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Revisiting Bioaccumulation Criteria for POPs and PBT Assessments

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EDITOR'S NOTE:

This paper represents 1 of 9 papers generated from a SETAC Pellston Workshop entitled "Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs," (January 2008, Florida, USA). The workshop objectives were to develop guidance and recommendations on the evaluation of substances fulfilling PBT and POP criteria, using scientific information such as experimental and monitoring data, and computer models.

ABSTRACT

Scientists from academia, industry, and government reviewed current international regulations for the screening of commercial chemicals for bioaccumulation in the context of the current state of bioaccumulation science. On the basis of this review, several recommendations were proposed, including a scientific definition for "bioaccumulative substances," improved criteria for the characterization of bioaccumulative substances (including the trophic magnification factor and the biomagnification factor), novel methods for measuring and calculating bioaccumulation properties, and a framework for screening commercial chemicals for bioaccumulative substances. The proposed framework for bioaccumulation screening improves current practices by reducing miscategorization, making more effective use of available bioaccumulation data that currently cannot be considered, reducing the need for animal testing, providing simpler and cheaper test protocols for animal studies in case animal studies are necessary, making use of alternative testing strategies, including in vitro and in silico metabolic transformation assays, and providing a scientific foundation for bioaccumulation screening that can act to harmonize bioaccumulation screening among various jurisdictions.

Keywords: Bioaccumulation Trophic magnification Food web PBT Models

INTRODUCTION

Forty-six years after Rachel Carson highlighted the dangers of widespread chemical use in *Silent Spring* (Carson 1962), the United States, Canada, the European Union, and several other countries are presently in the process of evaluating the environmental behavior of many thousands of current-use commercial chemicals as signatories of the 2004 ratified Stockholm Convention on Persistent Organic Pollutants (POPs) and under mandate of domestic regulations. The main goal of the Stockholm Convention on Persistent Organic Pollutants is to identify chemicals that, because of their lack of degradability, their ability to biomagnify in food chains, and their toxicity, can cause harmful concentrations in upper trophic level organisms and human beings (UNEP 2001). To achieve the goals of the convention, a set of criteria has been developed (van Wijk et al. 2009) that specifies the persistence, bioaccumulative capacity, toxicity, and significant adverse effects chemical substances must exert to be recognized as substances that should be eliminated from large-scale global production. Substances that meet these criteria are designated POPs under the Stockholm Convention. Twelve chemicals have currently been identified as POPs (UNEP 2001), and several others substances are currently being considered for a POPs designation (UNEP 2007).

To achieve the goals of the Stockholm Convention, various jurisdictions have developed chemical evaluation and classification schemes for commercial chemicals (Government of Canada, Council of the European Union 2006; USEPA 1976). These schemes have adopted the 4 fundamental criteria for evaluation (i.e., persistence, bioaccumulation, toxicity, and risk) of the Stockholm Convention on POPs but differ in the selection of some of the criterion values. A common strategy applied to the evaluation of the many thousands of commercial chemicals is to screen them for persistence (P), bioaccumulation (B), and toxicity (T). Chemicals that meet the criteria for P, B, and T are referred to as PBTs and are subject to risk assessment or risk characterization to determine whether they are harmful. Chemicals that do not meet the PBT criteria are not subjected to risk assessment unless there is additional information to justify a risk assessment. The role of screening chemicals for PBT is to improve the efficiency of the chemical evaluation process by minimizing the number of chemicals that require a more demanding, time-consuming, and costly evaluation of risk.

Although the POPs protocol was ratified relatively recently, in 2004, the criteria used to identify POPs and PBTs were

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formulated in the 1970s on the basis of the scientific knowledge at the time. Since the 1970s, major developments in policy, law, ethics, and science have occurred. For example, whereas UN policies focused on specific, relatively well researched chemical substances, current country-specific policies involve screening large numbers of substances for which relevant scientific data are often unavailable. In law, the burden of proof has shifted from governments, who were mandated to demonstrate that chemicals are harmful, to chemical producers, who must now demonstrate that substances are safe (Council of the European Union 2006). This change in the burden of proof has implications in terms of who will do environmental assessment and what the assessment endpoints are. Furthermore, ethical considerations to reduce, refine, or replace the use of animal tests in toxicology and ecotoxicology have gained growing public attention and support. For example, the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) legislation contains a specific chapter that addresses approaches for the avoidance of unnecessary testing (Council of the European Union 2006). Amidst these social changes, remarkable advances in the sciences took place, notably in the areas of analytical chemistry, computational chemistry, environmental modeling, information technology, and environmental chemistry and toxicology. The new technology and know-how make it possible to investigate a much wider array of chemical substances and to share scientific information better than was possible before. All of these developments have radically changed our perception of what POPs and PBTs are, what their properties are, how they can be identified, and how they can be managed. They justify taking a critical look at the frameworks, criteria, and methods that are used to evaluate substances. It is important that methods for chemical evaluation are scientifically sound and up to date with the current state of the science. It is equally important that such methods meet societal objectives and are effective, efficient in their allocation of resources, and consistent with current thinking on the environment.

Because an effective and efficient characterization of chemicals is in the interest of the environment, human health, and commerce, the Society of Environmental Toxicology and Chemistry (SETAC) organized a SETAC Pellston Workshop, "Science-based Guidance and Framework for the Evaluation and Identification of PBTs and POPs," held from 25 January to 1 February 2008 in Pensacola, Florida, USA (see Klečka and Muir 2009). The workshop involved scientists from academia, industry, and government. Seven study groups were convened to discuss persistence, bioaccumulation, toxicity, and risk. In this article, we document the deliberations of the bioaccumulation workgroup, which reviewed the current criteria and methods to identify bioaccumulative substances.

Bioaccumulation plays 2 roles in chemical evaluation initiatives. First, bioaccumulative properties of chemicals are considered in a chemical screening phase to identify potentially problematic substances. Second, the degree to which bioaccumulation occurs is considered in the assessment of chemical risk. Here, we address bioaccumulation in the former context, wherein bioaccumulation is viewed as an inherent property of the chemical that expresses the chemical's capacity to accumulate in organisms. Bioaccumulation in this context is independent of the actual chemical concentrations or chemical emissions in the environment. In Swackhammer et al. (2009), bioaccumulation is discussed in the context of exposure and risk assessment.

The objectives of this paper are to review the scientific rationale of criteria and methods to identify bioaccumulative substances and to make recommendations to improve the effectiveness and efficiency of chemical screening for bioaccumulative substances. In this paper, we strive to establish a sound scientific framework with broad support from international scientists in academia, government, and industry that can be use to harmonize chemical screening for bioaccumulation. To achieve this goal, we review current bioaccumulation regulations in relation to the current state of the science of bioaccumulation and make recommendations to improve the effectiveness and efficiency of bioaccumulation regulations.

A REVIEW OF BIOACCUMULATION REGULATIONS

Bioaccumulation is generally referred to as a process in which the chemical concentration in an organism achieves a level that exceeds that in the respiratory medium (e.g., water for a fish or air for a mammal), the diet, or both. The extent to which chemicals bioaccumulate is expressed by several quantities (Table 1), including the bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic or food web magnification factor (TMF).

Whether a substance is considered "bioaccumulative" in the regulatory context is determined by the regulation. In the text of the Stockholm Convention, there is no documented definition for a bioaccumulative substance. Chemicals are considered bioaccumulative under the Stockholm Convention if they meet the criteria listed in Annex D of the Stockholm Convention:

(i) Evidence that the bioconcentration factor or bioaccumulation factor in aquatic species for the chemical is greater than 5000 L/kg wet weight (ww) or, in the absence of such data, that log $K_{OW} > 5$;
(ii) Evidence that a chemical presents other reasons for concern, such as high bioaccumulation in other species, high toxicity, or ecotoxicity; or
(iii) Monitoring data in biota indicating that the bioaccumulation potential of the chemical is sufficient to justify its consideration within the scope of this Convention.

