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How error-prone bioaccumulation experiments affect the risk assessment of hydrophobic chemicals and what could be improved

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Abstract

Bioaccumulation is one of the three criteria for the PBT assessment of chemicals, where P stands for persistence, B for bioaccumulation, and T for toxicity, which is a cornerstone for the "Registration, Evaluation, Authorization, and Restriction of Chemicals" (REACH) in the EU. Registrants are required by REACH to submit data on bioaccumulation if the chemical is manufactured in and/or imported to the European Economic Area at more than 100 t/year. Most of the experimental bioaccumulation studies submitted were on the bioconcentration factor (BCF) and were conducted prior to 2012, before the OECD Test Guideline 305 on Bioaccumulation in Fish was updated. An analysis of the submitted data revealed that many of the experimental data, but also the data from QSARs and other calculation methods, underestimate the actual bioaccumulation potential of hydrophobic substances considerably. One of the main reasons in the nonexperimental studies is that the BCF is related there to the total concentration of the chemical in water and not to the dissolved chemical concentration. There is therefore an urgent need to reassess the bioaccumulation potential of the hydrophobic substances registered under REACH. Based on the model calculations in the present study, between 332 and 584 substances that are registered under REACH are likely to bioaccumulate in the aquatic environment—many more than have so far been identified in the B assessment. *Integr Environ Assess Manag* 2023;19:792–803. © 2022 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Bioaccumulation; bioconcentration factor; hydrophobic substances; risk assessment

INTRODUCTION

The assessment of a chemical's bioaccumulation potential is an important element of current hazard and risk assessment schemes—under the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in the EU and various national regulations, but also internationally under the Stockholm Convention on Persistent Organic Pollutants (POPs) (Gobas et al., 2009). Under REACH, bioaccumulation is one of the criteria for the PBT assessment and only if all three criteria are fulfilled, that is, a chemical is persistent (P), bioaccumulative (B), and toxic (T), it is further evaluated and potentially considered a Substance of Very High Concern.

The bioaccumulation potential of a substance can be concluded from various endpoints, including the bioconcentration factor (BCF), the bioaccumulation factor (BAF), the biomagnification factor (BMF), or the trophic magnification factor (TMF). The BCF and BAF are defined as ratios of the steady-state chemical concentrations in an aquatic water-respiring organism and the water, where the BCF is obtained through aqueous exposure only and the BAF through combined dietary and aqueous exposure (Gobas et al., 2009). The BMF is the ratio of the steady-state chemical concentrations in a water- or air-respiring organism and in the diet of the organism (Gobas et al., 2009). The exposure can be aqueous and/or dietary. The BCF is the most commonly used parameter and most regulations that deal with the bioaccumulation potential have thresholds for the BCF. Under REACH in the EU, a BCF > 2000 indicates a bioaccumulative substance, and a BCF > 5000 a very bioaccumulative substance. In addition to the BCF, Environment and Climate Change Canada and the Stockholm Convention use a logarithmic octanol–water partition coefficient (log K_{OW}) greater than 5 as a (screening) criterion

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for bioaccumulation (Gobas et al., 2009; Stockholm Convention, 2001). In the present study, the word bioaccumulation (potential) is used as a general term to describe the potential of a substance to accumulate chemicals. It is not specific to a certain exposure pathway and can be expressed as BCF, BAF, BMF, or TMF.

For hydrophobic substances, the determination of the bioaccumulation potential can be difficult. This is also one of the reasons why the OECD Test Guideline (TG) 305 for bioconcentration tests has been updated several times, including in 1996 and 2012 (OECD, 2012). Before 1996, there were five TGs (305 A–E) where, for example, TG 305 C was for flow-through tests and TG 305 D for static tests. These guidelines were consolidated into a single TG 305 in 1996, and a flow-through fish test where the fish are exposed to the test chemical through the water phase (aqueous exposure yielding a BCF) was recommended. However, it was recognized later on that testing of very poorly water-soluble substances may not be technically feasible via aqueous exposure (OECD, 2012).

Possible artifacts and shortcomings of studies that were conducted for hydrophobic substances following versions of the OECD TG 305 from before 2012 are listed in the Guidance on Information Requirements and Chemical Safety Assessment—Chapter R.11 of the European Chemicals Agency (ECHA) and are also explained by Ehrlich et al. (2011). These include "Difficulties in measuring the 'true' aqueous concentration due to sorption of the substances to particulate and dissolved (organic) matter; unstable concentration of the test substance in water and thus highly fluctuating exposure conditions; adsorption of the test substance to glass walls or other materials; volatilization; testing at concentrations clearly above the water solubility of the test substance, normally via the inclusion of dispersants or vehicles which would lead to an underestimation of the BCF; and determination of a BCF as the ratio between the concentration in fish and in water but under non steady-state conditions" (ECHA, 2017a). To overcome these shortcomings, various changes have been incorporated in the new TG 305, including the recommendation for a dietary exposure test for hydrophobic substances (log K_{OW} > 5) with a solubility less than approximately 0.01–0.1 mg/L (OECD, 2012). The new guideline also acknowledges the use of passive dosing systems and puts greater emphasis on the kinetic BCF (OECD, 2012).

Despite this development and the acknowledged shortcomings, many BCF values for hydrophobic substances from old OECD 305 studies are still used and accepted as "correct" (Miyata et al., 2022). In addition, there has been a debate whether highly hydrophobic chemicals (with log K_{OW} > 5–6) bioconcentrate less than what may be expected from their hydrophobicity due to a "hydrophobicity cutoff" or a "molecular size cutoff". However, it has been demonstrated that these cutoff criteria are not valid (Arnot et al., 2009; Geyer et al., 1992; Groh et al., 2017; Jonker & van der Heijden, 2007; Müller & Nendza, 2007) and originated in artifacts in the experimental data. A recent study suggests that "uptake of super hydrophobic chemicals from food does take place without any anomalies. It is slow but it will eventually result in a huge bioconcentration of these compounds when they are not metabolized" (Larisch & Goss, 2018). Further evidence comes from studies using fish (Schmieder et al., 1995), earthworms (Van Der Wal et al., 2004), and sediment-dwelling organisms (Kraaij et al., 2003), demonstrating that bioaccumulation in these species is correlated linearly with the octanol–water partition coefficient up to a log K_{OW} of 7–8.

