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# Bioconcentration Prediction under the Amended Chemical Substances Control Law of Japan

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Bioconcentration of organic chemicals in fish is considered to be one of the most important factors determining the fate of chemicals in the aquatic environment, and thus, the exposure levels to human and environmental organisms. Under the Chemical Substances Control Law of Japan, bioconcentration testing is, therefore, one of the principal requisites for the registration of a new chemical of which the intended volume is beyond the specified criterion. On the other hand, in order to reduce the burden of laboratory testing and also to provide early warning for possible hazards of a chemical, bioconcentration predictions utilizing a descriptor such as the 1-octanol/water partition coefficient can be partly applicable to registration purposes under the law.

This report presents an overall picture of applicable methods for the prediction, especially that based on restricted permeability of molecules, which we have partly contributed to the introduction of a new criterion through the national survey project funded by METI.

This paper is translated from R&D Report, "SUMITOMO KAGAKU", vol. 2006-I.

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## Introduction

At the International Conference on Chemicals Management (ICCM) of the United Nations Environment Programme (UNEP) in February 2006, the "Strategic Approach to International Chemicals Management (SAICM)" was adopted, and over one hundred countries around the world reached agreement to "minimize significant adverse effects from production and use of chemicals on human health and the environment by 2020." At the same time as the above policy was agreed upon, the International Council of Chemical Associations consisting of chemical industries from 52 countries announced Global Product Strategy (GPS), which is designed to improve a global chemical substances management and to support the SAICM with a voluntary industry action. Thus, the importance of ensuring safety of chemicals is increasing. Under these circumstances, Sumitomo Chemical has been proactively taking voluntary responsible care and maintaining our compliance to laws and regulations pertaining to chemicals.

In the SAICM, the persistent, bioaccumulative and toxic substances (PBT-substances) or persistent organic pollutants (POPs) are considered to be in high priority in risk management. The chemicals possessing

these properties are also under the control of many other regulations, such as the Stockholm Treaty. Therefore, safety measures to prohibit the manufacture/usage, to reduce discharge and to treat appropriately the aforementioned chemicals are required with international cooperation. In Japan, chemicals that possess these properties have been already strictly controlled under the Chemical Substances Control Law of Japan.

Although the Chemical Substances Control Law of Japan was greatly amended in 2004, evaluation of the chemical properties such as biodegradable and bioaccumulative potential relating to PBT-substances or POPs have been consecutively stipulated as an absolute requirement since its enactment in 1974. In particular, chemicals having high bioaccumulative potential are strictly controlled under the law. With respect to bioaccumulative potential evaluation, the bioconcentration test of chemicals have been partially exempted and displaced by prediction methods, for example using the logarithm of the 1-octanol/water partition coefficient as a descriptor, with compiling actual measured test data in consideration of cost-saving measures.

In this paper, we summarize the prediction methods for bioaccumulative potential that are permitted under

the current Chemical Substances Control Law of Japan. We especially focus upon the method based on restricted permeability of molecular size in the bioaccumulating process, which we have partly contributed to the introduction of a new criterion through a national survey project funded by the Ministry of Economy, Trade and Industry (METI).

## Amended Chemical Substances Control Law of Japan

Firstly, a summary of the amended Chemical Substances Control Law of Japan is described in this section. The law, originally enacted in 1973, was amended in 2004 for the second time. Primary modifications in the last amendment are as follows: (1) Introduction of prior evaluation systems and regulations against chemicals with certain ecotoxicity to encompass the perspective of prevention from the adverse effects of chemicals on ecosystems, as well as that of human health; (2) Reassessment of measures for some existing chemicals (persistent and highly bioaccumulative chemicals); (3) Introduction of a new pre-examination system that focuses on the possibility of release into the environment (intermediates, chemicals for use in closed systems, for export only or with total amounts equal to or less than 10 tons, which could be manufactured or imported without obligatory submission of toxicity data) and (4) Imposition of an obligatory reporting system for hazard information voluntarily

obtained by businesses.

Under the Chemical Substances Control Law, biodegradability of a chemical is evaluated first, according to the flow scheme for the application (Fig. 1) to evaluate the environmental effect of a chemical. When a chemical evaluated to be persistent, its bioaccumulative potential will be evaluated (if more than 1% of the degradation product(s) remain, all degradation products will be examined). As a result of this evaluation, if the chemical has been evaluated as having high bioaccumulative potential and harmful to higher predators, it will be strictly controlled as a “Class I Specified Chemical Substance,” which is the same classification as PCBs. This virtually means prohibition of manufacturing and importing. Such chemicals have been treated with extreme care since the enactment of the Chemical Substances Control Law. Furthermore, if a certain existing chemical substance demonstrates persistence and high bioaccumulative potential, even if its toxicity is unknown, it is designated as a “Type I Monitoring Chemical Substance” and is managed under certain controls. Thus, bioaccumulative potential is one of the crucial primary evaluation points in the Chemical Substances Control Law.

