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Toxic Ratio as an Indicator of the Intrinsic Toxicity in the Assessment of Persistent, Bioaccumulative, and Toxic Chemicals

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Persistence, bioconcentration, and toxicity (PBT) are important hazardous properties of organic chemicals. In PBT assessments, it is desirable that the three criteria P, B, and T are independent. However, this requirement is not fulfilled if an aqueous lethal concentration (LC_{50}) is used as T indicator because LC_{50} includes both bioconcentration and intrinsic toxicity. Indicators for intrinsic toxicity such as the internal lethal concentration (ILC) are independent of a chemical's bioconcentration potential. However, ILC_{50} data are scarce and difficult to measure. Therefore, the toxic ratio (TR) is proposed here as an alternative. TR is defined as the ratio of a chemical's LC_{50} estimated from a QSAR for baseline toxicity and the experimental LC_{50} value. TR can also be interpreted as a measure of the ILC relative to the ILC for baseline toxicity. A TR of 10 separates specifically toxic chemicals from baseline toxicants. With some 800 chemicals, the practicability of classifying chemicals in terms of TR is demonstrated. Employing TR as toxicity indicator leads to different T scores for 30% of the chemicals studied. The baseline toxicity of hydrophobic compounds with TR $<$ 10 does not receive a high T score but is still indicated by a high B score. The toxicity of specifically toxic hydrophilic substances is given additional emphasis by high TR values. These classification changes require that the interpretation of the B and T dimensions in PBT assessments is redefined.

Introduction

Persistent, bioaccumulative, and toxic chemicals (PBTs) have received growing attention since the late 1980s. In the OSPAR Convention of 1992, PBTs are mentioned in Annex 1 (*1*). In the United States, Canada, and the European Union, these substances are specifically regulated (*2*−*4*). In addition, different international organizations have been developing programs for the identification and regulation of PBTs or subgroups of PBTs; for example, persistent organic pollutants (*5*−*9*). Several PBT identification methods have been published (*2*, *10*−*13*), all of which are based on separate indicators for P, B, and T.

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An important question in the context of PBT assessments is whether external or internal concentrations should be used as toxicity indicator (*14*−*16*). We restrict our analysis of this question to acute toxicity in aquatic organisms with mortality as endpoint. The toxicity indicator often used for acute toxicity is the aqueous concentration that is lethal for 50% of the test organisms, LC_{50} (*17*−*20*). However, a toxicity indicator based on aqueous concentrations represents both the transfer of the chemical from the water to the organism (i.e., its bioconcentration tendency, possibly affected by biotransformation) and the chemical's intrinsic toxicity. Here, intrinsic toxicity refers to different modes of toxic action (narcosis or baseline toxicity, inhibition of enzymes, competition with receptors, various reactive mechanisms, etc.) and the corresponding different internal effect concentrations (*14*).

In other words, if LC_{50} values are used to quantify the T indicator, the T indicator also includes information on the bioconcentration of a chemical, but the relative importance of intrinsic toxicity and bioconcentration is different for different compounds and not discernible from the LC_{50} values. Here, we argue that, for reasons of transparency, the two indicators for B and T should be independent and that therefore internal effect concentrations should be used as T indicator in PBT assessments. With this argumentation, we follow the proposal presented by Gobas et al. (*15*). An approximation of the toxic concentration at a target site is the total internal effect concentration (IEC) of the pollutant, either on a lipid weight, dry weight, or wet weight basis (*21*). For 50% lethality of the test organisms, IEC is expressed as ILC_{50}, also called critical body residue (*22*) or lethal body burden (*23*).

In principle, internal concentrations as toxicity indicators have several advantages. For all baseline toxicants, ILC_{50} is similar: in studies on a variety of fish species, ILC_{50} was found only to vary between 2 and 8 mmol/kg_{ww} (ww, wet weight) (*22*−*28*). In addition, ILC_{50} values for acute baseline toxicity are largely independent of the test protocol (duration, species, uptake route) (*14*, *22*, *23*, *25*, *26*, *28*−*33*). When expressed in terms of ILC_{50}, baseline toxicity is the lowest toxicity a chemical can possibly have (i.e., baseline toxicants exhibit the highest ILC_{50} value). Therefore, chemicals with a specific mode of toxic action have a lower ILC_{50} than baseline toxicants and can be identified on that basis. Time dependence of ILC_{50} was reported (*34*, *35*) as the mode of toxic action possibly changes with the exposure regime (*36*). However, when acute toxicity is considered, this effect is negligible (*23*, *34*). Therefore, ILC_{50} can be considered as a useful indicator for the acute intrinsic toxicity of chemicals.