The first criterion is quantitative in nature and considered least ambiguous for its application (Kitano 2007). It has acted as a model for the development of criteria for bioaccumulation in several jurisdictions. The 2 remaining criteria are less quantitative in nature and include elements of risk, which are also addressed in the criteria relating to "adverse effects" in the Stockholm Convention. They are more difficult to operationalize considering the lack of a definition for bioaccumulation (Kitano 2007) and the apparent overlap in criteria of the Stockholm Convention. It is perhaps for these reasons, that criteria (ii) and (iii) have not been routinely used in chemical screening schemes for bioaccumulation other than in the Stockholm Convention. In a review of the application of bioaccumulation criteria in the Stockholm Convention, Kitano (2007) observed that 5 chemicals fulfilled the screening criteria for bioaccumulation despite their low BCFs (i.e., less than 5000). This illustrates that despite difficulties with their interpretation, criteria (ii) and

Table 1. Definitions of the bioconcentration factor (BCF), bioaccumulation factor (BAF), laboratory-based biomagnification factor (BMF), field-based BMF, trophic magnification factor (TMF), octanol–water partition coefficient, and octanol–air partition coefficient

Bioconcentration factor (BCF, L/kg wet weight [ww])	Ratio of the steady state chemical concentrations in an aquatic water-respiring organism (C_B, g chemical/kg ww) and the water (C_W, g chemical/L) determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water (but not in the diet).	$BCF = C_B/C_W$
Bioaccumulation factor (BAF, L/kg ww)	Ratio of the steady state chemical concentrations in an aquatic water-respiring organism (C_B, g chemical/kg ww) and the water (C_W, g chemical/L) determined from field data in which sampled organisms are exposed to a chemical in the water and in their diet.	$BAF = C_B/C_W$
Biomagnification factor—laboratory based (BMF, kg dry/kg ww)	Ratio of the steady state chemical concentrations in a water- or air-respiring organism (C_B, g chemical/kg ww) and in the diet of the organism (C_D, g chemical/kg dry) determined in a controlled laboratory experiment in which the test organisms are exposed to chemical in the diet (but not the water or air).	$BMF = C_B/C_D$
Biomagnification factor—field based (BMF, kg ww/kg ww)	Ratio of the steady state chemical concentrations in a water- or air-respiring organism (C_B, g chemical/kg ww) and in the diet of the organism (C_D, g chemical/kg ww) determined from field data in which sampled organisms are exposed to chemical in air, water, and diet.	$BMF = C_B/C_D$
Trophic magnification factor or food web magnification factor (TMF or FWMF, unitless)	The average factor by which the normalized chemical concentration in biota of a food web increases per trophic level. The TMF is determined from the slope (m) derived by linear regression of logarithmically transformed normalized chemical concentration in biota and trophic position of the sampled biota.	$TMF = 10^m$
Octanol–water partition coefficient (K_{OW}, unitless)	Ratio of the chemical concentrations in 1-octanol (C_O) and water (C_W) in an octanol–water system that has reached a chemical equilibrium.	$K_{OW} = C_O/C_W$
Octanol–air partition coefficient (K_{OA}, unitless)	Ratio of the chemical concentrations in 1-octanol (C_O) and air (C_A) in an octanol–air system that has reached a chemical equilibrium.	$K_{OA} = C_O/C_A$

(iii) have played an important role in identifying bioaccumulative substances and that criterion (i) alone has been deemed insufficient for identifying bioaccumulative substances.

Bioaccumulation regulations in Canada, Japan, the United States, and the European Union state specific criteria (listed in Table 2) to identify bioaccumulative substances. Table 2 illustrates a common approach among the regulations. All regulations identify bioaccumulative substances on the basis of the bioconcentration, bioaccumulation factor (defined relative to the chemical concentration in water), or octanol–water partition coefficient (K_{OW}). The use of data from field studies or dietary bioaccumulation experiments is not recognized in the criteria. The criteria apply to aquatic organisms (e.g., fish), whereas bioaccumulation measures in nonaquatic organisms are not considered. Table 2 further shows that regulatory criteria values do vary among the regulations.

The application of the regulations to the large number of chemicals in commercial use has presented difficulties for regulators and industrial manufacturers. First, information on the bioconcentration and bioaccumulation factors of chemicals is limited. For example, Arnot and Gobas (2006) reported that empirical bioconcentration and bioaccumulation factors were available for only 4% and 0.2%, respectively, of the chemicals on the Canadian Domestic Substances List, and only a fraction of the available data are of acceptable quality. The lack of information on bioconcentration and bioaccumulation factors caused the great majority of chemicals in Canada to be screened on the basis of the octanol–water partition coefficient. Although the octanol–water partition coefficient is a highly useful predictor for bioaccumulation in aquatic organisms, it is unable to account for any biotransformation of the chemical in organisms. Biotransformation reduces the degree of chemical bioaccumulation. Hence, screening on the basis of K_{OW} alone can produce false positives (i.e., chemicals considered to be bioaccumulative when in reality they are not). A second problem of the bioaccumulation regulations is that bioaccumulation data other than bioconcentration, bioaccumulation, or octanol–water partition coefficient are not readily usable for screening. For example, monitoring data of food web bioaccumulation of chemicals (e.g., trophic magnification factors) or data from dietary bioaccumulation experiments cannot be used for chemical screening. Also, available data on biotransformation rates cannot be readily considered in chemical screening because of the limitations of the screening criteria. This is unfortunate because field and laboratory data can provide insights into the bioaccumulation behavior of chemicals that are not obtained by the bioconcentration factor

Table 2. An overview of regulatory bioaccumulation assessment endpoints and criteria

Regulatory agency	Bioaccumulation endpoint	Criteria	Program
Environment Canada	K_{OW}	≥100000	CEPA 1999[a]
Environment Canada	BCF	≥5000	CEPA 1999
Environment Canada	BAF	≥5000	CEPA 1999
European Union "bioaccumulative"	BCF	≥2000	REACH[b]
European Union "very bioaccumulative"	BCF	≥5000	REACH
United States "bioaccumulative"	BCF	1000–5000	TSCA,[c] TRI
United States "very bioaccumulative"	BCF	≥5000	TSCA, TRI
United Nations Environment Program	K_{OW}	≥100000	Stockholm Convention[d]
United Nations Environment Program	BCF	≥5000	Stockholm Convention

[a] CEPA = Canadian Environmental Protection Act, 1999 (Government of Canada 1999, 2000).
[b] Registration, Evaluation, and Authorisation of Chemicals (REACH) Annex XII (European Commission 2001).
[c] Currently being used by the US Environmental Protection Agency in its Toxic Substances Control Act (TSCA) and Toxic Release Inventory (TRI) programs (USEPA 1976).
[d] Stockholm Convention on Persistent Organic Pollutants (UNEP 2001).

or octanol–water partition coefficient. A third problem is that bioconcentration tests are difficult to perform, especially for high K_{OW} chemicals that posses the largest apparent bioaccumulation potential. Creating aqueous solutions of extremely water insoluble substances poses particular experimental problems when conducting bioconcentration tests and causes problems in the interpretation of the results because the bioavailability of the chemical to the organism in the test is often in doubt. Also, for chemicals of high K_{OW} and low aqueous solubility, aqueous exposure is not the predominant route of chemical uptake in the environment. Furthermore, bioconcentration tests are considered to be time consuming and costly and require the use of live animals.