## Bioconcentration Test

### 1. Definition of Bioaccumulative Potential

The definition of “Highly Bioaccumulative” in the safety evaluation under the Chemical Substances Con-

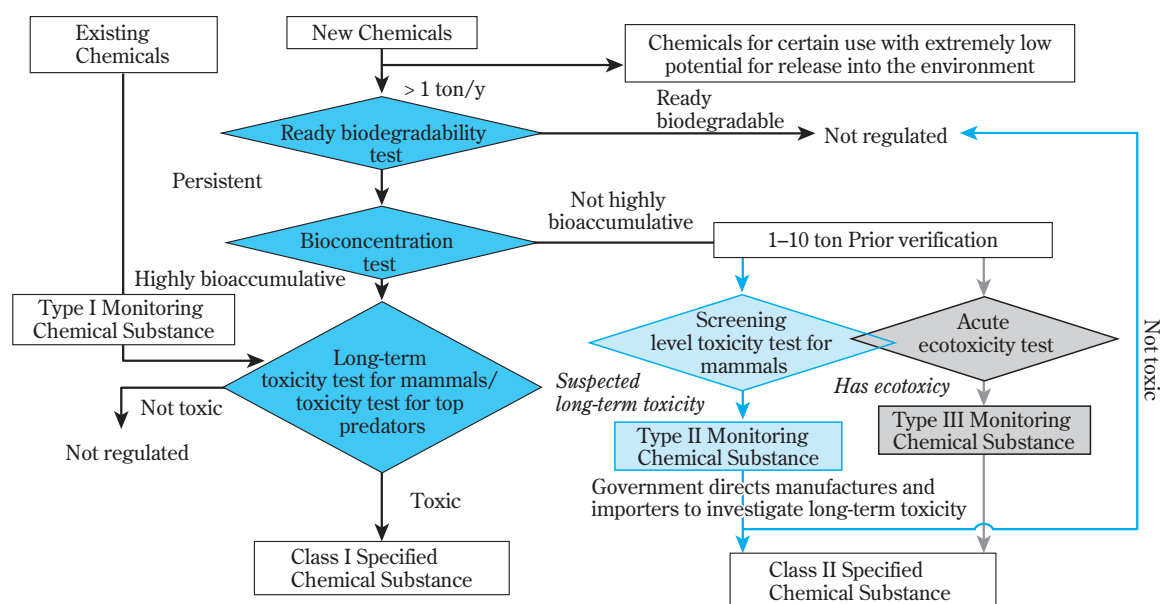


Fig. 1 Framework for evaluation and regulation under the Chemical Substances Control Law of Japan

Control Law suggests that a certain chemical has a tendency to accumulate in living organisms through the food chain or the biological actions within an organism. Definitions of frequently used terms related to bioaccumulative potential and accumulation are described below<sup>1)</sup>:

– Bioconcentration: A direct concentration process of a chemical into a fish body through the gill and surface of the body, from surrounding water. The bioaccumulative index obtained from this aspect is the Bioconcentration Factor (referred to as *BCF* hereafter). *BCF* can be obtained by using the formula shown below.

$BCF = \text{chemical concentration in a fish body} / \text{chemical concentration in water}$

– Bioaccumulation: A combined process of the above bioconcentration process and a process in which the chemical is taken orally through food.

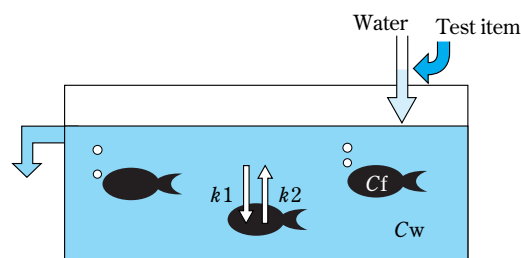
– Biomagnification: A bioaccumulation process via the food chain of the ecosystem. It indicates a phenomenon in which chemical concentration increases at higher trophic levels.