However, there are also several problems associated with the use of internal concentrations. ILC_{50} data are scarce and arduous to measure. Moreover, the test guidelines at national and international levels are designed for LC_{50} tests, and the adoption of ILC_{50} as toxicity indicator would require modifying them. Given the existing difficulties in finding appropriate toxicity data for many chemicals (*37*, *38*), changing the guidelines would exacerbate the lack of data.

Because of these problems, the approach presented by Gobas et al. is based on available LC_{50} data and bioconcentration factors (*15*). Here, we aim to further demonstrate the feasibility of the general concept proposed by Gobas et al. (*15*). To this end, we develop a similar method and illustrate it with a consistent set of 808 measured toxicity data for fathead minnow. We use the toxic ratio (TR; i.e., the ratio of the estimated LC_{50} for baseline toxicity of a chemical and its measured LC_{50}) as an estimate of the intrinsic toxicity that can be derived from LC_{50} and K_{ow} data. We also discuss the

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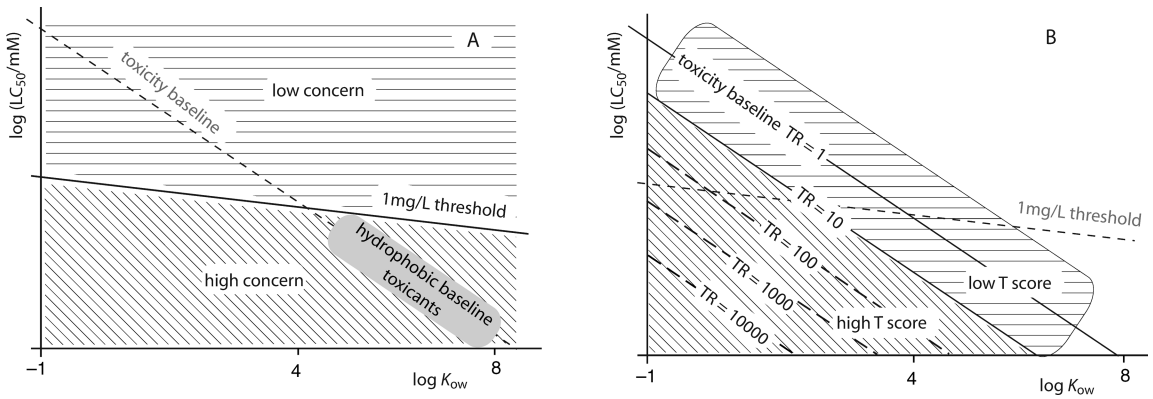

FIGURE 1. Toxicity assessment concepts based on external, aqueous concentrations (A) and internal concentrations (B). TR = toxic ratio.

assumptions that are necessary for interpreting the TR in this way, compare the two toxicity classifications in terms of TR and LC_{50}, and evaluate the implications of using the TR for the toxicity classification in a PBT assessment scheme. Note that it is the purpose of this paper to illustrate the general concept of using the TR in PBT assessments. The focus is not on deriving a QSAR for baseline toxicity from the toxicity data used or on an exhaustive analysis of the TR values obtained from the toxicity data.

