Journal of Environmental Biology **January 2008,** 29(1) 89-92 (2008)

Molecular size as a limiting characteristic for bioconcentration in fish

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(Received: November 17, 2006 ; Revised received: April 15, 2007 ; Accepted: May 04, 2007)

Abstract: *The relationships between the bioconcentration factor (BCF) of chemicals in fish and their size, as characterized by molecular weight (MW), effective cross sectional diameter (Deff), and maximum diameter (Dmax) have been investigated using an experimental data set of 737 new and 441 existing chemicals monitored by the Japanese Chemical Substances Control Law (CSCL). Substances with BCF ≥5000 (very high bioconcentration potential) typically have MW < 550, Deff < 1.1 nm and Dmax < 2.0 nm, respectively, and the substances with BCF ≥1000 (high bioconcentration potential) have MW < 550, Deff < 1.4 nm and Dmax < 2.9 nm, respectively. Therefore, the previously suggested threshold values for Deff (0.95 nm) and Dmax (1.5 nm) used for discriminating between bioconcentrative and non-bioconcentrative substances were found to be somewhat small. We found that many substances with BCF ≥1000 and Dmax ≥1.5 nm have Deff < 0.95 nm*

Key words: *Bioconcentration factor, Molecular size, Effective cross sectional diameter, Maximum diameter*
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Introduction

Non-degradable chemical substances released into the environment often accumulate in biota resulting in negative impacts on the environment and human health. Hence, bioconcentration plays an important role in the hazard assessment procedures for chemical safety. The tendency of chemicals to bioconcentrate is generally expressed as a bioconcentration factor (BCF), defined as the ratio of the chemical concentration in biota to its steady state environment. Generally, fish are used for BCF assessment and a chemical with a BCF ≥ 5000 is considered as having a very high bioconcentration potential and a BCF ≥ 1000 has a high bioconcentration potential.

Bioconcentration can be considered as the partitioning of substances between the lipid phase of an organism and the water phase. A number of linear relationships have been reported between the octanol/water partition coefficient and BCF in fish (Veith *et al*., 1979; Mackay, 1982; Meylan *et al*., 1999; Weisbrod *et al*. 2007; Gupta and Srivastava, 2006; Shukla *et al*., 2007). However, due to their limited ability to penetrate cell membranes, larger molecules frequently do not follow this relationship and so when discriminating between bioconcentrative and non-bioconcentrative chemicals, thresholds for molecular size have been proposed (*e.g.* chemicals with a molecular weight (MW) > 600 are too large to use in a standard bioconcentration calculation (Brooke *et al.,* 1986).

Some regulatory authorities specify MW as the criteria for low bioconcentrative chemical substances. Effective cross sectional diameter (Deff) is defined as the minimum diameter of infinite cylinders circumscribing a molecule. Opperhuizen *et al*. (1985) suggested that chemicals with Deff > 0.95 nm cannot penetrate cell membranes, as this corresponds to the pore diameter of a cell membrane, however Dimitrov *et al*. found that some chemicals with Deff > 0.95 nm can show high BCF (Dimitrov *et al*., 2002; Dimitrov *et al*., 2003). They ascribed this to an active transport mechanism and suggested a higher threshold value of about 1.5 nm for maximum diameter (Dmax), defined as the minimum diameter of spheres circumscribing a molecule. Although the active transport mechanism is not clear, they note that this size is similar to half the thickness of a lipid bilayerin cell membranes. A BCF prediction model based on the assumption of a maximum BCF with mitigating factors that reduce the BCF was recently developed (Dimitrov *et al*., 2005). In this model, Dmax is used one of the mitigating factors.

It was desirable to validate the reliability of these threshold values using as much experimental data as possible, particularly where the number of larger molecules in previous studies had been insufficient. In this study, we investigated the relationships between BCF and MW, Deff, Dmax (as indicators for molecular size), using a data set comprising 737 new and 441 existing chemical substances listed under the Japanese Chemical Substances Control Law (CSCL), (Chemicals Inspection and Testing Institute Japan, 1992) which, to our knowledge, is the largest data set used for such a study. Although the existing chemicals have been widely studied, the data for the new chemicals is generated for the first time and is of particular interest as it includes many larger molecules.