Based on these recent findings, one would expect all hydrophobic substances to bioaccumulate provided that they are not metabolized. However, of the bioaccumulation data submitted to ECHA that we analyzed in this study, only 24 out of 132 substances with experimental data and a log K_{OW} > 5 had an experimental BCF > 2000 (ECHA, 2021). Additional BCF values for these substances are much lower than 2000. The present study investigates therefore the bioaccumulation data submitted under REACH for neutral substances and tries to answer the question of why there are almost no substances exhibiting strong bioaccumulation in the ECHA database, although many hydrophobic substances have been registered (and are expected to bioaccumulate). This is done by combining a generic bioaccumulation model for fish with substance-specific biotransformation data. In addition, the methods used and study conditions in the studies in the ECHA database are analyzed to highlight where problems may have occurred. Finally, a stepwise approach is proposed to generate more reliable bioaccumulation data, especially for hydrophobic substances.

It is important to note here that the analysis was done with data from the ECHA database because these data are readily available for numerous substances, and these data are used for the regulation of the chemicals in the EU. However, it is to be expected that other large datasets containing data on bioaccumulation would exhibit similar problematic points, which would lead to similar conclusions.

METHODS

Substance data submitted under REACH

The nonconfidential substance data submitted under the REACH regulation were downloaded from the IUCLID website in April 2022 and contained the data from the ECHA website as of 15 September 2021. The data contained 26 081 registration dossiers (ECHA, 2021) for 23 184 substances. The isomeric simplified molecular-input line-entry system (SMILES) string that describes the chemical structure in line notation, and the Chemical Abstracts Service Registry Numbers™ (CAS RN) were not included in the IUCLID data. We therefore contacted ECHA and obtained a list of the registered substances with the available IUPAC names, EC numbers, CAS RN, molecular formulas, and SMILES notations in January 2021. Additional SMILES were obtained manually from the website in February and May 2022. The substances investigated in this study are

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those that are neutral organic chemicals, mono-constituent with one component and have a full registration (including previously notified substances [NONS]). Intermediates were not included. Also not included are those substances where CAS RN and SMILES notation did not correspond to each other. The respective substances will be published in an additional article. The final number of substances investigated was 5891.

Data processing of the data from the ECHA database

The data from the IUCLID website were obtained in the form of registration dossiers where each dossier had its own folder that contained the data in several xml documents. The data themselves, whether experimental, calculated, or read-across, were given in "studies". For each study, information on general points such as endpoint, study type, or reliability as well as on the methods used and the results were reported. Multiple results were possible in one study. For example, BCF values could be given at different points in time of an experiment, or the BMF value and the back-calculated BCF could be provided from the same experiment.

Extracting the data from the xml documents was done with Python 3.7.9. Up to eight results were saved per study, which covered 99% of all of the studies' available results. Forty-four studies (1%) had between 9 and 15 results. Here only the first eight results were saved. All studies that were rated in the registration dossier (by the registrants) as "3 (not reliable)" or which had been flagged as "disregarded due to major methodological deficiencies" were excluded from the analysis. A lipid normalization to 5% of body lipids was done for all study results that were reported as "not lipid-normalized" but where the lipid content was provided. Additionally, values were excluded from the analysis if only one value was given as lower or upper bound (qualifiers: $<$, $\leq$, $>$, $\geq$). If the values were given as a range, the mean of both was used in the analysis. For the BCF values, only the highest value from a study was selected and used in the analysis. This ensured that values taken in the course of an experiment but not representing the final (highest) BCF were not included.

Calculation of pK_a values

pK_a values were calculated with MarvinSketch 22.18 (Chemaxon, 2022). Substances were considered as ionized in the environment (at pH 7.4) if either the most acidic pK_a was lower than 5.4 or the most basic pK_a was greater than 9.4. This included 934 substances (16% of the investigated substances). Substances where the most acidic and/or the most basic pK_a values were between 5.4 and 9.4 were considered as partly ionized (690 substances).

Calculation of log K_{OW} with BIOVIA COSMOconf and COSMOtherm 2020

BIOVIA COSMO*conf* is a tool that can generate the most relevant conformers of a molecule. These conformers can be used later on in BIOVIA COSMO*therm* to estimate the physicochemical properties of a substance. Both programs use quantum chemistry calculations and are based on the COSMO-RS theory (Eckert & Klamt, 2002; Klamt, 1995). COSMO*therm* cannot calculate the bioaccumulation potential of a substance, but can calculate the log $K_{OW,}$ and the results of these calculations were used in the present study. Most other programs that are able to calculate the log K_{OW}, such as EPI Suite, OPERA, or SPARC, are based on a training set, and for substances that are poorly, or not at all, represented in the training set, the results are quite uncertain. COSMO*conf* and COSMO*therm*, on the other hand, derive their results from calculated differences between the physicochemical interactions of a molecule dissolved in different solvents and do not need substance-specific parameters. In the present study, this is especially useful for the hydrophobic substances because not so many measurements are available for them.

COSMO*conf* was run with the BP-TZVPD-FINE_COSMO + GAS parameterization. For molecules that failed in COSMO*conf* or that were too large to be completed in a reasonable time frame (~13% of the molecules), experimental data or those from OPERA and EPI Suite were used. For more details see the Supporting Information: 2.

Model for the bioaccumulation in fish

To evaluate the bioaccumulation data from the ECHA database, it is crucial to understand how the BAF and BCF are defined and calculated. Thus, if we define a fish as a single, well-mixed compartment with instantaneous equilibrium partitioning within the fish and with all uptake and elimination processes following first-order kinetics, the rate equation for the chemical in the fish surrounded by water can be defined as:

$$\frac{dC_F}{dt} = k_R C_W + k_D C_D - C_F \times (k_V + k_E + k_M + k_G), \quad (1)$$

where C_F is the concentration in fish, C_W the dissolved chemical concentration in water, and the k-values the rate constants: k_R for respiratory uptake, k_D for dietary uptake, k_V for respiratory loss, k_E for egestion, k_M for biotransformation, and k_G for growth dilution (Gobas, 1993; Mackay et al., 2018; OECD, 2012). The sum of the three loss process rate constants plus the growth dilution rate constant is also known as the elimination rate constant.

