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Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks

Part I: Bioaccumulation assessment

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Abstract

Aquatic and non-aquatic ecosystems differ with regards to metabolism as well as exposure and uptake routes. Current international and European regulatory criteria for Persistence, Bioaccumulation, and Toxicity (PBT) assessment of chemical substances are mainly based on toxicity and bioaccumulation data in aquatic species. In the literature, there is evidence that several persistent organic chemicals, which are not classified as bioaccumulative and/or toxic in aquatic organisms according to existing criteria, can biomagnify in non-aquatic food chains up to the top predators (including humans) and exert their toxicity. Therefore, the regulatory frameworks may fail to identify a number of substances that are bioaccumulative and/or toxic in non-aquatic organisms and related food chains (exposed through soil and food), but not in aquatic species. Based on these considerations, two reports were prepared on available criteria for non-aquatic organisms within PBT/vPvB frameworks: one on bioaccumulation assessment (Part I) and one on toxicity assessment (Part II). Specifically, the present document illustrates and discusses the outcomes of a regulatory and literature review on available criteria for bioaccumulation assessment in non-aquatic organisms at international and European level (Part I). This report could be used to support an eventual revision of guidance documents, e.g. for REACH (EU Regulation 1907/2006), as well as to promote the harmonisation of regulatory criteria for PBT/vPvB assessment.

Review of available criteria for non-aquatic organisms in PBT/vPvB frameworks

Part I: Bioaccumulation assessment

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Preface

This report has been prepared in the frame of an Administrative Arrangement between the Directorate-General Environment (DG ENV) and the Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP) on 'Scientific and technical support to safety assessment of chemicals'. One of the requests of DG ENV was to carry out a review of available criteria for bioaccumulation and toxicity assessment in non-aquatic organisms within current regulatory Persistent Bioaccumulative Toxic (PBT) and/or very Persistent very Bioaccumulative (vPvB) assessment frameworks.

Based on this request two reports were prepared on available criteria for non-aquatic organisms within PBT/vPvB frameworks: the present one on bioaccumulation assessment (Part I) and one on toxicity assessment (Part II) (Hartmann et al. 2014). An intermediate version of this report on bioaccumulation assessment was circulated to participants of the PBT Expert Group of the European Chemicals Agency (ECHA). Thereafter, the report has been amended, taking into consideration the comments and suggestions of the ECHA PBT Expert Group.

We would like to thank the experts of the ECHA PBT Expert Group and the colleagues from DG ENV, ECHA and the JRC for their useful comments.

Executive Summary

Current international and European regulatory criteria for bioaccumulation assessment under frameworks for identification of substances with Persistent Bioaccumulative Toxic (PBT) and/or very Persistent very Bioaccumulative (vPvB) properties are mainly focused on the aquatic compartment and use the Bioaccumulation Factor (BAF) or Bioconcentration Factor (BCF) in aquatic species and/or the octanol-water partitioning coefficient (K_{ow}) as metrics.

In the literature, there is evidence that several persistent organic chemicals, which are not considered as bioaccumulative in aquatic organisms according to existing criteria, can biomagnify in non-aquatic food chains up to the top predators, including humans. This occurs as non-aquatic organisms differ from aquatic ones in terms such as uptake and elimination mechanisms, diet, energy requirements, and feeding rates. Therefore, there is a risk that these regulatory frameworks for PBT/vPvB assessment neglect a group of substances that are bioaccumulative in the non-aquatic compartment.

Based on these considerations, the Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP), was asked by the Directorate-General Environment (DG ENV) to prepare a regulatory and literature review on available criteria for bioaccumulation and toxicity assessment in non-aquatic organisms to support the revision process of the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment* for implementation of the European chemicals legislation (REACH Regulation 1907/2006), as well as to promote the harmonisation of regulatory criteria for PBT/vPvB assessment at European level.

The literature review points out that there is consensus in the scientific community on the combined use of criteria based on predictive physico-chemical parameters such as K_{oa} (octanol-air partitioning coefficient) > 5 or 6 and $K_{ow} > 2$, which are easy to calculate and implement, and should therefore be considered within existing PBT/vPvB assessment frameworks, especially for screening purposes.

In a more detailed bioaccumulation assessment that is usually performed at higher tier, there is general agreement in the scientific community that all available bioaccumulation metrics need to be considered as complementary lines of evidence and evaluated in a weight of evidence approach (in line with the principles outlined in the REACH Annex XIII published in 2011). Specifically, it is recommended that both bioconcentration and biomagnification are investigated by means of multiple laboratory and field metrics and that aquatic and non-aquatic compartments are separately addressed. Specifically, BSAF (Biota/Soil Accumulation Factor) and BCF values in soil organisms such as earthworms seem to represent good indicators for the bioaccumulation potential at the base of non-aquatic food chains, despite several technical limitations. As food is the main exposure route for non-aquatic organisms and it is demonstrated that biomagnification can be higher in non-aquatic food webs compared to aquatic ones, field-based BMF (Biomagnification Factor) and TMF (Trophic Magnification Factor) values determined for non-aquatic food webs could be considered.

Furthermore, the elimination half-life has been proposed recently in the literature as an alternative bioaccumulation metric.

In conclusion, the scientific community has suggested several metrics to overcome the lack of consideration of the non-aquatic compartment in the regulatory PBT/vPvB assessment of substances. However, research efforts are still needed to improve the scientific understanding of the proposed metrics and define cut-off values. Moreover, the impact that the introduction of non-aquatic criteria in a regulatory context may have needs to be analysed in depth.

It is important to note that suggestions provided in this document should not be considered as an attempt to define a regulatory strategy on how to screen and/or assess substances based on their bioaccumulation potential in non-aquatic organisms nor to evaluate the impact of such a strategy on e.g. the REACH implementation process. Further elaboration is needed to integrate the knowledge summarised in the present document into the current regulatory framework. This could be the work for a dedicated expert group.

1 Background and scope

Current international and European regulatory criteria on bioaccumulation assessment are mainly based on the use of the Bioaccumulation Factor (BAF) or Bioconcentration (BCF) in aquatic species and the octanol-water partitioning coefficient (K_{ow}) (see Annex I, Glossary). BCF and BAF values are generally determined for aquatic organisms. BCF values are obtained under laboratory-controlled conditions and do not take the exposure route through the diet into account (Gobas et al. 2009; Ehrlich et al. 2011; Goss et al. 2013). BAF values are generally preferred as they are more ecologically relevant (field experiments, steady-state conditions, all exposure routes) than BCF values for the same species; however, BAF values are largely variable due to site-specific environmental conditions affecting their determination and less available than BCF values (Arnot and Gobas 2006; Weisbrod et al. 2009; Costanza et al. 2012). K_{ow} is a measure of the equilibrium partitioning of organic compounds between water and octanol, which is considered representative for the lipid in biota, and therefore only apply to estimation of aquatic bioaccumulation.

Nonetheless, there is evidence in the literature that several persistent organic chemicals, which are not classified as bioaccumulative in aquatic organisms according to K_{ow} /BCF/BAF-based criteria, can biomagnify in non-aquatic food chains up to the top predators, including humans (e.g. Kelly and Gobas 2001; Kelly and Gobas 2003; Kelly et al. 2007; Tonnelier et al. 2011). There is therefore a risk that regulatory frameworks may neglect a group of substances, which are bioaccumulative in non-aquatic organisms and related food chains.

A number of OECD Test Guidelines (TGs) are available to generate bioaccumulation data that can be used in a regulatory context. In 2012, the OECD TG 305 on *Bioaccumulation in Fish: Aqueous and Dietary Exposure* has been adopted (OECD 2012). This Test Guideline was updated in order to incorporate dietary exposure in fish and therefore enable determination of bioaccumulation potential of very poorly water soluble substances. The OECD TG 315 on *Bioaccumulation in Sediment-dwelling Benthic Oligochaetes* is also available (OECD 2008). In 2010, OECD recognised that extrapolation from aquatic bioaccumulation data to non-aquatic organisms is difficult, if not possible, and developed the OECD TG 317 on *Bioaccumulation in Terrestrial Oligochaetes* (OECD 2010), which may help with obtaining an indication of the bioaccumulation potential for low trophic levels of the non-aquatic food chains.

As far as the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation 1907/2006 (EC 2006) is concerned, the criteria for PBT (Persistent Bioaccumulative Toxic) and vPvB (very Persistent very Bioaccumulative) assessment presented in Annex XIII to the legal text was revised and published as European Regulation 253/2011 (EC 2011). The guidance document for the implementation of REACH of the European Chemicals Agency (ECHA) named *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment* (ECHA

2012a) is currently under review and its publication is expected soon³. A major change in the Annex XIII concerns the explicit requirement for a weight of evidence approach considering all available relevant information to derive a conclusion on P, B and T properties. Moreover, criteria for bioaccumulation assessment now specifically ask for considering bioaccumulation in terrestrial species, data from human body fluids or tissues and biomagnification in the food chain in addition to bioconcentration and/or bioaccumulation in aquatic species. However, threshold values are given for BCF and BAF criteria in aquatic species only.

The Joint Research Centre's Institute for Health and Consumer Protection (JRC-IHCP) was asked by the Directorate-General on Environment to support the review process of the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment* as well as to promote the harmonisation of regulatory criteria for PBT assessment at European level by preparing a state-of-the-art report on bioaccumulation and toxicity assessment in non-aquatic organisms. In this context, the present document is aimed to: i) review the legislative status of bioaccumulation assessment in different international and European regulatory frameworks and ii) present and discuss available criteria and approaches for screening and assessment of bioaccumulation potential of chemicals. The ultimate goal of the document is to provide initial suggestions on possible criteria that enable the identification of substances that may accumulate and biomagnify in non-aquatic environments in addition to the aquatic ones. Suggestions provided in this document should not be considered as an attempt to define a regulatory strategy on how to screen and/or assess substances based on their bioaccumulation potential in non-aquatic organisms nor to evaluate the impact of such a strategy on e.g. the REACH implementation process. Further elaboration would be needed to integrate the knowledge summarised in the present document into the current regulatory framework. This could be the work for a dedicated expert group.

An intermediate version of this document was made available as background document to the ECHA PBT Expert Group as well as to the PEG (Partner Expert Group) established for reviewing the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment*. Comments to the document were received by several experts from ECHA, Member State Competent Authorities as well as industry and were addressed accordingly.

In summary, Section 2 briefly describes the current use of criteria for bioaccumulation assessment in international and European regulations while their limitations are discussed in Section 3. Section 4 aims at illustrating and discussing a series of evidences on bioaccumulation in non-aquatic organisms which have been reported in the literature. Based on that, Section 5 focuses on possible criteria and approaches for the identification of bioaccumulative substances in non-aquatic organisms and discusses advantages and disadvantages of several literature proposals. In Section 6, on-going

³ Link to the ECHA website where last draft is available: <http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach>

initiatives that could provide more information and tools on bioaccumulation in non-aquatic environments in the near future are briefly illustrated. Finally, Section 7 explains the main conclusions. In order to ensure a common understanding of the issues tackled in the present document, Annex I contains a glossary of the key terms.

2 International and European regulatory criteria for bioaccumulation assessment

Official criteria that are used for bioaccumulation assessment of substances in several international and European regulatory frameworks are summarised in Table 1.

Bioaccumulation assessment is required to i) identify Persistent, Bioaccumulative and Toxic (PBT) and/or very Persistent very Bioaccumulative (vPvB) substances or Persistent Organic Pollutants (POPs), which correspond to PBT/vPvB substances that are also characterised by long-range transport potential, or to ii) classify the hazard to the environment of the substances in a more generic way. The assessment is generally based on the use of the Bioaccumulation Factor (BAF) or Bioconcentration Factor (BCF) in aquatic species or, in the absence of such experimental data, the logarithmic octanol/water partitioning coefficient ($\log K_{ow}$) (see Annex I, Glossary). Cut-off values for BAF/BCF in aquatic species and $\log K_{ow}$ are usually recommended. Some regulatory frameworks provide qualitative criteria for bioaccumulation assessment, which give the possibility of using data for non-aquatic organisms in a few cases as discussed below.

Under international regulatory frameworks for POPs identification such as the **United Nations Economic Commission for Europe (UN ECE) POP Protocol** (UN ECE 1996; 1998) and **United Nations Environment Programme (UNEP) Stockholm Convention** (UNEP 2001; 2009)⁴, a substance is classified as 'bioaccumulative' if the value of the BCF or BAF in aquatic species is greater than 5000 L/Kg or, in the absence of such experimental data, if the $\log K_{ow}$ is greater than 5.

Similar numerical criteria are applied for PBT substances identification in the **Canadian Environment Protection Act (CEPA)** (Government of Canada 1999; 2004) (see Table 1). Under the **Convention for the Protection of the marine Environment of the North-East Atlantic (OSPAR Convention)**, more stringent cut-off values (i.e. one order of magnitude lower for both BCF and $\log K_{ow}$) were chosen for determining whether a substance has the 'liability to bioaccumulate' (OSPAR 1992; 2010) (see Table 1), meaning that more substances will fulfil the criteria of being liable to bioaccumulate. This is probably due to the fact that chemicals of concern under OSPAR are used off-shore and directly released into the environment (as suggested by Moermond et al. (2011)).

⁴ In 2001, twelve POPs (called 'the dirty dozen' and including pesticides, industrial chemicals and by-products) were listed under Annex A (elimination), B (restriction) or C (unintentional production) of the Stockholm Convention: i.e. aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs). In 2009, nine new POPs were added to the initial list: i.e. chlordecone, alpha hexachlorocyclohexane (α -HCH), beta hexachlorocyclohexane (β -HCH), lindane, pentachlorobenzene, hexabromodiphenyl ether and heptabromodiphenyl ether, pentachlorobenzene, perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride, tetrabromodiphenyl ether and pentabromodiphenyl ether. In 2011, Annex A was amended to include technical endosulfan and its related isomers. (Source: <http://chm.pops.int/Convention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx>)

In spite of that, not only quantitative criteria based on BAF/BCF or $\log K_{ow}$ but also qualitative criteria can be used for bioaccumulation assessment in some frameworks. These criteria give some flexibility to the classification schemes and allow for consideration of additional evidences such as high (eco)toxicity and monitoring data. Under the **UN ECE POP Protocol**, it is explicitly stated that if the bioaccumulation potential is significantly lower than the numerical criteria, other factors such as high toxicity of the substance that may raise concern within the scope of the protocol can be considered (UN ECE 1998). Under the **UNEP Stockholm Convention** (UNEP 2001; 2009), two qualitative criteria are given: i) information suggesting other reasons of concern such as evidence of bioaccumulation in other species than the aquatic ones, high toxicity and high ecotoxicity; and ii) monitoring data in biota. In this framework, it is therefore explicitly required to consider bioaccumulation in other species than the aquatic ones as additional evidence. In 2007, Kitano prepared a *Discussion Paper on Bioaccumulation Evaluation* for the Third Meeting of the POPs Review Committee where he pointed out the role of the qualitative criteria in the screening of bioaccumulation properties for substances that do not exceed the cut-off values defined for BCF/BAF and/or $\log K_{ow}$. In this case, Kitano suggested that several pieces of information such as BCF values, half-life, biomagnification, toxicity/(eco)toxicity, monitoring in biota and human body as well as exposure in development stages should be considered in a weight of evidence approach to derive a conclusion on the bioaccumulation properties of a substance (Kitano 2007). In the Stockholm Convention it is also stated that the criteria for POPs identification are meant for screening and should not be applied in a rigid way but in an integrative and balanced approach considering all available information.