Materials and Methods

The bioconcentration test, established by the CSCL, is conducted on chemicals that are not biodegradable and hence

substances that readily undergo biodegradation are absent from the dataset used for this evaluation.

The CSCL bioconcentration test is conducted as a part of the 305C method, established by "The Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals" (OECD, 1982). The test fish (carp) are exposed to two concentrations of the test chemical in water, under flow-through conditions, where the higher concentration was one-hundredth of the threshold incipient median tolerance limit (TLm) and the lower concentration was one-thousandth of the TLm.

Herein, we selected the BCF data for 1178 chemicals with well-defined chemical structures (*e.g.* polymers, mixtures or metal compounds were not used) from the CSCL test report. The arithmetical mean of the last three BCF values, at the lower concentration, was used as the average BCF value for each of the substances. This dataset contained 27 substances with BCF $\geq$ 5000, 45 substances with BCF between 1000 and 5000 and 1106 substances with BCF < 1000. The molecular weight ranges of the data set are from 16 to 1736.

OASIS Forecast, version 4.31 beta was used to calculate Dmax and Deff, where the conformers are generated by a genetic algorithm (Mekenyan *et al.*, 1999) and the geometry optimization is conducted by MOPAC calculation with the AM1 Hamiltonian. For molecular size calculations, SYBYL standard atomic radii were used.

Results and Discussion

Fig. 1-3 show graphical plots of log BCF against our three chosen indicators for molecular size (MW, Deff, Dmax), where all distributions are approximately temple bell shape and an upper limit in BCF with increasing size was observed. The peaks of each graph fall at about 300 (MW), 0.8 nm (Deff) and 1.3 nm (Dmax), respectively, which are similar to those previously reported. However, clear threshold values cannot be determined from these Figures.

Substances with BCF$\geq$ 5000 (very high bioconcentration potential) typically have MW < 550, Deff < 1.1 nm and Dmax < 2.0 nm, respectively, and substances with BCF $\geq$1000 (high bioconcentration potential) have MW < 550, Deff < 1.4 nm and Dmax < 2.9 nm, respectively. As Fig. 1 demonstrates, there were no substances with a BCF $\geq$1000 that had a MW > 600, the previously suggested threshold value; however, as only a small number of chemicals with MW > 600 were tested (9%), designating a low bioconcentrative potential using this threshold value would not be reasonable. Fig. 2 reveals substances with a larger Deff than the previously recommended threshold value of 0.95 nm that show BCF $\geq$1000, which would indicate a penetration mechanism other than passive diffusion. Similarly, Fig. 3 shows some substances, with a larger Dmax than the previously suggested threshold value of 1.5 nm, that show BCF $\geq$1000. Hence, we conclude that the current Deff and Dmax values are too low.

For compounds with BCF $\geq$ 5000, defined as having a very high bioconcentration potential, examination of Fig. 1 shows a maximum MW cut-off of 550. A total of 137 compounds have a MW > 550, with a mean BCF of 7.2., *i.e.*, these 137 compounds would not be expected to have a very high concentration potential (presumably they have low bioconcentration potential), if MW were used as a predictor, and MW > 550 was the cut-off value. Similar examination of Fig. 2, shows a maximum Deff cut-off of 1.1 nm, leaving 190 compounds with Deff > 1.1 nm, with a mean BCF value of 13.8. Finally, Fig. 3 establishes a Dmax cut-off of 2.0 nm, to give 252 compounds with Dmax > 2.0 nm and a mean BCF of 9.6. If a BCF

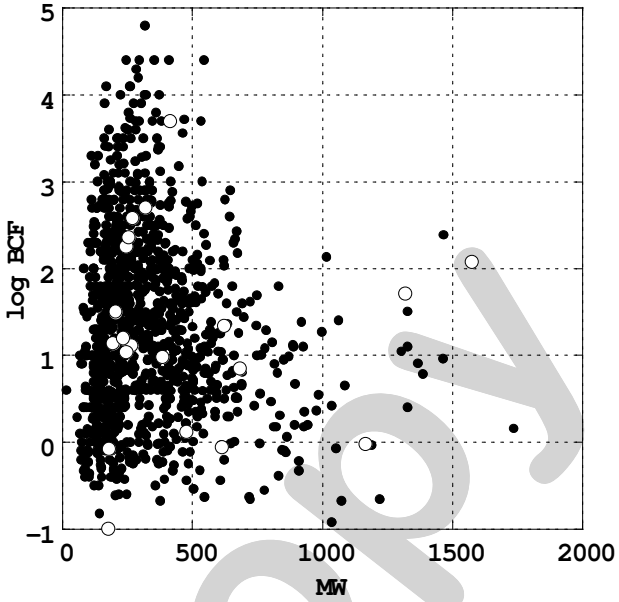

Fig. 1: Graphical plot of log BCF against MW for 1178 CSCL chemicals. Open circles represent compounds containing tetrafluoroethylene subunits. Closed circles represent all other compounds