Steady-state BAF and BCF. Under steady-state conditions, Equation (1) can be rearranged to calculate the BAF and BCF, respectively. Both, BCF and BAF are defined as the concentration in fish divided by the dissolved chemical concentration in water (ECHA, 2017b):

$$\mathrm{BAF} = \frac{C_F}{C_W} = \frac{k_R C_W + k_D C_D}{C_W \cdot (k_V + k_E + k_M + k_G)}, \quad (2)$$

$$\mathrm{BCF} = \frac{C_F}{C_W} = \frac{k_R}{k_V + k_E + k_M + k_G}. \quad (3)$$

Kinetic BAF and BCF. The BCF that is calculated in Equation (3) via the rate constants is also known as the

kinetic BCF. The kinetic BCF is a steady-state value; however, it can be calculated before the test system has reached its steady state, because the rate constants do not change over time (see Equation 3).

Determination of the chemical concentration in water. It is important to note here that the commonly used liquid–liquid extraction (LLE) yields the total chemical concentration in water (C_{WT}) and not the freely dissolved chemical concentration (C_W) (OECD, 2017). However, fish can only absorb substances that are freely dissolved (Black & McCarthy, 1988); therefore, the BCF should also refer to this concentration. Not using the freely dissolved chemical concentration in water has been recognized as an important issue that leads to an underestimation of the BCF (Böhm et al., 2016; Ehrlich et al., 2011; Geyer et al., 2005; Müller & Nendza, 2007), and the OECD Test Guideline 305 for Bioaccumulation in Fish: Aqueous and Dietary Exposure (OECD TG 305) recommends the use of solid-phase microextraction (SPME) to obtain the freely dissolved chemical concentration (OECD, 2012). Semipermeable membrane devices may also be used (Ehrlich et al., 2011). Another option (that is not included in the OECD TG 305) would be to back-calculate the dissolved chemical concentration from the total chemical concentration in water using the concentrations of dissolved and particulate organic carbon that are contained in the water:

$$C_W = \phi \times C_{WT}, \tag{4}$$

$$\phi = 1/(1 + f_{POC} \times 0.35 \times K_{OW} + f_{DOC} \times 0.035 \times K_{OW}), \tag{5}$$

where ϕ is the fraction of the total chemical concentration in the water that is freely dissolved, f_{POC} is the fraction of particulate organic carbon, and f_{DOC} the fraction of dissolved organic carbon (Arnot & Gobas, 2003). The OECD TG 305 permits a maximum value of 5 mg_C/L for particulate matter, which is defined as the organic carbon not passing a 0.45 µm filter (POC), and 2 mg_C/L for organic carbon in the filtrate (DOC; OECD, 2012). Using Equation (5) and the values for DOC and POC permitted in the OECD TG 305, one can see that ϕ is decreasing with increasing hydrophobicity, so that substances with a log K_{OW} > 7 are almost completely particle bound, and greater than a log K_{OW} of 5 a substantial reduction in C_W as compared with C_{WT} is expected (Figure 1). This means that for highly hydrophobic substances the BCF and BAF would be artificially low if they were based on the total concentration of the substance in water.

Model development. In the present study, the model of Arnot and Gobas (2003) was used to calculate BCF values. Six different QSARs were used to predict the biotransformation rate constants, and the geometric means of the predicted values were used in the Arnot and Gobas model. Additionally, an alternative model for the respiratory uptake (equation of Sijm et al., 1995) was used in the model

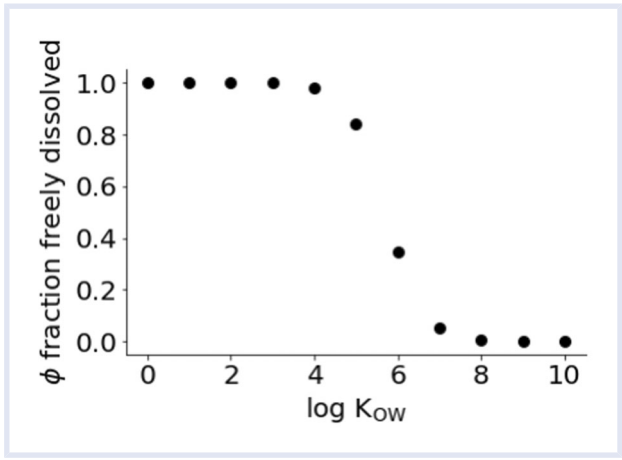

FIGURE 1 Dependence of the fraction of the total chemical concentration in water that is freely dissolved (ϕ) on the log K_{OW} using 5 mg_C/L for the f_{POC} and 2 mg_C/L for the f_{DOC}, respectively

of Arnot and Gobas (2003) for comparison purposes. More details are provided in the Supporting Information: 1.

RESULTS

There are 2955 organic mono-constituent substances with full registration in the ECHA database with information on bioaccumulation, including experimental data for 627 of the substances and data from (Q)SARs, read-across or model calculations for 2614 substances. The 1011 experimental studies include more than 1731 individual results. Of these, 1588 results reported the BCF, 15 the BAF, 74 the BMF, 11 the biota-sediment accumulation factor (BSAF), and 43 "other" (Table 1).

Experimental BCF values from the ECHA database

There are 764 studies that were labeled as experimental studies yielding a BCF value. Nine of them stated that dietary exposure was used, whereas for the other ones the route was either given as aqueous exposure or was not stated at all. Examining the dietary exposure studies in more detail revealed that only four of them (EC numbers: 252-021-1, 201-618-5, 410-610-2, and 274-581-6) were purely dietary exposure studies according to OECD TG 305 that gave dietary BMF values and were then converted into BCFs. Two other studies used a mixture of dietary and aqueous exposure and calculated an endpoint based on the water concentration of the test substance, which they called BCF but that should correctly be termed a BAF (EC numbers: 255-460-7, 249-720-9). The three remaining studies (according to the information available on the ECHA website in May 2022) had no dietary exposure at all (EC numbers: 228-846-8, 204-496-1, 206-581-9).