Methods and Data Selection

Assessment Concept. In a PBT assessment method, the three indicators for persistence, bioaccumulation, and toxicity should be independent. This requirement is based on the fact that a strong but not explicit correlation between two indicators makes it difficult to evaluate which property actually leads to a high score. It is more informative to decision makers if the different properties of a chemical are specified independently. However, in the present practice of expressing acute ecotoxicity in terms of LC_{50}, the T indicator, being based on external exposure concentrations, is a function of both the intrinsic toxicity and the bioconcentration potential of the substance. This point is illustrated in Figure 1A with the hydrophobic baseline toxicants. A threshold value used to identify chemicals of high toxicological concern in terms of LC_{50} is an aqueous substance concentration of 1 mg/L (*17*−*20*). In Figure 1A, this value is converted into units of mol/L by using the relationship between molecular mass versus K_{ow} derived from 799 chemicals with log K_{ow} from −0.5 to 7.5 (see Supporting Information). The toxicity baseline, on the other hand, indicates the relationship between log LC_{50} and log K_{ow} for narcotic chemicals (*21*, *39*, *40*) (diagonal line in Figure 1A). Because the toxicity baseline is below the 1 mg/L line for log K_{ow} > 4 (Figure 1A), hydrophobic compounds are automatically classified as of high toxicological concern, even if these chemicals are merely baseline toxicants.

As already mentioned, ILC_{50} as an approximation of the target site concentration is not influenced by the bioconcentration tendency of a chemical. However, because ILC_{50} is not available for many chemicals, we propose to use the toxic ratio as an indicator that corresponds to internal concentrations and can be used as a measure of the intrinsic toxicity. TR is defined as the ratio of two LC_{50} values (*41*, *42*). The numerator is the LC_{50} for baseline toxicity ($LC_{50,bl}$), which can be derived from the K_{ow} with quantitative structure activity relationships (QSAR) (*21*); the denominator is the experimentally measured LC_{50} ($LC_{50,exp}$):

$$TR = \frac{LC_{50,bl}}{LC_{50,exp}} \quad (1)$$

Here, it is assumed that $LC_{50,bl}$ can be derived from the K_{ow}, which implies that steady state has been reached between water and the lipid phase of the organism and no biotransformation occurs. It is further assumed that this condition is fulfilled for $LC_{50,exp}$, too. If these conditions are not fulfilled, the K_{ow} predicts too low $LC_{50,bl}$ values and TR might be underestimated.

Under these assumptions, the internal concentration can be related to LC_{50} by the bioconcentration factor, BCF (*26*, *29*, *39*, *43*):

$$ILC_{50} = BCF \times LC_{50} \quad (2)$$

BCF has units of L/kg_{ww}, LC_{50} is given in units of mmol/L (mM) and ILC_{50} in $mmol/kg_{ww}$. Equation 2 or equivalently $LC_{50} = BCF^{-1} \times ILC_{50}$ is the mathematical expression of the mixed nature of external exposure concentrations such as LC_{50}. The steady-state assumption of eq 2 might not be fulfilled for compounds of high hydrophobicity, which have slower bioconcentration kinetics, or if net uptake is slower due to an increased elimination rate (e.g., by biotransformation or other pseudo-elimination processes).

With eq 2, TR is also equal to the ratio of two internal concentrations:

$$TR = \frac{BCF \times LC_{50,bl}}{BCF \times LC_{50,exp}} = \frac{ILC_{50,bl}}{ILC_{50,exp}} \quad (3)$$

$ILC_{50,bl}$ is the internal lethal concentration for 50% of the test organisms for baseline toxicity, and $ILC_{50,exp}$ is the corresponding measured internal lethal concentration. BCF in eq 3 can be estimated as (*44*)

$$BCF = 1 + \rho^{-1} f_{fat} K_{ow} \quad (4)$$

or

$$BCF = \rho^{-1} f_{fat} K_{ow} \quad \text{for } \log K_{ow} > 1 \quad (5)$$

if it is assumed that there is no biotransformation of the chemical in the organism. ρ (in kg/L) is the density of the organism and $f_{fat} = 0.05$ is the average fat content of fathead minnow (*30*). In the following, ρ is assumed to be 1 kg/L.

Under these assumptions, TR provides a scale for $ILC_{50,exp}$ relative to $ILC_{50,bl}$ (eq 3) but can be calculated from measured LC_{50} data and K_{ow}-derived LC_{50} values for baseline toxicity (eq 1). An additional advantage of TR is that it is a dimensionless value.