Considering the purposes of the regulations under the Chemical Substances Control Law, it is desirable to conduct a test that can evaluate either the above biomagnification or bioaccumulation. However, it is technically difficult to conduct the test within a laboratory and attain accurate results. Moreover, as chemicals are quite often discharged finally to the water system, *BCFs* obtained from the “bioconcentration test method using fish” for evaluation of bioconcentration are often utilized as bioaccumulative indices under many regulations including the Chemical Substances Control Law. In the Chemical Substances Control Law, if the *BCF* of the chemical is more than 5,000, it is determined to be “highly bioaccumulative” (meaning that the potential exists for biomagnification).

## 2. Fish Bioconcentration Test

Fig. 2 depicts a conceptual model for a bioconcentration test adopted in the Chemical Substances Control Law. In this test, the *BCF* of the chemical is obtained as follows: fish (usually, carp) are exposed to flowing water in which the chemical being tested is kept at consistent concentrations (the concentrations are usually set to two concentrations at less than 1/100 of the fifty percent lethal concentration [LC<sub>50</sub>], with a 10 times concentration ratio to each other) for at least 28 days. When the chemical concentration in

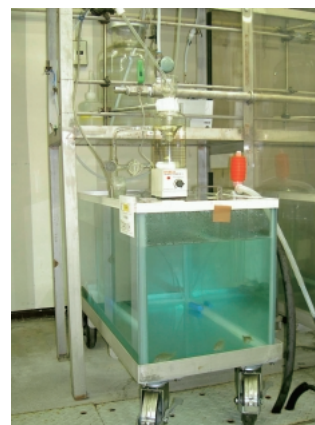
the fish body becomes constant, the *BCF* can be determined from the comparison in the ratio between the concentration in the fish body and the concentration in the water (see Fig. 3 for the testing system). However, as it requires 5–6 months to complete a whole test, and sometimes the development of analysis methods are of extreme difficulty, an improvement in the efficiency of this test is desirable.



$k_1$  and  $k_2$ : Uptake and depuration rate constants

$C_w$  and  $C_f$ : Concentrations of a chemical in the water and fish

**Fig. 2** Conceptual model for a bioconcentration test



**Fig. 3** An experimental apparatus for bioconcentration test

## Bioaccumulative Potential Prediction Methods

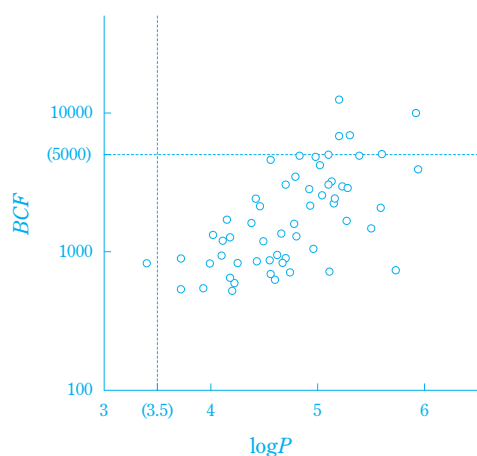
As we mentioned previously, since measurement data has been compiled over the years, the Chemical Substances Control Law has permitted several prediction methods instead of conducting the bioconcentration test. In this paper, firstly, we will explain about the method that utilizes the logarithm of the 1-octanol/water partition coefficient as a descriptor (flask shaking method: After this method was approved, some detailed procedures were amended in December 2002. Then the high-performance liquid

chromatography [HPLC] method was also approved.), which was adopted in March 1987 via notification by a director of the Bureau. Secondly, we will describe the method using molecular weight threshold values that we have been involved with in related research (notified by a director of the Bureau in September 2004) and the principle concept of the values.

## 1. Prediction Methods Based on Hydrophobicity

### (1) 1-Octanol/Water Partition Coefficient (Flask Shaking Method)

Since bioconcentration processes can be considered as phenomena in which chemicals distribute from water to lipids in fish<sup>1)</sup>, they have high similarity to 1-octanol/water partition behavior. The logarithm value of 1-octanol/water partition coefficient ( $\log P$ ) is treated as a typical physiochemical index that represents hydrophobicity of the chemicals. It is also widely known that it has good correlation with the logarithm of the  $BCF$ .<sup>2)</sup> In the flask shaking method, which is the most basic method of all  $\log P$  measuring methods, after achieving equilibrium for a chemical that had been added to 1-octanol and water by shaking the container,  $\log P$  can be obtained from the logarithm of the concentration ratio of the chemical distributed between the two solvents. The concentration potential of the chemical can be predicted through such a simple method. As a result, testing time and cost can be reduced significantly, moreover with no fish. However, this prediction method cannot be applied to organometallic compounds and surface-active substances. If  $\log P$  is determined to be lower than 3.5 from



**Fig. 4** Relationship between  $BCF$  and  $\log P$  of existing chemicals under the Chemical Substances Control Law of Japan (57 chemicals within the  $\log P$  range 3 – 6)

a test result, then the chemical is considered to be “not highly bioaccumulative” under the Chemical Substances Control Law, and the bioconcentration test using fish will thereby be exempt from evaluation (Fig. 4). Therefore, application of this prediction method is usually evaluated prior to conducting bioconcentration tests using fish.