Twenty-three of the aqueous exposure studies were conducted according to the updated OECD TG 305 from 2012 (OECD, 2012), 329 of the studies according to one of the older versions of OECD TG 305, 19 studies were conducted according to one of the guidelines from the US EPA, and for 388 studies no test guideline was provided.

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TABLE 1 Number of available results for the endpoint bioaccumulation in the ECHA database for organic mono-constituent substances with full registration

Endpoint	All organic mono-constituent substances	Experimental studies	(Q)SARs and calculation	Read-across and others
BCF	6470	1588	3836	1046
BAF	415	15	372	28
BMF	77	74	0	3
BSAF	12	11	0	1
Other	844	43	342	459

Note: Studies that were flagged as not reliable or that they should be disregarded due to major methodological deficiencies were disregarded. Studies with reference substances different from the registered substance were only counted for "read-across and others".
Abbreviations: BAF, bioaccumulation factor; BCF, bioconcentration factor; BMF, biomagnification factor; BSAF, biota-sediment accumulation factor.

Two substances for which a BCF study was conducted according to the updated OECD TG 305 had a water solubility in the ECHA database below 0.01 mg/L (EC numbers: 221-573-5 and 223-445-4), and also for five additional substances we calculated with COSMO*therm* a water solubility below 0.01 mg/L (EC numbers: 274-570-6, 422-600-5, 620-097-9, 829-608-1, 208-762-8). These seven substances should have been tested with a dietary exposure study. Overall, it appears that many of the available studies were conducted using old guidelines and/or methods that are inadequate according to current knowledge.

Data waivers were also included in 210 of the dossiers. Most of them concerned substances with a log $K_{OW} < 3$, for which no aqueous bioaccumulation data are required. However, there were also 33 dossiers without acceptable data waivers or without any data at all. The respective substances, their log $K_{OW,}$ and the data waiver justification are given in Supporting Information: Table S3. Often, the justification for the data waiver was "study scientifically not necessary/other information available" or "the study does not need to be conducted because direct and indirect exposure of the aquatic compartment to the substance is unlikely." Six times it was also stated that the substance is readily biodegradable and therefore not bioaccumulative. However, the Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c states very clearly that "biodegradability does not preclude a bioaccumulation potential" (ECHA, 2017b). The likelihood that a biodegradable chemical is also metabolized is given but bacterial degradation has a larger reaction range than metabolism, which is typically a simple oxidation by monooxygenases in its first step followed by a conjugation.

The BCF data themselves are shown in Figure 2A and Supporting Information: Figure S4. Figure 2A shows the experimental BCF values for organic mono-constituent substances with full registration from the ECHA database, plotted against the log K_{OW} from COSMO*therm*. The experimental BCF values plotted against the "best available" log K_{OW} from the ECHA database are shown in Supporting Information: Figure S4. In both plots, there is an increasing trend of the BCF with increasing log K_{OW} until a log K_{OW} of approximately 5–6 and a decreasing trend thereafter. Only 23 out of 243 experimental BCF values are greater than the threshold of 2000. The next sections investigate whether

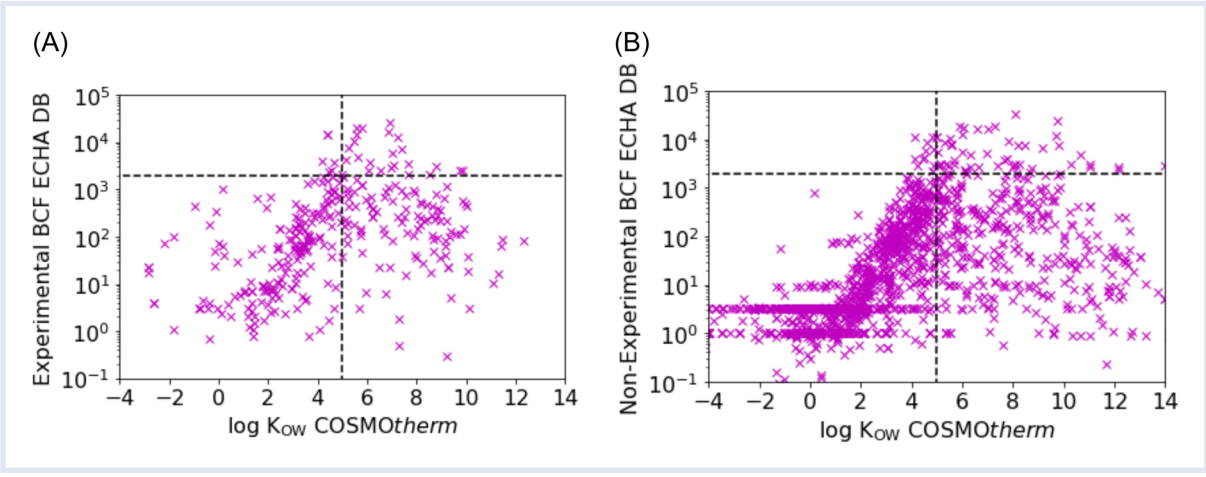

FIGURE 2 (A) Experimental and (B) nonexperimental bioconcentration factor (BCF) values for organic monoconstituent substances with full registration from the ECHA database, plotted against the log K_{OW}. K_{OW} values were calculated using COSMO*therm* 2020. The horizontal dashed line indicates a BCF of 2000, the vertical dashed line a log K_{OW} of 5