Under the **European REACH Regulation 1907/2006** (EC 2006), a framework for PBT and vPvB assessment including both quantitative and qualitative criteria is provided in Annex XIII (EC 2011)⁵. REACH Annex XIII explicitly requires that all available information in registration dossiers and open literature shall be considered in a weight of evidence approach to draw a conclusion on the P, B and T properties of a substance. In case only few data is available, a reduced dataset can be used to screen for bioaccumulation properties and eventually conclude that the substance does not have a bioaccumulation potential. The screening dataset includes: i) the $\log K_{ow}$; and ii) other suitable and reliable information. If, based on screening information, the substance shows bioaccumulation properties, additional data needs to be considered and eventually generated before deriving a conclusion. The dataset that is required for a proper bioaccumulation assessment includes: i) bioaccumulation in aquatic species; ii) other information on the bioaccumulation potential such as bioaccumulation study in terrestrial species, human body fluids or tissues, detected high levels in

⁵ In the frame of REACH, the assessment of PBT and/or vPvB profiles is necessary to identify Substances of Very High Concern (SVHC) to be included in the REACH Candidates List for Authorisation. So far, 16 out of 73 chemicals on the list have been included due to their PBT and/or vPvB properties: i.e. anthracene and several types of anthracene oil, musk xylene, pitch coal tar, trybutiltin oxide (TBTO), alkanes C10-13 chloro, hexabromocyclododecane (HBCDD), decabromodiphenyl ether (DecaBDE), and four perfluorocarboxylic acids (i.e. hencosafluoroundecanoic acid, heptacosafuorotetradecanoic acid, tricosafuorododecanoic acid, pentacosafuorotridecanoic acid). (Source: <http://echa.europa.eu/web/guest/candidate-list-table>).

biota, chronic toxicity study on animals, toxicokinetic behaviour; and iii) information on the ability of the substance to biomagnify possibly based on the Biomagnification Factor (BMF) or Trophic Magnification Factor (TMF) (see Annex I, Glossary). These requirements point out that a comprehensive assessment is necessary and bioaccumulation in other species, in addition to the aquatic ones, as well as magnification through the food chains need to be investigated before drawing a conclusion on the bioaccumulation potential of a substance. To this end, REACH explicitly requires a weight of evidence approach. The numerical criteria given in Annex XIII concern bioaccumulation in aquatic species only and correspond to two cut-off values: if the BCF/BAF value is higher than 2000 L/Kg, then the substance is classified as 'bioaccumulative' (B); if the value is higher than 5000 L/Kg, then the substance is classified as 'very bioaccumulative' (vB) (see Table 1). No threshold values are given for the remaining criteria mentioned in Annex XIII. ECHA developed a Guidance Document for implementation of PBT/vPvB assessment under REACH (ECHA 2012a) where additional numerical criteria are suggested. For example, it is stated that $\log K_{ow}$ lower than or equal to 4.5 can be used as screening criterion for classification of the substance as 'not bioaccumulative' (not B, not vB). Moreover, in the Guidance Document values of BMF higher than 1 are considered as convincing evidence that the substance can biomagnify in food chains. Additional indicators are proposed in the Guidance Document, provided that they are used in combination with other information. For example: the maximum molecular length of a substance higher than 4.3 nm may indicate a low bioaccumulation potential; a molecular weight higher than 1100 g/mol or in the range of 700-1100 g/mol is an indicator that the aquatic BCF of the substance is lower than 2000 L/Kg (not B) or lower than 5000 L/Kg (not vB), respectively; no uptake by mammals may suggest that the substance does not pass through the gills membranes and consequently does not bioaccumulate in fish. However, the Guidance Document does not give any specific indication on how the available data and indicators could be assessed and integrated in a weight of evidence approach (as also pointed out by Solomon et al. (2013)). It has to be underlined that the Guidance Document is currently under review in order to align it with the current Annex XIII provisions; consequently, the abovementioned additional indicators and criteria may undergo modifications and more information on how to apply the weight of evidence approach may be provided. The consultation procedure of the updated Guidance Document is ongoing and draft versions are downloadable from the ECHA website⁶. Publication is expected soon.

As pointed out by Moermond et al. (2011), the BCF/BAF threshold value used under UNEP and UN ECE international frameworks equals the vB criterion in REACH, which means REACH is more protective as it also provides a lower threshold value for the B criterion. The **US Toxic Substances Control Act (TSCA)** (US EPA 1998; 2002) distinguishes between substances that show a 'tendency to accumulate in organisms' (BCF/BAF: 1000-5000 L/Kg) and those ones with 'properties consistent with substances widely acknowledged to be bioaccumulative' (BCF/BAF > 5000 L/Kg). In this case the highest cut-off value is the same as the vB criterion under REACH while the lowest cut-off value used

⁶ <http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach>

in the US is more stringent than the B criterion applied in REACH (i.e. BCF/BAF: 1000-5000 L/Kg vs BCF/BAF > 2000 L/Kg).

The regulatory requirement of considering all available relevant information in a weight of evidence approach in the current REACH Annex XIII has introduced dissimilarities in the PBT assessment frameworks at European level (Moermond et al. 2011). For example, whereas the **Biocidal Products Regulation (BPR) 528/2012** (EC 2012) explicitly refers to REACH Annex XIII and its provisions, the **Plant Protection Products (PPPs) Regulation 1107/2009** (EC 2009) does not require a weight of evidence approach but relies on numerical criteria for bioconcentration in aquatic organisms only (see Table 1). As concluded by Moermond et al. (2011) and recalled in Solomon et al. (2013) as well as in Rauert et al. (2014), these differences in criteria (in terms of both threshold values and approach) create a challenge to harmonise the PBT/vPvB assessment of substances at European level. Solomon et al. (2013) advocates the use of a formal, quantitative and transparent process of weight of evidence in categorisation of pesticides under the PPPs Regulation 1107/2009. In addition, the Authors recognise the need of developing criteria for bioaccumulation and toxicity in non-aquatic organisms, which could be applied when pesticides exhibit persistence in soil (not aquatic) compartment. Similarly, Rauert et al. (2014) recommend that all available information is considered in a weight of evidence approach under all regulatory frameworks.

Under existing regulatory frameworks for hazard classification and labelling, such as the **Globally Harmonised System (GHS) of Classification and Labelling of chemicals** (UN 2013) and the **European Regulation on 'Classification, Labelling and Packaging (CLP) of substances and mixtures'** (EC 2008), the 'potential of a substance to bioconcentrate' in aquatic organisms (along with aquatic toxicity data and degradability) is considered in the procedure for classification of a substance as hazardous for the environment. In these frameworks, the recommended cut-off values for log K_{ow} and BCF in aquatic species are one order of magnitude more stringent than the ones used for bioaccumulation assessment under POP/PBT/vPvB frameworks, meaning that more substances will fulfil the criteria of having potential to bioaccumulate.

Table 1. Overview of quantitative and qualitative criteria for bioaccumulation assessment in different international and European regulatory frameworks. POP = Persistent Organic Pollutant; PBT = Persistent Bioaccumulative Toxic; vPvB = very Persistent very Bioaccumulative; BCF = Bioconcentration Factor; BAF = Bioaccumulation Factor; K_{ow} = octanol-water partitioning coefficient; BMF = Biomagnification Factor; TMF = Trophic Magnification Factor.

Assessment Framework	Regulation	Bioaccumulation Criteria	Reference
POPs identification	UN ECE POP Protocol	BCF or BAF (aquatic) > 5000 Or: Log K_{ow} > 5 Alternatively, if the bioaccumulation potential is significantly lower than above, other factors such as high toxicity of the substance, that make it concern within the scope of the protocol.	UN ECE 1996; 1998
POPs identification	UNEP Stockholm Convention Annex D Information requirements and screening criteria Article 8 (3)	BCF or BAF (aquatic) > 5000 Or: Log K_{ow} > 5 Or: Other reasons of concern (e.g. high bioaccumulation in other species, high toxicity, ecotoxicity) Or: Monitoring data in biota 'The Committee shall examine the proposal and apply the screening criteria specified in Annex D in a flexible and transparent way, taking all information provided into account in an integrative and balanced manner.'	UNEP 2001; 2009

Table 1. (cont.)

Assessment Framework	Regulation	Bioaccumulation Criteria	Reference
POPs identification	EU Plant Protection Product Regulation (1107/2009)	BCF or BAF (aquatic) > 5000 Or: Log K_{ow} > 5 Or: Other reasons of concern (e.g. high bioaccumulation in other non-target species, high toxicity or ecotoxicity)	EC 2009
PBT substances identification	OSPAR Convention	Log K_{ow} \geq 4 Or: BCF (aquatic) \geq 500	OSPAR 1992; 2010
PBT substances identification	Canadian Environmental Protection Act (CEPA)	BAF (aquatic) \geq 5000 Or: BCF (aquatic) \geq 5000 Or: Log K_{ow} \geq 5	Government of Canada 1999; 2004
PBT/vPvB substances identification	US Toxic Substances Control Act (TSCA)	Tendency to accumulation in organisms: BCF or BAF (aquatic): 1000-5000 Properties consistent with substances widely acknowledged to be bioaccumulative (ban criteria): BCF or BAF (aquatic) \geq 5000	US EPA 1998; 2002

Table 1. (cont.)

Assessment Framework	Regulation	Bioaccumulation Criteria	Reference
PBT/vPvB substances identification	Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS)	<p>B: BCF or BAF (aquatic) > 2000</p> <p>Or:</p> <p>Log K_{ow} > 4.2</p> <p>Substances with vB properties are defined and identified according to criteria provided by Stockholm Convention on POPs</p>	<p>Australian Government 2013</p> <p>EPHC 2009</p>
PBT/vPvB substances identification	EU REACH Regulation (1907/2006) Annex XIII	<p>Screening based on weight of evidence:</p> <p>Log K_{ow}</p> <p>And:</p> <p>Other suitable and reliable information</p> <p>Assessment based on weight of evidence:</p> <p>Bioaccumulation in aquatic species:</p> <p>B: BCF or BAF (aquatic) > 2000</p> <p>vB: BCF or BAF (aquatic) > 5000</p> <p>And:</p> <p>Other information on bioaccumulation potential (e.g. bioaccumulation study in terrestrial species, human body fluids or tissues, detected high levels in biota, chronic toxicity study on animals, toxicokinetic behaviour)</p> <p>And:</p> <p>Information on the ability to biomagnify (where possible: BMF or TMF)</p>	<p>EC 2006; 2011</p>

Table 1. (cont.)

Assessment Framework	Regulation	Bioaccumulation Criteria	Reference
PBT/vPvB substances identification	EU Plant Protection Product Regulation (1107/2009)	B: BCF (aquatic) > 2000 vB: BCF (aquatic) > 5000	EC 2009
PBT/vPvB substances identification	EU Biocidal Products Regulation (528/2012)	In accordance with Annex XIII to REACH Regulation 1907/2006	EC 2012
Hazard classification and labelling	UN Globally Harmonised System (GHS)	Log K_{ow} \geq 4 Or: BCF (aquatic) \geq 500	UN, 2013
Hazard classification and labelling	EU CLP Regulation (1272/2008)	Log K_{ow} \geq 4 Or: BCF (aquatic) \geq 500	EC 2008

3 Limitations of the current regulatory criteria for bioaccumulation assessment

According to current legislations in Canada, the US, and the EU as well as international conventions and protocols (see Table 1), screening and assessment of bioaccumulation potential is usually addressed by means of two quantitative criteria (in addition to other criteria for which no cut-off value is provided): 1) BCF or BAF data in aquatic species greater than a certain value; or, in absence of such experimental/empirical data, 2) $\log K_{ow}$ greater than a certain value.

Several authors have recently reviewed the limitations of these regulatory metrics and related criteria. Solutions to fulfil the gaps have also been proposed in the scientific literature. A summary of the current understanding is provided in the following sub-sections.

3.1 Highly hydrophobic substances

As reviewed by Ehrlich et al. (2011), the linear relationship between $\log K_{ow}$ and BCF seems not to apply to highly hydrophobic substances as BCF levels tend to either level off or decline at $\log K_{ow}$ higher than 5.5-6. This phenomenon is known as 'hydrophobicity cut-off' and is still under debate. Some Authors mechanistically explained this phenomenon with the steric factors due to excessive molecular size and consequent reduction of gill permeation ability of the substances or cellular uptake. Based on this assumption, chemicals with $\log K_{ow}$ above 8 or 10 are commonly considered as not likely to bioaccumulate (Dimitrov et al. 2004). ECHA also concludes that the aquatic BCF of a substance is unlikely to be > 2000 L/Kg (i.e. higher than the threshold value for classification of a substance as 'bioaccumulative' under REACH) if the available $\log K_{ow}$ is > 10 (ECHA 2012a).

Various criteria based on molecular size and related cut-off values have been proposed in the literature to explain reduced bioconcentration for larger and more hydrophobic substances such as molecular weight (> 700 - 1000 g/mol), effective diameter (> 0.95 nm), and maximum diameter (> 1.5 nm) (Dimitrov et al. 2003; 2004; Nicholson et al. 2009). Arnot et al. (2009) critically review the proposed criteria and conclude that cut-off values based on molecular size are not supported by the available data and should not be used in a regulatory context. The Authors suggest that an integrated, holistic approach is applied to account for competing rates of uptake and elimination in an organism to assess bioaccumulation rather than applying molecular attributes (including consideration of bioavailability, dissociation in water, biotransformation, and dietary exposure) (Arnot et al. 2009). For example, Arnot and Gobas (2003) explain the reduction in BAF values with increasing K_{ow} for very hydrophobic substances (i.e. $\log K_{ow} > 7.5$) by the reduction in bioavailability in water. Based on the results obtained from their BAF-QSAR model, Arnot and Gobas (2003) indeed argue that BAF values decrease at high K_{ow} due to an increase in the chemical's sorption coefficient to particulate and dissolved organic carbon, not because of steric factors or lack of biomagnification.

Other Authors have challenged the 'hydrophobicity cut-off' phenomenon and ascribed it to experimental artefacts, i.e. non-equilibrium conditions and 'third phase' effect (as argued by Jonker and van der Heijne (2007) and subsequently critically discussed in Yang and Zeng (2008) and in Jonker and van der Heijden's reply (2008)). Specifically, Jonker and van der Heijne (2007) conclude that bioaccumulation of very hydrophobic compounds does occur and is controlled by hydrophobicity up to $\log K_{ow}$ values of 7.5 at least (if BCF is properly measured and the test is properly conducted, e.g. by using a passive sampler and prolonging exposure time). The Authors suggest that the current regulatory criteria and the assumption of the 'hydrophobicity cut-off' are reconsidered, as the actual bioaccumulation for compounds with $\log K_{ow} > 6$ may be underestimated (Jonker and van der Heijne 2007).

3.2 Biotransformation

Metabolism appears to be the most important factor mitigating bioconcentration (Dimitrov et al. 2005). Ehrlich et al. (2011) point out that other processes such as retarded elimination, bioactivation, and production of metabolites with comparable bioaccumulation potential as the parent compound may occur and should be considered in addition to metabolism. Bioaccumulation potentials estimated from K_{ow} do not take into account any biotransformation of chemicals in organisms such as excretion, depuration, metabolism, thus potentially leading to false positives (e.g. Gobas et al. 2009). A K_{ow} -based approach is therefore mainly applicable for poorly metabolised organic chemicals.

