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PBT assessment under REACH: Screening for low aquatic bioaccumulation with QSAR classifications based on physicochemical properties to replace BCF *in vivo* testing on fish

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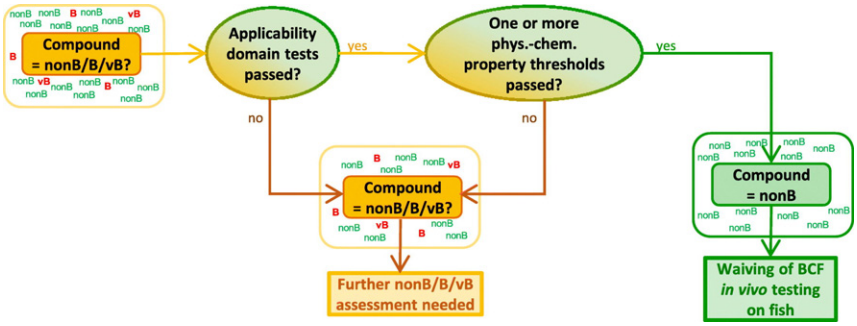
HIGHLIGHTS

- BCF waiving scheme to screen for absence of PBT properties
- Identification of low bioaccumulation potential based on physicochemical properties
- Reliable QSAR classifications with 100% sensitivity (no false negatives)
- Prediction confidence based on similarity with nonB and B/vB compounds
- Contribution to the 3Rs by reduction of BCF *in vivo* testing on fish by at least 50%

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic bioconcentration factors (BCFs) are critical in PBT (persistent, bioaccumulative, toxic) and risk assessment of chemicals. High costs and use of more than 100 fish per standard BCF study (OECD 305) call for alternative methods to replace as much *in vivo* testing as possible. The BCF waiving scheme is a screening tool combining QSAR classifications based on physicochemical properties related to the distribution (hydrophobicity, ionisation), persistence (biodegradability, hydrolysis), solubility and volatility (Henry's law constant) of substances in water bodies and aquatic biota to predict substances with low aquatic bioaccumulation (nonB, BCF < 2000).

The BCF waiving scheme was developed with a dataset of reliable BCFs for 998 compounds and externally validated with another 181 substances. It performs with 100% sensitivity (no false negatives), >50% efficacy (waiving potential), and complies with the OECD principles for valid QSARs. The chemical applicability domain of the BCF waiving scheme is given by the structures of the training set, with some compound classes explicitly excluded like organometallics, poly- and perfluorinated compounds, aromatic triphenylphosphates, surfactants. The prediction confidence of the BCF waiving scheme is based on applicability domain compliance, consensus modelling, and the structural similarity with known nonB and B/vB substances.

Compounds classified as nonB by the BCF waiving scheme are candidates for waiving of BCF *in vivo* testing on fish due to low concern with regard to the B criterion. The BCF waiving scheme supports the 3Rs with a possible reduction of >50% of BCF *in vivo* testing on fish. If the target chemical is outside the applicability domain of the BCF

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waiving scheme or not classified as nonB, further assessments with *in silico*, *in vitro* or *in vivo* methods are necessary to either confirm or reject bioaccumulative behaviour.

1. Introduction

The accumulation of chemicals in aquatic biota is of major concern for environmental hazard and risk assessment. The internal concentration of contaminants in organisms may increase by accumulation to a level that causes toxic effects, even if the external concentration remains below the critical limit. Also an exposure for a short time may produce high internal concentrations that persist in the organism much longer than in the surrounding water. Because of their elevated and lasting level in living tissues, bioaccumulative substances may evoke potentially chronic effects, not only in the organisms directly exposed but also in species at higher levels in the food chain, including humans. Bioaccumulation is therefore an important link between the pollution of surface waters and human exposure to xenobiotic substances.

National and international chemical legislations require bioaccumulation assessments mainly based on bioconcentration factors (BCFs). Within the European Union, the REACH regulation concerning the registration, evaluation, authorisation and restriction of chemicals (European Commission, 2006) requests BCF studies for chemicals produced or imported above 100 tonnes per year (Annex IX). For substances produced or imported between 10 and 100 tonnes per year, BCF studies are not required but BCFs are needed for PBT (persistent, bioaccumulative, and toxic) and vPvB (very persistent, very bioaccumulative) assessments. The criteria for bioaccumulative (B) and very bioaccumulative (vB) substances are BCFs above 2000 and 5000, respectively. Similarly, the Stockholm Convention on persistent organic pollutants (UNEP, 2015) and Environment Canada's Persistence and Bioaccumulation Regulations (CEPA, 2016) use a threshold of BCF > 5000 to identify bioaccumulative substances.