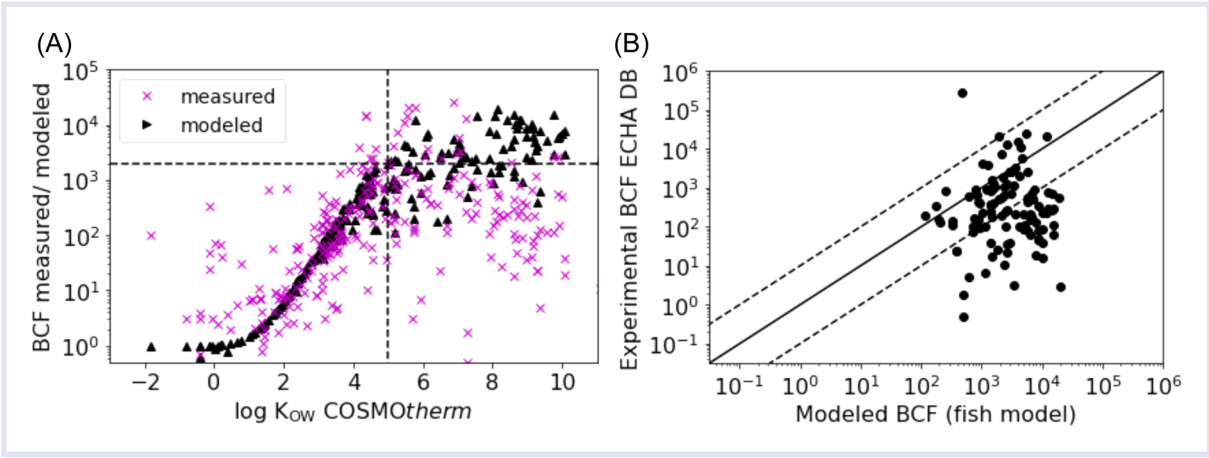

FIGURE 3 (A) Experimental bioconcentration factor (BCF) values from the ECHA database (magenta crosses) and modeled BCF values with Equation (3) (black triangles). (B) Experimental BCF values versus modeled BCF values for substances with a log K_{OW} > 5. The fish weight in the model was 10 g. The horizontal dashed line in (A) indicates a BCF of 2000, the vertical dashed line a log K_{OW} of 5. The solid line in (B) indicates the 1:1 line, the dashed line the deviation of a factor of 10

these data are realistic. For this analysis, the log K_{OW} values of COSMO*therm* were used because some dossiers in the ECHA database contain questionable log K_{OW} values (e.g., for CAS RN: 4051-63-2), and there is also a systematic bias for the highly hydrophobic substances to underestimate the log K_{OW} (more details will be provided in a separate publication).

Modeled versus experimental BCF values for substances from the ECHA database. The comparison of the 243 experimental BCF values from the ECHA database and the modeled BCF values is shown in Figure 3A in relation to the log K_{OW}. Figure 3B shows the experimental versus the modeled BCF values for the hydrophobic substances (log K_{OW} > 5) only. For substances that are partly ionized in the environment (pH 7.4), only the neutral fraction was considered in the modeled BCF value. The potential bioaccumulation of the ionized species was not considered. The modeled and measured BCF values agree very well for the more hydrophilic substances (log K_{OW} < 5) but not for the hydrophobic ones (Figure 3A). Approximately 50% of the hydrophobic substances (55 out of 117 substances) show a BCF value in the ECHA database that is more than 10 times lower than what was modeled (Figure 3B). The next section will therefore test the reliability of the model to clarify the origin of the deviations.

Modeled versus experimental BCF values for test substances. Two groups of substances were used to test the reliability of the model: (i) 22 substances that are known to bioaccumulate (acknowledged POPs under the Stockholm Convention) and (ii) seven substances that are readily biodegradable, with a log K_{OW} > 3. Substances in the latter group are less likely bioaccumulative although the mechanisms of biodegradation in the environment might be different from the one for the biotransformation in the organism. The results reveal that the model is capable of predicting correctly the high BCF values of the substances from the first group as well as the low BCF of those from the second group that were not expected to bioaccumulate (Figure 4). Substances that are more likely to bioaccumulate in the terrestrial environment (log K_{OW} between 2 and 5, log K_{OA} > 5; Kelly et al., 2007) were (as expected) not captured by the model. Details on the test substances are provided in the Supporting Information: 2.

This benchmarking exercise indicates that the model is a reliable tool for identifying bioaccumulative substances and that there are indeed substantial problems with the experimental BCF studies for the hydrophobic chemicals. Some of the experimental problems were already pointed out in the introduction. The next section will examine more closely those points that are also visible in the data from the ECHA database.

Potential issues in the experimental BCF studies. First, there are 128 BCF values for substances with a calculated log K_{OW} > 5 of which 124 were measured via aqueous

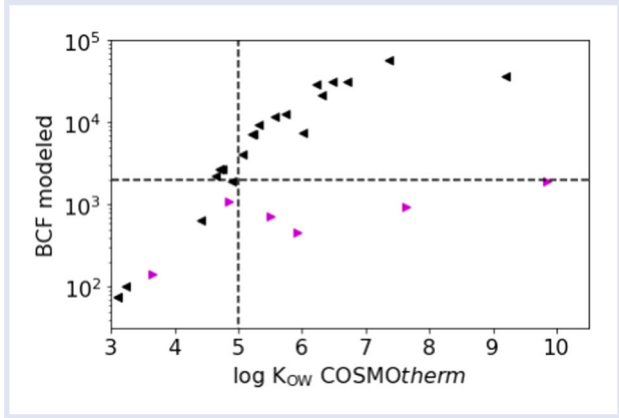

FIGURE 4 Modeled bioconcentration factor (BCF) values for acknowledged persistent organic pollutants (POPs) (black triangles) and ready-biodegradable substances (magenta triangles) over log K_{OW} values from COSMO*therm*. The fish weight was 10 g. The horizontal dotted line corresponds to a log K_{OW} of 5, the vertical dotted line to a BCF of 2000

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exposure and only four via dietary exposure. The updated OECD TG 305 recommends dietary exposure tests for substances with a log $K_{OW} > 5$ and a water solubility below approximately 0.01–0.1 mg/L for various reasons (OECD, 2012). One is that the uptake may be limited by the low exposure concentrations that occur because of the low water solubilities of the substances.

Second, it is apparently very difficult to maintain sufficiently constant water concentrations if the solubility of the substance is very low. An important point here is also that the water concentration may not only have contained the dissolved fraction of a chemical, but also the fraction that was bound to particulate or dissolved organic matter in the test system. The OECD TG 305 does not require a "back-calculation" from total to freely dissolved concentration. In the ECHA database, TOC levels are given only in some studies, and their results are not exported in IUCLID. It was therefore not possible to correct the BCF values for the TOC content in water.