The possibility to use bioaccumulation models to determine a cut-off value for metabolism (k_m) to be applied to BCF/BAF for correction, has been proposed and discussed in the literature (e.g. Arnot and Gobas 2003; Nichols et al. 2007; Arnot et al. 2008; Cowan-Ellsberry et al. 2008; Nichols et al. 2009). Based on the fact that the currently available k_m values are highly context-specific and the relationship between k_m and actual metabolism is quite complex, Arnot et al. (2008) conclude that the best option would be to apply k_m estimates on a chemical by chemical basis when *in vivo* bioaccumulation data are also available.

3.3 Dietary exposure

Both experimentally determined aquatic BCF values and predicted K_{ow} values quantify the extent to which the chemical exchange between water and biota occurs, and are therefore based on the steady state lipid-water partitioning approach. However, studies (e.g. Connolly and Pedersen 1988; Gobas et al. 1993; 1999) demonstrate that this is not the only mechanism driving bioaccumulation as also food digestion and absorption cause ingested chemicals to magnify in the gastrointestinal tract against the thermodynamic gradient if the elimination/metabolism rates are low (dietary bioaccumulation). Consequently, the use of criteria based on BCF or K_{ow} values can underestimate the real bioaccumulation and generate false negatives. This particularly applies to highly hydrophobic substances (high K_{ow} , low solubility) for which exposure via diet or ingestion of e.g. soil/sediment gains more importance than via water (Ehrlich et al. 2011).

Recently, the OECD 305 Test Guideline (TG) *Bioaccumulation in Fish: Aqueous and Dietary Exposure* has been adopted (OECD 2012). This Test Guideline has been updated in order to incorporate dietary exposure and therefore enable determination of bioaccumulation potential of very poorly water soluble substances. The test allows for the determination of a laboratory dietary BMF. In Annex 8 'Approaches to estimate tentative BCFs from data collected in the dietary exposure study' suggestions are given in case a BCF_k needs to be derived from the depuration rate measured in the dietary study. Specifically, Annex 8 suggests that a combination of models are applied to obtain a range of values for the uptake rate (k_1) and related BCF. It is also recommended that these estimations are treated in a weight of evidence approach along with the laboratory dietary BMF value and other information (e.g. molecular size) in order to obtain an overall picture of the bioaccumulation potential of the substance (OECD 2012). An equation to calculate BCF_k from BMF was proposed by Weisbrod et al. (2009). This is a rough estimation of BCF_k but may facilitate the application of the dietary test results in a regulatory context (Ehrlich et al. 2011).

3.4 Air-respiring organisms

The K_{ow} and aquatic BAF/BCF values do not take into account bioaccumulation potential in air-respiring organisms such as birds, mammals and humans, which is based on the organism-to-air exchange (Gobas et al. 2009; Ehrlich et al. 2011; Goss et al. 2013). Moreover, intake via food is generally the dominant route of exposure in non-aquatic organisms (Solomon et al. 2013). Another important factor to account for is that birds, mammals and humans are homeotherms⁷. Several Authors (e.g. Branue et al. 1989; Fisk et al. 2001; Hop et al. 2002) report that homeotherms have higher energy requirements, feeding rates, trophic positions, longer life time and different biotransformation abilities than poikilotherms⁸ (including fish), and therefore extrapolation from fish-related bioaccumulation data to other organisms should not be made (Martin et al. 2003a). In particular, Kelly et al. (2007) explain that higher biomagnification of certain organic compounds in air-breathing organisms is due to the greater ability to absorb and digest their diet, which is related to differences in digestive tract physiology and body temperature. The state-of-the-art on this subject is covered under Section 4 and 5.

3.5 Proteinophilic substances

Proteinophilic substances are substances that tend to accumulate in protein-rich tissues rather than lipids. Some Authors suggest that the traditional equilibrium partitioning may not be fully appropriate for substances that are amphiphilic (i.e. both hydrophobic and oleophobic) and show

⁷ Organisms that maintain their body temperature at a constant level by its metabolic activity, regardless the external influence (e.g. birds, mammals).

⁸ Organisms that cannot regulate their body temperature except by behavioural means (e.g. reptiles, fish).

different mechanisms of bioaccumulation as well as target organs (e.g. Martin et al. 2003a; 2003b; Houde et al. 2006; Conder et al. 2008; Haukas et al. 2007; Ehrlich et al. 2011; Goss et al. 2013; Nh and Hungerbuhler 2013). Specifically, perfluorinated acids (e.g. PFOS) were found to preferentially accumulate in protein-rich tissues such as blood, liver and kidney rather than lipids and to have affinity to plasma albumin and hepatic proteins such as fatty acids-binding proteins (e.g. Martin et al. 2003a; 2003b; Jones et al. 2003; Conder et al. 2008; Ng and Hungerbuhler 2013). In addition to that, K_{ow} is problematic to measure due to the tendency of surfactants to aggregate at the aqueous-organic interface (Martin et al. 2003a; Conder et al. 2008). OECD (2002) states as follows: 'Due to the surface-active properties of PFOS and the test protocol itself, PFOS forms three layers in octanol/water and hence, an n-octanol/water (K_{ow}) partition coefficient cannot be determined. Consequently, the various physicochemical properties (e.g., bioconcentration factor, soil adsorption coefficient), which can usually be estimated for conventional organic compounds utilizing K_{ow} equations, cannot be estimated, and a calculated (estimated) log K_{ow} cannot be trusted. Even if the log K_{ow} were known, it may not be appropriate for predictive purposes, e.g., bioconcentration. Studies on laboratory rats indicate that PFOS does not bioconcentrate in the lipid fraction. Instead, it tends to bind to certain proteins'.

Martin et al. (2003a; 2003b) report that half-lives, uptake rates and BCF values of perfluorinated acids increase with increasing length of the perfluoroalkyl chain with perfluorosulfonates (PFSAs) showing higher values than the corresponding perfluorocarboxylates (PFCAs) of equivalent perfluoroalkyl chain length. Based on these findings, the Authors suggest that the hydrophobicity of these substances could be better predicted by the perfluoroalkyl chain length (Martin et al. 2003a; 2003b) rather than K_{ow} . They also considered the Critical Micelle Concentration (CMC) (as initially suggested by Tolls and Sijm (1995)) and found that bioaccumulation potential of perfluorinated acids increase with decreasing CMC. However, as neither hydrophobicity nor CMC alone can explain the highest bioaccumulation potential of PFSAs relative to PFCAs of equivalent chain length, the Authors recommend that the acid function of the substance is also considered as determining parameter (Martin et al. 2003a; 2003b). Similar results and considerations from different studies have been reported in subsequent reviews (Houde et al. 2006; Conder et al. 2008). Conder et al. (2008) explicitly state that K_{ow} -based regulatory criteria are inappropriate for predicting bioaccumulation potential of perfluorinated acids. Similarly, Kitano (2007) also concludes that both BCF and K_{ow} are not applicable as bioaccumulation potential descriptors to PFOS.

However, the scientific debate on the appropriateness of the equilibrium partitioning approach to characterise bioaccumulation potential of perfluorinated acids is still on-going and further empirical investigation is needed to fully understand the underlying mechanism and develop validated models.

Some Authors have recently attempted to include protein associations in bioaccumulation modelling of perfluorinated acids. For example, Kelly et al. (2009) suggest that kinetics of perfluorinated acids may be more accurately represented by two different parameters, which are the protein-water partitioning coefficient (K_{pw}) and the protein-air partitioning coefficient (K_{pa}). In addition, the Authors propose that biomagnification is evaluated by TMFs based on protein-normalised concentrations in

food web organisms. Ng and Hungerbuhler (2013) have recently developed a bioconcentration model in fish that for the first time explicitly considers interactions of perfluorinated acids with proteins including: i) binding to serum albumin in blood and interstitial fluids; ii) binding to cytosolic Fatty Acid Binding Proteins (FABPs) in the liver; and iii) renal clearance facilitated by Organic Anion Transporters (OATs). The Authors remark that deep understanding and modelling of protein binding is key for providing the full picture on the bioaccumulation potential of perfluorinated acids (Ng and Hungerbuhler 2013).

Different views are reported by Webster and Ellis (2011) and Armitage et al. (2013). According to these Authors, the traditional equilibrium partitioning approach and related models are appropriate for perfluorinated acids. Webster and Ellis (2011) remark that: i) the amphiphilic/charged/surface active nature of surfactants is a property of the anion but not of the neutral species; ii) bioaccumulation of the neutral species is dominant due to larger K_{ow} compared to the one of the anionic species (regardless the relative fractions in the medium); and iii) octanol is a sufficient surrogate of any organic phase and K_{ow} can therefore be used to predict the bioaccumulation potential of any substance regardless its preference for lipids or protein-rich tissues. Webster and Ellis (2011) algebraically demonstrate that standard hydrophobically-driven equilibrium partitioning models are valid for perfluorinated acids and usable without modification. Their model for BCF prediction is based on two physico-chemical properties: pK_a (as measured in the study performed by Burns et al. (2008)) and K_{ow} of the neutral species (adjusted by the fraction of neutral species at biological pH). More recently, a mechanistic model to predict bioconcentration of ionogenic organic chemicals in fish has been proposed by Armitage et al. (2013). This model relies on the existing mechanistic framework for neutral organic chemicals (i.e. bioaccumulation as net result of uptake and elimination), which was modified to account for: i) dissociation and relative fractions of neutral and charged species; ii) transport of charged species across the gill (as illustrated by Shore et al. (1957)); and iii) preferential sorption of charged species to phospholipids (as demonstrated by: Lehmler and Bummer (2004); Lehmler et al. (2006); Xie et al. (2010a; 2010b)). This study highlights the importance of considering the interaction of charged species with phospholipids for prediction of bioaccumulation of ionogenic organic chemicals but also calls for further empirical investigations (Armitage et al. 2013). The Authors state that interaction with site-specific proteins can be included in the model using the volume fraction of proteins and estimated distribution ratios for the charged species (Armitage et al. 2013). However, it is recommended that the effect of competition for binding sites with endogenous and exogenous ligands as well as differences among tissues are considered when protein binding is modelled (as previously noted by: Jones et al. (2003); Han et al. (2005)).

3.6 Data availability

The availability of experimentally measured BCF and BAF values in the literature is limited (Arnot and Gobas 2006; Arnot and MacKay 2008; Gobas et al. 2009). However, in a regulatory context like the European REACH Regulation and Biocidal Products Regulation (BPR), screening criteria can be used to decide whether additional information on the bioaccumulation potential must be generated.

3.7 Technical issues

Bioconcentration tests are difficult to perform, especially for highly hydrophobic, very poorly water soluble organic substances (e.g. adsorption to test containers); moreover, bioconcentration tests are time consuming (e.g. for highly hydrophobic substances several months may be necessary to reach the steady state) and costly (Gobas et al. 2009).

4 Evidence of bioaccumulation in non-aquatic organisms

Both log K_{ow} and BCF/BAF criteria in European and international legislation largely apply to aquatic (or water-respiring) organisms without taking into account that certain substances might have a larger bioaccumulation potential in non-aquatic food chains than in aquatic ones or be bioaccumulative only in non-aquatic (or air-respiring) organisms, including humans.

In the past, studies on bioaccumulation in non-aquatic organisms were sporadic. Some examples are: Salisbury et al. (1992); Thomas et al. (1992); Landers et al. (1995); and Wong (1995). According to a recent review (Gobas et al. 2009), more systematic research has been carried out in the last years, including experimental studies and models aimed to support the identification of bioaccumulative substances in non-aquatic food webs and thus demonstrate the limits of the current regulatory bioaccumulation assessment (e.g. Kelly and Gobas 2001; 2003; Gobas et al. 2003; Armitage and Gobas 2007; Kelly et al. 2007; Tonnelier et al. 2011). The following paragraphs report and discuss the main literature findings in the field of bioaccumulation in non-aquatic food webs, including examples of substances that show higher and/or different bioaccumulation potential in non-aquatic organisms (including humans) compared to aquatic ones (see Table 2 for a summary).

The research and monitoring carried out for POP in remote areas like the Arctic Sea and Northern Canada indicates differences in bioaccumulation patterns of POPs and organochlorides between poikilotherms (including fish, crustaceans) and homeotherms (including air-breathing organisms such as birds and mammals) in various food chains.

Among the others, Fisk et al. (2001) calculated TMF and BMF values for several POPs measured in different species of a **Canadian Arctic marine food web**. The Authors underline that TMF values for food webs including only poikilotherms (i.e. zooplankton and fish) are lower than TMF for food webs including both poikilotherms and homeotherms (i.e. seabirds and/or mammals). BMF values are also much higher in seabirds and mammals than in fish and zooplankton. The greater bioaccumulation showed by homeotherms is attributed to their greater energy requirements and subsequent feeding rates, suggesting that birds tend to bioaccumulate contaminants more than mammals (Fisk et al. 2001).

Hop et al. (2002) calculated TMF and BMF values for several POPs in the **Barents Sea** and confirm the results previously reported by Fisk et al. (2001) for the Canadian Arctic. Specifically, the higher biomagnification of β -hexachlorocyclohexane (β -HCH), oxychlordan, *cis*-chlordan, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), and polychlorinated biphenyl (PCB) from 47 to 153⁹ in homeotherms than in poikilotherms is related to higher energy requirements, higher trophic

⁹ Authors do not report K_{ow} values of investigated substances.

position, longer life-span, and negligible direct exchange with seawater across respiratory surfaces (Hop et al. 2002).

Borgå et al. (2001) studied biomagnification patterns of organochlorides along a Barents Sea food chain and found lower concentrations of organochlorides such as Σ HCHs ($\log K_{ow} = 3.9^{10}$) and hexachlorobenzene (HCB) ($\log K_{ow} = 5.2$), Σ Chlordanes ($\log K_{ow} = 6.1-6.4$), dichlorodiphenyltrichloroethane (Σ DDTs) ($\log K_{ow} = 5.7$), Σ PCBs ($\log K_{ow} = 28-153$) in pelagic crustaceans and fish than in sea birds (i.e. one to three orders of magnitude less). The low concentrations in crustaceans and fish are attributed to their ability to eliminate contaminants by diffusion to water. It is also noticed that the organochlorine pattern change moving up the food chain. Specifically, the relative contribution of α -HCH and γ -HCH to Σ HCHs, HCB, and *cis*-chlordane and *trans*-nonachlor to Σ Chlordanes decreases while the relative contribution of their metabolites (i.e. β -HCH and oxychlordane) as well as more persistent compounds (i.e. Σ DDTs and Σ PCBs) increases. These results reflect the different elimination potentials between poikilotherms such as crustaceans and fish, which eliminate contaminants through direct diffusion via water, and homeotherms such as seabirds, which exhibit higher metabolic rates and consequently higher ability to metabolise contaminants (Livingstone 1992; Livingstone et al. 1992; Borga et al. 2001). Hoekstra et al. (2003), who studied trophic transfer of organochlorides in an Arctic marine food web located in Alaska, also support this conclusion.

In a review of the mechanisms and models of intestinal absorption and bioaccumulation of organic chemicals in wildlife and humans, Kelly et al. (2004) underline that BMFs in **homeotherms** are higher than those in poikilotherms organisms and follow different relationships with the physico-chemical properties of chemicals for two main reasons: i) efficient gastrointestinal absorption; and ii) very slow respiratory elimination to the air. Accordingly, the Authors recommend further investigations into the mechanisms of bioaccumulation in homeotherms and reconsideration of the current regulatory initiatives where the fundamental processes controlling biomagnification in air-breathing homeotherms are not fully recognised (Kelly et al. 2004).