A REVIEW OF BIOACCUMULATION SCIENCE

The phenomenon that man-made chemicals can bioaccumulate in food webs was first brought to the attention of the scientific community in a paper by Woodwell (1967). Woodwell argued that ecological cycles can concentrate pollutants to levels at which they may be harmful to animal and human lives. His work showed that concentrations of DDT in biota of a salt water marsh increased with increasing trophic position. He proposed that the magnification phenomenon occurs as a result of biomass conversion in food chains. To confirm the occurrence of DDT bioaccumulation in food webs, Hamelink et al. (1971) conducted experiments in farm and artificial ponds and observed the bioaccumulation of the DDT concentration from water to aquatic biota but could not confirm that the magnification of DDT in the food chain was due to predator-prey relationships. The authors proposed that the DDT bioaccumulation was the result of DDT exchange between the water and fats of the organism caused by differences in solubilities of DDT in water and fat. Increases in DDT concentrations between trophic levels were explained by differences in fat content among the organisms. Bioaccumulation due to a solubility-controlled water–animal exchange was later referred to as "bioconcentration," and the degree of bioconcentration was expressed by the bioconcentration factor ($BCF = C_B/C_W$), the ratio of chemical concentrations in animal (C_B) and water (C_W) at steady state. This work was followed by a number of experimental studies involving the bioaccumulation of hydrophobic organic chemicals in fish under controlled laboratory conditions, wherein fish were exposed to test chemicals via the water (e.g., Neely et al. 1974; Veith et al. 1979). These studies showed excellent correlations between the bioconcentration factor and the octanol–water partition coefficient (K_{OW}), a commonly used descriptor of lipid–water exchange of pharmaceuticals at the time. The significance of this work was that the bioaccumulation of chemicals in nature became recognized as a predictable phenomenon. These studies fostered the current regulatory "lipid–water partitioning" approach that became the theoretical basis of current methods to identify bioaccumulative chemicals.

However, field studies of the bioaccumulation of DDT, PCBs, and other persistent halogenated organic chemicals in the Great Lakes in the late 1980s and early 1990s showed that chemical concentrations in biota expressed either on a lipid weight basis or in terms of fugacities were greater than expected from lipid–water partitioning and also increased with increasing trophic position (Connolly and Pedersen 1988). The results meant that lipid–water partitioning alone is unable to explain the distribution of chemicals in food webs and that an additional magnification process occurs that causes chemical transport from a low lipid–normalized concentration (or fugacity) in the prey to a higher concentration (or fugacity) in the predator. This process was referred to as biomagnification. Biomagnification is fundamentally different from the bioconcentration process in that it involves chemical transport against the thermodynamic gradient (i.e., from a low fugacity in the prey to a higher fugacity in the predator), whereas bioconcentration involves equilibrium partitioning in which the fugacity in the organism can at the most achieve that in the water. Laboratory and field studies to determine how predators can biomagnify chemicals in their prey showed that food digestion and absorption cause ingested chemical to "concentrate" in the gastrointestinal tract (Gobas et al. 1993, 1999). This gastrointestinal magnification process can cause chemicals to biomagnify in

food webs if the rates of chemical elimination and metabolic transformation are low. The implication of these studies is that, currently, 2 processes are recognized to contribute to chemical bioaccumulation. They are bioconcentration (i.e., chemical exchange between the respiratory medium and the organism) and biomagnification (i.e., chemical magnification from dietary ingestion). Chemicals with the greatest capacity to bioaccumulate are those subject to both bioconcentration and biomagnification.

Earlier bioaccumulation studies predominantly focused on chemical distribution in aquatic food webs. Only in the last few years has the bioaccumulation behavior of organic chemicals in terrestrial food webs been studied systematically. These studies produced new insights into the bioaccumulation process with some important consequences for identifying bioaccumulative substances in nonaquatic food webs. One of the key observations was that less hydrophobic (i.e., more hydrophilic) chemicals such as chlorobenzenes and lindane, which have K_{OW}s and BCFs in fish experiments far below the regulatory criteria of 100000 (for K_{OW}) and 5000 L/kg ww (for BCF), were found to exhibit a high degree of biomagnification in lichen–caribou–wolf (Kelly and Gobas 2001, 2003) and marine mammalian food chains (Kelly et al. 2007) in northern Canada. Also, perfluorinated sulfonic acids such as perfluorooctane sulfonate (PFOS), which with a calculated $K_{OW} < 100{,}000$ does not biomagnify in laboratory tests with fish (Martin et al. 2003, 2004), show a high degree of biomagnification in birds and marine mammals (Martin et al. 2004; Tomy et al. 2004; Houde et al. 2006). These findings indicate that hydrophobic (high K_{OW}) chemicals are not the only chemicals with biomagnification potential and that bioaccumulation behavior in fish and aquatic piscivorous food chains cannot serve as a universal model for the bioaccumulation behavior of chemicals in wildlife and humans beings. Current bioaccumulation criteria and assessment schemes therefore fail to identify many substances that are bioaccumulative in wildlife and in human subsistence and agricultural food chains (Kelly et al. 2007; Czub and McLachlan 2004).

Also, there is ample evidence that many substances that are expected to be bioaccumulative on the basis of their hydrophobicity have no or less bioaccumulative capacity than expected because they are quickly biotransformed (Weisbrod et al. 2007). Such chemicals can be incorrectly identified as being bioaccumulative if evaluated on the basis of the octanol–water partition coefficient alone. Biotransformation is therefore an important phenomenon that should be appropriately recognized in chemical evaluation schemes. However, including biotransformation in chemical evaluation frameworks is challenging. Currently, no standard test methods for measuring or calculating biotransformation rates exist. Computational methods to calculate such rates are in their infancy, in that they lack the empirical data to develop quantitative structure–activity relationships (QSARs). Furthermore, variation in biotransformation capacity can be substantial among biota. In addition, biotransformation can produce metabolites (e.g., dichlorodiphenyldichloroethylene [DDE]), which in some cases can be bioaccumulative themselves. However, methods for including metabolic transformation in the bioaccumulation evaluation process have recently emerged. For example, Arnot et al. (2008) developed methods for back-calculating biotransformation rates from in vivo bioconcentration data. Han et al. (2007) applied bioassays involving fish hepatocytes to measure in vitro hepatic metabolic transformation rates. Nichols et al. (2006, 2007) showed that physiology-based pharmacokinetic models can be useful in translating in vitro biotransformation rates into in vivo biotransformation rates that can be used in food web bioaccumulation models, and Cowan-Ellsberry et al. (2008) showed that this approach is feasible and can improve the estimation of the BCF for chemicals that are metabolized rapidly.

REVIEW CONCLUSIONS

The review of criteria and methods for identifying "bioaccumulative" in the context of the current state of the science of bioaccumulation produced several conclusions.

1. The lack of a definition for a bioaccumulative substance (a) impedes the application of highly relevant scientific data on bioaccumulation, (b) provides a barrier to the application of new methodologies for determining and assessing bioaccumulation, and (c) contributes to the lack of global harmonization of regulatory bioaccumulation screening initiatives.
2. The BCF is no longer recognized to be a good descriptor of the biomagnification capacity of chemical substances. In addition, the BCF is determined in bioconcentration tests, which are (a) difficult to perform for very poorly water soluble organic chemicals with high bioaccumulation potential, (b) time consuming, and (c) costly. Bioaccumulation evaluations carried out under the Stockholm Convention for POPs show that the BCF was unable to identify the bioaccumulative capacity of several substances.
3. The criteria for BCF, BAF, and K_{OW} currently used in the screening of chemical bioaccumulation are applicable to water-respiring organisms of aquatic food webs but are inadequate for identifying chemicals that biomagnify in air-respiring organisms of food webs (e.g., terrestrial and agricultural food webs) such as mammals, birds, and human beings.
4. The shortage of empirical BCF and BAF data for the many thousands of commercial chemicals implies that the great majority of commercial chemicals can be expected to be screened with the use of the K_{OW} criterion. Because K_{OW} is a chemical property that does not recognize biological and environmental factors (e.g., biotransformation, membrane permeation rates, bioavailability) controlling the degree of chemical bioaccumulation, bioaccumulation screening on the basis of K_{OW} can be expected to produce many false positives.
5. Because current bioaccumulation evaluation schemes are no longer based on up-to-date and sound science information, nor do they consider all relevant scientific information from field and laboratory studies and are unable to include existing and new methodologies for identifying bioaccumulative substances, the credibility of the regulatory approach is at stake. A high level of credibility is required to ensure that industry, government, and academia productively participate in the timely evaluation of chemical substances. Therefore, it is important that the current criteria and methods used for evaluation of commercial chemicals for their bioaccumulative properties are updated.

