maximum cross-sectional diameter can be used to explain the significant scatter around the maximum of the $\log(BCF)/\log(K_{ow})$ curve. The chaotic collision of molecules with the cell membrane surface at different angles could explain the significance of this geometric characteristic, instead of generally accepted effective diameter. The drop in bioconcentration of chemicals at a maximum cross-sectional diameter of about 1.5 nm is an indication of a switch of the mechanism of uptake of chemicals into cells above this threshold. The value of this transition point can be used as an additional parameter of hydrophobicity for regression modeling of the BCF variation. Conformational flexibility tends to further increase the significance of entropy to cell permeability, which leads to additional decreases of BCF. The effect of this structural characteristic, however, needs to be further evaluated in order to be used for quantifying the bioconcentration of chemicals.

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