The question of whether chemicals with $\log K_{ow} < 5$ (i.e. classified as 'not bioaccumulative' in aquatic species according to the Stockholm Convention on POPs as illustrated in Table 1) can exhibit an ability to biomagnify in non-aquatic food webs was first explicitly addressed by Kelly and Gobas (2001)¹¹. A field study showed that some persistent but less hydrophobic substances can substantially biomagnify in the **Arctic lichens-caribou-wolf terrestrial food chain** despite of their low $\log K_{ow}$ and therefore in contrast to what occurs in aquatic food chains. Specifically, the Authors compared the bioaccumulation of organic compounds with different $\log K_{ow}$ values to investigate

¹⁰ Authors report $\log K_{ow}$ values compiled from the literature.

¹¹ Authors state that K_{ow} values were compiled from the literature (available as Supporting Information).

congener-specific bioaccumulation patterns. β -HCH ($\log K_{ow} = 3.81$) shows evidence of bioaccumulation in the whole Arctic lichens-caribou-wolf food chain. In particular, it appears that caribou and wolves do not efficiently eliminate β -HCH. 1,2,4,5 tetrachlorobenzene (1,2,4,5 TCB) ($\log K_{ow} = 4.70$) also shows evidence of biomagnification in the whole Arctic lichens-caribou-wolf food chain.

Subsequently, Kelly and Gobas (2003)¹² developed a mechanistic model to predict BMF values of POPs in the Arctic lichens-caribou-wolf terrestrial food chain. The model's results show that moderately hydrophobic and non-metabolisable organic compounds such as β -endosulfan ($\log K_{ow} = 3.7$), β -HCH ($\log K_{ow} = 3.8$) and 1,2,4,5 TCB ($\log K_{ow} = 4.6$) can biomagnify in the terrestrial mammals food chain and in some cases exhibit greater BMF values than more hydrophobic substances. The Authors suggest that the negligible respiratory elimination via exhalation to the air of these organic compounds may be responsible for this phenomenon (Kelly and Gobas 2003).

In a review of mechanisms and models of intestinal absorption and bioaccumulation of organic chemicals in wildlife and humans, Kelly et al. (2004) report a list of polar non-volatile compounds that do not biomagnify in aquatic organisms (i.e. $\log K_{ow} < 5$) but may substantially biomagnify in **air-breathing organisms**, unless they are sufficiently metabolised at a significantly high rate or depurated by urinary excretion. The list include: HCHs, endosulfan, atrazine, bis-4-chlorophenyl sulfone (BCPS), trischlorophenyl methanol (TCPMeOH), perfluorooctane sulfonate (PFOS)¹³.

In 2007, Kelly et al. measured concentrations of organic contaminants of varying hydrophobicity in a piscivorous food web (water-respiring organisms only), a terrestrial food web (air-breathing organisms only) and a marine mammalian food web (including water- and air-breathing organisms) from northern Canada. Compounds characterised by low estimated $\log K_{ow}$ values¹⁴ such as β -HCH ($\log K_{ow} = 3.8$), 1,2,4,5-TCB ($\log K_{ow} = 4.7$), β -endosulfane ($\log K_{ow} = 3.7$), dicofol ($\log K_{ow} = 3.5$), musk xylene ($\log K_{ow} = 4.1$), trifluralin ($\log K_{ow} = 4.4$), and tetradifon ($\log K_{ow} = 4.6$) do not biomagnify in the piscivorous food web but **in the terrestrial food web and in the air-breathing organisms of the marine mammalian food web**. The Authors report that air-breathing organisms exhibit greater ability to absorb and digest their diet than water-respiring organisms, which is related to differences in digestive tract physiology and body temperature (Kelly et al. 2007).

Humans being at the top of both aquatic and non-aquatic food webs are particularly exposed to persistent pollutants in the environment (Czub and McLachlan 2004a). A mechanistically-based, non-steady state model developed by Czub and McLachlan (2004a; 2004b) predicts less hydrophobic but

¹² Authors state that K_{ow} values are calculated at 20°C and results are comparable to reported values in the literature.

¹³ Authors do not report K_{ow} values of investigated substances.

¹⁴ Authors state that K_{ow} values were compiled from the literature and temperature-corrected.

non-volatile substances such as atrazine, mecoprop, 2,4-dichlorophenoxyacetic acid, γ -HCH, and phenanthrene as bioaccumulative in humans despite their $\log K_{ow}$ lower than 5¹⁵. The Physiologically Based Toxicokinetic model for screening of chemicals with human bioaccumulative potential developed by Tonnelier et al. (2011) confirms high bioaccumulation potential in humans for PCBs¹⁶ ($\log K_{ow}$ ranging from 6.6 to 7.65), DDTs ($\log K_{ow} = 6.91$), and PFOS ($\log K_{ow} = 6.28$), in line with the available literature. In addition, pesticides such as emamectin ($\log K_{ow} = 5.0$), buprofezin ($\log K_{ow} = 4.3$), fenvalerate ($\log K_{ow} = 6.2$), parathion ($\log K_{ow} = 3.83$), cypronidil ($\log K_{ow} = 4.0$), pyraclostrobin ($\log K_{ow} = 5.45$), fipronil ($\log K_{ow} = 4.0$), and bromacil ($\log K_{ow} = 2.11$) show high bioaccumulation potential in humans despite their low BCF values in fish (< 1700 Kg/L) and relatively low $\log K_{ow}$ values (most of them lower than 4.3).

Some of the chemicals mentioned above are classified as bioaccumulative and currently banned or restricted under the **Stockholm Convention on POPs** (UNEP 2001; 2009) (see Section 2, footnote 4). PCBs, DDTs, chlordane, and HCB are part of 'the dirty dozen', which is the original list compiled in 2001. A few substances such as PFOS, C-Octabromodiphenyl ether (Octa-BDE), lindane, α -HCH and β -HCH were added later in 2009. Endosulfan (and its isomers) was included in 2011. Many of these substances are pesticides, which are strongly related to terrestrial food chains. It is indeed pointed out that substances like PCBs and DDTs were most affecting non-aquatic organisms and the effects were more evident on birds and mammals rather than fish (Gobas et al. 2003). In a *Discussion Paper on Bioaccumulation Evaluation* prepared for the POPs Review Committee Third Meeting in 2007, Kitano points out that substances such as lindane, α -HCH, β -HCH, and OctaBDE fulfil the screening criteria of the Stockholm Convention on POPs despite their low BCF values (< 5000 L/Kg) as they show evidence of bioaccumulation in non-aquatic organisms. The following evidences are reported in the Kitano's discussion paper: i) detection of high concentrations of substances having BAF/BCF values < 5000 L/kg in tissues of Arctic seabirds and mammals; ii) detection in breast milk and placenta tissues in mammals; iii) detection in bird eggs; iv) detection in breast milk and placenta tissues in humans; v) BMF values higher than 1 for different trophic levels including upper trophic levels (e.g. mammals) of marine and terrestrial food webs; vi) high half-life in humans; vii) high soil-organism bioaccumulation factor.

Substances such as 1,2,4,5-TCB, oxychordane, dicofol, musk xylene, trifluralin, and tetradifon are still not covered by the Stockholm Convention on POPs. However, musk xylene is a Substance of Very High Concern (SVHC) included in the **REACH Candidate List for Authorisation** because of its vPvB properties. According to ECHA (2008), the vB assignment is based on a key study reporting a number of BCF values in fish, some of them greater than 5000 Kg/L. Bioaccumulation in terrestrial organisms

¹⁵ Authors do not report $\log K_{ow}$ values for the investigated substances. However, Figure 6 in Czub and McLachlan (2004b) shows these substances as characterised by $\log K_{ow}$ lower than 5.

¹⁶ Physico-chemical properties including $\log K_{ow}$ are estimated by EPI Suite v4.0 (US EPA 2011).

was estimated but not taken into account in the conclusion. Some recent publications also confirm musk xylene's bioaccumulation potential in aquatic organisms.

Table 2. List of substances for which there is evidence of bioaccumulation in non-aquatic organisms (and homotherms) as reported in the scientific literature. K_{ow} = octanol-water partitioning coefficient.

Substance name	Log K_{ow}	Evidence on bioaccumulation in non-aquatic organisms (and homeotherms)	Reference
β -hexachlorocyclohexane (β -HCH)	Not reported	Evidence of higher biomagnification in homeotherms than poikilotherms in Barents Sea and Canadian Arctic	Fisk et al. 2001 Hop et al. 2002
	3.81	Evidence of bioaccumulation in Arctic lichens-caribou-wolf food chain	Kelly and Gobas 2001
	3.8	Predicted biomagnification in Arctic lichens-caribou-wolf food chain	Kelly and Gobas 2003
	3.8	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
	< 5	Predicted as bioaccumulative in humans	Czub and McLachlan 2004a;b
Σ hexachlorocyclohexanes (Σ HCH)	3.9	Higher concentrations in sea birds than pelagic crustaceans and fish in Barents Sea	Borga et al. 2001
	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
Oxychlorane	Not reported	Higher biomagnification in homeotherms than poikilotherms in Barents Sea and Canadian Arctic	Fisk et al. 2001 Hop et al. 2002
<i>cis</i> -chlorane	Not reported	Higher biomagnification in homeotherms than poikilotherms in Barents Sea and Canadian Arctic	Fisk et al. 2001 Hop et al. 2002
Σ chloranes	6.1-6.4	Higher concentrations in sea birds than pelagic crustaceans and fish in Barents Sea	Borga et al. 2001

Table 2. (cont.)

Substance name	Log K_{ow}	Evidence on bioaccumulation in non-aquatic organisms (and homeotherms)	Reference
p,p'-dichlorodiphenyldichloro ethylene (p,p'-DDE)	Not reported	Higher biomagnification in homeotherms than poikilotherms in Barents Sea and Canadian Arctic	Fisk et al. 2001 Hop et al. 2002
Dichlorodiphenyltrichloro ethane (Σ DDTs)	5.7	Higher concentrations in sea birds than pelagic crustaceans and fish in Barents Sea	Borga et al. 2001
Polychlorinated biphenyl (PCB) from 47 to 153	Not reported	Higher biomagnification in homeotherms than poikilotherms in Barents Sea and Canadian Arctic	Fisk et al. 2001 Hop et al. 2002
Polychlorinated biphenyl (PCBs)	28-153	Higher concentrations in sea birds than pelagic crustaceans and fish in Barents Sea	Borga et al. 2001
Hexachlorobenzene (HCB)	5.2	Higher concentrations in sea birds than pelagic crustaceans and fish in Barents Sea	Borga et al. 2001
1,2,4,5 tetrachlorobenzene (1,2,4,5 TCB)	4.70	Evidence of bioaccumulation in Arctic lichens-caribou-wolf food chain	Kelly and Gobas 2001
	4.6	Predicted biomagnification in Arctic lichens-caribou-wolf food chain	Kelly and Gobas 2003
	4.7	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
β -endosulfan	3.7	Predicted biomagnification in Arctic lichens-caribou-wolf food chain	Kelly and Gobas 2003
	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
	3.7	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
Atrazine	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
	< 5	Predicted as bioaccumulative in humans	Czub and McLachlan 2004a;b

Table 2. (cont.)

Substance name	Log K _{ow}	Evidence on bioaccumulation in non-aquatic organisms (and homeotherms)	Reference
bis-4-chlorophenyl sulfone (BCPS)	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
Trischlorophenyl methanol (TCPMeOH)	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
Perfluorooctane sulfonate (PFOS)	< 5	May substantially biomagnify in air-breathing organisms	Kelly et al. 2004
Dicofol	3.5	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
Musk xylene	4.1	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
Trifluralin	4.4	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
Tetradifon	4.6	Evidence of biomagnification in terrestrial food web and air-breathing organisms of marine mammalian food web from Northern Canada	Kelly et al. 2007
Mecoprop	< 5	Predicted as bioaccumulative in humans	Czub and McLachlan 2004a;b
2,4-dichlorophenossiacetic acid	< 5	Predicted as bioaccumulative in humans	Czub and McLachlan 2004a;b
Phenantrene	< 5	Predicted as bioaccumulative in humans	Czub and McLachlan 2004a;b
Emamectin	5.0	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Buprofezin	4.3	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011

Table 2. (cont.)

Substance name	Log K _{ow}	Evidence on bioaccumulation in non-aquatic organisms (and homeotherms)	Reference
Fenvalerate	6.2	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Parathion	3.83	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Cypronidil	4.0	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Pyraclostrobin	5.45	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Fipronil	4.0	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011
Bromacil	2.11	High bioaccumulation potential in humans despite low BCF values in fish (< 1700 Kg/L)	Tonnelier et al. 2011

5 Literature criteria for screening and assessment of bioaccumulation in non-aquatic organisms

The evidences reported in Section 4 and summarised in Table 2 suggest that current international and national regulatory systems, where only quantitative bioaccumulation criteria based on $\log K_{ow}$ and/or aquatic BCF/BAF data are considered for screening purposes (see Table 1), may fail to identify a number of substances that have the potential of biomagnifying in non-aquatic food chains but not in aquatic ones. In Europe there has been improvement in the bioaccumulation assessment requirements under the REACH Regulation as additional qualitative criteria including 'bioaccumulation in other organisms such as terrestrial organisms' and 'detected levels in human tissues' have been formally mentioned in the revised REACH Annex III (see Table 1). However, these criteria are subject to interpretation as no cut-off value is provided and there is currently no clear guidance on how to apply them in a weight of evidence approach (see Section 2).

Several Authors (e.g., Kelly and Gobas 2001; 2003; Gobas et al. 2003; Kelly et al. 2004; Armitage and Gobas 2007; Kelly et al. 2007; Kitano 2007; Gobas et al. 2009; McLachlan et al. 2011; Tonnelier et al. 2011) have therefore highlighted the need of incorporating new regulatory criteria or adjusting the existing ones, thus enabling the identification of substances that are potentially bioaccumulative in non-aquatic organisms (including humans) but not in aquatic ones. The following paragraphs describe and discuss the criteria and related threshold values, which have been suggested in the scientific literature (see Table 4 for a summary).

5.1 Screening criteria

The statistical analysis on the data collected in the experimental work in Canadian Arctic lichen-caribou-wolf food chains carried out by Kelly and Gobas (2001) reveals the important relationship between K_{oa} and BMF values in terrestrial mammals. The study shows statistically significant increases in the BMF values in wolves (but not in caribou due to their ruminant digestive system) with increasing K_{ow} and K_{oa} . The data suggest that wolves have an efficient digestive system and high uptake rate through the gastrointestinal tract, which is counterbalanced by elimination via air (exhalation). Consequently, non-metabolised hydrophobic substances with high K_{oa} are eliminated slower via air (compared to substances with low K_{oa}) and hence exhibit higher biomagnification (Kelly and Gobas 2001). Moreover, it is pointed out that the increase of BMF values with K_{oa} is less than proportional. This phenomenon is ascribed to the expected reduction in uptake with increasing K_{ow} of the substances (see Section 3.1), which seems to occur at a lower rate than the reduction in elimination via air with increasing of K_{oa} and thus resulting in a less than proportional relationship between BMF and K_{oa} (Kelly and Gobas 2001). The Authors indicate gastrointestinal absorption efficiencies (strongly dependent on K_{ow}) and lipid-to-air elimination rates (strongly dependent on K_{oa}) as two important parameters affecting POPs bioaccumulation and call for additional research that enables the identification of a regulatory threshold.