Verhaar et al. classified organic substances in four groups with respect to different TR values (*42*). They proposed a value of TR = 10 that separates their groups 1 and 2 (inert and less inert chemicals, corresponding to narcosis I and II chemicals), on one hand, and groups 3 and 4 (reactive and specifically acting chemicals), on the other hand (*42*). Here,

TABLE 1. Comparison of the Two Toxicity Assessment Concepts Using LC_{50} and TR

toxicity definition	indicator	threshold value	toxicity definition high vs low toxicity score
present	LC_{50} (mg/L)	$LC_{50} = 1$ mg/L	$LC_{50} < 1$ mg/L vs $LC_{50} > 1$ mg/L
proposed	TR (−)	TR = 10	chemical with specific mode of action (TR > 10) vs baseline toxicant (TR ≥ 10)

we adopt this threshold value of TR = 10; all chemicals with TR values below 10 are classified as baseline toxicants and receive a low score for the T dimension. Narcotics I and II are not distinguished here because their mode of toxic action is the same, namely nonspecific disturbance of structure and function of biological membranes (*45*, *46*). Narcotics I and II fall on different correlation lines between log LC_{50} and log K_{ow} because octanol is not a perfect surrogate for membrane lipids. However, the differences are smaller than 1 order of magnitude; therefore, the two groups can be included in the one group of baseline toxicants.

Chemicals with TR ≥ 10 are assumed to have a specific mode of toxic action and receive, therefore, a high score for the T dimension. There are also chemicals with TR values below 0.1, which means that their measured LC_{50} is considerably higher than the estimated $LC_{50,bl}$. In such cases, often nominal concentrations are reported while bioavailable concentrations are significantly lower due to sorption to food, faeces, or other organic material or due to losses through degradation or evaporation. These chemicals are excluded from the toxicity classification.

The general definition of TR, its interpretation as a measure of ILC_{50} relative to $ILC_{50,bl}$, and the selection of the threshold between baseline toxicants (low T score) and specifically acting substances (high T score) are the core part of the assessment concept proposed here, see Table 1. The objectives of the following analysis are to compare the two toxicity classifications in terms of LC_{50} and TR in a plot of log LC_{50} versus log K_{ow} and to discuss the implications of using TR.

The reference point of the TR scale is the ILC_{50} for narcotic chemicals (TR = 1). In the log K_{ow}-log LC_{50} plot, this reference point is indicated by the linear relationship between log LC_{50} and log K_{ow} that has been established for narcotic chemicals (*22*, *26*, *29*, *39*, *47*−*50*):

$$\log LC_{50} = a \log K_{ow} + b \qquad (6)$$

(diagonal line in Figure 1B). If octanol is assumed to be an ideal surrogate of the body lipids, the slope of this line is −1 (*14*, *26*, *39*, *49*, *50*); measured relationships often show somewhat lower slopes, see next section. Conceptually, the narcotic chemicals represented by this line have the highest possible ILC_{50} (namely, $ILC_{50,bl}$), and therefore, a TR of 1. All chemicals lying on a line parallel to the toxicity baseline have the same TR (dashed lines parallel to the toxicity baseline in Figure 1B). From the intercept *b* of eq 6, $ILC_{50,bl}$ can be derived (*14*, *26*, *39*, *50*) (see Supporting Information).

Figure 2 visualizes the differences between both toxicity definitions. The lines indicate the two toxicity thresholds presented in Figure 1A and 1B and the threshold for bioconcentration, for which log $K_{ow} = 4$ is used (*17*, *51*). The toxicity and bioconcentration characteristics of the six resulting fields are listed in Table 2.

