Including Bioconcentration Kinetics for the Prioritization and Interpretation of Regulatory Aquatic Toxicity Tests of Highly Hydrophobic Chemicals

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Supporting Information

ABSTRACT: Worldwide, regulations of chemicals require short-term toxicity data for evaluating hazards and risks of the chemicals. Current data requirements on the registration of chemicals are primarily based on tonnage and do not yet consider properties of chemicals. For example, short-term ecotoxicity data are required for chemicals with production volume greater than 1 or 10 ton/y according to REACH, without considering chemical properties. Highly hydrophobic chemicals are characterized by low water solubility and slow bioconcentration kinetics, which may hamper the interpretation of short-term toxicity experiments. In this work, internal concentrations of highly hydrophobic chemicals were predicted for standard acute ecotoxicity tests at three trophic levels, algae, invertebrate, and fish. As demonstrated by comparison with maximum aqueous concentrations at water solubility, chemicals with an octanol–water partition coefficient (Kow) greater than 10^6 are not expected to reach sufficiently high internal concentrations for exerting effects within the test duration of acute tests with fish and invertebrates, even though they might be intrinsically toxic. This toxicity cutoff was explained by the slow uptake, i.e., by kinetics, not by thermodynamic limitations. Predictions were confirmed by data entries of the OECD’s screening information data set (SIDS) (n = 746), apart from a few exceptions concerning mainly organometallic substances and those with inconsistency between water solubility and Kow. Taking error propagation and model assumptions into account, we thus propose a revision of data requirements for highly hydrophobic chemicals with log Kow > 7.4: Short-term toxicity tests can be limited to algae that generally have the highest uptake rate constants, whereas the primary focus of the assessment should be on persistence, bioaccumulation, and long-term effects.

INTRODUCTION

Regulations of chemicals worldwide require a base set of ecotoxicity data for registration of chemicals. For example, short-term toxicity tests on invertebrates (mostly Daphnia) and growth inhibition tests on algae are required for any chemicals with an annual tonnage greater than 1 ton. Acute toxicity on fish is required for chemicals with an annual tonnage greater than 10 tons under European REACH.1 In other areas of the world, there are similar data requirements for chemical regulations on the evaluation of short-term ecotoxicity based on tonnage,2−4 although specific data requirements differ. Under REACH, if no or low exposure is expected, the effect assessment can be waived (Exposure Based Adaptation and Triggering of Information Requirements under REACH).5 Currently, no regulation considers the properties of chemicals that might be crucial in exhibiting short-term effects in aquatic organisms.

Ecotoxicity of hydrophobic organic chemicals using short-term tests is often explained by baseline toxicity which is apparent when xenobiotic chemicals accumulate greater than the critical body burden (CBB) or critical body residue (CBR).6−11 In this theory, toxic effects become apparent because chemical species accumulated in the cell membranes disable many important membrane functions regardless of the type of chemicals.12−14 Thus, baseline effects become apparent

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increasing hydrophobicity. This issue is the main motivation for the present study.

In this paper, we propose a kinetic toxicity cutoff that can be used for regulatory purposes and help to reduce unnecessary animal experiments for chemical registration and management and to refine data requirements for chemical registration. Conventional aquatic toxicity tests using algal growth inhibition, immobilization of aquatic invertebrates, and mortality of fish were chosen because they form the base set of environmental hazard data required for registration. Time-dependent accumulation of chemicals during the acute toxicity tests was modeled within the limit of water solubility. We then propose water solubility limits ($S_{\text{ow}}$) below which acute baseline toxicity is not expected. Using the inverse relationship between water solubility and bioconcentration ratio, we propose critical bioconcentration ratio values ($BCR_{\text{crit}}$) as proxies for the toxicity cutoff above which no acute toxicity is expected unless the test chemical has a specific toxic mode-of-action with a TR > 10, which as argued above, is not likely for very hydrophobic chemicals. Finally, the proposed $BCR_{\text{crit}}$ was evaluated and confirmed using toxicity data collected and evaluated for high production volume chemicals under the Screening Information Data Set (SIDS) by the Organisation for Economic Co-operation and Development (OECD). The SIDS toxicity data were not prescreened to include only chemicals with baseline toxicity, and the obtained findings do thus apply to chemicals with either baseline toxicity or limited excess toxicity (i.e., TR ≤ 10) due to other causes.