RECOMMENDATIONS

A definition for bioaccumulative substances

Currently, there is not a scientific definition for a "bioaccumulative" substance in the text of the Stockholm Convention or in the regulations for evaluating commercial chemicals for bioaccumulation in Canada, the European Union, and the United States. Chemicals are considered "bioaccumulative" if they meet specific criteria outlined in the previous section. Because substances are only deemed bioaccumulative if they meet the stated criteria, improvements in the understanding of what makes chemicals bioaccumulative cannot be readily considered in the evaluation process. This means that other bioaccumulation criteria and alternative and new methods of identifying bioaccumulative substances cannot be included in the evaluation process. The latter limits regulators, scientists, and chemical manufacturers to take advantage of available data and methods to determine the bioaccumulative nature of commercial chemical substances. Considering the large numbers of chemicals that require evaluation, this is a significant impediment to achieving the goals of the regulations.

During the development of the Stockholm Convention, discussions took place on the rationale for the bioaccumulation criteria. These discussions were not formally documented, as far as we know. However, personal records of some of the participants are still available. From these records it appears that the goal of the bioaccumulation criteria was to identify chemicals that have the capacity to biomagnify in food chains. Biomagnification is the phenomenon wherein chemical concentrations in biological organisms increase with increasing trophic level. These substances were considered to be of concern because they have the capacity to reach their highest concentrations in upper trophic level organisms and humans. High concentrations increase the possibility of health effects in biota and humans.

The rationale for identifying the most troublesome bioaccumulating chemicals as those that biomagnify in the food chains is still applicable to date. Workgroup discussions produced unanimous agreement on this issue and produced the following definition for a bioaccumulative substance: A substance is considered bioaccumulative if it biomagnifies in food chains.

Biomagnification is defined as the phenomenon wherein the normalized concentration (or fugacity) of the chemical in biological organisms increases with increasing trophic position. This definition refers to "normalized" chemical concentrations to account for differences in body composition of organisms in food chains. Predators (e.g., harbor seals) can differ substantially in body composition (e.g., in lipid content) from their prey (e.g., fish). Hence, comparing chemical concentrations in predators and prey organisms can amount to comparing apples and oranges. To enable comparison of chemical concentrations among a wide array of organisms, chemical concentrations need to be expressed on a common basis, such that the concentrations can be compared to determine the occurrence of biomagnification. Expressing chemical concentrations in terms of their corresponding fugacities is a well grounded and thermodynamically based method to accomplish this (Connolly and Pedersen 1988; Mackay and Fraser 2000). A number of authors have applied this normalization successfully to express the degree of biomagnification of organic substances in food webs (e.g., Kelly and Gobas 2001, 2003). Other methods include lipid normalization of chemical concentrations (Mackintosh et al. 2004). Lipid normalization involves dividing the wet weight–based chemical concentration in a biological sample by the lipid content of that sample. The result is a chemical concentration in the lipids of organisms that can be compared among different organisms, as long as animal lipids are comparable in their ability to dissolve the chemical substance. More recent normalization methods recognize the contribution of the lipid, protein, carbohydrate, and water fraction of animal tissues (Kelly et al. 2007, 2008). The relative solubility of chemicals in proteins, carbohydrates, and water is expressed relative to that in lipid to derive a lipid-equivalent chemical concentration (g chemical/kg equivalent lipid; deBruyn and Gobas 2007), which is comparable to but differs from lipid-normalized concentrations in that they include the contribution of proteins, carbohydrates, and water in organism tissues to absorb the chemical substance. The latter is important when evaluating chemical concentrations in organisms with a low lipid content (e.g., mussels) or high carbohydrate content (algae, plants) or for substances that bind strongly to proteins (e.g., perfluorinated sulfonic acids) or that ionize in aqueous solution.

Trophic position expresses predator–prey relationships in food webs. It can be determined from analyses of the intestinal contents of organisms by a simple model (Vander Zanden and Rasmussen 1996). Stable nitrogen isotope ratios in animal tissues provide an alternative method to determine trophic status (Vander Zanden et al. 1997). Typically, N^{15}/N^{14} ratios in animal tissues increase with increasing trophic position in food webs. N^{15}/N^{14} ratios therefore provide a useful empirical surrogate for trophic position.

Criteria for bioaccumulative substances

After establishing a definition for bioaccumulative substances, the workgroup addressed criteria that can be used to identify bioaccumulative substances. The criteria that were discussed recognize different types of information that can be used for evaluation, such as information from field studies, laboratory experimentation, food web modeling, structure-property relationships and molecular computation. The weight of evidence of information obtained by different routes of investigation was evaluated and included in a tiered evaluation scheme that is presented below.

Trophic magnification factor

Workgroup members agreed that information from field studies provides the most conclusive evidence of the ability of chemicals to biomagnify in food webs. Field studies involving the collection and analyses of biota of different trophic levels can document the change in chemical concentrations in a food web and hence the occurrence of biomagnification. The most relevant measure of biomagnification in food webs is the Trophic Magnification Factor (TMF) or Food web Magnification Factor (FWMF) (Fisk et al. 1998; Mackintosh et al. 2004). The TMF can be determined from a correlation between appropriately normalized chemical concentrations in biota and the trophic positions of the sampled biota. Normalized chemical concentrations are first expressed on a logarithmic basis and then plotted as a function of trophic position. Linear

regression is then used to calculate the slope m, which is used to derive the TMF as

$$TMF = 10^{m} \quad (1)$$

No change of the chemical concentration over several trophic levels produces a slope m of 0 and a TMF of 1, i.e., magnification of the chemical at each trophic level by a factor of 1 or no biomagnification. An increase in the logarithm of normalized chemical concentrations with increasing trophic level produces a $m > 0$ and a $TMF > 1$, which indicates the occurrence of biomagnification. For example, if $m = 1$, then $TMF = 10$ and the chemical concentration magnifies 10 times for each unit increase in trophic level. A drop of the logarithm of normalized chemical concentration with increasing trophic level, produces a $m < 0$ and a $TMF < 1$, indicating trophic dilution, i.e. the opposite of biomagnification when chemical concentrations drop with increasing trophic level.

To derive a TMF, it is important to express observed chemical concentrations on a normalized basis to account for differences in chemical concentrations among organisms of a food web due to differences in (body) composition of organisms or sampled tissues. Normalization provides an appropriate theoretical foundation for the comparison of chemical concentrations. For example, comparing concentrations in whale blubber samples to those in fish muscle samples can produce large differences in the concentrations of fat soluble substances as the fat content of blubber can be 80 to 100% while the muscle tissue of fish may be as low as 2%. In this example, differences in concentrations between whale blubber and fish muscle tissues do not only reflect differences in trophic position but also differences in fat composition. Only, if the chemical concentrations are normalized to the fat content of the samples, differences in concentrations due to trophic status become apparent. Lipid or lipid-equivalent normalization are appropriate techniques for many fat soluble substances (Mackintosh et al. 2004; Kelly et al. 2007). However, normalization based on protein content, organic carbon or carbohydrate content can also be required (deBruyn and Gobas 2007). For example, chemical concentrations in phytoplankton and plant materials are often best expressed on an organic carbon basis, while substances with a high protein binding capacity (e.g., perfluorinated sulfonic acids) are best normalized to protein content.