### (2) 1-Octanol/Water Partition Coefficient (HPLC Method)

In 2005, the HPLC method was approved by the Chemical Substances Control Law as one of the  $\log P$  measurement methods that can be applied for evaluation of chemicals. In the HPLC method,  $\log P$  values are estimated by several primary standard chemicals, for which  $\log P$  values are already known and their retention times are compared from reversed-phase HPLC analysis. This method has enabled the measurement of  $\log P$  of mixtures and chemicals that are not of high purity, a result that cannot be achieved using the conventional flask shaking method. Therefore, as shown in the flask shaking method, bioconcentration tests using fish will be exempted if  $\log P$  is determined to be lower than 3.5 by the HPLC method. Furthermore, as with the flask shaking method, the HPLC method cannot be applied to organometallic compounds.

Before the adoption of the HPLC method to the Chemical Substances Control Law, a ring test was conducted by several research institutes including our company. Then, the reliability of the method was evaluated. It is important to appropriately select primary standard chemicals in order to achieve accurate results through this method. Thus, it is desirable to take other properties of a chemical, such as hydrogen bond properties, into account.<sup>3)</sup>

### (3) Limits of Prediction Methods Based on Hydrophobicity

Many studies that prove the following fact have been reported thus far: When chemical molecules are sterically bulky, the chemicals have low bioaccumulative potential. This phenomenon cannot be easily explained only by observing the hydrophobicity of the chemical, which has been described previously in this paper. Based on bioconcentration mechanisms, in order for a chemical to be absorbed into a fish body, it must permeate a biomembrane. Therefore, it can be expected that by thoroughly analyzing previous test examples

and theory behind this permeation process, a new prediction method that explains the above phenomenon can be developed.

## 2. Prediction Method Based on Steric Effects

We were entrusted with a survey project to investigate the correlation between the bioaccumulative potential of a chemical and its molecular size by the Chemicals Evaluation and Research Institute, Japan. This project was conducted as a part of the Ministry of Economy, Trade and Industry's outsourced survey project entitled "Chemical Substances Safety Enhancement/International Regulation Measures, etc. (Chemical Substance Testing Method Development, etc.)." During this survey, we conducted detailed research on known findings and attempted to propose a certain threshold value from the result. A summary of this survey will be introduced in the following sections. The survey was conducted in 2003 and the results have already been published.<sup>4)</sup> We have excerpted the following article from the report.

### (1) Membrane Transport Mechanisms

Several mechanisms by which chemicals are transported through living organisms have been reported as shown in Table 1.<sup>5), 6)</sup> Factors that greatly affect the bioconcentration processes are "solvent drag" and "simple diffusion."

**Table 1** Membrane transport mechanisms

A	Solvent drag
B	Diffusional transport B-1 : Simple diffusion B-2 : Facilitated diffusion B-2-a : Channel transport B-2-b : Carrier transport
C	Energy-dependent transport C-1 : Cotransport, antiport C-2 : Active transport C-2-a : Primary active transport C-2-b : Secondary active transport
D	Cytosis D-1 : Pinocytosis D-2 : Phagocytosis

### (i) Effect of Molecular Size During Chemical Transportation Through Solvent Drag

The concept of transport mechanisms is as follows: Biomembranes contain pores and channels. A chemical is transported through these pores and chan-

nels, exiting the biomembrane together with the water flow that is proportional to the pressure difference created when the water flows through the membrane. The transport coefficient  $P_s$  of spherical chemicals can be obtained from the following equation:

$$P_s = (Ndp^2 / (12N_A\eta dcm)) \cdot (1 - dc/dp)^2 \cdot \{ \int 2\pi\beta d\beta \cdot [1/F(dc/dp, \beta)] 2\pi\beta d\beta \} \quad (\text{Eq. 1})$$

In the above equation,  $dp$  is the pore diameter,  $dc$  is the diameter of the chemical,  $dm$  is the thickness of the membrane,  $N_A$  is Avogadro's Number,  $N$  is the number of pores,  $\eta$  is the viscosity,  $F(dc/dp, r)$  is the sphere size correction formula, and  $\beta$  is the variable of integration (interval  $[0, (dp-dc)/2]$ ). The first term of the right-hand side of the equation represents Stokes Einstein hydrostatic interactions, the second term represents hydrostatic interactions of steric constraints based on the diameters of the chemical molecules and the pores, and the third term represents hydrostatic interactions (frictional actions) between the pores and spherical particles. From the above equation, the following facts can be theoretically proven: the steric size of the chemical determines the size of  $P_s$ ; and when the diameter of the chemical is larger than that of a pore on the membrane, transportation does not proceed.