**Methods**

**Theoretical Model for Toxicokinetics.** Three conventional short-term ecotoxicity tests used for regulatory purposes are algal growth inhibition, acute immobilization of invertebrates, and acute fish mortality. According to test protocols, it is desired to maintain constant exposure concentration of a test chemical during the test. Lipid-normalized concentration in the organism is often modeled assuming uniform distribution in the body and first-order respiratory uptake and depuration kinetics by the following differential equation

$$\frac{d(M_o C_o)}{dt} = k_M M_o C_o - k_M M_o C_o,$$

where $M_o$ is the lipid mass of the test organism (i.e., algae, invertebrate, and fish) [kg/$L$], $C_o$ is lipid-normalized concentration of the chemical in organism [mmol/kd/$L$], $C_o$ is the aqueous free concentration maintained constant [mmol/L], and $k_o$ [L/kgd/$L$] and $k_d$ [d$^{-1}$] are lipid-normalized respiratory uptake and depuration rate constants. Whereas negligible growth in the organism is expected for invertebrate and fish tests because organisms are not fed during the tests, volume growth is not negligible for algal growth inhibition tests in which exponential growth of the organism is desired. Assuming exponential growth, $M_o$ can be written

$$\frac{dM_o}{dt} = k_g M_o,$$

where $k_g$ is the first-order growth rate constant [d$^{-1}$] of algae. Plugging $M_o$ from eq 2 into eq 1, the following equation is obtained:
shows various relationships between log $K_{ow}$ and the logarithm of water solubility ($\log S$) in the literature for different chemical classes. The intercept of regression varied depending on the data set used for the derivation of relationships, but the slope was between $-1.49$ and $-0.92$ for most of studies. The most comprehensive model based on 1450 different organic chemicals by Meylan et al. used in Wskowin v1.42 reported a slope of $-1.02$ (Table 1). The unit slope is consistent with the concept of the general solubility eq (eq 6) proposed by Ran and Yalkowsky, which includes both the contribution by hydrophobicity (expressed as $K_{ow}$) and the entropy of fusion (expressed as melting point MP).}

$$\log S = 0.5 - 0.01(\text{MP} - 25) - \log K_{ow}$$

For those cases where the slope is $<-1$, the deviation from 1 is likely caused by the positive correlation between the melting point and $\log K_{ow}$. Using the average melting point in the data set evaluated for hydrophobic chemicals with $\log K_{ow}$ greater than 4.0 of 112 ($\pm 105^\circ$C ($n = 75$, Table S2), the general solubility equation for hydrophobic organic chemicals of concerns can be simplified to

$$\log S = -\log K_{ow} - 0.37$$

If we assume that $K_{ow}$ is a surrogate for lipid–water partitioning and has approximately the same value as the lipid-normalized BCR ($k_{ow}/k_d$), then $C_{w,max}$ in mmol L$^{-1}$ is given by

$$C_{w,max} = S \left(10^{2.63} \frac{\text{mmol mol}^{-1}}{K_{ow}} \approx 10^{2.63} \frac{\text{BCR}}{k_{ow}/k_d}\right)$$

Inserting eq 8 into eqs 4 and $S$, the maximum concentration of a chemical ($C_{o,max}$ in mmol kg$^{-1}$) in the organism can be expressed by $S$ or BCR as follows:

$$C_{o,max}(t) \approx 10^{2.63} \left[1 - \exp\left(-\frac{k_u}{BCR} t\right)\right]$$

$$C_{o,max}(t) \approx \frac{k_u}{BCR} \times 10^{2.63} \left[1 - \exp\left(-\left(k_g + \frac{k_u}{BCR}\right) t\right)\right]$$

When $C_{o,max}(t)$ equals the critical body residue (CBR), eqs 9 and 10 are then solved for $S$ or BCR to obtain the critical values of $S$ ($S_{CR}$) or BCR ($BCR_{CR}$) below or above which $C_{o,max}$ cannot achieve the CBR.