Trophic position can be determined by conducting analyses of the intestinal contents of organisms. A trophic positioning model is then used to assign a numerical value for the trophic position. Stable N^{15}/N^{14} nitrogen isotope ratios in animal tissues provide an alternative method to determine trophic status. Typically, N^{15}/N^{14} nitrogen isotope ratios in animal tissues increase with increasing trophic position in food webs. N^{15}/N^{14} nitrogen isotope ratios therefore provide a useful and inexpensive empirical measure or surrogate for trophic position. Several authors have suggested that increases in the N^{15}/N^{14} nitrogen isotope ratio of 0.34% to 0.38% correspond with a 1-unit increase in trophic position.

When using the TMF as a measure of biomagnification, an appropriate criterion for identifying bioaccumulative substances is when:

$$TMF > 1 \quad (2)$$

This criterion will be met if the slope m of the correlation between the logarithm of normalized chemical concentrations and trophic position is significantly greater than 0. A statistical significance test often focuses on reducing the probability of type I errors, i.e., false positives, i.e., chemicals that appear to biomagnify while in reality they do not. However, in chemical screening it may be preferable to reduce false negatives (i.e., chemicals that do not appear to biomagnify while in reality they are).

It is crucial that in the characterization of the TMF both aquatic and terrestrial food webs are considered. This is due to the fact that chemicals can exhibit fundamentally different TMFs in aquatic and terrestrial food webs due to differences in bioaccumulation mechanism between water and air breathing organisms. Kelly and others (2001, 2007) have documented several substances that do not show evidence of biomagnification in aquatic food webs involving water breathing organisms (i.e., a $TMF \leq 1$) while terrestrial and marine mammalian food webs including air-breathing organisms demonstrate a high degree of biomagnification (i.e., $TMF > 1$).

The biomagnification factor

While the workgroup acknowledges the high weight of evidence of field bioaccumulation data involving organisms of multiple trophic levels, it recognizes that data of this kind are unavailable for many chemicals that require evaluation. The workgroup therefore proposed a second criterion for identifying bioaccumulative substances based on evidence of biomagnification in a single trophic relationship. This criterion applies the biomagnification factor or BMF derived either under controlled laboratory conditions or based on field data. The BMF is the ratio of appropriately normalized chemical concentrations in a specific organism and that in the organism's diet or prey at steady-state.

$$BMF = C_{predator}/C_{prey} \quad (3)$$

It can be measured by conducting experiments under controlled laboratory conditions where test organisms are exposed to a constant chemical concentration in their diet. During an uptake phase, chemical concentrations are followed over time, ideally until the concentration in the organism no longer changes with time (i.e., steady-state). The ratio of the chemical concentrations in the test animals and their diet at steady-state is the BMF. For the same reasons as discussed earlier for the TMF, it is important that chemical concentrations are normalized to account for differences in composition of the organism and the diet. If a steady-state cannot be reached in the experiment, the uptake phase is followed by a depuration phase where the organisms are exposed to uncontaminated food. The rate of decline in chemical concentration over time measured in the depuration phase can then be used to derive the chemical uptake rate from the concentration-time data in the uptake phase. In a fashion similar to that used in the OECD bioconcentration test protocol (OECD 1996), the ratio of uptake and elimination rates can be used to derived the steady-state biomagnification factor.

An alternative to the BMF measured in laboratory experiments is the BMF determined in field studies. The field derived BMF is the ratio of normalized chemical concentrations in predator and prey for a well characterized predator-prey trophic interaction. The field and laboratory derived BMFs differ in the sense that the field BMF involves exposure of the predator to chemical in both the respiratory

medium and the diet while the BMF derived in laboratory tests only includes dietary uptake of the chemical.

The criterion that can be used to indicate the capability of the chemical to biomagnify is

$$\mathrm{BMF} > 1 \quad (4)$$

A BMF statistically greater than 1 indicates that the chemical is a probable bioaccumulative substance. Because it is possible that a chemical can biomagnify in certain organisms (e.g., fish) but not in others (e.g., mammals and birds), for example due to greater capability of higher trophic level organisms to biotransform chemicals, the BMF provides less conclusive information than the TMF. The statistical significance level of the BMF criterion requires special consideration as BMFs are typically subject to substantial variability. An appropriate significance level should be set to provide the corresponding level of acceptability of type I and II errors.

Biomagnification factors in both air and water breathing organisms must be considered because certain chemicals which do not biomagnify in water breathing organisms can biomagnify in air breathing organisms and visa versa. Depending on the physical-chemical properties of the substances, certain chemicals have the propensity to biomagnify in terrestrial food chains while others biomagnify in water breathing organisms of aquatic food chains, while yet others can biomagnify in organisms of all food webs.

Standardized protocols for determining laboratory based BMFs, like the OECD 305 standard bioconcentration test, do currently not exist. However, a number of authors have applied dietary bioaccumulation studies to measure the BMF in fish and mammalian species as well as to approximate the BCF (Bruggeman et al. 1981; Parkerton 2004). These methods administer food, which is ''artificially'' (through spiking) or ''naturally'' (through partitioning) contaminated with test chemicals, to the test organisms over a specified duration while simultaneous chemical uptake via the respiratory and other routes is avoided. The chemical concentrations in the test organisms are measured over time during this uptake period. After the uptake period is completed, non-contaminated food is administered to the test organisms and the chemical concentration in the organisms is followed over time. As detailed in the supporting information, the concentration measurements during the uptake and depuration phase of the biomagnification experiment can be used to derive the BMF. We recommend the use of rainbow trout and rats in BMF tests because of the long-term experience with these commonly used test species and because of access to data previously collected for these test animals that can be used in the construction of BMF data bases for decision making. The biomagnification test avoids the dissolution of the chemical substance in water or air that occurs in bioconcentration and aerial exposure tests. Aqueous and gaseous exposure can pose particular experimental challenges for very hydrophobic and poorly volatile substances. Among the many chemicals in commerce, very hydrophobic and poorly volatile substances are the most susceptible to bioaccumulate in food chains and hence often a priority for testing. The BMF test is considered to be a simpler and more reliable and perhaps cheaper alternative to the BCF test for these substances. When characterizing the BMF by using field concentration data, it is important to include both a mammalian or bird species (i.e., representing air breathing organisms) and a submerged aquatic species such as fish (i.e., representing water-breathing organisms). If a BMF test is to be included in chemical screening frameworks, it is important that a standard protocol for the biomagnification test is developed.

The bioconcentration factor

While the BCF is generally used to characterize bioaccumulative or B substances, the workgroup concluded that the BCF is not a good surrogate for the BMF or TMF in terrestrial food webs and that in many cases, the BCF is not a reliable indicator of chemical biomagnification in aquatic food chains. These conclusions are supported by the observations and arguments of Kitano (2007), who reported that the UN POPRC assessed 5 chemicals to be bioaccumulative in the context of the Stockholm Convention while the BCF criterion was not met. As discussed above, the Stockholm Convention on POPs considers bioaccumulation criteria in addition to the BCF. However, criteria other than the BCF are not considered in chemical screening frameworks other than that of the Stockholm Convention.