The four fields A, C, D, and E are classified equally by the two toxicity definitions and also with regard to bioconcentration. However, the toxic potential of the chemicals in two fields, B and F, is evaluated differently by the two toxicity definitions (Figure 2, Table 2). An LC_{50}-based criterion does not cover the toxic potential of the B field chemicals. They are specifically toxic but have such a low K_{ow} that their LC_{50} values still lie above 1 mg/L. On the other hand, chemicals

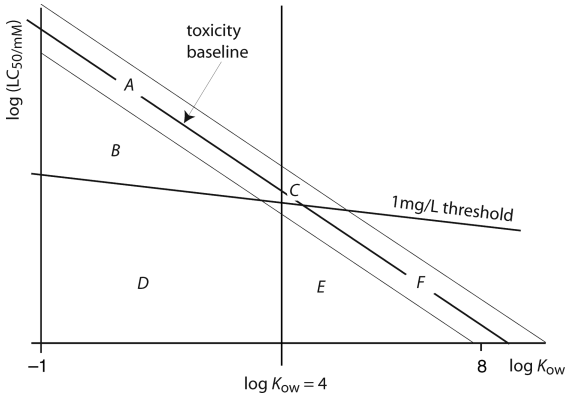

FIGURE 2. Different categories of high and low toxicity, specific and nonspecific modes of toxic action, and high and low bioconcentration potential. The different fields are defined in Table 2.

TABLE 2. Nomenclature for the Two Toxicity−Bioaccumulation Categories of the Two Assessment Concepts and Shares of Different Categories[a]

category	present toxicity indicator	proposed toxicity indicator	bioaccumulation indicator	share of chemicals (in %)
A	$LC_{50} > 1$ mg/L	TR < 10	log $K_{ow} < 4$	53.8
B	$LC_{50} > 1$ mg/L	TR ≥ 10	log $K_{ow} < 4$	**24.5**
C	$LC_{50} > 1$ mg/L	TR < 10	log $K_{ow} \geq 4$	5.3
D	$LC_{50} \leq 1$ mg/L	TR ≥ 10	log $K_{ow} < 4$	5.7
E	$LC_{50} \leq 1$ mg/L	TR ≥ 10	log $K_{ow} \geq 4$	1.9
F	$LC_{50} \leq 1$ mg/L	TR < 10	log $K_{ow} \geq 4$	**8.4**

[a] In % of the 808 chemicals with TR ≥ 0.1. Chemicals in categories B and F are classified differently by the two concepts (in bold).

in field F are classified as highly toxic because of a low LC_{50} that is caused by their high bioconcentration factors.

TR, in contrast, separates the intrinsic toxicity from the bioconcentration factor of a substance. Compounds that are relatively hydrophilic but have TR ≥ 10 receive a high T score (field B). On the other hand, hydrophobic baseline toxicants are only flagged for their high bioconcentration potential. Because of this bioconcentration potential (and possibly because of a high persistence), the substances of the F field are still of concern. This is according to the approach proposed in the EU White Paper (*52*) that defines the group of vPvB chemicals (i.e., very persistent and very bioaccumulative substances) as problem chemicals.

It is important to keep in mind that the concept presented here is restricted to acute mortality, according to the LC_{50} data used. Effects that are not covered by the LC_{50} data such as, for example, the photoinduced acute toxicity of polycyclic aromatic hydrocarbons (*53*) or endocrine disruption cannot be assessed by the proposed TR classification. In principle, however, chronic ecotoxicity, which is also not addressed in the present analysis, could be handled in a similar way as eqs 1 and 2 can be applied to chronic endpoints as well. In this case, an understanding of the temporal response of the ILC_{50} is required so that it can be ensured that concentrations of equal exposure duration are employed and that biotransformation does not affect the results.