In short-term toxicity tests, exposure durations are typically 72, 48, and 96 h for algae, invertebrate, and fish, respectively. For algal growth inhibition tests, it is expected that algae reproduce exponentially during the test period with a minimum growth rate constant of 0.92 d$^{-1}$. Lipid-normalized respiratory uptake rate constants ($k_u$) for hydrophobic organic chemicals are affected by limited diffusive mass transfer in the aqueous boundary layer as well as by the physiology of respiratory systems of test organisms. It is generally acknowledged that lipid-normalized $k_u$ (L kg$^{-1}$ d$^{-1}$) tend to decrease with increasing size due to the decrease in surface-to-volume ratio. Although $k_u$ may decrease with $\log K_{ow}$ due to reduced bioavailability, particularly in algal tests, where dissolved organic matter reduces freely dissolved concentration, dependence of $k_u$ on hydrophobicity was not considered because $k_u$ in the model is based on free concentration. Table 2 shows the ranges of uptake rate constants of $k_u$ during conventional ecotoxicity reported in the literature with more details for various aquatic organisms with the organism size reported in Table S3. Average lipid contents of 2.4 ± 1.7, 1.9 ± 0.5, and 6.7 ± 5.4% for algae, invertebrates, and fish (Table S4) were used to calculate $k_u$ from whole-body uptake rate constants. $k_u$ ranged from 22 to 2.5 × 10$^5$ L kg$^{-1}$ d$^{-1}$ for fish weighing between 0.0010 and
240 g. The range of $k_o$ for the ecotoxicity assessments should be narrower around 1000 L kg$^{-1}$ d$^{-1}$ because it is recommended to use small fish.9

Critical Body Burden of Aquatic Organisms. The theoretical maximum concentration in the test organism needs to be compared with the CBR of test species. Many researchers suggested a range of values of CBR, and it is accepted between 2 and 8 mmol kg$^{-1}$ wet weight.5,9,33,54 In a recent review by McCarty et al.,9 data from the Environmental Residue Effects Database were evaluated to derive critical body residues causing baseline toxicity in aquatic test species. The median value including fish, invertebrates, and algae was 54 mmol kg$^{-1}$ (n = 161 for 29 narcotic chemicals). Despite the variability of reported toxicity values, this median value is in good agreement with other studies.6,10,54 Thus, it was used regardless of species in the following analysis.

Evaluation of Literature Toxicity Data. For the evaluation of the proposed model, screening information data sets (SIDS) by OECD were used to test the proposed model for short-term toxicity. Data required for chemical hazard assessment and OECD-wide agreed conclusions on chemical hazards were provided by member countries through the OECD Cooperative Chemicals Assessment Programme. Among data compiled at http://webnet.oecd.org/hpv/ui/SponsoredSubstances.aspx26 as of Dec 30, 2014, inorganic substances, mixtures, and strong electrolytes were excluded for the evaluation. Finally, for the evaluation of the model, 746 organic substances were selected that covered a wide range of physicochemical properties and molecular structures. These chemicals did not a priori have a certain mode of toxic action. Baseline toxicity was thus initially assumed during the model development, whereas its final assessment and confirmation were not limited to baseline toxicity.

Classification of Ecotoxicity Test Results. A summary of the reported toxicity values in OECD SIDS is presented in (Tables S5–7) including chemical properties, test methods, organisms used, and reported toxicity values. Although the data in SIDS have been evaluated through OECD review processes, many toxicity data were reported above the aqueous solubility of chemicals (see Tables S5–7 for details). Thus, all results were classified into the following four groups: (1) toxicity value reported is below the aqueous solubility of the test chemical and the test chemical exhibits toxicity according to the test protocol, (2) toxicity value reported is above the aqueous solubility in the data set, indicating that experimental result has low reliability, (3) toxicity is not observed within the range of chemical concentration tested, indicating that the test chemical did not exhibit toxicity under the test conditions, and (4) no result is found in the report and these chemicals were excluded for the evaluation. Among 746 organic chemicals in the database, the sizes of data for group 1–3 were 514 (69%), 578 (77%), and 579 (78%) for algae, invertebrate, and fish tests, respectively.

### Results and Discussion

Theoretical Toxicity Cutoff and Experimental Results. In order to evaluate the kinetic toxicity cutoff in conventional short-term ecotoxicity tests, it was assumed that the maximum body concentration ($C_{\text{on,max}}$) should exceed the critical body residue of 54 mmol kg$^{-1}$. Figure 1 illustrates the changes in body concentration of hypothetical chemicals during a conventional 96 h short-term test for acute mortality of fish (Figure 1a) and during a conventional 72 h algae growth inhibition test (Figure 1b). Although the lipid-normalized respiratory uptake rate constant ($k_o$) varies with the physiology of respiration of the test organisms as well as chemical properties of test substances,5,32,54 1000 L kg$^{-1}$ d$^{-1}$ was assumed for the small fish used for the short-term mortality test for the purpose of illustration. Three representative curves in Figure 1a are shown for BCR ($k_o$/k$_d$) = 10$^4$ L kg$^{-1}$ mol$^{-1}$ or $S = 10^{-3}$ (solid line), BCR = 10$^{3}$ or $S = 10^{-2}$ (dashed line), and BCR = 10$^2$ or $S = 10^{-1}$ (dotted line). All three curves approach to the same steady-state concentration of 430 mmol kg$^{-1}$ in fish test, whereas they approach to different values in algae growth inhibition due to the exponential growth. Accumulation half-lives are also noted.