The reason for the poor ability of the BCF to identify bioaccumulative substances is that the BCF quantifies chemical bioaccumulation from water but not from the diet. Dietary bioaccumulation is responsible for biomagnification in food webs. Dietary bioaccumulation originates in the gastro-intestinal tract where food digestion and absorption concentrate the chemical in the GIT. This phenomenon cannot be measured in bioconcentration tests where dietary exposure of the test organism is deliberately avoided. The BCF determined in fish bioconcentration tests is therefore not a measure of dietary bioaccumulation. However, under certain conditions, the BCF in fish can be a surrogate for the occurrence of chemical biomagnification in aquatic food chains. For example, bioaccumulation modeling (Arnot and Gobas 2003) and laboratory experiments (Fisk et al. 1998) show that chemicals with BCFs $<$ 5000 L/kg ww and a log $K_{OW} < 5$ exhibit no biomagnification in fish with a lipid content of 5%. This is because the rate of chemical depuration in fish is sufficiently high to counteract chemical magnification in the intestines and hence prevent biomagnification in fish. However, for more hydrophobic substances (i.e., log $K_{OW} \geq 6$), the bioconcentration test is problematic and the BCF is subject to considerable experimental error, which often artificially lowers the BCF (Arnot and Gobas 2006). Hence, for such chemicals, BCFs measured to be less than 5000 L/kg ww are not necessarily indicative of the sufficiently high depuration rates required to prevent dietary biomagnification in fish. Also, relatively high BCFs are not necessarily indicative of the occurrence of biomagnification. The latter has been observed for certain chemicals (e.g., esters) which are quickly degraded in the intestinal tract after ingestion but which are more slowly degraded by organisms after aqueous exposure. Such chemicals are subject to chemical transformation in the intestines, which lowers dietary absorption and causes a reduction or absence of biomagnification. However, this is not revealed in bioconcentration tests because bioconcentration tests exclude dietary exposure. Fish bioconcentration tests therefore have a limited capacity to identify bioaccumulative substances in aquatic food webs and are difficult to perform for poorly water soluble substances with the greatest potential for biomagnification.

Fish bioconcentration tests do not have the capacity to identify bioaccumulative substances in food webs that include

air-breathing organisms. This is because chemical depuration rates in fish can have little resemblance to the depuration rates in mammals. Respiratory elimination in fish and mammals occurs to different media (i.e., water and air) and their rates are controlled by different chemical characteristics. The latter has been demonstrated in field studies which showed that while certain chemicals lacked the capacity to biomagnify in water-breathing organisms of aquatic food webs, they showed a high degree of biomagnification in air-breathing organisms of terrestrial and marine mammalian food webs (Kelly and Gobas 2001; Kelly et al. 2007). Differences in metabolic capacity between fish and mammals can further contribute to differences in depuration rates between fish and mammals that can interfere with the correct interpretation of fish bioconcentration test results.

Because bioconcentration tests are essentially incapable of measuring biomagnification in food webs, the workgroup recommends that bioconcentration test results are treated with the greatest of care to identify bioaccumulative substances in chemical screening initiatives. The application of the often-used criterion that identifies that chemicals are bioaccumulative

$$\mathrm{BMF} > 5000 \quad (5)$$

can play a useful role in bioaccumulation screening in water-respiring organisms. However, substances that are transformed at high rates in the intestinal tract but are transformed at much lower rates elsewhere in the organism can be miscategorized by this criterion. For chemicals with a log $K_{OW} \leq 5$, BCFs $\leq$ 5000 L/kg ww indicate a lack of biomagnification potential in water-respiring organisms. However, for chemicals with a log $K_{OW} > 6$, observed BCFs below 5000 L/kg ww are often insufficient to proof the lack of biomagnification potential in water-respiring organisms of aquatic food webs. Guidelines have been proposed for the evaluation of the results from bioconcentration tests (Arnot and Gobas 2006). Application of those guidelines will reduce the probability of miscategorization chemicals for their bioaccumulation behavior in fish. However, those guidelines are insufficient to avoid errors that occur when BCF data are used to identify bioaccumulation potential in air-breathing organisms.

K_{OW} and K_{OA}

Arnot and Gobas (2006) reported that empirical bioaccumulation data were available for only approximately 4% of all the chemicals that required chemical screening in Canada. This illustrates the need for alternative criteria and methods to carry out bioaccumulation screening in a timely fashion. The REACH program's objective to minimize the need for animal studies, further points out the need to develop criteria and methods for bioaccumulation evaluation that do not involve animal testing or sampling.

The octanol–water partition coefficient has played a key role in predicting the bioaccumulation potential of chemicals in fish for many years and has been adopted in bioaccumulation screening worldwide. The octanol–water partition is a physical chemical property that can be measured without the need to include animals. In addition, reliable methods are available to calculate the octanol–water partition coefficient from chemical structure. This means that the octanol–water partition coefficient can be derived for most organic chemicals in commerce.

The workgroup concluded that the K_{OW} is a highly useful chemical specific descriptor of the bioaccumulation potential of chemicals in fish and many other water breathing aquatic organisms. The octanol–water partition coefficient expresses the tendency of chemical substances to partition from water into the lipids of fish. A chemical with a high octanol–water partition therefore has a high potential for bioaccumulation. However, whether this potential is realized depends on a number of other factors, including the rate of chemical biotransformation, the rate of membrane permeation, the bioavailability of the chemical and the rate of growth of the animal. For example, if a chemical is biotransformed by the organisms at a sufficiently high rate, then the chemical will not be able to distribute between water and the fat of the organisms as expected from the octanol–water partition coefficient. Also, a low rate of chemical permeation across membrane systems can produce a smaller degree of bioaccumulation than anticipated from K_{OW}. As a result, the octanol–water partition coefficients can only express the potential for chemical bioaccumulation and only in water respiring aquatic organisms. Despite its limitations, K_{OW} can play a key role in chemical screening. A review of documented BCFs and BAFs for organic chemicals shows that non-ionizable, non-polar organics, chemicals with a log $K_{OW} < 4$ do normally not biomagnify in water respiring organisms of aquatic food webs (Arnot and Gobas 2006). Also, there is no evidence that these substances exhibit BCFs > 5000. This implies that large numbers of chemicals can be quickly screened for bioaccumulation in aquatic systems based on K_{OW} alone.

To evaluate the potential of chemicals to bioaccumulate in food webs that include air-respiring organisms, the workgroup recommends that the octanol–air partition coefficient (K_{OA}) is used. The octanol–air partition coefficient expresses the tendency of chemicals to partition between lipids and air. Empirical methods for the determination of K_{OA} are available (Harner and Mackay 1995) and K_{OA} is included in chemical data bases such as the Environmental Fate Data Base (EFDB) in EPISuite (USEPA 2004) and in Handbooks (Mackay et al. 1999). A chemical with a high K_{OA} eliminates slowly by exhalation in air-respiring organisms (Gobas et al. 2003). A lower elimination rate of the chemical implies a higher degree of bioaccumulation. Field observations and bioaccumulation models (Kelly and Gobas 2001, 2003; Arnot and Gobas 2003; Czub and McLachlan 2004; Armitage and Gobas 2007; Kelly et al. 2007) indicate that chemicals with log $K_{OA} > 5$ to 6 can biomagnify in air-respiring organisms, even the chemical's log $K_{OW} < 5$. The data and models also show that chemicals with a low K_{OW} (i.e., log $K_{OW} < 2$) are quickly eliminated by urinary excretion in air-respiring organisms, causing chemicals with a high K_{OA} not to biomagnify, even though their elimination rate from exhalation is low. Comparable K_{OA} cut-offs for the biomagnification potential were proposed by Czub and McLachlan (2004) on the basis of the models of chemical distribution in human agricultural food chains. As with K_{OW}, the K_{OA} can only reveal whether the chemical possesses the physicochemical partitioning characteristics that make it possible to biomagnify. The K_{OA} does not reveal the chemical's ability to permeate through membranes or to be biotransformed in organisms. Hence, criteria based on K_{OA} can overestimate the actual biomagnification capacity if the chemical is biotransformed at a sufficiently high rate or if the chemical has a low rate of membrane permeation. For the

evaluation of chemical biomagnification potential in terrestrial food webs involving air-breathing organisms, we expect chemicals with log $K_{OA} < 5$ not to have the inherent capacity to biomagnify in terrestrial food webs. These substances can be excluded from further evaluation of the bioaccumulation potential in terrestrial food chains and might not require BMF tests in air-respiring organisms.