Based on these findings, the Authors used the same dataset to develop models for bioaccumulation of POPs in the Arctic terrestrial food web, and in a couple of follow-up papers explicitly propose that $\log K_{oa}$ is included as a bioaccumulation predictor in current regulatory systems (Kelly and Gobas 2003; Gobas et al. 2003). The models demonstrate that:

- Predicted BAF values (lichens, caribou and wolves) increase with increasing K_{oa} ;
- Predicted BAF values increase with trophic level, thus highlighting the importance of dietary uptake and biomagnification in the terrestrial food chains;
- $\log K_{oa}$ is a better predictor of BMF values in non-aquatic organisms due to the importance of animal-to-air exchange: relatively hydrophilic and non-metabolised chemicals exhibit greater BMF values than more hydrophobic substances due to higher K_{oa} and hence negligible respiratory elimination;
- Non metabolised chemicals with $\log K_{oa}$ lower than 5 do not biomagnify in terrestrial food chains (air respiration is the main route of elimination);
- Chemicals with $\log K_{ow}$ lower than 5 (i.e. not bioaccumulative in aquatic organisms according to the Stockholm Convention criteria) can biomagnify in terrestrial food chains;
- Chemicals with $\log K_{oa}$ higher than 5 can biomagnify in terrestrial food chains if $\log K_{ow}$ is higher than 2 and metabolism is low (substances are very poorly eliminated into air as well as urine and bile);
- Chemicals with high K_{oa} but $\log K_{ow}$ lower than 2 have a reduced biomagnification potential due to the efficient urine excretion;
- BMF values in terrestrial food chains can be much greater than those in aquatic food chains (generally one order of magnitude greater);
- BMF values for non-aquatic organisms do not exhibit a 'hydrophobicity cut-off' (i.e. no BMF maximum at $\log K_{ow} \sim 7$ and drop off with increasing K_{ow}) as it is the case for aquatic organisms. This is because the dietary uptake efficiency in mammals and birds does not show the same reduction as in fish (this phenomenon is known as 'hydrophobicity cut-off' and is discussed in Section 3.1).

Kelly et al. (2007) measured and compiled concentrations of organic contaminants of varying K_{ow} in a piscivorous food web (water-respiring organisms only), a terrestrial food web (air-respiring organisms only) and a combined marine mammalian food web (including water- and air-respiring organisms) from northern Canada. The study confirms previous results, i.e.:

- Less hydrophobic compounds ($\log K_{ow} < 5$) do not biomagnify in the piscivorous food web but show a high degree of biomagnification in the terrestrial food web and in air-breathing organisms of the marine mammalian food web;

- Air-breathing organisms exhibit higher BMFs than those in water-respiring organisms because of their greater ability to absorb and digest their diet;
- The relationship between the BMF and chemical properties is controlled by the rate of elimination (in water-respiring organisms elimination becomes sufficiently slow for substances with $\log K_{ow} < 5$ whereas in air-respiring organisms this occurs when $\log K_{oa} > 5$ and $\log K_{ow} > 2$).
- In the terrestrial food web non-metabolised chemicals with a $\log K_{ow}$ between 2 and 10 and $\log K_{oa} > 6$ can biomagnify (in contrast to the piscivorous food web where biomagnification does not occur for super-hydrophobic organic substances with $\log K_{ow} > 8$, which are absorbed at very slow rate);
- Simulations of human dietary exposure of contaminants to the indigenous Inuit population of Northern Canada shows that biomagnification can occur for chemicals with a $\log K_{ow}$ in the range 2-5 and $\log K_{oa} > 6$.

In addition to previous studies, a mechanistic terrestrial food chain bioaccumulation model for POPs was developed and applied to the soil-earthworms-shrews system by Armitage and Gobas (2007). The results demonstrate that:

- Substances with $\log K_{oa} < 5$ do not biomagnify in terrestrial vertebrates;
- Substances with $\log K_{ow} < 2$ have no bioaccumulation potential;
- Very hydrophobic substances with $\log K_{ow}$ between 8 and 12 show BMF values greater than 1 in this system;
- Substances with $\log K_{ow}$ between 4 and 10 and $\log K_{oa} > 7$ exhibit the greatest BMF values.

According to the 2001-2007 literature, **there seems to be a consensus in the scientific community on the importance of considering K_{oa} as criterion to identify substances that may bioaccumulate/biomagnify in non-aquatic food chains including humans.** Specifically, two main implications from a regulatory point of view can be pointed out.

The first implication is that substances characterised by **$\log K_{ow}$ in the range 2-5 and $\log K_{oa} > 5$** represent a new group of potentially bioaccumulative substances that may not be identified by the criteria used e.g. under the Stockholm Convention (i.e. bioaccumulative if $\log K_{ow} > 5$) or the Canadian Environmental Protection Act (i.e. bioaccumulative if $\log K_{ow} > 5$) and currently suggested in the ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment* (i.e. non bioaccumulative if $\log K_{ow} < 4.5$) (ECHA 2012a). According to Gobas et al. (2003), the 67% (i.e. two thirds) of approximately 12000 organic substances in the Canadian Domestic Substances List have low K_{ow} but high K_{oa} and are therefore neglected even if they have the potential to bioaccumulate in non-aquatic organisms. This implication may therefore have a large impact if considered in a screening/prioritisation exercise or incorporated into the regulatory criteria for PBT/vPvB assessment.

The second implication is that categorising very hydrophobic organic chemicals with a $\log K_{ow} > 9-10$ as being non bioaccumulative due to the slow rate of absorption in aquatic organisms may neglect their high bioaccumulation potential in terrestrial food chains (Kelly and Gobas 2003; Gobas et al. 2003; Armitage and Gobas 2007). In particular, Armitage and Gobas (2007) report BMF higher than 1 for organic chemicals with $\log K_{ow}$ in the range 8-12 with maximum BMF values for organic chemicals with $\log K_{ow}$ in the range 4-10 and $\log K_{oa}$ higher than 7. However, **the bioaccumulation/biomagnification potential of highly hydrophobic substances in both aquatic and non-aquatic organisms and related cut-off values would need further research and discussion before specific provisions may be explicitly included into a regulatory framework.**

Finally, it has to be pointed out that K_{oa} is also based on the equilibrium partitioning assumption to lipids. Therefore, **K_{oa} -based cut-off values are also affected by the same limitations as K_{ow} -based ones in relation to substances that partition into other tissues, e.g. proteins, and may not be universally applicable as a screening method for the non-aquatic compartment** (this issue is discussed in Section 3.5).

5.2 Assessment criteria

In January 2008 an international workshop sponsored by the Society of Environmental Toxicology and Chemistry (SETAC) was held in Pensacola (FL, USA). The workshop aimed at discussing scientific aspects related to persistence, bioaccumulation, toxicity and long-range transport in order to provide recommendations on how to use the available scientific information and foster the advancement of a sound scientific foundation for the regulatory criteria used to identify POP and PBT substances (Klečka et al. 2009). A series of three articles were published to address methods for the evaluation of the bioaccumulation potential including predictive approaches, *in vivo* laboratory tests and field studies (i.e. Nichols et al. 2009; Weisbrod et al. 2009; Gobas et al. 2009). One more article discussed the use of measurement data for the evaluation of exposure to wildlife and humans (Swackhamer et al. 2009).

Nichols et al. (2009) reviewed the use of predictive approaches to investigate Adsorption Distribution Metabolism Elimination (ADME) processes and assess bioaccumulation including Quantitative Structure-Activity Relationships (QSARs) models, mass-balance models, food web bioaccumulation models and *in vitro* systems. The discussion centres on bioaccumulation assessment for fish but the Authors state that the same principles apply to bioaccumulation assessment for other animals, including terrestrial wildlife and humans (Nichols et al. 2009). The Authors acknowledge the 2001-2007 literature led by Kelly, Gobas and co-workers addressing air-breathing organisms and suggesting K_{oa} as an additional criterion for bioaccumulation assessment. In addition to that, the Authors suggest that modelling efforts for both aquatic and non-aquatic organisms consider multi-media fate information to determine the medium of exposure based on the mode of entry into the environment (Nichols et al. 2009). The Authors conclude that a weight of evidence approach is

applied considering both *in vitro* data and computational prediction models, which is generic and applicable to both aquatic and non-aquatic organisms.

Weisbrod et al. (2009) reviewed *in vivo* laboratory and field metrics that can be used to assess bioaccumulation in PBT substances and POPs identification. In particular, the Authors considered several bioaccumulation metrics that may be related to non-aquatic species:

- Biota Soil/Sediment Accumulation Factor (BSAF);
- Bioaccumulation Factor (BAF);
- Biomagnification Factor (BMF);
- Trophic Magnification Factor (TMF); and
- Tissue residues vs Critical tissue residues.

A conservative trigger value of 1 is recommended for BSAF, BMF and TMF because values above 1 represent amplification above the expected bioaccumulation due to simple equilibrium partitioning (Weisbrod et al. 2009).

The Authors state that **results from soil exposure laboratory tests or field studies, which are typically reported as BSAF values, are useful for indicating whether the substance can accumulate from soil into organisms at the base of some food chains. A BSAF value > 1 indicates the substance may be bioaccumulative.** However, the Authors point out that **a BSAF value < 1 does not necessarily indicate a correlative BAF value will be low as field-based BAF values reflect steady-state conditions with multiple exposure routes.** Moreover, **BSAF values with soil organisms do not provide any insight into the potential of a substance for biomagnification along the food chain.** Consequently, BSAF may be less likely to raise concern than other bioaccumulation metrics that inherently account for more exposure routes and trophic levels (Weisbrod et al. 2009). From a technical point of view, BSAF values are also subject to several limitations: i) 'aging' of the spiked soil; ii) non-equilibrium conditions may prevail due to spiking procedures; iii) BSAF is an endpoint composed of at least four underlying measurements (chemical concentration in soil/sediment, organic carbon fraction in soil/sediment, chemical concentration in biota, lipid fraction in biota), which causes considerable propagation of error; and iv) BSAF is a ratio between two non-polar phases (organic carbon and lipid), which renders it relatively insensitive to differences in compound's hydrophobicity (Weisbrod et al. 2009).

In this context, it is worth to mention that there are tools such as the OECD Test Guideline (TG) 317 on *Bioaccumulation in Terrestrial Oligochaetes*, which are already available (OECD 2010). As extrapolation from aquatic bioaccumulation data to non-aquatic organisms is difficult, if not possible, and earthworms (*Oligochaetes*) represent a food source for several organisms including vertebrates e.g. foxes and gulls (OECD 2010), the OECD TG 317 may help with obtaining an indication of the bioaccumulation potential for low trophic levels of the non-aquatic food chains. In the OECD TG 317, various uptake routes are considered (i.e. not only direct contact with chemicals in the soil solution

through the outer skin but also exposure to soil-bounded chemicals via ingestion of soil particles) and results can be reported as BAF ($K_{g_{soil}}/K_{g_{worm}}$) and, additionally, as BSAF ($K_{g_{soilorganiccarbon}}/K_{g_{wormlipidcontent}}$). The OECD TG 317 is applicable to both organic chemicals, including very hydrophobic substances with $\log K_{ow} > 6$, and metals.

The current version of the ECHA 'Guidance on information requirements and chemical safety assessment. Chapter R.7c: Endpoint-specific Guidance' advises that BSAF may be a misleading indicator as it also reflects sorption behaviour and suggests that **BCF based on freely dissolved chemical concentrations in soil pore water could be a better choice** (ECHA 2012b). A mechanistic approach for estimating bioconcentration of organic chemicals in earthworms was proposed by Jager in 1998. More recently, the model has been tested and assessed by Brooke and Crookes (2007). ECHA suggests its application under the REACH Regulation (ECHA 2012b; 2012c¹⁷). According to ECHA, BCF data from sediment-dwelling organisms may also be used as surrogate for terrestrial earthworms data on a case-by-case basis provided that any differences in organic carbon and pore water content between sediment and soil is taken into account (ECHA, 2012b).

Finally, Solomon et al. (2013) have recently concluded that BSAF and BCF in soil could be used as criteria to assess bioaccumulation in non-aquatic organisms provided that a normalisation procedure is established to account for the matrix influence.

BAF determined in the field is a more ecologically relevant metric as it reflects steady-state conditions and bioaccumulation from multiple routes of exposure, including the diet. For this reason, BAF values are usually higher than corresponding BCF values for the same species (Arnot and Gobas 2006; Weisbrod et al. 2009) and should be preferred over BCF, when available (Arnot and Gobas 2006). A drawback is that BAF data are largely variable and affected by a number of site-specific environmental conditions (Weisbrod et al. 2009) as well as analytical factors (Arnot and Gobas 2003; Gobas et al. 2009). Moreover, BAF data are not available for most chemicals in commerce (Arnot and Gobas 2006). It is worth to notice that a QSAR-based model that could be used for regulatory purposes was developed by Arnot and Gobas (2003) (see also Costanza et al. (2012)). The model can be adapted to consider metabolic rate. However, it only predicts bioaccumulation potential in aquatic food webs and caution is required when it is applied to charged or ionic compounds (Arnot and Gobas 2003). It needs to be pointed out that all consulted literature sources that discuss the use of field-based BAF as regulatory criteria refer to aquatic species.

BMF is considered as a more powerful metric as it gives an insight into dietary exposure and biomagnification potential of a substance, which seems to be a major concern for higher trophic levels in both aquatic and terrestrial food webs. According to Weisbrod et al. (2009), **substances with BMF values higher than 1 exhibit potential for biomagnification and substances with BMF values**

¹⁷ A consultation procedure is currently on-going to update the guidance documents. Link to the ECHA website where the last drafts are available: <http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach>.

lower than 1 may undergo trophic dilution. However, the Authors point out that **BMF values focus on single trophic relationships and may largely vary among studies and species, especially if field-based** (Weisbrod et al. 2009).

TMF is the most comprehensive metric for understanding the biomagnification potential of a substance as it represents the average increase or decrease of concentration levels in a food chain: a TMF higher than 1 indicates that the substance biomagnifies in the food web (i.e. concentration increases with trophic level); a TMF lower than 1 indicates that the substance undergoes trophic dilution (Weisbrod et al. 2009). However, the Authors highlight that TMF values are also affected by some assumptions (Weisbrod et al. 2009). For example, one assumption is that all organisms have similar metabolism efficiency but e.g. poikilotherms and homeotherms are known to have different metabolic capabilities. The consequence is that the TMF value may overestimate or underestimate the degree of biomagnification in poikilotherms and homeotherms, respectively (Weisbrod et al. 2009).

The Authors also suggest the use of contaminant body burden (or tissue residue) as field metric for bioaccumulation assessment. These values can be considered as surrogate for the internal dose and can be compared to known critical body burdens (or critical tissue residues) derived from laboratory acute or chronic toxicity studies, thus allowing calculation of hazard quotients and prediction of risk (Weisbrod et al. 2009). Weisbrod et al. (2009) propose 0.1 or 0.01 as threshold value when acute or chronic critical body burdens (or critical tissue residues) are used but do not discuss such numbers.