### Table 2. Range of Uptake Rate Constants ($k_u$) in the Literature (Detailed Data in Table S3)

<table>
<thead>
<tr>
<th>organism size (g)</th>
<th>reported $k_u$ (L kg$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>algae (dry weight)</td>
<td>$2 \times 10^{-12}$ – $4 \times 10^{-11}$</td>
</tr>
<tr>
<td>invertebrate</td>
<td>$1.6 \times 10^{-3}$ – $9 \times 10^{-3}$</td>
</tr>
<tr>
<td>fish</td>
<td>$1.0 \times 10^{-3}$ – 240</td>
</tr>
</tbody>
</table>

**Figure 1.** Whole body concentration in fish ($C_o$) as a function of exposure time during (a) a conventional 96-h short-term toxicity test and (b) a 72-h algae growth inhibition test. For a visualization, uptake rate constant ($k_o$) was assumed 1,000 L kg$^{-1}$ d$^{-1}$ for fish and $5 \times 10^6$ L kg$^{-1}$ d$^{-1}$ for algae. Three representative curves were drawn at BCR ($k_o$/k$_d$) = 10$^4$ or $S = 10^{-3}$ (solid line), BCR = 10$^3$ or $S = 10^{-2}$ (dashed line), and BCR = 10$^2$ or $S = 10^{-1}$ (dotted line). All three curves approach to the same steady-state concentration of 430 mmol kg$^{-1}$ in fish test, whereas they approach to different values in algae growth inhibition due to the exponential growth. Accumulation half-lives are also noted.
was $10^{-5.37}$ during 4 days, the typical duration of the acute test. Although the steady-state body residue concentration is 430 mmol kg$^{-1}$ for all cases, more hydrophobic chemicals cannot reach this steady-state value because accumulation half-life increases with BCR. Accumulation half-lives are 6.9, 69, and 690 days for BCR values of $10^1$, $10^3$, and $10^6$, respectively. For an algae growth inhibition test, $k_u$ and $k_d$ were assumed to be $5 \times 10^6$ L k$^{-1}$ g$^{-1}$ d$^{-1}$ and 0.92 d$^{-1}$, respectively. As shown, the internal steady state was reached in a much shorter exposure time than fish with the accumulation half-lives of 0.0014, 0.014, 0.12 days for BCR values of $10^1$, $10^3$, and $10^6$, respectively. Because of the dilution effects due to the exponential growth, the steady-state concentration differed with BCR.

Figure 2 shows the relationships between the lipid-normalized respiratory uptake rate constant ($k_u$) and critical bioconcentration ratio ($BCR_{crit}$) for fish, invertebrate, and algae. The lines cover the reported range of $k_u$ values in Table 2. Although the same equation (eq 9) was used for fish and invertebrates, $BCR_{crit}$ for invertebrates was slightly lower because test duration for invertebrate (48 h) is shorter than that for fish (96 h). For algae, $BCR_{crit}$ was lower than that of fish or invertebrates at the same $k_u$ because of growth dilution. However, because of their higher surface to volume ratios, algae can have much higher $k_u$ values and thus much higher $BCR_{crit}$ values compared to fish and invertebrates (Figure 2).

Because BCR can be considered as a property solely determined by hydrophobicity or thermodynamic partitioning, it is reasonable to relate BCR with $K_{ow}$. As shown in the previous analysis and illustrated in Figures 1 and 2, short-term baseline toxicity on fish and daphnids are not likely to be observed for chemicals with log $K_{ow}$ greater than 6.0 or water solubility less than $10^{-6.37}$ mol L$^{-1}$. Table 3 presents classification of SIDS data according to the criteria defined above based on the reported $K_{ow}$ ranges. For all test species, fraction of organic chemicals classified as group 2 or 3 dramatically increased with increasing log $K_{ow}$. Most chemicals with log $K_{ow}$ above 6 that correspond to the theoretical BCR were classified as group 2 or 3, suggesting that this theoretical evaluation is well supported by the existing set of short-term toxicity data. The percentage of chemicals with log $K_{ow}$ greater than 6.0 classified as group 2 or 3 was above 80% for all tests. Since the SIDS data were not limited to baseline toxicity, this observation applies more broadly and also to chemicals with limited excess toxicity (e.g., TR $\leq 10$).