Third, substances with a log $K_{OW} > 5$ and, at the same time, a steady-state BCF < 2000 (dark blue bars in Figure 5) had only in 30% of the cases an exposure time longer than 30 d. This indicates that the exposure time may have been too short in some of these experimental studies, all of which were experiments that attempted to reach steady state and did not derive the kinetic BCF. With a careful determination of the steady state, such cases should never occur. However, OECD TG 305 accepts deviations of 20% between two successive analyses of the fish concentrations, and this makes it difficult to determine whether or not the steady state was reached, especially if 2 d were chosen as test interval and not 7 d as recommended for substances that are slowly taken up. It could be argued that substances with a high biotransformation rate and thus a low BCF do not require long exposure times because they reach steady state faster. However, according to the analysis in Supporting Information: 1, Section S5, biotransformation was not the reason for the short exposure times.

Most of the points mentioned here as well as in the introduction lead to reported BCF values that are likely lower than they are in reality. The answer to the question of why almost no substances displaying strong bioaccumulation in the ECHA database is, consequently, that for many of the substances, the bioaccumulation potential has been underestimated and, contrary to the conclusions in the REACH dossiers, the substances are bioaccumulative. Our model predicts that up to 66 out of 120 substances with log $K_{OW} > 5$ and experimental data in the ECHA database have a BCF > 2000. Taking all mono-constituent substances registered under REACH into account, between 332 and 584 substances are expected to bioaccumulate (BCF > 2000) in the aquatic environment.

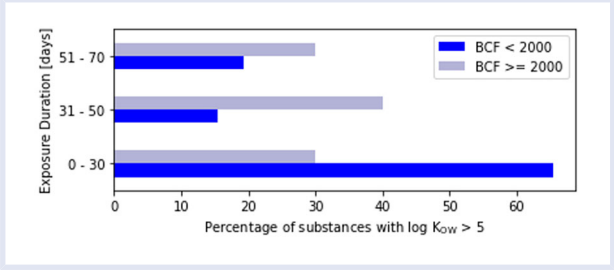

FIGURE 5 Exposure duration for studies that calculated the bioconcentration factor (BCF) value at steady state for substances with log $K_{OW} > 5$

Experimental BMF values from the ECHA database

The ECHA database includes 72 experimental BMF values. Half of them were OECD TG 305 studies according to the updated guideline from 2012, six were OECD TG 305 studies according to the guideline from before 2012, and nine studies were either not conducted according to any guideline or the guideline was not stated. Only three of the 72 BMF values are greater than 1. To check the plausibility of the BMF values, BCF values were modeled for these substances. The results for the 24 substances that could be modeled (out of 25) are shown in Figure 6.

Of the 24 substances, eight have a modeled BCF > 2000. Although a BCF and a BMF are not the same—the BCF describes an increase in concentration between water and fish whereas the BMF describes an increase in fugacity (and concentration) from food to fish that is possible resulting from the decomposition of the food in the digestive tract (Mackay et al., 2018)—they are still related through the elimination rate in the fish. A substance can only bioaccumulate (in a fish) if the elimination is slower than the uptake. For this reason, the modeled BCF values also indicate the BMF.

An explanation of the low BMF values reported in the ECHA database could be growth rates of the fish, which were significantly higher than the sum of the other elimination rates. OECD TG 305 recommends the use of growth-corrected depuration rate constants; however, it is also stated that for very slowly depurating substances tested in fast growing fish, the derived growth-corrected depuration rate constants may be very small and so errors in the

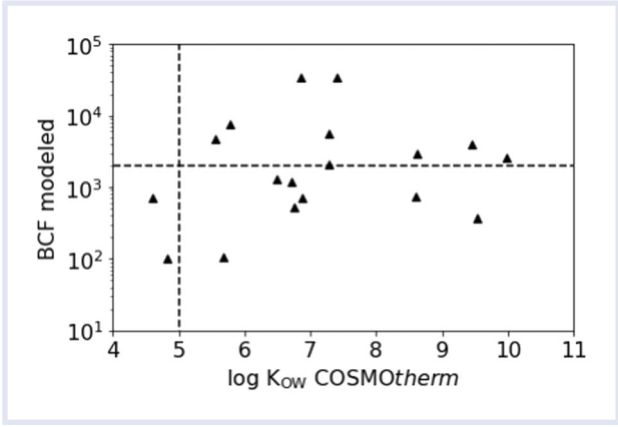

FIGURE 6 Modeled bioconcentration factor (BCF) values using Equation (3). The fish weight was assumed to be 10 g. The horizontal dotted line corresponds to a log K_{OW} of 5, the vertical dotted line to a BCF of 2000

determination of the rate constants can become critical (OECD, 2012). Because mostly juvenile fish were used in the tests, it may well be that the growth rates have led to problems in the determination of the other elimination rate constants.

Nonexperimental BCF values from the ECHA database

The nonexperimental BCF values are shown in Figure 2B. As with the experimental data, there is an increasing trend of the BCF values with increasing octanol–water partition coefficient until a log K_{OW} of approximately 6 and a decreasing trend thereafter. However, when the BCF values are calculated with the model presented in the Supporting Information: 1, an increasing trend with increasing log K_{OW} is also observed for substances with a log $K_{OW} > 5$ (Supporting Information: Figure S4). To investigate the differences, we first determined which QSARs and/or models were used in the ECHA database. This was done by searching for specific keywords in the free text field "Method no Guideline" in the IUCLID data. The field "Method no Guideline" is available to the registrants to describe the methods used if the study was not conducted according to a specific guideline. It was included in the IUCLID data until 2020 (but not in 2021). The IUCLID data from September 2020 were therefore used here. The analysis revealed that almost half of the datapoints were obtained from EPI Suite, and approximately 20% from OASIS Catalogic and other parabolic relationships (Table 2).

Models in EPI Suite (BCFBAF). There are two models implemented in EPI Suite that give BCF values. One is a regression-based model and the other is based on the Arnot–Gobas model. The regression-based model is based on experimental BCF data and predicts the BCF for neutral substances based on the log K_{OW} (Meylan et al., 1999). Compounds with a log $K_{OW} < 1$ are assigned an estimated BCF of 3.1. For compounds with a log K_{OW} between 1 and 7, the BCF increases with increasing log K_{OW}. However, for substances with a log $K_{OW} > 7$, a decreasing BCF with increasing log K_{OW} is assumed. This is not necessarily surprising also given the experimental data from Figure 3B. However, considering how many problems there are with BCF tests of hydrophobic substances, almost all of which result in BCF values that are too low, it is very likely that the decreasing trend reflects experimental artifacts.