Weisbrod et al. (2009) propose benchmark values or approximate maximum values for several bioaccumulation metrics. Table 3 illustrates the benchmark values for metrics that are relevant for non-aquatic organisms. Data that exceed these values or limits should be critically evaluated for potential errors in their derivation (Weisbrod et al. 2009).

Table 3. Benchmark values for bioaccumulation metrics that are relevant for non-aquatic organisms (Modified from: Weisbrod et al. 2009). BSAF = Biota Soil/Sediment Accumulation Factor. BMF = BioMagnification Factor. TMF = Trophic Magnification Factor. OC = Organic carbon. LC = Lipid Content.

Bioaccumulation metrics	Measurement unit	Benchmark values
Laboratory Invertebrates BSAF	$K_{g_{soil/oc}}/K_{g_{biota/c}}$	3
Mammal BMF	$K_{g_{prey}}/K_{g_{mammal}}$	100
Avian BMF	$K_{g_{prey}}/K_{g_{avian}}$	10
TMF	-	10

In order to overcome the limitations of the current legislative schemes on bioaccumulation assessment, Gobas et al. (2009) propose a scientific definition for 'bioaccumulative substances' and a tiered framework outlining how various metrics can be used for identifying 'bioaccumulative' substances. According to the Authors, 'A substance is considered bioaccumulative if it biomagnifies in food chains. Biomagnification is defined as the phenomenon wherein the normalised concentration (or fugacity) of the chemical in biological organisms increases with increasing trophic position' (Gobas et al. 2009). Based on this definition, various criteria using different types of information (e.g. field studies, laboratory tests, food web modelling, QSARs) are discussed and included in a tiered framework structured into five steps representing decreasing availability of experimental data. According to Gobas et al. (2009), the selection of an appropriate food web and the use of more comprehensive parameters such as the Trophic Magnification Factor (TMF) and the Biomagnification Factor (BMF) for both aquatic and non-aquatic food chains would ensure that bioaccumulation in non-aquatic organisms is also assessed. Consequently, Step 1 of the framework involves the selection of appropriate food webs that should include both water- and air-respiring organisms and, specifically, upper trophic level organisms such as raptors, whales and humans. Step 2 asks for characterisation of the TMF, which represents the most conclusive evidence of the ability of a substance to biomagnify in food webs (Gobas et al. 2009; Conder et al. 2012). A TMF > 1 is therefore the proposed criterion to 'confirm' that a substance is bioaccumulative (Gobas et al. 2009). If the TMF cannot be calculated because of lack of relevant field studies, the BMF should be used (Step 3). The BMF is less comprehensive since it focuses on one trophic relationship. Consequently, a BMF > 1 is used to identify a 'probable' bioaccumulative substance. Rainbow trout and rats are considered as suitable species for a BMF test (Gobas et al. 2009). In Step 4 available BCF or BAF values are used to determine whether a substance can biomagnify in water-respiring organisms of aquatic food webs. In this case, a BCF and/or BAF value higher than 5000 L/Kg indicates a 'possible' bioaccumulative substance. A standard protocol on how to perform a bioconcentration study with fish exists (OECD TG 305: OECD 2012). This protocol has been recently updated to consider dietary exposure in addition to aqueous exposure when testing bioaccumulation in fish. A standard protocol on how to test bioaccumulation in soil organisms (i.e. earthworms) (OECD 2010) and sediment-dwelling organisms (OECD 2008) is also available. Step 5 is applied when experimental/empirical data to assess bioaccumulation potential are not available and is based on the evaluation of physico-chemical properties of the substance (e.g. K_{ow} and K_{oa}) as well as the application of predictive models as suggested e.g. by Nichols et al. (2009). Criteria used in Step 5 can only indicate a 'potential' bioaccumulative substance.

It has to be pointed out that the definition of 'bioaccumulative' substance as proposed by Gobas et al. (2009) has some limitation as it only considers substances that biomagnify in food chains as bioaccumulative. According to the tiered framework, a substance that does not exhibit biomagnification properties, i.e. TMF or BMF < 1, is not considered as bioaccumulative. However, lack of biomagnification along the food chain or at some trophic levels does not necessarily mean that a substance fails to meet criteria for bioaccumulation based on BAF/BCF values (ECHA 2012a). Consequently, the proposed criteria and tiered framework tend to neglect the case of a substance

that exhibits bioaccumulation properties in lower trophic levels due to e.g. bioconcentration processes but undergo trophic dilution along the food chain. Moreover, a substance may or may not exhibit biomagnification properties based on the specific trophic level and species that is investigated when a BMF or TMF is determined. To this end, **it is recommended that multiple trophic levels are represented in the data set used to assess biomagnification, and not just a single ratio** (Swackhamer et al. 2009). For the same reason, **extrapolation of biomagnification data from aquatic to terrestrial food webs and vice versa cannot be justified** (Swackhamer et al. 2009).

There could also be the case when an increase in contaminant concentration up to a food chain is detected (i.e. TMF > 1) but the concentration levels in the analysed organisms are not sufficient to cause adverse effects. In this context, an analysis of the toxicological significance of tissue residues via calculation of hazard quotients may be helpful (e.g. as suggested by Weisbrod et al. (2009)).

Another point to be raised is that the tiered framework according to Gobas et al. (2009) focuses on the use of field metrics as the most conclusive criteria for bioaccumulation assessment, which are expensive and time-consuming and may not be the most suitable and pragmatic metrics for a screening approach. To this end, the Authors suggest that for screening large number of substances the framework is used in reverse order. This means starting from an initial phase (Step 5) where substances are prioritised based on their physico-chemical properties and results from food web bioaccumulation models up to the use of empirically determining BMF and TMF values (Step 1) for a reduced sub-set of compounds that need in-depth investigation before drawing a conclusion on their bioaccumulation potential.

In November 2009, the international Lab-Field Bioaccumulation Workshop sponsored by the International Life Sciences Institute Health and Environmental Sciences Institute (ILSI HESI), the US Environmental Protection Agency, and SETAC was held in New Orleans, Louisiana (USA). The workshop aimed at: i) comparing laboratory and field measurements of bioaccumulation endpoints; ii) evaluating the reasons why laboratory and field bioaccumulation data may not align; and iii) exploring the measurement and application of TMFs (Burkhard et al. 2012a). The results of the workshop are summarised in a series of five articles.

An important outcome of the workshop is a clear distinction between the terms 'bioaccumulation' and 'biomagnification'. Burkhard et al. (2012b) clarified that 'bioaccumulation' causes increased chemical concentrations in an organism compared to its ambient environment through all exposure routes and 'biomagnification' only quantifies increases between an organism and its prey due to dietary absorption.

As food is the main contributor to the uptake of hydrophobic contaminants for benthic, pelagic and terrestrial organisms (Selck et al. 2012), and dietary exposure seems to be the driver of accumulation at higher trophic levels such as birds, marine mammals, and humans (Conder et al. 2012), the workshop participants concluded that **all available data and metrics (not only BCF and/or K_{ow}) should be used in a weight of evidence approach to assess bioaccumulation potential of chemicals**

in a regulatory context (Borgå et al. 2012; Burkhard et al. 2012b; Conder et al. 2012; Selck et al. 2012). To this end, Burkhard et al. (2012b) propose the use of **fugacity ratio as a common dimensionless standard metric for integration of data about different species and food webs, including non-aquatic ones, and comparison of results from laboratory and field data**. The conversion to a fugacity ratio would also overcome the issue of defining scientifically sound cut-off values for each metric (Burkhard et al. 2012b). However, **this approach is not universally applicable** and cannot be used for e.g. ionic organic chemicals such as PFOS. Moreover, this approach only detects chemicals that biomagnify (Burkhard et al. 2012b). Though as explained by the Authors, fugacity ratios higher than 1 indicate biomagnification and chemicals that biomagnify do bioaccumulate; however, fugacity ratios lower than 1 indicate lack of biomagnification but chemicals may still bioaccumulate to unacceptable concentrations at lower trophic levels and additional evidence may be needed to derive a conclusion.

The framework proposed by Gobas et al. (2009) was also discussed at the workshop mentioned above, and TMF was acknowledged as the most conclusive metric to assess biomagnification in food webs (Borgå et al. 2012; Burkhard et al. 2012b; Conder et al. 2012). However, Borgå et al. (2012) along with Conder et al. (2012) critically analysed the application of TMF for regulatory screening and assessment of bioaccumulation potential. First, **both TMF and BMF measure biomagnification through trophic transfer, which is one aspect of the bioaccumulation process** according to the definitions agreed on at the workshop (Conder et al. 2012). In addition, it is important to consider that **TMF values can only be calculated for chemicals that have been in commerce long enough to be released, detected and quantified in environmental samples** (Borgå et al. 2012), and hence not applicable to e.g. emerging contaminants or for a pro-active evaluation of new substances to be placed on the market. To this end, it is also necessary that appropriate analytical techniques are available (Borgå et al. 2012), which may not always be the case. Moreover, a TMF implies sampling efforts and sacrifice of animals, which is in contrast with the reduction of animal use as promoted by REACH (Conder et al. 2012). Consequently, TMF may not be the most suitable metric for screening purposes. Another consideration is that the TMF is an 'estimation' of biomagnification in the food web that is investigated in the study as the true value and it can also be determined if all organisms and trophic levels are considered (Conder et al. 2012). **It is therefore recommended that the TMF value is used for regulatory purposes only if accompanied by the statistical analysis of its variability and a statistical power analysis of the study performed** (Conder et al. 2012). An aspect to be taken into account when using TMF is the relevance of the food web, especially when conclusions from multiple studies are available. It is indeed acknowledged by both studies performed, i.e. by Borgå et al. (2012) as well as by Conder et al. (2012), that TMF values are influenced by various biological factors such as: i) different metabolic rates between poikilotherms and homeotherms that lead to higher TMF values in food webs including homeotherms, especially if they are apex predators; ii) different biotransformation abilities that lead to e.g. higher TMF values in food webs including avian or mammals predators; iii) uptake across respiratory surfaces that affect accumulation at low trophic levels; iv) size and age in fish; and v) sex in mammals. Consequently, biomagnification of a chemical in terrestrial ecosystems can be much higher than in some aquatic food webs (Borgå et al. 2012).

Taking into account the paucity of TMF data in terrestrial ecosystems Borgå et al. (2012) suggest that **TMF values for food webs containing water-respiring organisms only are evaluated separately from the ones containing air-breathing organisms**. Burkhard et al. (2012b) also point out that TMF reflects an average change between trophic levels in food chains and is therefore less variable than BMF. However, in some cases TMF may obscure biomagnification relationships between individual trophic levels of particular interest (Conder et al. 2012). Therefore, it is suggested that BMF values are also considered when biomagnification potential is evaluated (Conder et al. 2012). Finally, TMF does not take into account the initial transfer of chemicals from environmental compartments such as water, sediment and soil into organisms while BCF or BAF do, which can indicate bioaccumulation even when TMF values are lower than 1 (Conder et al. 2012). Accordingly, Conder et al. (2012) conclude that TMF is the most accurate biomagnification metric and should be incorporated into the regulatory framework. Based on the above considerations, field-based **TMF cannot be used for screening purposes but should be considered in a step-by-step process in cases when the available information suggests bioaccumulation and more information is needed on biomagnification potential at higher trophic levels (including humans). Supporting lines of evidence could be BMF (to evaluate individual relationships between trophic levels) and BCF/BSAF/BAF (to estimate initial transfer from abiotic compartments into biota)**. Conder et al. (2012) also evaluated the applicability of other metrics (i.e. BMF_{TL}^{18} , $BSAF_{TL}$, BAF_{TL} , BCF, laboratory BAF, food web bioaccumulation models, K_{ow} , and K_{oa}) as alternatives when the TMF cannot be applied or is not available, and suggest that **none of the metrics is applied individually but all contribute as multiple lines of evidence to a more comprehensive and holistic assessment of the biomagnification potential**.

In conclusion, the regulatory use of TMF is recommended by several Authors but research efforts on improving the scientific understanding and defining measurement methods are needed (Bukhard et al. 2013). In order to overcome the limitation of field-based TMF, which can be measured only when the substance is already on the market and released into the environment, and of laboratory-based TMF, which are complex, expensive and time consuming, Solomon et al. (2013) have recently emphasised the use of model-based TMF (i.e. TMF values estimated by food web models) for screening of biomagnification potential of substances.

Concerning non-aquatic food webs, Muller et al. (2011) point out that the selection of the species to be used to calculate trophic levels and of the appropriate trophic enrichment factor need further study. Moreover, standard operating procedures as well as guidance on how to use TMF in combination with other laboratory and field metrics (e.g. BCF, BAF, BSAF, BMF) in a weight of evidence approach are fundamental (Bukhard et al. 2013).

It is worth to notice that Jonker (2011) questions the meaningfulness of TMF and BMF as their variation could merely reflect different affinity of chemicals for different lipid compositions across

¹⁸ TL = Trophic Level-normalised.

species, and therefore may not be related to their hydrophobicity or bioaccumulation potential. In this case, **species-specific BAF may result more valuable in risk assessment** (Jonker 2011).

More recently, Goss et al. (2013) have suggested the **elimination half-life of a chemical (EL_{0.5}: the time at which the 50% of a chemical is eliminated by an organism) as an alternative metric to the regulatory BCF as well as to the BMF and TMF**. The Authors argue that BMF and TMF only reflect exposure through diet, depend largely on biological and ecological factors rather than chemical's properties, are difficult to normalise and standardise, and are not applicable to new chemicals and consequently not suitable for hazard screening. On the contrary, EL_{0.5} accounts for all exposure routes, is directly linked to the physico-chemical properties of substances, reflects susceptibility of chemicals to undergo biotransformation, can be applied to both water-respiring and air-breathing organisms, is less variable than BMF and TMF values and is practical as usually measured in bioaccumulation and bioconcentration tests (Goss et al. 2013). It therefore represents an appropriate metric for bioaccumulation screening in a tiered approach. The Authors demonstrate that EL_{0.5} can be linked to BMF and proposes a mathematic procedure to derive a threshold level for EL_{0.5} that is equivalent to BMF equal to 1. Specifically, the Authors propose an **EL_{0.5} value of 70 d as criterion that keep the BMF smaller than 1 under physiological conditions that are realistic and representative for humans**. The Authors suggest that a tiered approach is used for bioaccumulation assessment. In Tier 1 biotransformation is neglected and chemicals that are screened out are those whose physico-chemical properties allow exhalation and urination to efficiently compensate for the intake. According to Goss et al. (2013), EL_{0.5} < 70 d correspond to log K_{ow} < 1.3 and log K_{oa} < 4.5 in air-breathing organisms, which are criteria similar to the ones proposed by Kelly, Gobas et al. but more stringent than the ones used under REACH. For water-respiring organisms, this leads to log K_{ow} < 6, which is a less stringent than the value used under REACH (Goss et al. 2013). This highlights **the importance of assessing bioaccumulation in aquatic and non-aquatic organisms separately, even if the metric is the same** (Goss et al. 2013). It is also underlined that although EL_{0.5} can be in principle applied to inorganic compounds, the scientific understanding on their behaviour is currently so limited that it would not be feasible (Goss et al. 2013). In Tier 2, biotransformation is taken into account for those chemicals that are not screened out in Tier 1. The biotransformation rate constant may be estimated *in silico* and/or *in vitro*. In this tier, the occurrence of metabolites that may be of regulatory concern needs also to be addressed (Goss et al. 2013). Chemicals that are not screened out in Tier 1 and 2 are tested *in vivo* in Tier 3. Goss et al. (2013) recommend that the EL_{0.5} is measured for various reference animals in standardised tests, which may be more problematic for non-aquatic organisms.