**Evaluation of Uncertainties of the Proposed Model.**

The four assumptions made to propose the short-term toxicity cutoff are (1) for chemical accumulation kinetics, test organisms behave as a single compartment; (2) the lipid-based bioconcentration ratio (BCR = $k_u/k_d$) can be approximated by the octanol–water partition coefficient ($K_{ow}$); (3) the general solubility eq (eq 6) is applicable for all test chemicals; and (4) the critical body residue to produce a toxic end point is 54 mmol kg$^{-1}$.

Uncertainties associated with those assumptions are as follows:

1. Although accumulated concentration of organic chemicals in the body may vary with dynamic absorption, distribution, metabolism, and excretion processes, it is reasonable to assume that the concentration of HOCs in the lipid compartment throughout the body is constant especially when baseline toxicity is evaluated. Chemical concentration in target lipids may differ from that in nontarget lipids. However, it was shown that lipid-normalized concentration based on total lipids is a fairly good estimate for concentration in membrane lipids.

2. Relationships between the octanol–water partition coefficient ($K_{ow}$) and lipid–water partition coefficient ($K_{lipw}$) have been evaluated by many researchers as recently reviewed by Endo et al. In their analysis, a simple regression model between $K_{ow}$ and $K_{lipw}$ for 181 compounds showed that the deviation for most compounds did not exceed 0.8 log unit.

3. Root-mean-square errors of general solubility equation were reported as 0.52 log units when measured values of $K_{ow}$ were used.

![Table 3. Classification of Reported Short-Term Toxicity Data for Fish, Invertebrates, and Algae](image-url)

<table>
<thead>
<tr>
<th>test species</th>
<th>range of log $K_{ow}$</th>
<th>group 1: $LC_{50} &lt; S$ (i.e., low reliability)</th>
<th>group 2: $LC_{50} &gt; S$ (i.e., low reliability)</th>
<th>group 3: no toxicity up to highest tested conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>fish</td>
<td>log $K_{ow} &lt; 4$</td>
<td>379</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>4 $\leq$ log $K_{ow} &lt; 6$</td>
<td>31</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>6 $\leq$ log $K_{ow} &lt; 8$</td>
<td>1</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>8 $\leq$ log $K_{ow}$</td>
<td>2</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>invertebrates</td>
<td>log $K_{ow} &lt; 4$</td>
<td>373</td>
<td>13</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>4 $\leq$ log $K_{ow} &lt; 6$</td>
<td>32</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>6 $\leq$ log $K_{ow} &lt; 8$</td>
<td>7</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>8 $\leq$ log $K_{ow}$</td>
<td>4</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>algae</td>
<td>log $K_{ow} &lt; 4$</td>
<td>296</td>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>4 $\leq$ log $K_{ow} &lt; 6$</td>
<td>32</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>6 $\leq$ log $K_{ow} &lt; 8$</td>
<td>4</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>8 $\leq$ log $K_{ow}$</td>
<td>3</td>
<td>7</td>
<td>18</td>
</tr>
</tbody>
</table>

*aGroup 1: $LC_{50} < S$. Group 2: toxicity value reported above aqueous solubility in the dataset, indicating that experimental result has low reliability. Group 3: toxicity was not observed within the range of chemical concentration tested, indicating that the test chemical did not exhibit toxicity under the test conditions.
4. As analyzed by McCarty et al., the 95% confidence interval of critical body residues for 161 observations was 0.179—18.0 mmol kg⁻¹ wet weight or 0.824—431 mmol kg⁻¹ dry weight, based on total lipids. Uncertainties associated with CBR include those related with assumption 1.

If assumptions 2—4 are independent, the overall uncertainty of the proposed model could be estimated by the propagation of errors in case of multiplication or division. With the representative uncertainties of assumptions 2, 3, and 4 of 0.8, 0.52, and 1.0 log units, the overall uncertainty of the proposed model would be 1.38 log unit, meaning that a conservative cutoff at log $K_{ow}$ of 7.4 largely would take those model assumptions and error propagation into account. The comparison of the developed model using the SIDS data was used as a reality check, which in turn reduced its dependency on the initial assumptions.