### (ii) Effect of Molecular Size on Simple Diffusion

In general, the membrane transport coefficient  $P_m$  in non-electrolytic lipid bilayer membranes can be shown by the distribution/diffusion model as below. It can be considered as a function of the distribution coefficient  $K_m$  and the diffusion coefficient  $D_m$ .

$$P_m = D_m \cdot K_m / dm \quad (\text{Eq. 2})$$

At first, with respect to the diffusion coefficient of a chemical in water  $Daq$ , the experimental formula has been reported as follows:<sup>7)</sup>

$$Daq = 1.326 \times 10^{-4} / (\eta^{1.4} \nu^{0.598}) \quad (\text{Eq. 3})$$

In the above formula,  $\eta$  is the viscosity of the water, and  $\nu$  is the molecular volume ( $\text{cm}^3/\text{mol}$ ) of the chemical. Also, it has been confirmed that the steric bulkiness of the molecule affects the diffusion coefficient  $D_m$  of the chemical contained within the membrane. For example, by using the molecular weight

$MW$ , the following relationship has been experimentally derived:<sup>8)</sup>

$$D_m \propto 1/MW^{1/2} \quad (\text{Eq. 4})$$

On the other hand, diffusion of the chemical in the lipid bilayer membranes differs from that in the continuous liquid. The suppression of  $D_m$  due to steric molecular volume can be observed more obviously in a biomembrane than in an artificial lipid membrane because the space between the molecules in the biomembrane is smaller due to the lipid components of the biomembrane being more densely packed.<sup>9), 10)</sup>

Viewing the distribution/diffusion relationship in a membrane more microscopically for a chemical having a rather large coefficient of transport, it is necessary to consider effects from the unstirred water layer on the membrane surface. The unstirred water layer is a water layer in which fluidity has been depressed. In an actual biomembrane, sugar chains such as glycolipids and glycoproteins composing the membrane are present in the layer, thus further reduction of the fluidity will be observed. The unstirred water layer can reach a thickness of  $100\ \mu\text{m}$ , making it even more difficult for a hydrophobic chemical to transport. Thus, the coefficient of transport  $P_{\text{obs}}$  in the simple diffusion process observed can be generally expressed as follows<sup>11)</sup>:

$$\begin{aligned} P_{\text{obs}} &= 1/(1/P_m + 1/P_{\text{aq}}) \\ &= D_m \cdot D_{\text{aq}} \cdot K_m / (d_m \cdot D_{\text{aq}} + 2d_{\text{aq}} \cdot K_m \cdot D_m) \end{aligned} \quad (\text{Eq. 5})$$

In the above equation,  $P_{\text{aq}}$  is the coefficient of transport of the unstirred water layer,  $D_{\text{aq}}$  is the coefficient of diffusion of the chemical in the water and  $d_{\text{aq}}$  is the thickness of the unstirred water layer. For a chemical having low hydrophobicity, it will be  $d_m \cdot D_{\text{aq}} \gg 2d_{\text{aq}} \cdot K_m \cdot D_m$ . Therefore,  $P_{\text{obs}}$  can be expressed by equation (2). On the other hand, for a chemical having high hydrophobicity,  $d_m \cdot D_{\text{aq}} \ll 2d_{\text{aq}} \cdot K_m \cdot D_m$ . Therefore, the coefficient of transport can be expressed by equation (6). In other words, diffusion in the unstirred water layer may become an obstacle for chemical uptake.<sup>12)</sup> In this case also, based on experimental formulae such as (3), it is obvious that the steric size of molecules such as molecular weight can be a cause of restriction for chemical uptake.

$$P_{\text{obs}} = D_{\text{aq}} / (2d_{\text{aq}}) \quad (\text{Eq. 6})$$

Thus, from the observation of the biomembrane transport mechanism, it can be concluded that the steric size of the chemical can serve as a cause of restriction for uptake of a chemical.