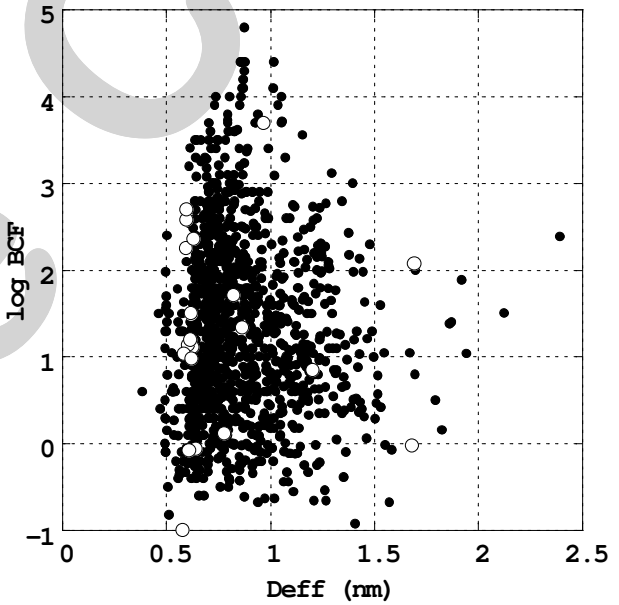

Fig. 2: Graphical plot of log BCF against Deff for 1178 CSCL chemicals. Open circles represent compounds containing tetrafluoroethylene subunits. Closed circles represent all other compounds

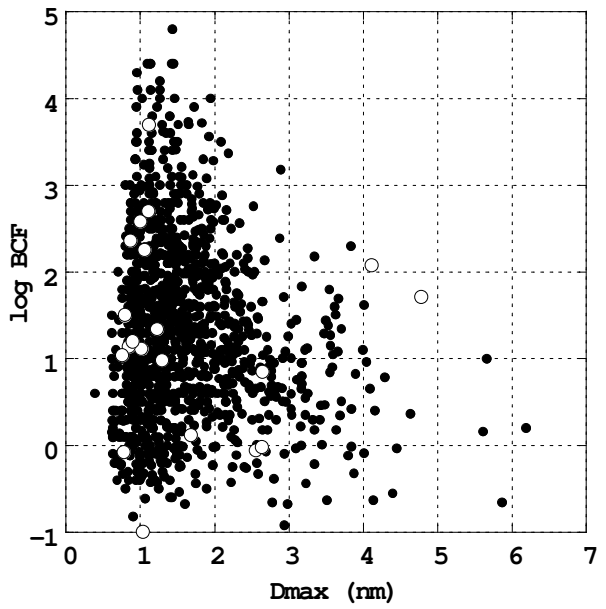

Fig. 3: Graphical plot of log BCF against Dmax for 1178 CSCL chemicals. Open circles represent compounds containing tetrafluoroethylene subunits. Closed circles represent all other compounds

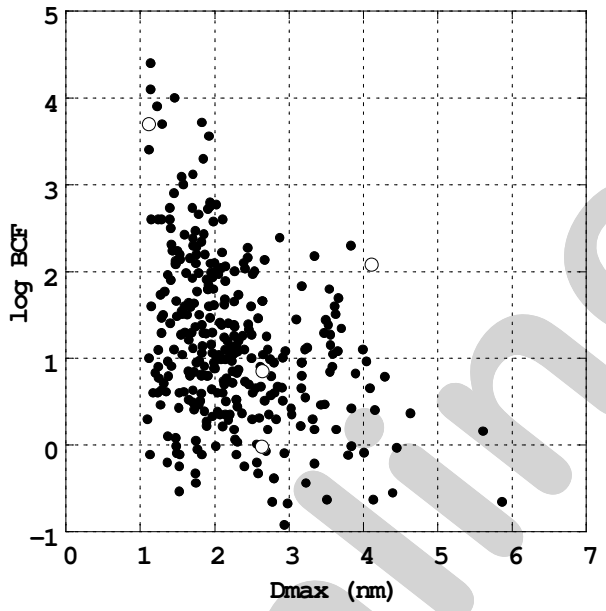

Fig. 4: Graphical plot of log BCF against Dmax for CSCL chemicals having Deff ≥ 0.95 nm. Open circles represent compounds containing tetrafluoroethylene subunits. Closed circles represent all other compounds