**Outliers of the Proposed Model.** There are a few outlier chemicals that exhibited short-term toxicity on test organisms even though their log $K_{ow}$ values are greater than 6.0 (Table S8). There are 7, 11, and 3 chemical species that had reported toxicity values for algae, invertebrates, and fish, respectively, sorted by decreasing log $K_{ow}$. Values of log $K_{ow}$ in Table S8 are those reported in the SIDS publications. Four outliers are organotin compounds. For these compounds, it is difficult to clarify whether the deviation is mainly due to an excess toxicity of these biocides or to inaccurate log $K_{ow}$ values. The log $K_{ow}$ values of these compounds were estimated using the KOWWIN program. Because of the metallic character of the central tin in organotin compounds, the estimated log $K_{ow}$ values using KOWWIN tend to greatly overestimate log $K_{ow}$. Experimental log $K_{ow}$ of tributyltin (CAS reg. no. 688-73-3) was measured as 4.10 at higher pH, and the logarithm of lipid–water partition coefficient was also measured as ~4 near pH 7, whereas the calculated value using the group contribution method in KOWWIN is 7.35. In addition, log $K_{ow}$ values for those organotin compounds, although not measured, should be much lower based on the reported water solubility values and do not obey the general relationship (eq 7). pH-dependent chemical speciation and complexion with biotic ligands also make it difficult to predict the toxicity of organotin compounds in terms of baseline effects that act as uncouplers, inhibiting mitochondrial electron transport and ATPase. Thus, the proposed concept of BCRcrit is not rebutted by them.

Other deviating chemicals were esters, phenol, or thiols with long alkyl chain, undecylbenzene, octamethyltrisiloxane, and 1,1-(1,1-dimethyl-3-methylene-1,3-propanediyl)bisbenzene (Table S8). The reported water solubility values of two chemicals (3,7,11,15-tetramethyl-1-hexadecen-3-ol and decanedioic acid, bis(2,2,6,6-tetramethyl-4-piperidinyl) ester) were higher than expected using the general solubility equation. The other seven (6 in Daphnia immobilization test and 3 in algae growth inhibition) are all liquids at room temperature. Although their water solubility values are close to those predicted by the general solubility equation, they deviate from eq 7 assuming an average melting point. In addition, those chemicals at the borderline of short-term toxicity cutoff might not be classified as baseline toxicants with TR less than 10.

**Recommendation for Data Requirement.** The recent chemical regulations to protect human health and the environment share a “no data, no market” philosophy. For highly hydrophobic chemicals, however, ecotoxicological data are requested based on tonnage, although it is difficult to provide reliable results due to their intrinsic chemical properties. Major concerns with those highly hydrophobic chemicals are not short-term effects such as mortality but rather long-term chronic toxicity and food-chain magnification even if their environmental level is very low. As shown, results from short-term toxicity tests are mostly “no toxic effects observed” or unreliable. They give very limited information for management purposes compared to resources invested. It is also against animal welfare and 3R principles of replacing, reducing, and refining animal testing. Thus, the current decision tree should be revised. During the preregistration of chemicals under EU REACH, approximately 10% of organic chemicals were highly hydrophobic with log $K_{ow}$ greater than 6. For those chemicals, it would be more reasonable to refine their physicochemical properties such as partition coefficients and to evaluate their environmental persistence and bioaccumulation potential and waive acute toxicity testing when they exceed the BCRcrit in lieu of assessment of bioaccumulation and persistence. This does not fully apply to algal growth inhibition tests. Despite their short test duration, high internal steady-state concentrations can be reached even for rather hydrophobic chemicals as shown in Figure 1b and Figure 2, and they include several generations of cells and provide also sublethal and chronic effects. For such tests, we propose to limit rather than waive the testing of highly hydrophobic organic chemicals. Instead of carrying out full dose response testing, we suggest limit tests carried out exactly at the solubility limit, which are feasible with recently developed passive dosing techniques.

In lieu of acute toxicity testing, it remains vital to obtain more reliable log $K_{ow}$ values using recently updated and developed robust determination methods to assess bioaccumulation and biomagnification, to test for long-term chronic toxicity, and to test environmental degradability if used in significant quantity and if potential bioaccumulation is suspected.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03942.

Definitions of symbols and abbreviations; melting points of hydrophobic organic chemicals with log $K_{ow}$ > 4.0; uptake rate constants for various aquatic organisms in the literature; lipid contents of test organisms reported in the literature; toxicity data from OECD SIDS; outliers of the proposed model along with their physicochemical properties and LC50/EC50 values

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**Notes**

The authors declare no competing financial interest.

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