In February 2011, an international workshop on 'Moving Bioaccumulation Assessments to the Next Level: Progress Made and Challenges Ahead' was organised by the ILSI Health and Environmental Sciences Institute. According to the workshop summary report available online (ILSI HESI 2011), participants agreed that bioaccumulation in non-aquatic organisms and food chains is a major research interest and recommended that: i) existing bioaccumulation models are expanded to

include non-aquatic organisms; ii) a consensus terrestrial food web bioaccumulation model is developed; and iii) the metabolism rate in non-aquatic species is further investigated.

As a follow-up, a specific workshop on 'Terrestrial Bioaccumulation' was organised by the ILSI Health and Environmental Sciences Institute in January 2013. The outcomes of the workshop were presented in a dedicated session at the SETAC North America 34th Annual Meeting in Nashville on the 17th -21st November 2013. In summary, **Biota-Soil Accumulation Factors (BSAF) and BMF are confirmed to be robust metrics for evaluating the bioaccumulation potential in non-aquatic food webs** (Conder et al. 2013). However, several aspects still need further investigation and optimisation including: i) the selection of target terrestrial species; ii) spatial and temporal issues; iii) non-lethal sampling; iv) statistical analysis that accounts for variation in BSAF and BMF estimates from field data; iv) mesocosm studies to bridge the gap between laboratory and field data (Conder et al. 2013; Arblaster et al. 2013). **Concerning the BMF, predatory birds seem to exhibit higher statistical robustness and may therefore provide more definitive assessments of the biomagnification potential, if studies and analytical approaches will be optimised** (Arblaster et al. 2013).

5.3 Human-specific criteria

Since humans are air-respiring organisms and top predators in both aquatic and terrestrial food chains, regulatory bioaccumulation criteria based on aquatic species, or referring to a specific medium, may not be appropriate for humans as we consume food of different origins (Czub and McLachlan 2004b). Moreover, biomagnification of a compound does not necessarily lead to exposure to humans: although it is reasonable to assume that humans are significantly exposed to persistent, hydrophobic organic chemicals via e.g. fish consumption, some compounds may bioaccumulate in lower trophic levels but be metabolised by mammalian enzyme systems or accumulate in food webs by different mechanisms (Swackhamer et al. 2009).

According to McLachlan et al. (2011), the criteria suggested in Section 5.1 and 5.2 (e.g. K_{oa} , BMF, TMF) may be of limited value for humans because bioaccumulation is still assessed in isolation, without considering it as a part of the whole environment. To this end, the Authors support **the use of an integrative multimedia approach to assess bioaccumulation in humans that takes into account partitioning properties, biotransformation and multimedia exposure** (Czub and McLachlan 2004b; McLachlan et al. 2011). Specifically, Czub and Lachlan (2004b) linked a human bioaccumulation model for POPs (i.e. ACC-HUMAN; Czub and Lachlan 2004a) to a multimedia model and calculated a new parameter named **Environmental Bioaccumulation Potential (EBAP)**. **EBAP is defined as the ratio between the quantity of the body burden of a certain chemical in a human organism and the quantity of the chemical in the whole environment ($m^2/organism$)**. The Authors suggest that the EBAP could support a comparative risk assessment and the identification/prioritisation of potentially bioaccumulative substances in humans. The results from this study show that only chemicals with $\log K_{oa}$ greater than 6 (i.e. less volatile compounds that are inefficiently eliminated via exhalation) can accumulate in humans regardless the nature of their diet

(aquatic rather than agricultural). When the agricultural diet only is taken into account, chemicals with $\log K_{ow}$ in the range 2-9 and $\log K_{oa}$ in the range 6-10 are found to be potentially bioaccumulative in humans. These results show discrepancies with the $\log K_{ow}$ -based method: while $\log K_{ow}$ identifies hydrophobic but relatively low volatile substances (i.e. $\log K_{ow} > 5$ and $\log K_{oa} < 6$) as bioaccumulative, the EBAP shows that in humans they can be eliminated via respiration; while $\log K_{ow}$ does not consider less hydrophobic and non-volatile substances (i.e. $\log K_{ow}$ 2-5 and $\log K_{oa} > 5$) as bioaccumulative, EBAP predicts them as bioaccumulative through the agricultural food web.

Similarly, in another study Czub et al. (2008) linked the zonally averaged global transport model Globo-POP with the Arctic version of ACC-HUMAN to calculate the **Arctic Contamination-Bioaccumulation Potential (AC-BAP)** for the Inuit indigenous sub-population through a marine diet. This parameter is defined as the ratio between human body burden of the chemical and the quantity of chemical cumulatively emitted to the global environment (Czub et al. 2008). The study shows that persistent chemicals characterised by $\log K_{ow}$ in the range 3.5 and 8.5 and $\log K_{oa}$ higher than 6 obtained AC-BAP values of at least 10% of the maximum value (Czub et al. 2008).

Another finding pointed out by Czub and Lachlan (2004b) is that **EBAP values were nearly constant over a large spectrum of partitioning properties of organic contaminants, thus indicating that partitioning properties are not the primary determinants of bioaccumulation in humans.**

In a more recent study, Mc Lachlan et al. (2011) have shown that **biotransformation is a more significant determinant of bioaccumulation in humans and substances with similar partitioning properties may have a different bioaccumulation potential because of metabolism and/or excretion.** The Authors calculated a new parameter named **multimedia Bioaccumulation Factor (mmBAF) ($m^2/organism$)**, which is equivalent to EBAP but covers non-persistent chemicals in addition to persistent ones. According to Mc Lachlan et al. (2011), mmBAF values also show to slightly vary over a large spectrum of partitioning properties but turn out to vary by 9 orders of magnitude over a large spectrum of biotransformation constants.

A recent development has also been the implementation of the poly-parameter Linear Free Energy Relationship (pp-LFER) in the ACC-HUMAN human bioaccumulation model (Undeman et al. 2011). This approach tried to overcome the traditional modelling paradigm based on a single linear relationship between the lipid-water partitioning and the octanol-water partitioning by considering all possible intermolecular interactions due to different human tissues (fat, muscle, blood, skin, liver, brain and lungs). However, the implementation of pp-LFER showed little benefit in terms of results and uncertainties.

A Physiologically Based Toxicokinetic model for screening of human bioaccumulative substances was developed by Tonnelier et al. (2011). The model confirms the weak influence of $\log K_{ow}$ on human BCF values, as already demonstrated by Czub and Mc Lachlan (2004a; 2004b) and McLachlan et al. (2011), and shows a strong discrepancy between BCF values for fish and humans. This also suggests that new criteria should be investigated and used for bioaccumulation assessment in humans. The

Authors propose an approach describing the **bioaccumulation potential as a function of hepatic clearance and renal excretion**. Both parameters can easily be obtained through *in vitro* testing (Tonnelier et al. 2011) although test guidelines would be needed to ensure application in a regulatory context. **The hepatic clearance appears to be the main driving factor in the study of concern** (Tonnelier et al. 2011).

In line with the abovementioned, that means elimination and biotransformation as key determining parameters of bioaccumulation in humans, Goss et al. (2013) propose that the **whole body total elimination half-life (HL_T) and whole body biotransformation half-life (HL_B) are considered as metrics for bioaccumulation assessment**. To this end, Arnot et al. (2014) have recently developed and validated screening-level QSARs for estimation of *in vivo* HL_T and HL_B for organic chemicals in humans.

Table 4. Criteria and related threshold values for screening and assessment of bioaccumulation/biomagnification potential in non-aquatic organisms (including humans) as proposed in the scientific literature.

Criteria	Threshold value	Description	Reference
Octanol-air partitioning coefficient (K _{oa}) & octanol-water partitioning coefficient (K _{ow})	Log K _{oa} > 5 & Log K _{ow} > 2 (no 'hydrophobicity cut-off')	Identification of non-metabolised hydrophobic organic substances that have the potential to biomagnify in non-aquatic food chains (humans excluded)	Kelly and Gobas 2003; Gobas et al. 2003
	Log K _{oa} > 6 & Log K _{ow} : 2-10	Identification of non-metabolised hydrophobic organic substances that have the potential to biomagnify in non-aquatic food chains and air-breathing organisms of aquatic food-chains (humans excluded)	Kelly et al. 2007
	Log K _{oa} > 6 & Log K _{ow} > 2 (no 'hydrophobicity cut-off')	Identification of non-metabolised hydrophobic organic substances that have the potential to biomagnify in humans	Kelly et al. 2007
	Log K _{oa} > 5 & Log K _{ow} : 2-12	Identification of non-metabolised hydrophobic organic substances that have the potential to biomagnify in non-aquatic food chains (humans excluded)	Armitage and Gobas 2007
	Log K _{ow} > 1.3 & Log K _{oa} > 4.5	Identification of non-metabolised substances that have the potential to biomagnify in air-breathing organisms	Goss et al. 2013

Table 4. (cont.)

Criteria	Threshold value	Description	Reference
	Log K _{ow} 2-9 & Log K _{oa} > 6	Identification of substances that have the potential to biomagnify in humans regardless the nature of their diet (aquatic or agricultural)	Czub and Lachlan 2004a; b
	Log K _{ow} 2-9 & Log K _{oa} 6-10	Identification of substances that have the potential to biomagnify in humans if agricultural diet is considered	
Biota-Soil Accumulation Factor (BSAF) in soil earthworms	BSAF > 1	Indication that a substance can bioaccumulate from soil into organisms at the base of the non-aquatic food chain (uptake via dermal contact with soil pore water and soil particles ingestion).	Weisbrod et al. 2009; OECD 2010; ECHA 2012b; Conder et al. 2013
Bioconcentration Factor (BCF) in soil earthworms	-	Indication that a substance can bioaccumulate from soil into organisms at the base of the non-aquatic food chain (uptake via dermal contact with soil pore water)	Jager 1998; ECHA 2012b; c
Bioconcentration Factor (BCF) in sediment-dwelling earthworms	-	Indication that a substance can bioaccumulate from soil into organisms at the base of the non-aquatic food chain (uptake via dermal contact with sediment pore water)	ECHA 2012b
Bioaccumulation Factor (BAF) in soil earthworms (laboratory-based)	-	Indication that a substance can bioaccumulate from the surrounding environment into the considered organism (steady-state conditions, all uptake routes including the diet)	OECD 2010

Table 4. (cont.)

Criteria	Threshold value	Description	Reference
Biomagnification Factor (BMF) in non-aquatic organisms	BMF > 1	Indication that a substance can biomagnify in the considered trophic relationship	Weisbrod et al. 2009; Gobas et al. 2009; Conder et al. 2013;
	BMF < 1	<p>Indication that a substance can undergo trophic dilution in the considered trophic relationship</p> <ul style="list-style-type: none"> ○ Suitable species: rats ○ More statistically robust results for: predatory birds ○ Multiple trophic relationships to be considered 	Swackhamer et al. 2009 Gobas et al. 2009 Arblaster et al. 2013 Swackhamer et al. 2009
Trophic Magnification Factor (TMF) in non-aquatic food chains	TMF > 1	Indication that a substance can biomagnify along the considered non-aquatic food chain	Weisbrod et al. 2009; Gobas et al. 2009; Borga et al. 2011;
	TMF < 1	<p>Indication that a substance can undergo trophic dilution along the considered non-aquatic food chain</p> <ul style="list-style-type: none"> ○ Upper trophic level organisms such as raptors, mammals and humans to be included ○ Accompanied by the statistical analysis of its variability and a statistical power analysis of the study performed 	Burkhard et al. 2011b; Conder et al. 2011; Solomon et al. 2013 Gobas et al. 2009 Conder et al. 2011
Elimination half-life (EL _{0.5}) in non-aquatic organisms (<i>in vivo</i>)	EL _{0.5} > 70 d	Indication that exhalation and urination and biotransformation cannot compensate for the intake and the substance has the potential to biomagnify in non-aquatic food chains	Goss et al. 2013
	EL _{0.5} ≤ 70 d	Indication that exhalation and urination (and, biotransformation, if determined) can easily compensate for the intake and the substance does not have the potential to biomagnify in non-aquatic food chains	

Table 4. (cont.)

Criteria	Threshold value	Description	Reference
Environmental Bioaccumulation Potential (EBAP) (predicted)	-	Identification of persistent substances that have the potential to bioaccumulate in humans taking into account partitioning properties, biotransformation and multimedia exposure (both aquatic and agricultural diet)	Czub and Lachlan 2004a; b
Arctic Contamination-Bioaccumulation Potential (AC-BAP) (predicted)	-	Identification of substances that have the potential to bioaccumulate in Arctic indigenous populations taking into account partitioning properties, biotransformation and multimedia exposure (marine diet)	Czub et al. 2008
Bioaccumulation Factor (mmBAF) (predicted)	-	Identification of persistent and non-persistent substances that have the potential to bioaccumulate in humans taking into account partitioning properties, biotransformation and multimedia exposure (both aquatic and agricultural diet)	Mc Lachlan et al. 2011
Hepatic clearance and renal excretion (<i>in vitro</i>)	-	Identification of substances that have the potential to bioaccumulate in humans	Tonnellier et al. 2011
Whole body total elimination half-life (HL_T) and whole body biotransformation half-life (HL_B) (<i>in vivo</i> or predicted by QSAR)	-	Identification of substances that have the potential to bioaccumulate in humans	Goss et al. 2013; Arnot et al. 2014

6 Ongoing and future initiatives

An OECD project named 'Harmonisation of OECD Environmental Assessment Practices for Pesticides with Persistent, Bioaccumulative, Toxic and Long-Range Transport (PBT/LRT) Characteristics', chaired by the US Environmental Protection Agency (EPA), is currently ongoing. Project goals are: i) to facilitate a common understanding of the unique issues for environmental assessment of pesticides with PBT/LRT characteristics; ii) to develop a set of definitions of key concepts; and iii) to harmonise both empirical and model-based methods (Brady 2011). Assessing bioaccumulation in terrestrial ecosystems is one of the issues identified by OECD (Brady 2011). The project's working group will consider two country-specific guidance documents, which are under development, as basis for harmonisation. The first document is the US EPA PBT/LRT pesticides risk assessment guidance, which builds on the US EPA PBT White Paper (US EPA 2008). US EPA is currently evaluating several terrestrial bioaccumulation models and using the K_{oa} criteria as a 'flag' of potential terrestrial bioaccumulation for those pesticides that are characterised by negligible biotransformation rates (Keith Sappington personal communication 31 January 2012). The validation of an earthworm fugacity model is also among the US EPA tasks. The second document is the German UBA internal guidance on hazard assessment of PBT/LRT substances, including pesticides, biocides, industrial chemicals, pharmaceuticals, etc. Currently, the working group is drafting a series of white papers on the subject, which will prepare the ground for the OECD harmonised guidance (Keith Sappington personal communication 04 September 2013).

The ECHA *Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment* (ECHA 2012a) is currently under review in order to align it with the REACH Annex XIII criteria published in 2011. The consultation procedure is still ongoing and draft versions of the updated Guidance Document are downloadable from the ECHA website¹⁹. Publication is expected soon. The ECHA PBT Expert Group has also selected a list of issues with relevance for the PBT/vPvB assessment of substances under REACH, which require further investigation. Among them, terrestrial bioaccumulation is one of the priorities in terms of development of screening triggers as well as testing and assessment strategies (Peltola-Thies et al. 2014).