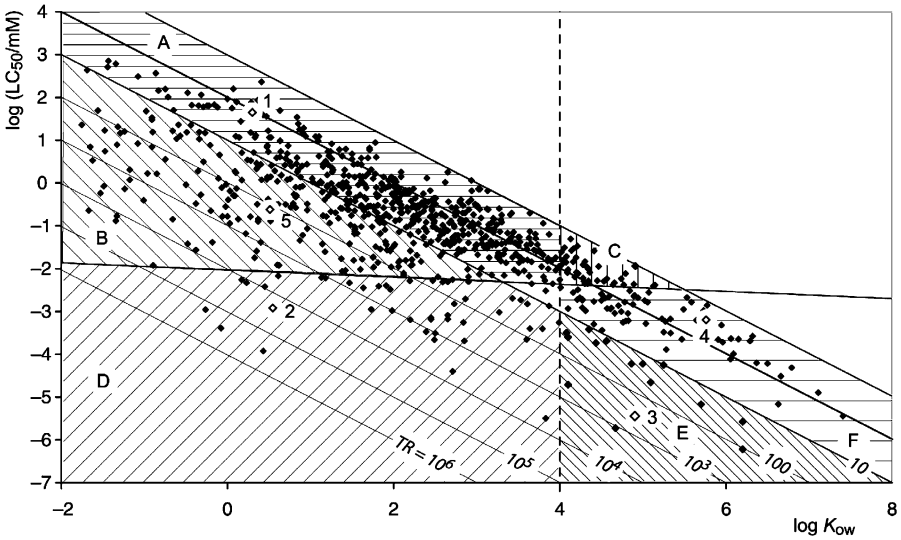

FIGURE 3. 96-h LC_{50} for fathead minnow and K_{ow} data for 808 chemicals. Toxicity baseline according to eq 7. Letters refer to the fields defined in Table 2, numbers to the following compounds: (1) 2-butanone, (2) hydroquinone, (3) endrin, (4) *n*-nonylphenol, and (5) epichlorohydrin.

Chemical Data. Values for K_{ow} and LC_{50} were retrieved from two databases: the U.S. EPA fathead minnow database (FHM) and the Environmental, Health and Safety (EHS) database developed at ETH Zürich (*54*). The first database contains mainly calculated K_{ow} values and measured 96-h LC_{50} values for fathead minnow (*Pimephales promelas*) as well as information on the mode of toxic action for some 600 industrial organic compounds (*55*−*57*). The second database contains environmental, health and safety data for a broad set of organic chemicals, mainly high production volume chemicals. The EHS database combines physicochemical parameters and toxicity data from several existing databases: the International Uniform Chemical Information Database (IUCLID) from the European Chemicals Bureau (*58*); IGS−Check from the Swiss National Emergency Operations Center (*59*); Aquire from the U.S. EPA (*60*); and Chemfate and Biodeg (*61*, *62*) as well as Epi Suite (*63*, *64*), a property estimation program, from Syracuse Research Corporation.

After a selection of suitable chemicals (see Supporting Information), the resulting data set contained 644 chemicals from EHS, 152 from FHM, and 49 with LC_{50} from FHM and K_{ow} from EHS. All toxicity data for the 845 chemicals were transformed into units of mM. For both K_{ow} and LC_{50}, the geometric mean was calculated when more than one value was found for the same chemical.

Derivation of the Toxicity Baseline. The toxicity baseline describing the relationship between K_{ow} and the LC_{50} for narcosis is required for the calculation of TR according to eq 1. Here, this relationship is derived from the toxicity data (96-h LC_{50} *P. promelas*) of 29 chemicals identified as baseline toxicants (narcosis I, A level of confidence) in the FHM database. For a comparison to existing expressions for the toxicity baseline, see Supporting Information. For reasons of simplicity, we assume a slope of −1 in the log LC_{50}-log K_{ow} relationship, see preceding section. Under this assumption, the 29 narcosis I compounds with an A level of confidence from the FHM database yield

$$\log (LC_{50,bl}/\text{mM}) = -\log K_{ow} + 2.00 \quad n = 29,\ r^2 = 0.88 \tag{7}$$

(see Supporting Information for regression line). The band defined by a factor of 10 in both directions of this line contains 90% of the 281 chemicals classified as either narcosis I or narcosis II compounds in the FHM database. This shows that the toxicity baseline in eq 7 in combination with a factor of 10 is sufficient to include most of the narcotic chemicals. Narcosis I and II have the same mode of toxic action (*21*, *45*, *46*) and are therefore used here as one set of reference compounds to be represented by the toxicity baseline.

Equation 7 is used to calculate $LC_{50,bl}$ and, in combination with the measured LC_{50} data of the 845 chemicals, their TR values. The TR values of 808 chemicals are indicated by the positions of the (log K_{ow}, log LC_{50}) points in relation to the diagonal lines of the TR scale in Figure 3; 37 chemicals with TR values below 0.1 were excluded.

