Bioconcentration Prediction under the Amended Chemical Substances Control Law of Japan

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 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:

- **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.

\[
BCF = \frac{\text{chemical concentration in a fish body}}{\text{chemical concentration in water}}
\]

- **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 [LC50], 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.

### 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, 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. 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 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.

(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

![Fig. 4](image-url) 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)
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. We have excerpted the following article from the report.

(i) Membrane Transport Mechanisms

Several mechanisms by which chemicals are transported through living organisms have been reported as shown in Table 1. Factors that greatly affect the bioconcentration processes are “solvent drag” and “simple diffusion.”

<table>
<thead>
<tr>
<th>A</th>
<th>Solvent drag</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Diffusional transport</td>
</tr>
<tr>
<td>B-1</td>
<td>Simple diffusion</td>
</tr>
<tr>
<td>B-2</td>
<td>Facilitated diffusion</td>
</tr>
<tr>
<td>B-2-a</td>
<td>Channel transport</td>
</tr>
<tr>
<td>B-2-b</td>
<td>Carrier transport</td>
</tr>
<tr>
<td>C</td>
<td>Energy-dependent transport</td>
</tr>
<tr>
<td>C-1</td>
<td>Cotransport, antiport</td>
</tr>
<tr>
<td>C-2</td>
<td>Active transport</td>
</tr>
<tr>
<td>C-2-a</td>
<td>Primary active transport</td>
</tr>
<tr>
<td>C-2-b</td>
<td>Secondary active transport</td>
</tr>
<tr>
<td>D</td>
<td>Cytosis</td>
</tr>
<tr>
<td>D-1</td>
<td>Pinocytosis</td>
</tr>
<tr>
<td>D-2</td>
<td>Phagocytosis</td>
</tr>
</tbody>
</table>

(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 $D_m$.

$$P_m = D_m \cdot K_m/d_m \quad (Eq. 2)$$

At first, with respect to the diffusion coefficient of a chemical in water $D_{aq}$, the experimental formula has been reported as follows: $7)$

$$D_{aq} = 1.326 \times 10^{-4}/(\eta^{1.4} \nu^{0.588}) \quad (Eq. 3)$$

In the above formula, $\eta$ is the viscosity of the water, and $\nu$ is the molecular volume ($cm^3/mol$) 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.
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.

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. In addition, it is considered that uptake via direct bioconcentration through the gills is more important than uptake through digestive organs. 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. 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. 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). Thus, blood and the external

\[ P_{obs} = \frac{Daq}{2d_{aq}} \]  

(Eq. 6)
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 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>
<thead>
<tr>
<th>No.</th>
<th>Researcher (Year)</th>
<th>Compound</th>
<th>Fish species</th>
<th>Threshold [Related BCF]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zitko et al. (1976)</td>
<td>11 halogenated biphenyls and 2 halogenated benzenes (MW: 223–546)</td>
<td>Atlantic salmon</td>
<td>MW 600 [BCF 0]</td>
</tr>
<tr>
<td>2</td>
<td>Anliker et al. (1988)</td>
<td>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)</td>
<td>Unexplained</td>
<td>MW 500 [BCF &lt; 100]</td>
</tr>
<tr>
<td>3</td>
<td>Dimitrov et al. (2003)</td>
<td>610 nonionic compounds, 84 ionic compounds (MW: Unexplained, logP : −3.98–13.98)</td>
<td>Unexplained</td>
<td>MW 700 [BCF &lt; 100]</td>
</tr>
<tr>
<td>4</td>
<td>Martin et al. (2003)</td>
<td>5 perfluorocarboxylates, 2 perfluorosulfonates (MW: 400–714, Number of fluorine atoms: 13–27, Perfluoroalkyl chain length: 6–13)</td>
<td>Rainbow trout</td>
<td>Uncertain (MW &gt; 700)</td>
</tr>
<tr>
<td>5</td>
<td>Brooke et al. (1986)</td>
<td>ca. 30 Chlorohydrocarbons (MW: ca.100–ca.400)</td>
<td>Unexplained</td>
<td>Uncertain [Maximum BCF at around MW 300]</td>
</tr>
</tbody>
</table>
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.\textsuperscript{18)} 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.\textsuperscript{24)} 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 $MV \approx 400 – 500 \text{ cm}^3/\text{mol}$.\textsuperscript{2)} 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.\textsuperscript{25)} Since this discovery was initially reported, a variety of $Deff$ threshold values have been reported. On the other hand, for the maximum diameter ($D_{max}$: 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 $D_{max}$ larger than 14.7 Å (1.47 nm) do not have high bioaccumulative potential ($BCF > 5000$) from the BCF analyses conducted on 694 chemicals using a variety of fish species.\textsuperscript{20)}

(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.\textsuperscript{26)}
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.

References

8) F. A. P. C. Gobas and H. A. Morrison, “Handbook of property estimation methods for chemicals, Environmental and health sciences”, R. S. Boeth-