The second model in EPI Suite that yields BCF and BAF values is the model of Arnot and Gobas (2003). In contrast to the definition that we use here, Arnot and Gobas (2003) define the BCF and BAF based on the total chemical concentration in water (Equations S7 and S8 in the Supporting Information: 1). However, this definition leads to a systematic and substantial underestimation of the BCF and BAF values for hydrophobic substances. Based on this analysis, we argue that the BCF and BAF values that are derived from the models in EPI Suite for substances with a log $K_{OW} > 5$ should not be used in the risk assessment of these substances.

Catalogic or other parabolic relationships. A BCF model is also implemented in Catalogic (2022). It consists of two major components: a model for predicting the maximum potential for bioaccumulation based on the log K_{OW} and a set of mitigating factors that account for the reduction in the bioaccumulation potential of chemicals based on chemical (molecular size, ionization, and water solubility) and organism (metabolism)-dependent factors (Dimitrov et al., 2005; LMCasis, 2022). The maximum potential for bioaccumulation is described in a parabolic relationship where

TABLE 2 Analysis of the field "Method no Guideline" in the IUCLID data from September 2020 for studies that reported bioaccumulation data (BCF, BAF, BMF, others) from "(Q)SARs" or "calculation (if not (Q)SAR)"

Model	Keyword in "Method no Guideline"	No. of studies
One of the two models in EPI Suite	2000 US Environmental Protection Agency; BCF BAF v3.00; Estimation Programs Interface SuiteTM; epi; BCFBAF; Arnot–Gobas; BCFWIN; Meylan	1485
OASIS Catalogic or other parabolic relationships	Equations are used for substances with log $K_{OW} < 1$, between 1–7 and > 7; parabolic relationship; CATALOGIC	634
ACD/Labs	ACD	60
PBT Profiler or QSAR Toolbox	QSAR Toolbox version 3.1; PBT profiler	121
Model of G. D. Veith et al. (1979) and (1980)	Veith; 0.85 log K_{OW} – 0.7; 0.85. Log K_{OW} – 0.7	31
NaN	NaN	183
Models for substances with log $K_{OW} < 6$	Relationship applies to substances with a log $K_{OW} < 6$; Linear model to estimate BCF for neutral, nonionized chemicals with a log K_{OW} of 1.0–6.0	87
Other		682

Abbreviation: BCF, bioconcentration factor.

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the highest BCF is obtained for substances with a log K_{OW} of 6–7 (Dimitrov et al., 2005). The parabolic curve was apparently chosen because it represents the underlying experimental BCF values. However, because of the various problems with the experimental BCF tests of hydrophobic substances, models that use these data as a basis, most probably reflect experimental artifacts. We therefore also argue that BCF values coming from any model based on a parabolic relationship between BCF and log K_{OW} are not suitable for the risk assessment of hydrophobic substances because these models might underestimate the bioaccumulation potential of the substances.

Models of G. D. Veith et al. (1979 and 1980). G. D. Veith et al. (1979) proposed the linear relationship log BCF = 0.85 × log K_{OW} − 0.7 for organic chemicals. The correlation was obtained for substances with a log K_{OW} between 1 and 7. Similarly, G. D. Veith et al. (1980) proposed as relationship log BCF = 0.76 × log K_{OW} − 0.23. Here, we were not able to obtain information on the applicability domain of the equation. Because both equations consider only the hydrophobicity of the substances, but not the metabolism, they should only be used as a first approximation but not in the actual risk assessment. This also applies to data from ACD/Labs that use for substances with a log K_{OW} between −1 and 9 the relationship from G. D. Veith et al. (1980) to estimate the BCF (E. Kolovanov, ACD/Labs, personal communication, 14 April 2022).

Conclusion on nonexperimental results. Given that 65% of the datapoints originate either from regression-based models that mimic the experimental data (and their shortcomings) or from a model that bases the BCF and BAF on the total water concentration, it is not surprising to also see a large discrepancy between the nonexperimental BCF data in the ECHA database and our modeled BCF values for the hydrophobic substances. From this, it can be concluded that the available models might be useful for the bioaccumulation assessment of substances with log K_{OW} < 5, but not for assessment of the hydrophobic substances.

To facilitate an easier evaluation of the bioaccumulation potential of the substances registered under REACH, the calculated BCF values that are based on the model in this study are provided in the Supporting Information: 2 for all monoconstituent organic substances with full registration under REACH.

DISCUSSION

Accuracy of the BCF data for hydrophobic substances in the ECHA database

According to the one-compartment model for fish that was used in this study, 50% of the experimental BCF values for hydrophobic chemicals in the ECHA database are underestimated by more than a factor of 10. The model and model parameters also carry uncertainties and more research is needed, for example, to better define the uptake rate constants (and/or transfer efficiencies) for hydrophobic substances. However, Figure 3A shows that the model predicts the BCF values well for substances with a log K_{OW} between 0 and 5. Because it has been demonstrated that the suggested "hydrophobicity cutoff" and the "molecular size cutoff," which would result in very hydrophobic or very large molecules not being able to bioaccumulate, are caused by artifacts in the experimental data (Arnot et al., 2009; Geyer et al., 1992; Groh et al., 2017; Jonker & van der Heijden, 2007; Larisch & Goss, 2018; Müller & Nendza, 2007), it can safely be assumed that the BCF model used here is also suitable for hydrophobic substances.

Also, the argument that the log K_{OW} is not a good descriptor for the partitioning into membranes for large molecules, as suggested by Gobas et al. (1988) and others (Dulfer & Covers, 1995), was disproved. According to the studies of Jonker and van der Heijden (2007), Endo et al. (2011), and Endo et al. (2013), the log K_{OW} describes the partitioning into membrane lipids up to a log K_{OW} of at least 8.3 very well. The estimation of partitioning into storage lipids seems to be less precise (Endo et al., 2011) especially for compounds with long alkyl chains. However, the log K_{OW} rather underestimates the partitioning into storage lipids for the hydrophobic substances (Endo et al., 2011), which would lead to too low BCF values in the model.