Bioaccumulation models

Bioaccumulation models have played a key role in evaluating the bioaccumulation potential of commercial chemicals in Canada (Environment Canada 2003; Arnot and Gobas 2006). Bioaccumulation models can calculate bioconcentration factors, bioaccumulation factors, biomagnification factors, and the trophic magnification factor of chemicals in various food webs, including aquatic (e.g., Arnot and Gobas 2004) and terrestrial (e.g., Armitage and Gobas 2007) food webs. The models use the properties of the chemical but also include algorithms to calculate the effect of several biological and environmental factors on the degree of bioaccumulation. They include several classes of organisms such as plants, fish, mammals, birds, and humans. The models also recognize the role of membrane permeation, bioavailability, fecal egestion, growth of the animals and the biochemical composition of the organisms (e.g., lipid content). They also include environmental parameters such as temperature, the organic carbon content of bottom and suspended sediments, and suspended particle concentrations in water and air. The bioaccumulation models can also take into account the effect of metabolic transformation on the bioaccumulation capacity. However, the models are yet unable to predict in vivo biotransformation rates of chemicals. Biotransformation rates can be determined experimentally and then included in the model calculations, as discussed in Cowan-Ellsberry et al. (2008).

Because bioaccumulation models recognize biological and environmental factors, they are more refined tools for identifying potentially bioaccumulating substances than chemical properties, such as K_{OW} and K_{OA}. To identify potentially bioaccumulative substances, the models can calculate the BMF and TMF, which can be compared with the criterion values discussed earlier (i.e., BMF or TMF > 1 indicates the potential for biomagnification).

FRAMEWORK FOR APPLICATION

Recommendations for a framework for identifying bioaccumulative substances are shown in Figure 1. The proposed framework includes a 5-step process and can incorporate data from field studies, standard laboratory tests, bioaccumulation models, and physicochemical property data.

Step 1 involves the selection of appropriate food webs for the assessment process. Historically, aquatic food webs, including phytoplankton, zooplankton, benthic invertebrates, and fish, have been considered. However, such food webs do not provide an adequate model for evaluation. The primary objective of the bioaccumulation evaluation is to identify chemicals that can biomagnify in food webs and reach relatively high concentrations in upper-trophic level organisms such as raptors, whales and humans. Food webs considered in the evaluation should include such organisms. Examples of relevant food webs are the grass–cow–human food chain considered in Czub and McLachlan (2004) and soil–worm–shrew–raptor in Armitage and Gobas (2007). At a minimum, the food webs considered in the bioaccumulation evaluation should include water- and air-respiring biota because of the fundamental differences in the bioaccumulation behavior of these organisms. The selection of the food web will have important implications for the design of field studies to measure the TMF or for the development of laboratory-based test protocols for determining the BMF.

Step 2 involves the characterization of the TMF of the compound. This step can only occur if TMF data exist or appropriate field studies are conducted. If the TMF criterion is met (i.e., TMF > 1), the chemical substance should be considered bioaccumulative. The TMF is the most conclusive evidence of the bioaccumulative nature of the compound. Hence, TMF > 1 confirms the bioaccumulative capacity of the substance. The TMFs in both aquatic food webs, including water-respiring organisms, and nonaquatic food webs, including air-respiring organisms, have to be considered.

Step 3 determines whether the substance is a probable bioaccumulative substance. It involves the determination of the BMF through either controlled laboratory or field studies. This evaluation is meant to be applied if reliable TMF data are not available or if such data cannot be determined (e.g., cases in which the chemical substance is not yet commercialized or concentrations in the environment are below detection limits). This step involves conducting a standard biomagnification test of the chemical under controlled laboratory conditions, an analysis of field bioaccumulation data to determine the chemical's BMF, or both. If BMF > 1, the chemical is considered a probable bioaccumulative substance. Biomagnification tests have been carried out by a number of investigators in both fish and mammalian species (e.g., rats), but a protocol for a standardized biomagnification test does currently not exist. If the BMF is used in bioaccumulation screening, it is important that standardized test protocols are developed for the biomagnification test. Also, guidance is required for the derivation of the BMF from field concentration data. It is further imperative that the BMFs are determined in both water- and air-respiring organisms because of their different elimination mechanisms that affect the BMF. Rainbow trout and rats are considered to be suitable species for BMF tests.

Step 4 applies available data on BCFs, determined under controlled laboratory conditions, or BAFs, derived from concentration data collected in field studies to determine whether it is possible for the chemical to biomagnify in water-respiring organisms of aquatic food webs. Step 4 takes advantage of BCF and BAF data that are often required by regulatory agencies. However, data on the BCF and BAFs of substances are considered to be less conclusive in revealing the bioaccumulative nature of substances than data on the BMF or TMF.

For substances with log $K_{OW} < 6$, for which standard OECD 305 experimental protocols often provide reliable information on the BCF, a BCF ≥ 5000 L/kg ww indicates that the chemical posses a sufficiently low rate of chemical depuration that dietary biomagnification is possible. However, it should be stressed that the BCF test does not provide information on biotransformation in the gastrointestinal tract after ingestion. A high rate of chemical reaction in the intestinal tract can prevent the occurrence of biomagnification, even if the BCF is high. Examples of chemicals that behave in this way are phthalate esters and polybrominated biphenyls, which are biotransformed in the intestines of fish

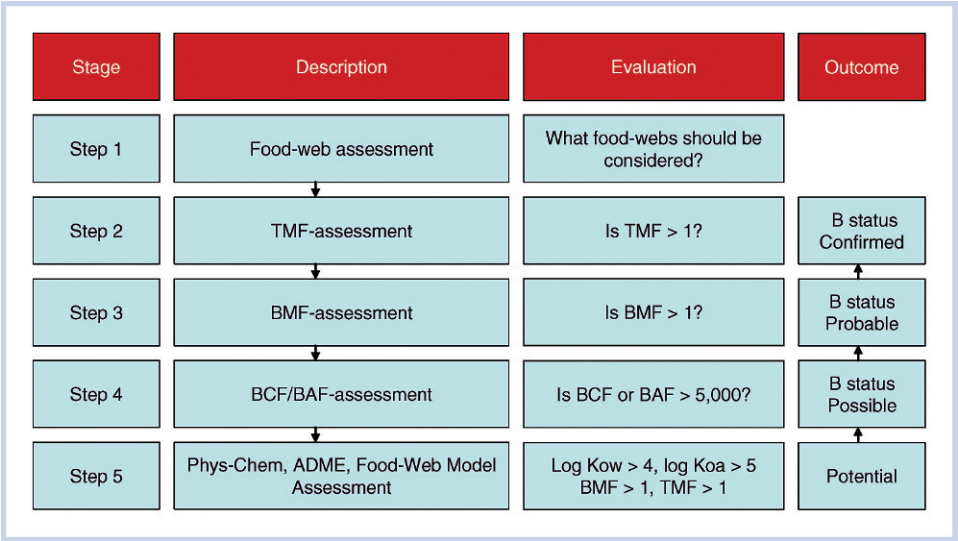

Figure 1. Outline of a B-assessment framework. Society of Environmental Toxicology and Chemistry (SETAC) Pellston Workshop on PBT/POPs (persistence [P], bioaccumulation [B], and toxicity [T]/persistent organic pollutants) assessments; 27–31 January 2008; Pensacola, Florida, USA.

(Stapleton et al. 2004) and do not biomagnify in aquatic food chains (Macintosh et al. 2004; Kelly et al. 2008). For substances with log $K_{OW} < 6$, an observed BCF < 5000 L/kg ww indicates that the depuration rate of the chemical is sufficiently high to prevent the occurrence of biomagnification in fish. The lipid content of the organism can have a significant effect on the outcome of BCF tests. The BCF criterion value of 5000 L/kg ww is an appropriate value for small fish with a lipid content of 5%. For test fish with a lipid content less than 5%, the criterion value should be reduced, whereas for fish with a lipid content greater than 5%, the criterion value should be increased. To recognize differences in biochemical composition among test organisms in bioconcentration tests, the criterion value can be expressed on a lipid equivalent basis. The wet weight-based criterion value of 5000 L/kg ww corresponds with a lipid equivalent-based criterion value of 5000/5% or 100000 L/kg equivalent lipid. The BCF cannot be used to measure the degree of biomagnification in mammals, birds, humans, and other air-respiring organisms because these organisms cannot eliminate chemicals by respiring water.