value >1000 is defined as a high bioconcentration potential, the same analysis as above would give the same MW cut-off of 550. However, the Deff cut-off increases to 1.4 nm, leaving 41 compounds with Deff > 1.4 nm, with a mean BCF value of 8.3. *i.e.* 41 compounds would not be expected to have a high concentration potential, if Deff were used as a predictor and Deff > 1.4 nm was the cut-off value. Likewise, Fig. 3 establishes a Dmax cut-off of 2.9 nm, to give 81 compounds with Dmax > 2.9 nm, and a mean BCF of 4.8. Clearly MW should not be used as a predictor, as substances with BCF > 5000 and the substances with BCF > 1000 could not be distinguished. If Deff and Dmax are compared, for both BCF > 5000 and BCF > 1000, using Dmax as the cut-off gave a larger number of compounds with a lower mean BCF than by using Deff, and thus compounds with a low BCF can be most accurately classified using the Dmax threshold values as predictors.

We found that many substances with BCF ≥ 1000 and Dmax ≥1.5 nm have Deff < 0.95 nm, implying that cell penetration of a stick-shaped molecule with a base diameter < 0.95 nm is somewhat independent of the length of the longer axis. Fig. 4 is a graphical plot of log BCF against Dmax for compounds with Deff ≥ 0.95 nm, and shows that substances with BCF ≥ 1000 all have Dmax < 2.0 nm. If we define compounds with Deff ≥ 0.95 nm and Dmax ≥ 2.0 nm as low bioconcentrative substances, all 169 substances correctly fall in the BCF < 1000 domain, with a mean BCF of 7.6. Thus, it would be much more accurate (and safer) to use both Deff and Dmax values to specify low BCF substances.

It can be seen in Fig. 1-3 that some large compounds can still show a relatively high BCF, possibly due to adsorption into the fish epidermis. For example, one compound with MW = 1574 shows BCF = 120, with, according to the test report, 20% of the substance concentrated in the fish epidermis.

It was noted that low-density compounds, such as perfluorides, show BCF values that seem overly high when using MW as a determining factor (Martin *et al*., 2003; Yakata *et al*., 2003). The relationships between BCF and MW, Deff and Dmax for compounds containing a tetrafluoroethylene subunit are highlighted in Fig. 1-3 (open circles). It can be seen that the open circles are found in the small molecular domain in Fig. 2 (Deff) and 3 (Dmax) in comparison to Fig. 1 (MW).

MW is normally used with only homogenous series of chemicals, as it cannot reflect steric information. On the other hand, Deff is related to the size of cell membranes and is considered to be a better predictor than MW in discriminating between bioconcentrative and non-bioconcentrative substances. However, there are many substances with a BCF ≥ 1000 and a Deff > 0.95, as shown in Fig. 2 and reported by Dimitrov *et al*. Dimitrov *et al*. (2003). This implies the existence of a penetration mechanism other than passive diffusion. The most likely alternative mechanism is cytosis. We speculate that Dmax would affect the speed of penetration by cytosis.

Dimitrov *et al*. calculated the probability of a chemical crossing the cell membrane based on increases in its maximum diameter (Dimitrov *et al*., 2005). According to their calculation, the probability for a molecule with a Dmax of 2.0 nm is about 0.1. On the other hand, there are many substances with a BCF ≥ 10 that had a Dmax > 2.0 nm, as shown in Fig. 3. It is likely that high-energy conformers with small Dmax may penetrate to the cell membrane, as compounds with Dmax > 2.0 nm have conformational flexibility. More detailed consideration of conformers with conformational flexibility is necessary.

Acknowledgment

This work was supported by the NEDO grant for the Acceleration of safety inspection for existing chemical substances project.

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