## (2) Structure of Fish Gills and Permeability

Although uptake of chemicals into fish may occur not only through gills but also through skin and digestive organs, the permeability of both hydrophobic and hydrophilic chemicals through skin is relatively low, because they have quite high tissue density and a composition consisting of multiple layers.<sup>13)</sup> In addition, it is considered that uptake via direct bioconcentration through the gills is more important than uptake through digestive organs.<sup>14), 15)</sup> In particular, in freshwater fish, it can be considered that water absorption through digestive organs almost never occurs due to osmoregulation. For this reason, gills are thought to be a primary uptake pathway. As gills have thin membranes with an area 2–10 times larger than the body surface, and a structure with high diffusion efficiency, it can be concluded that the gill is the site through which chemicals can be readily absorbed.

### (i) Structure of Fish Gills

Gills are the primary respiratory organ that efficiently perform gas exchange through a thin epidermis with a developed capillary bed and increased surface area due to projections and pleats.<sup>16)</sup> Epidermis cells in the gills are referred to as respiratory cells and their primary function is gas exchange. In addition, in the epidermis of the gills, which is composed of respiratory cells, these cells are connected with tight junctions. Therefore, it is considered that chemicals cannot readily permeate by the simple diffusion mechanism.<sup>17)</sup> Gill tissues are also composed of chloride cells, which are non-respiratory cells, and a small amount of mucous cells, as well as respiratory cells. These chloride cells are present as single cells in freshwater fish and are also connected with tight junctions to the adjacent respiratory cells. However, in saltwater fish, several chloride cells are intertwined, sharing a common aperture. Therefore, these cells are considered to be a complex in which they are bonded with each other in short and shallow tight junctions (leaky junctions).<sup>16), 17)</sup> Thus, blood and the external

medium virtually come into contact at this junction area, forming leaky and deep cavities. In freshwater fish, these leaky junctions of chloride cells do not exist.

#### (ii) Chemical Transport Pathways in Fish Gills

**Table 2** depicts the relationship between gill structure, transport pathways and transport mechanisms. From this table, it is clear that hydrophobic chemicals, for which there are concerns of bioaccumulative

**Table 2** Various pathways through a gill in consideration of its morphological structure

	Respiratory Pathway	Nonrespiratory Pathway (through chloride cell etc.)
Transcellular pathway	<ul style="list-style-type: none"> <li>• Related compounds : Small molecules (Hydrophobic and hydrophilic molecules)</li> <li>• Estimated mechanism : Simple diffusion in the membrane of respiratory cells</li> <li>• Estimated controlling factor : Hydrophobicity and the steric size of molecules</li> </ul>	<ul style="list-style-type: none"> <li>• Related compounds : Hydrophilic macromolecules</li> <li>• Estimated mechanism : Energy-dependent active transport or vesicular transport through the tubulovesicular system of chloride cells</li> <li>• Estimated controlling factor : Uncertain</li> </ul>
Paracellular pathway	<ul style="list-style-type: none"> <li>• Related compounds : Hydrophilic small molecules (excluding ions)</li> <li>• Estimated mechanism : Flux or Diffusion in aqueous phase through the deep tight junction between respiratory cells</li> <li>• Estimated controlling factor : Steric size of molecules</li> </ul>	<ul style="list-style-type: none"> <li>• Related compounds : Hydrophilic small molecules (including ions) and hydrophilic macromolecules</li> <li>• Estimated mechanism : Flux or Diffusion in aqueous phase through a shallow tight junction (a leaky junction) or the tubular reticulum in chloride cells.</li> </ul>

potential, can be taken into fish bodies through a transcellular pathway in respiratory cells. This process is controlled by the simple diffusion mechanism. Also, as uptake of chemicals having high steric size is restricted, it can be considered that bioaccumulation is low. Furthermore, in the event that a chemical transports through chloride cells or through a paracellular pathway, it suggests that macromolecules such as polysaccharides can be absorbed. However, these macromolecules are all hydrophilic and these chemicals seem to not be bioaccumulative.

As described above, uptake of a hydrophobic chemical from water to fish occurs primarily through the respiratory cell membrane of the gills, which is controlled by the simple diffusion mechanism. From this fact, it can be theoretically elucidated that the steric size of the chemical molecule can serve as a limiting factor for uptake, and thereby threshold values can be defined based on this steric size.

#### (3) Examples of Definitions of Threshold Values Based Upon the Steric Size of Molecules

There are a variety of descriptors for steric effects of chemicals. Below are examples for evaluating bioaccumulative potential of chemicals using some typical descriptors.