The standard experimental determination of BCFs according to the OECD Test Guideline 305 (Bioaccumulation in fish: aqueous and dietary exposure (OECD, 2012)) uses more than 100 fish and costs about 50,000 €. Considering that measured BCFs are available for <5% of the tens of thousands of commercial substances that require evaluation (OECD, 2016a; Weisbrod et al., 2007), it is obvious that testing all the BCFs is neither desirable with regard to animal welfare (Directive 2010/63/EU on the protection of animals used for scientific purposes (European Commission, 2010)) nor are these tests feasible because of insufficient laboratory capacities and limited economic resources. Approaches that support the 3Rs principles (replacement, refinement, and reduction of animal testing (Russel and Burch, 1959)) address several options to reduce the BCF *in vivo* testing on fish. Some savings are possible with reduced test design, for example, testing of only one test concentration or a reduced number of sample points (OECD, 2012; OECD, 2016b; Springer et al., 2008). Integrated testing and tiered assessment strategies aim to use also alternative data and non-guideline methods for the evaluation of the bioaccumulation potential of chemicals in fish (de Wolf et al., 2007; Lillicrap et al., 2016; Lombardo et al., 2014).

Screening tools can furthermore reduce BCF *in vivo* testing on fish based on the rationale that, with regard to bioaccumulation assessments under REACH, substances with BCF below 2000 have low testing priority because they classify as non-bioaccumulative (nonB) and, thus, they cannot be PBT/vPvB. On the contrary, substances with unknown BCF may be potentially B/vB and have higher testing priority to either confirm or reject bioaccumulative behaviour. Since most (>80%) chemicals are nonB, reliable screening for low aquatic bioaccumulation can direct the limited resources, as efficiently as possible, towards the substances with high testing priority and support to postpone or waive the BCF *in vivo* testing on fish for the low priority chemicals.

Screening for nonB substances may be based on quantitative structure-activity relationships (QSARs) describing the dependence of low aquatic bioaccumulation on structural features and physicochemical properties (Nendza et al., 2013). QSAR classifications can identify nonB compounds being candidates for the waiving of BCF *in vivo* testing on fish. However, waiving of BCF *in vivo* testing on fish can only be accepted if the classification as nonB is plausible and reliable. If there is any doubt about the classification as nonB, the chemicals should be assessed by further *in silico*, *in vitro* and/or *in vivo* methods.

It is the objective of this study to improve the identification of nonB chemicals being candidates for waiving BCF *in vivo* testing on fish. Based on earlier work (Nendza and Herbst, 2011; Nendza and Müller, 2010), we aim for a predictive model, the BCF waiving scheme, based on QSAR classifications using physicochemical properties to classify chemicals as either nonB (low testing priority) or potentially B/vB (high testing priority). The BCF waiving scheme shall (1.) provide reliable nonB classifications according to an external validation, (2.) perform better than the existing thresholds for waiving BCF studies, and (3.) inform about prediction confidence based on applicability domain (AD) compliance, consensus modelling, and the structural similarity with known nonB and B/vB substances.

2. Material and methods

2.1. Bioconcentration data

A training set with reliable BCF data for 998 discrete organic compounds was compiled from Arnot et al. (2009), CAESAR (2011), Dimitrov et al. (2005a), EURAS (2007), Fu et al. (2009), Strempel et al. (2013). The data were quality controlled regarding test substance identity and chemical structures, test protocol variation (*e.g.* exposure concentrations and pH) and represent wet-weight-based, steady-state BCF. If available, BCFs determined by the kinetic method were used. The mean value was calculated in the case of multiple data for the same chemical. The final dataset covers a log BCF range between −1 and 6, with 829 nonB (83.0%), 62 B (6.2%), and 107 vB (10.7%) compounds. The compounds are chemically diverse, including industrial chemicals and pesticides, and their molecular weights range between 46 and 1471 g/mol. The training set contains relevant contaminants, *e.g.* high production volume (HPV) chemicals and priority substances under the Water Framework Directive (WFD), and is detailed in the supplementary material (SI_1 Chemicals and data).

An external validation set with BCF data for another 181 compounds was collected from EChemPortal.[2] The search criteria were “Bioaccumulation: aquatic/sediment” and “Study result type: experimental result”. About 5000 results were retrieved for almost 1000 chemicals. Removing compounds without unique chemical structures (*e.g.* UVCBs) and inorganic chemicals resulted in BCF and BAF values for 475 discrete organic chemicals. 228 of these chemicals were included already in the training set. 66 chemicals had ambiguous BCF or BAF values (*e.g.*, it was not clear if the value referred to BCF or log BCF). The remaining 181 chemicals cover a log BCF range between 0 and 6 with 168 nonB (92.8%), 9 B (5.0%), and 4 vB (2.2%) compounds. The highest (worst case) value was used in the case of multiple data for the same substance to challenge the BCF waiving scheme. The external validation set is detailed in the supplementary material (SI_1 Chemicals and data).

[2] http://www.echemportal.org/, accessed 25.08.2016.