It is therefore reasonable to assume that the large difference between the experimental and modeled BCF values for hydrophobic substances originate mainly from experimental problems and artifacts and not from model uncertainties. This is supported by the small benchmarking simulation in Figure 4. It would therefore be very important to take a closer look at the 309 and 556 substances identified by the model in the present study with a BCF > 2000. Some of these substances are NONS and might not be on the market in the EU anymore, but the other substances should be evaluated very carefully.

It is also important to note that not only the experimental values are subject to errors but also the ones that are based on QSARs and calculations. There is no section in the Guidance on Information Requirements and Chemical Safety Assessment—Chapter R.11 (ECHA, 2017a) that addresses QSARs for bioaccumulation although the same guidance document contains chapters on QSARs for persistence and toxicity. It seems that the registrants have used whatever estimation method was available or preferable. We therefore strongly recommend that guidance should also be provided by ECHA for nonexperimental methods for quantifying bioaccumulation. Even if the QSAR values are rarely used for the risk assessment of the substances under REACH, it is still important to correct them because they might influence the prioritization of the compliance check or the substance evaluation.

Definition of the BCF and BAF

It is important to emphasize that the definitions of BCF and BAF should not be based on the total chemical concentration in water but on the dissolved one. This finding is

not new, but it is still not always implemented, and it is also not stated very clearly in the OECD TG 305. However, OECD TG 305 recommends that SPME be used to analyze the concentration in water (which gives the dissolved chemical concentration in water); further, Equations A5.1 and A5.4 also point to the dissolved chemical concentration in water as reference. Gobas and coworkers (Gobas & Lo, 2016; Gobas et al., 2020; Saunders et al., 2020; Trowell et al., 2018) but also Arnot and coworkers (Arnot & Gobas, 2003, 2004, 2006; Nichols et al., 2013) have interpreted the OECD TG 305 differently and use the total chemical concentration in water as a basis for the BCF. However, this is conceptually incorrect and the consequence is that the bioaccumulation potential of many highly hydrophobic substances has been underestimated—not only in experimental studies (Saunders et al., 2020), but also through the Arnot–Gobas model, which is included in the BCFBAF module in EPI Suite. Also the just recently developed Toxicokinetic Framework and Analysis Tool for Interpreting OECD TG 305 Dietary Bioaccumulation Tests (Gobas et al., 2020) bases the back-calculated BCF values on the total water concentration and thus underestimates the bioaccumulation potential of the hydrophobic substances.

The way forward

We have demonstrated in the present study that the experimental BCF values based on aqueous exposure very often underestimate the bioaccumulation potential for hydrophobic substances. Reasons are, inter alia, the very low freely dissolved concentration in water and the adsorption of the substances to suspended particles, glass walls, and other materials (Ehrlich et al., 2011; Gobas et al., 2009). As an alternative to the experimental BCF tests, it is proposed in OECD TG 305 to perform dietary exposure tests and to determine the growth-corrected elimination rate constant from the depuration phase (OECD, 2012). With this elimination rate constant and a calculated uptake rate constant, the kinetic BCF can be determined. However, the high growth rate of small fish may prevent the growth-corrected elimination rate constant from being determined reliably, because the measurement uncertainties of the growth rate may be greater than the calculated elimination rate constant. This would then lead to uncertain BCF values. The latter point can only be circumvented with extremely slow-growing fish which are rarely used in laboratory studies.

According to our analysis, a paradigm shift is required for a meaningful assessment of the bioaccumulation potential of hydrophobic substances. This could be done in a weight-of-evidence (WoE) approach using data from different sources. An example of such a WoE approach has been published by Arnot et al. (2022). Evidence in such an approach could come from models—like the one used in the present study for neutral compounds—with substance-specific values for the log K_{OW} and the biotransformation rate. Even if the model only represents reality in a simplified way, it can help to identify potentially bioaccumulative substances in a WoE approach. Supporting evidence could also come from existing biomonitoring studies in humans, mammals, and fish or newly generated data in approaches like "Food web on ice," which is a pragmatic approach to identifying the trophic magnification from historical data (Kosfeld et al., 2021). Monitoring studies have the advantage that they reveal directly what is happening in the environment. However, they also include variability of food chains and species, and the results can therefore differ between food chains. The data are still very useful in a WoE approach.

A disadvantage of monitoring studies is that they can only look at substances that have already been released into the environment. Read-across to new chemicals could be a viable option together with the biotransformation potential measured by in vitro biotransformation assays with fish liver S9 fractions (Krause & Goss, 2018; OECD, 2018a, 2018b, 2018c; Trowell et al., 2018). Such in-vitro data are also often used to calibrate BCF models (Mansouri & Williams, 2017; Nichols et al., 2007, 2013). Care should be taken here when in-vitro data are extrapolated as some of the available models (e.g., Trowell et al., 2018) relate the BCF to the total and not the dissolved chemical concentration in water.

Additional evidence could also come from experimental data using invertebrate species such as the freshwater amphipod *Hyalella azteca* (Kosfeld et al., 2020; Schlechtriem et al., 2019) or sediment-dwelling benthic oligochaetes (OECD, 2008). However, when such data are used, it has to be considered that they might overestimate the bioaccumulation potential of the substances because certain metabolic pathways are not present in these organisms compared with fish or humans (Schlechtriem et al., 2019). A combination of the data from the three areas mentioned above should facilitate a relatively fast and inexpensive but still accurate assessment of the bioaccumulation potential of hydrophobic substances. Tests with fish should, in our opinion, be performed only for the validation of models (but then slow-growing fish are needed) or to prepare liver fractions for in vitro biotransformation assays.

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AUTHOR CONTRIBUTIONS

Martin Scheringer did the conceptualization and acquired funding. Juliane Glüge was responsible for data curation, the formal analysis and wrote the original draft. All authors

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worked on the methodology and reviewed and edited the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and calculations are provided in the Supporting Information: 2. Additional Information is provided in the Supporting Information: 1.

SUPPORTING INFORMATION

Supporting Information 1 includes additional tables and graphics that support the statements in the main article. Supporting Information 2 includes the modeled data.

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