##### (i) Molecular Weight

Based upon the results obtained from bioaccumulative potential evaluations of 13 chemicals such as

**Table 3** Threshold values with regard to MW proposed for the uptake of several compounds

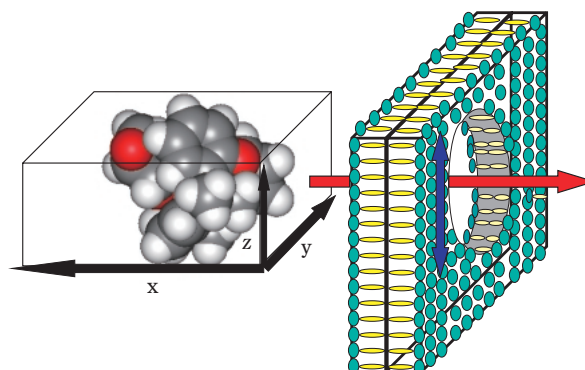
No.	Researcher (Year)	Compound	Fish species	Threshold [Related BCF]
1	Zitko <i>et al.</i> (1976) <sup>18)</sup>	11 halogenated biphenyls and 2 halogenated benzenes (MW: 223~546)	Atlantic salmon	MW 600 [BCF 0]
2	Anliker <i>et al.</i> (1988) <sup>19)</sup>	23 disperse dyestuffs, 16 halogenated aromatic hydrocarbons, 2 organic pigments, 1 optical brightener and 1 triazine herbicide (MW: 147~944, logP : 1.77~11.20)	Unexplained	MW 500 [BCF < 100]
3	Dimitrov <i>et al.</i> (2003) <sup>20)</sup>	610 nonionic compounds, 84 ionic compounds (MW: Unexplained, logP : -3.98~13.98)	Unexplained	MW 700 [BCF < 100]
4	Martin <i>et al.</i> (2003) <sup>21)</sup>	5 perfluorocarboxylates, 2 perfluorosulfonates (MW: 400~714, Number of fluorine atoms: 13~27, Perfluoroalkyl chain length: 6~13)	Rainbow trout	Uncertain (MW > 700)
5	Brooke <i>et al.</i> (1986) <sup>22)</sup>	ca. 30 Chlorohydrocarbons (MW: ca.100~ ca.400)	Unexplained	Uncertain [Maximum BCF at around MW 300]
6	Yakata <i>et al.</i> (2003) <sup>23)</sup>	7 Aryl fluoroalkyl ethers (MW: 292~498)	Carp	Uncertain [Maximum BCF at MW 300~430]

halogenated biphenyls using salmon as test species, Zitko *et al* proposed to adopt a molecular weight (*MW*) of 600 as an uptake limitation threshold value.<sup>18)</sup> **Table 3** depicts the results of a number of analyses that have been conducted on a variety of chemicals since Zitko's evaluations were reported. It can be considered that most of the maximum *MW* threshold values are close to 700. However, in chemicals having high fluorine content, such as perfluorinated acids, the *BCF* seems to be high even if *MW* exceeds 700. The primary reason for this is the high density of fluorine atoms. Therefore, when comparing such a chemical with hydrogen using the van der Waals radius as a standard, its density is approximately 14 times higher than that of hydrogen<sup>24)</sup>. This fact suggests that when using molecular weight as a descriptor, the steric size of molecules is overestimated. Apart from fluorine atoms, bromine atoms and iodine atoms also have relatively high densities. However, the reason why such an anomalous example has been seen only in chemicals containing fluorine is because that is relatively small and can be contained in a molecule in high proportions.

#### (ii) Other Descriptors for Steric Size

With respect to molecular volume (*MV*), relationships between *BCF* and 128 chemicals having diverse structures have been measured using 14 fish species. In this analysis, *BCF* decreased in proportion to an increase in *MV*. It was assumed that the uptake limitation threshold value was close to *MV* 400–500 cm<sup>3</sup>/mol.<sup>2)</sup> Next, with respect to the effective cross sectional diameter (*Deff*: the second shortest length of the three dimensions (length, width and height) of the smallest rectangular solid into which can fit the three-dimensional structure optimized using the molecular mechanics method etc. See **Fig. 5**), it has been discovered that a chemical for which *Deff* exceeds 0.95 nm cannot permeate through a biomembrane. This conclusion was obtained from observation of the relationship of *BCF* and 13 halogenated aromatic hydrocarbons, using guppies.<sup>25)</sup> Since this discovery was initially reported, a variety of *Deff* threshold values have been reported. On the other hand, for the maximum diameter (*Dmax*: the longest length among the three dimensions (length, width and height) of the smallest rectangular solid into which can fit the three-dimensional structure that has been optimized using the molecular mechanics method etc.), it has been reported that

chemicals having *Dmax* larger than 14.7Å (1.47nm) do not have high bioaccumulative potential (*BCF* > 5000) from the *BCF* analyses conducted on 694 chemicals using a variety of fish species.<sup>20)</sup>



The minimum diameter of the circular cylinder circumscribing the conformation of a molecule which is optimized by molecular mechanics etc., or the second-longest axis of the box (y axis) which circumscribes the optimum conformation. The diameter is also expressed as the “minimal internal cross section”.