In the interpretation of BAF data, it should be recognized that BAFs reflect chemical exposure in fish via the respiratory and dietary routes, whereas the BCF is measured under conditions that exclude dietary exposure. Exposure conditions in the field can have a significant effect on the value of the BAF. For example, disequilibria between chemical concentrations in water and sediment (Gobas and Maclean 2003) can favor dietary uptake over aqueous uptake in fish (Gobas 1993), especially if fish feed on benthic invertebrates that receive their contaminant body burdens from the sediments. In addition, biomagnification of the chemical in prey organisms can cause a high degree of dietary uptake. In such cases, the BAF can be substantially greater than the corresponding BCF in the same fish species (Arnot and Gobas 2006). The BAFs can also be difficult to measure because concentrations of potentially bioaccumulative substances in natural waters are often very low. In addition, chemicals can sorb to particulate matter, which can cause the bioavailable chemical concentration in the water to be overestimated and the BAF be underestimated. Experimental error should therefore be carefully considered when interpreting field-derived BAFs.

Step 5 involves the application of physicochemical property data, bioaccumulation models, or both to determine the bioaccumulation potential of the chemical. This step might be required if empirical bioaccumulation data are not available for bioaccumulation screening. The most relevant physicochemical property data to assess the bioaccumulation behavior in water-respiring organisms is the octanol–water partition coefficient. For air-respiring organism, the K_{OA} and K_{OW} are required to assess the bioaccumulation behavior. On the basis of current understanding of the bioaccumulation behavior of chemicals in fish, chemical substances with log $K_{OW} < 4$ lack the potential to biomagnify in water-respiring organisms of aquatic food webs. Substances with greater log K_{OW} do have an inherent biomagnification potential in fish but might not achieve this potential because of a high depuration rate (e.g., because of biotransformation) or a slow rate of uptake (e.g., because of a slow rate of membrane permeation), which are not simply related to K_{OW}. In many air-respiring organisms, chemicals with log $K_{OA} < 5$ also lack the potential for biomagnification because of their relatively high rate of elimination by exhalation. Substances with log $K_{OA} \geq 5$ that exhibit a slower rate of elimination by exhalation and with log $K_{OW} \geq 2$, which are eliminated slowly by urinary excretion, have an inherent biomagnification potential in air-respiring organisms. A high rate of metabolic transformation, a slow rate of dietary uptake (e.g., because of slow membrane permeation), or both can prevent the chemical's biomagnification potential from being achieved in the environment. The chemical properties can play an important role in screening large numbers of chemicals for their bioaccumulation behavior. Because they are relatively accessible, K_{OW} and K_{OA} data can identify large numbers of chemicals that lack bioaccumulative potential and for which animal testing for bioaccumulation can be avoided.

Bioaccumulation models can also be applied to determine the chemical's potential to be bioaccumulative. The models use K_{OW} and K_{OA} but also additional biological and environmental properties and characteristics for a more detailed and refined estimate of the biomagnification potential of chemicals. The most important contribution of food web models to the assessment of the biomagnification potential of chemicals is their ability to assess it with

extremely high K_{OW} and K_{OA} values, for which empirical methods to determine bioaccumulation are particularly challenging.

For screening large numbers of chemicals, the framework is likely to be used in reverse order, wherein physicochemical property data and food web bioaccumulation models are used to determine which chemicals have bioaccumulative potential. For chemicals with bioaccumulative potential, BCF and BAF data can be explored to determine whether their bioaccumulative nature is possible. An empirical BMF laboratory test or predator–prey concentration ratios in field studies can be used to determine whether the substance is a probable bioaccumulative substance. Conclusive confirmation regarding the bioaccumulative nature of the substance is gained from the TMF in field studies involving food webs including water- and air-respiring organisms.

CONCLUSIONS

To improve current regulatory frameworks for the evaluation of commercial chemicals for bioaccumulation and to take advantage of new methodologies for determining bioaccumulation behavior, it is essential that bioaccumulation screening initiatives adopt a clear scientific definition for bioaccumulative substances with broad scientific support. Currently, regulatory frameworks for chemical screening for bioaccumulation include criteria to identify bioaccumulative substances but do not contain a definition for a bioaccumulative substance. This approach contributes to disparities in chemical evaluation schemes between countries, prevents the use of high-quality scientific data in the screening process, and impedes the application of new methods for assessing the bioaccumulation behavior of commercial chemicals. The implications are a lack of harmonization among countries, inefficient use of resources and capital, unnecessary animal testing, miscategorization of chemicals, and possible effects on the environment and the economy.

Workgroup discussions involving scientists from academia, industry, and government produced unanimous agreement on the definition for a bioaccumulative substance (i.e., a substance is considered bioaccumulative if it biomagnifies in food chains). Biomagnification is defined as the phenomenon in which the normalized concentration (or fugacity) of the chemical in biological organisms increases with increasing trophic position. A similar definition is also believed to have guided the development of the bioaccumulation criteria of the Stockholm Convention on POPs, although there are no records that document this definition.

Adopting the above definition for a bioaccumulative substance implies that several previously unconsidered measures and criteria can be used to identify bioaccumulative substances. They include the trophic magnification factor (TMF) derived from data from field studies and the biomagnification factor (BMF) derived in laboratory tests or from field data. They also include the K_{OA}, which is a predictor for the bioaccumulation potential of chemicals in air-respiring organisms (e.g., mammals, birds, humans) just as K_{OW} is for water-respiring organisms (e.g., fish). Adopting the scientific definition in regulatory frameworks further opens the door to the application of food web bioaccumulation models, in vitro assays and other in silico methods (e.g., models and QSARs) to assess the bioaccumulative nature of chemical substances.

The workgroup developed a framework for bioaccumulation screening aimed at identifying bioaccumulative substances. The framework associates the greatest weight of evidence to the trophic magnification factor, a measure of food web biomagnification derived from field data. The framework further includes controlled laboratory biomagnification tests, which are expected to be simpler and cheaper than current bioconcentration tests and which can be performed for water- and air-respiring organisms. The framework includes criteria for the K_{OW} and K_{OA} to determine the biomagnification potential for chemicals that can quickly be used to identify many thousands of commercial chemicals that generally lack biomagnification potential and that do not require animal testing. The outcome of food web bioaccumulation models is included in the framework to further refine the screening for chemicals without biomagnification potential. Food web bioaccumulation is particularly effective in evaluating the bioaccumulation behavior of chemicals with very high K_{OW}, which lack biomagnification potential because of a very slow rate of dietary uptake, and for metabolizing chemicals for which biotransformation rate data are known. The framework for screening also includes criteria for the BCF to make efficient use of available data. However, the workgroup stresses that BCFs determined in standard bioconcentration tests are inadequate predictors of biomagnification in fish and should be interpreted with caution and only for chemicals for which bioconcentration tests are feasible. The framework further includes screening for bioaccumulation that is not limited to fish or aquatic food webs that include water-respiring organisms but also includes terrestrial food chains and air-respiring organisms because of some fundamental differences in the bioaccumulation mechanisms of water- and air-respiring organisms.

The workgroup believes that adopting the proposed framework for bioaccumulation screening has several advantages over currently used screening methodologies. The advantages include 1) better characterization of bioaccumulative substances by reducing miscategorization, 2) more effective use of available bioaccumulation data that currently cannot be considered, 3) a reduction in animal testing, 4) simpler and possibly cheaper test protocols for animal studies when case animal studies are necessary, 5) the application of alternative testing strategies, including in vitro and in silico methods that can be used to screen large numbers of chemicals, and 6) better harmonization in screening among various jurisdictions.

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