¹⁹ <http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach>

7 Conclusions

It has been demonstrated through experimental and modelling studies published in peer-reviewed literature that persistent but low hydrophobic and poorly metabolised organic chemicals can biomagnify in non-aquatic food webs and accumulate in human blood and tissues. Since current criteria for bioaccumulation assessment in international and European legislative frameworks aimed to identify substances with PBT/vPvB properties are mainly based on cut-off values for BCF/BAF measured in aquatic species and K_{ow} , there is the risk that these substances are not classified as bioaccumulative and consequently not to be properly addressed from a regulatory viewpoint.

In order to overcome these limitations and fill the gap, several Authors have proposed new metrics and models as well as generated data, which could be used and incorporated into the regulatory criteria to improve the way the bioaccumulation assessment for the non-aquatic compartment is currently performed.

The literature review points out that there is consensus in the scientific community on the **combined use of criteria based on predictive physico-chemical parameters such as $K_{oa} > 5$ or 6 and $K_{ow} > 2$** , which are easy to calculate and implement and should therefore be considered within existing PBT/vPvB assessment frameworks, especially **for screening purposes**. However, the impact of introducing this in a regulatory context such as e.g. the EU REACH Regulation needs to be evaluated more deeply, e.g. via quantification of the additional number of substances that could be identified as potentially bioaccumulative due to consideration of the non-aquatic compartment. Moreover, the assumption of the 'hydrophobicity cut-off' for substances with $\log K_{ow} > 9-10$ has also been questioned in some studies as the examined substances seem to exhibit bioaccumulation potential in non-aquatic organisms. It is therefore important that the applicability of the 'hydrophobicity cut-off' to non-aquatic bioaccumulation is further investigated and clarified. However, the proposed screening approach does not cover those substances that partition into other tissues than lipids, e.g. proteins, and is therefore not universally applicable.

Regarding bioaccumulation in humans, several modelling approaches suggest that biotransformation and elimination rather than partitioning properties are the primary determinants. These findings could be incorporated in a screening approach through the **application of models that take biotransformation and elimination processes in humans as well as multi-media exposure into account**.

In a more detailed bioaccumulation assessment that is usually performed at higher tier, there is general agreement in the scientific community that all available bioaccumulation metrics need to be considered as complementary lines of evidence and evaluated in a weight of evidence approach (in line with the principles outlined in the REACH Annex XIII published in 2011). Specifically, it is recommended that both **bioconcentration and biomagnification are investigated by means of multiple laboratory and field metrics and that aquatic and non-aquatic compartments are separately addressed**. The transfer of substances from abiotic compartments to organisms at lower

trophic levels, which is mainly driven by bioconcentration processes, could be assessed through laboratory metrics such as BCF and BSAF and/or field metrics such as BAF determined with relevant soil organisms such as earthworms. Specifically, **BSAF and BCF values in earthworms seem to represent good indicators for the bioaccumulation potential at the base of non-aquatic food chains, despite several technical limitations.** As food is the main exposure route for non-aquatic organisms and it is demonstrated that biomagnification can be higher in non-aquatic food webs compared to aquatic ones, **field-based BMF and TMF values determined for non-aquatic food webs could be considered.** However, further research, e.g. to determine appropriate multiple trophic levels and representative species, is necessary before BMF and TMF can be applied in a regulatory context. The availability of standardised protocols on how to conduct the experiment is also an issue. Therefore, it is recommended in the literature that TMF and BMF values are **accompanied by a statistical analysis of their variability and a statistical power analysis of the study performed.** It is also important to take into consideration that field values are costly and time consuming and can only be determined for those substances that are on the market / released into the environment since a long time. **For emerging contaminants or substances that are not yet on the market the application of predictive food web models could be considered.**

Finally, **the elimination half-life has been recently proposed in the scientific literature as an alternative bioaccumulation metric** to BCF/BAF/BSAF and BMF/TMF. Such a metric seems to show several advantages as it considers all exposure routes, is directly linked to the physico-chemical properties of the substance, reflects the susceptibility of the substance to undergo biotransformation, is less variable than field metrics, and can be applied to both aquatic and non-aquatic organisms. However, several aspects of the elimination half-life needs to be further investigated, e.g. how to select the representative species of a certain trophic level or food web, and how to define the most appropriate threshold values.

In conclusion, the scientific community has suggested several metrics to overcome the lack of consideration of the non-aquatic compartment in the regulatory PBT/vPvB assessment of substances. However, **research efforts are still needed to improve the scientific understanding of the proposed metrics and their cut-off values.** Moreover, the **impact that the introduction of non-aquatic criteria in a regulatory context may have needs to be analysed in depth.**

It is important to note that suggestions provided in this document should not be considered as an attempt to define a regulatory strategy on how to screen and/or assess substances based on their bioaccumulation potential in non-aquatic organisms nor to evaluate the impact of such a strategy on e.g. the REACH implementation process. **Further elaboration is needed to integrate the knowledge summarised in the present document into the current regulatory framework. This could be the work for a dedicated expert group.**

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9 Annexes

9.1 Annex I - Glossary

Note: this list explains expressions used in this report, some of them are common in scientific literature, but not necessarily defined in legal texts.

Non-aquatic organisms

This term refers to all organisms that occur and live in terrestrial environments and are air-breathing, including top predators and humans. In the context of this report, the expression 'non-aquatic organisms' is preferred to other terms used in the literature, such as 'terrestrial organisms', 'soil organisms' and 'air-breathing organisms', as it more clearly encompasses humans as part of the food web.

Persistent Bioaccumulative Toxic/very Persistent very Bioaccumulative (PBT/vPvB)

PBT substances are substances that are persistent, bioaccumulative and toxic while vPvB substances are characterised by high persistence and high tendency to bioaccumulate but not necessarily proven toxicity (ECHA 2012a). Because of their very low degradability, PBT/vPvB substances tend to remain in the environment for a long time and may accumulate and magnify in organisms' tissues including top predators and humans. The accumulation of PBT/vPvB substances is difficult to reverse as the reduction or cessation of release into the environment will not necessarily result in a decrease in the concentration level (ECHA 2012a). Moreover, the effects of such an accumulation over extended periods are not possible to predict though laboratory testing (ECHA 2012a).

Bioconcentration

Bioconcentration is the increase in concentration of a substance in or on an organism (or specified tissue) relative to the concentration of the substance in the test medium (OECD 2012). For aquatic organisms, bioconcentration is the net accumulation of a chemical in an organism that results from direct contact with water only, such as through gill membranes or other external surfaces (US EPA 2003; 2007). Bioconcentration excludes chemical accumulation from other exposure routes and sources such as ingestion of organisms and sediment (US EPA 2008). Although not routinely defined for terrestrial (air-breathing) organisms, an analogous measure of bioconcentration would be the net accumulation of a chemical that results from direct contact with air or soil only, such as through respiration or dermal uptake (US EPA 2008). In the OECD Test Guideline 317 (OECD 2010), bioconcentration in terrestrial Oligochaetes is defined as the increase in concentration of a substance in or on an organism relative to the concentration of the substance in the surrounding medium. The increase in concentration is due to the uptake of the substance exclusively from the surrounding medium via both the body surface and ingested soil.

Bioaccumulation

Bioaccumulation is the net accumulation of a chemical in an organism from all possible exposure routes (respiration, diet, dermal) and sources (water, soil/sediment, air and diet) (Spacie et al. 1995; US EPA 2003; 2007). Bioaccumulation results from both bioconcentration and biomagnification processes (OECD, 2010).

Biomagnification

Biomagnification can be defined as the increase in concentration of a substance in or on an organisms (or specified tissue) relative to the concentration of the substance in the food (OECD 2012). The increase in concentration may occur along a series of predator-prey associations in a food web, primarily through the mechanism of dietary accumulation (trophic transfer) (US EPA 2008).

Steady State

The steady state is defined as the equilibrium between the uptake and elimination processes that occur simultaneously during the exposure phase (OECD 2010). The steady state is reached by a system when rates of chemical movement between phases and reactions within phases are constant so that concentrations of the chemical in the phases of the system are unchanged over time (US EPA 2008). A system at steady state is not necessarily at equilibrium; steady-state conditions often exist when some or all of the phases of the system have different activities or fugacities for the chemical (US EPA 2008).

Bioconcentration Factor (BCF) [$L/Kg_{ww}; m^3/Kg_{ww}; Kg_{soil/ww}/Kg_{ww}$]

Ratio of the steady state concentration of a substance in an aquatic water-respiring organism (C_b : $g_{chemical}/Kg_{ww}$) and the concentration in water (C_w : $g_{chemical}/L$) determined in a controlled laboratory experiment in which the test organisms are exposed to a substance in the water (but not in the diet): $BCF_w = C_b/C_w$ [L/Kg_{ww}] (Gobas et al. 2009). BCF values > 1 indicate that the concentration/accumulation in the water-respiring organism is greater than that of the medium from which the chemical was measured (US EPA 2008). For terrestrial organisms, an analogous measure of a BCF would be the ratio of the concentration of a substance in the organism (C_b) and the concentration in air (or soil) (C_a : $g_{chemical}/m^3$; C_s : $g_{chemical}/Kg_{soil/ww}$), in situations where the organism is exposed via air or soil only: $BCF_a = C_b/C_a$ [m^3/Kg_{ww}]; $BCF_s = C_b/C_s$ [$Kg_{soil/ww}/Kg_{ww}$].

Biota-Soil/Sediment Accumulation Factor (BSAF) [$Kg_{soil/sediment/ww}/Kg_{biotaww}$]

This parameter measures the bioconcentration of chemicals in sediment/soil dwelling organisms. It corresponds to the ratio between the lipid normalised concentration of a substance in the organism and the organic content-normalised concentration in the soil/sediment matrix at steady state (OECD 2010). BSAF is essentially equivalent to BCF_{lipid}/K_{OC} (Weisbord et al. 2009).

Bioaccumulation Factor (BAF) [L/Kg_{ww}; m³/Kg_{ww}; Kg_{soilww}/Kg_{ww}]

Ratio of the steady state concentration of a substance in an aquatic water-respiring organism (C_b : g_{chemical}/Kg_{ww}) and the steady state concentration in water (C_w : g_{chemical}/L) determined from field or laboratory data in which sampled organisms are exposed to the substance in both the water and their diet: $BAF_w = C_b/C_w$ [L/Kg_{ww}] (Gobas et al. 2009). BAF values > 1 indicate that the concentration/accumulation in the organism is greater than that of the medium from which the chemical was measured (US EPA 2008). For terrestrial organisms, an analogous measure of a BAF would be the ratio of the concentration of a substance in a terrestrial organism (C_b : g_{chemical}/Kg_{ww}) to its concentration in air (or soil) (C_a : g_{chemical}/m³; C_s : g_{chemical}/Kg_{soilww}), in situations where both the organism and its food are exposed to the substance: $BAF_a = C_b/C_a$ [m³/Kg_{ww}]; $BAF_s = C_b/C_s$ [Kg_{soilww}/Kg_{ww}].

Biomagnification Factor - laboratory based (BMF) [Kg_{dietw}/Kg_{ww}]

In general, this factor represents the concentration of a substance in a predator relative to the concentration in the predator's prey or food at steady state (OECD 2012). The laboratory-based BMF is the ratio of the steady state concentration of a substance in a water- or air-respiring organism (C_b : g_{chemical}/Kg_{ww}) and the steady state concentration in the diet of the organism (C_d : g_{chemical}/Kg_{dw}) determined in a controlled laboratory experiment in which the test organisms are exposed to the substance in the diet (but not in the water or air): $BMF = C_b/C_d$ [Kg_{dw}/Kg_{ww}] (Gobas et al. 2009). Since the exposure to the aqueous phase is carefully avoided, a BMF value from a laboratory test cannot be compared with a BMF value from a field study (in which both water and dietary exposure may be combined) (OECD 2012).

Biomagnification Factor - field based (BMF) [Kg_{dietww}/Kg_{ww}]

In general, this factor represents the concentration of a substance in a predator relative to the concentration in the predator's prey or food at steady state (OECD 2012). The field-based BMF is the ratio of the steady state concentration of a substance in a water- or air-respiring organism (C_b : g_{chemical}/Kg_{ww}) and the steady state concentration in the diet of the organism (C_d : g_{chemical}/Kg_{dietww}) determined from field data in which sampled organisms are exposed to the substance via air, water, and the diet: $BMF = C_b/C_d$ [Kg_{dietww}/Kg_{ww}] (Gobas et al. 2009).

Trophic Magnification Factor (TMF) [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: $TMF = 10^m$ (Gobas et al. 2009).

Octanol-water partitioning 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: $K_{ow} = C_o/C_w$ (OECD 1995; 2004; 2006; Gobas et al. 2009).

Octanol-air partitioning 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: $K_{oa} = C_o/C_a$ (Gobas et al. 2009).

Metabolisation rate constant (k_m)

Reaction constant related to the half-life of a compound in an organism ($t_{1/2}$): $k_m = \ln 2/t_{1/2}$

9.2 Annex II - Abbreviations

AC-BAP	Arctic Contamination Bioaccumulation Potential
ADME	Absorption, Distribution, Metabolism, Elimination
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BCPS	bis-4-chlorophenyl sulfone
BMF	Biomagnification Factor
BSAF	Biota Soil/Sediment Accumulation Factor
CEPA	Canadian Environmental Protection Act
CLP	Classification Labelling Packaging
CMC	Critical Micelle Concentration
DDT	Dichlorodiphenyltrichloroethane
EBAP	Environmental Bioaccumulation Potential
ECHA	European Chemicals Agency
EL _{0.5}	Elimination half-life
EU	European Union
FABPs	Fatty Acid Binding Proteins
GHS	Globally Harmonised System
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HL _B	whole body biotransformation half-life
HL _T	whole body total elimination half-life
ILSI	International Life Sciences Institute

JRC-IHCP	Joint Research Centre's Institute for Health and Consumer Protection
K_{aw}	protein-air partitioning coefficient
K_{oa}	octanol-air partitioning coefficient
K_{ow}	octanol-water partitioning coefficient
K_{pw}	protein-water partitioning coefficient
LC	Lipid Content
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OATs	Organic Anion Transporters
OC	Organic Carbon
Octa-BDE	C-Octabromodiphenyl ether
OECD	Organisation for Economic Cooperation and Development
OSPAR	Convention for Protection of the marine Environment of the North-East Atlantic
p,p'-DDE	p,p'-dichlorodiphenyldichloroethylene
PBT	Persistent Bioaccumulative Toxic
PCB	Polychlorinated biphenyl
PEG	Partner Expert Group
PFCAs	Perfluorocarboxylates
PFOS	Perfluorooctane sulfonate
PFSAs	Perfluorosulfonates
pK_a	Acid dissociation constant
POP	Persistent Organic Pollutant
pp-LFER	poly-parameter Linear Free Energy Relationship
PPP	Plant Protection Product

QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SETAC	Society of Environmental Toxicology and Chemistry
SVHC	Substances of Very High Concern
TCB	Tetrachlorobenzene
TCPMeOH	Trischlorophenyl methanol
TG	Test Guideline
TMF	Trophic Magnification Factor
TSCA	Toxic Substances Control Act
UN ECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
US	United States
US EPA	United States Environmental Protection Agency
vPvB	very Persistent very Bioaccumulative

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