**Fig. 5** Effective cross sectional diameter (*Deff*)

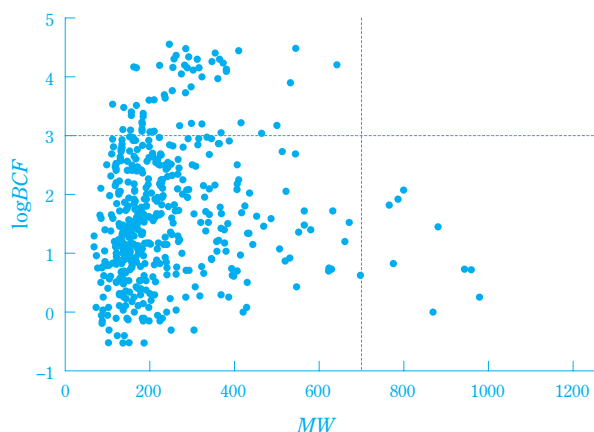
#### (iii) Determining the Most Effective Steric Size Descriptor

As described above, the validity of many descriptors has been reported. Considering the fact that there are rigid chemicals having a small degree of conformational freedom and flexible chemicals having a larger degree of freedom, it is not easy to theoretically determine the most effective descriptor. However, from the finding that these descriptors have a correlation with each other and taking the readiness of calculation and high reproducibility into account, it can be considered that molecular weight (*MW*) is an extremely effective descriptor.

#### (4) Verification of Chemicals Using Existing Test Data Under the Chemical Substance Control Law of Japan

**Fig. 6** depicts the relationship between *BCF* and molecular weight for test data of existing chemicals under the Chemical Substance Control Law. As shown in the figure, in the area where molecular weight exceeds 700, chemicals having high bioaccumulative potential are not observed. Also, for new chemicals designated by the Chemical Substance Control Law (763 new chemicals for which the structure can be specified), the same results have been reported.<sup>26)</sup>





**Fig. 6** Relationship between  $\log BCF$  and  $MW$  of existing chemicals under the Chemical Substances Control Law of Japan

#### (5) Proposed Uptake Limiting Threshold Values

Considering the theory of the uptake process of hydrophobic chemicals and the properties of respiratory cell membranes of fish gills, the following facts can be theoretically explained: since the steric size of chemical molecules can serve as an uptake limitation factor, a threshold value can be defined based on the steric size. From bioconcentration tests using fish, no possibility for bioaccumulative potential has been observed for chemicals with molecular weights exceeding 700. Thus, a molecular weight of 700 can be proposed as a threshold value for the uptake of chemical molecules. However, since some exceptional cases exist, such as those using perfluorinated acid, extra precautions are required when applying an uptake threshold value based on a molecular weight descriptor.

#### Threshold Value Stipulated Under the Chemical Substances Control Law of Japan

Considering the perspective of steric hindrance of biomembrane transport as described above, the following notification was released by a director of the Bureau in 2004: “For chemicals with molecular weights of more than 800, they are not considered to be chemicals that can readily accumulate within organisms.” As a result, regardless of the  $\log P$  value, any chemical having a molecular weight that exceeds the threshold value is determined to be a chemical having low bioaccumulative potential, thereby the bioconcentration test using fish shall be exempted. (However, the threshold value of chemicals containing more than two halogens is set to be more than 1000. A chemical with

a novel structure for which bioaccumulative potential is difficult to predict may not be subject to exclusion based on the threshold value.)

The introduction of this method using threshold values into the bioaccumulative potential evaluation stipulated under the Chemical Substances Control Law has enabled us to conduct simpler evaluations for bioaccumulative potential on a variety of chemicals, which were previously more complex and difficult to evaluate.

#### Conclusion

While regulations pertaining to safety evaluation of chemicals are becoming more diverse and comprehensive, in order to implement the regulations more flexibly and effectively, the sharing of test data and results from analogical evaluations using a “category approach” and the application of Quantitative Structure Activity Relationships (QSAR), are being widely investigated. It is extremely important to analyze theoretically the profound principles for developing new analogical evaluation methods with high accuracy and wide applicability. We shall continue to devote ourselves to developing safer chemicals by proactive evaluation with high accuracy and efficiency.

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