In addition, eq 7 is used to determine the internal concentration for narcosis, $ILC_{50,bl}$, which is obtained as (see Supporting Information):

$$ILC_{50,bl} = \rho^{-1} f_{fat} \times 10^{2.00}\ \text{mM} = 5.00\ \text{mmol/kg}_{ww} \quad \text{with } f_{fat} = 0.05 \tag{8}$$

Under the assumption that the slope in eq 7 is equal to −1, $ILC_{50,bl}$ is constant for chemicals with log K_{ow} > 1. On the other hand, if the slope of the relationship between log LC_{50} and log K_{ow} is determined as a regression parameter, a value of −0.87 is obtained, which is similar to the slopes found in other toxicity baselines (*4*, *26*, *40*, *56*, *65*) (see Supporting Information). In this case, $ILC_{50,bl}$ is not exactly constant for different values of K_{ow} but varies between 3.2 (log K_{ow} = 1) and 13.8 mmol/kg (log K_{ow} = 6). The lines of constant TR then also have a slope of −0.87, which however does not strongly affect the classification of the chemicals in Figure 3 (for comparison, a version of Figure 3 with a slope of −0.87 is given in the Supporting Information). Here, the aim is to demonstrate the feasibility of the TR-based approach in general; the approach can similarly be applied with somewhat different equations for the toxicity baseline.

Results and Discussion

Classification of Chemicals. Figure 3 shows the log K_{ow}-log LC_{50} points along with the TR values of the 808 chemicals studied. All five categories described in Figure 2 and Table 2 contain substances. Most of the chemicals lie close to the toxicity baseline: 68% of the substances are classified as not specifically toxic (10 > TR ≥ 0.1), 32% as specifically toxic (TR ≥ 10) (with a slope of −0.87, these percentages are 72% and 28%). This corresponds to values reported by Nendza and Russom (*55*) and Russom et al. (*56*), who identified about 70% of the FHM substances as narcotics.

The fraction of chemicals of low toxicological concern is higher when LC_{50} is used as toxicity indicator: 84% of the substances have an LC_{50} greater than 1 mg/L.

About half of the chemicals (54%) are in the A field and are thus identified by both methods and with respect to B as of low concern (Figure 3 and Table 2; the percentages obtained for the different fields with a slope of −0.87 are given in the Supporting Information). For example, 2-butanone (Figure 3, substance 1), a baseline toxicant of low hydrophobicity (*42*), lies in field A.

The two fields identified as of high concern by both methods (C and D) represent around 11% of the substances studied. They contain chemicals having LC_{50} below 1 mg/L and acting specifically toxic such as hydroquinone, an oxidant and redox cycler (*66*), and endrin, an organochlorine insecticide acting on the nervous system (*67*) (Figure 3, substances 2 and 3).

The chemicals in fields B and F are assessed differently. In field F, the substances are baseline toxicants (TR < 10) but have $LC_{50} \leq 1$ mg/L. A total of 8% of the substances is such hydrophobic baseline toxicants, for example, 4-nonylphenol (Figure 3, substance 4). On the basis of their LC_{50} values, they are considered as highly toxic and receive high scores in the B *and* T dimensions in a PBT assessment using LC_{50} values. If, in contrast, TR is used instead of LC_{50}, these chemicals are flagged only because of their high bioconcentration potential. This, however, should also be interpreted as an indication of high baseline toxicity. Note that certain compounds, including the example mentioned above (4-nonylphenol), are also of concern because of their potential endocrine disruptive effect. Such nonlethal effects are covered neither by the LC_{50} data nor by the TR values based on these LC_{50} data.

Field B contains hydrophilic substances acting by specific modes of action (TR ≥ 10) but with $LC_{50} > 1$ mg/L (24.5% of the chemicals). In this group there are reactive chemicals such as epichlorohydrin (Figure 3, substance 5), some of which are potential mutagens. These chemicals receive no high toxicity scores if $LC_{50} < 1$ mg/L is used as T criterion. It is only in additional toxicity tests that these substances are labeled as hazardous. For example, epichlorohydrin is considered as dangerous for water in Germany because the International Agency for Research on Cancer classifies it as probably carcinogenic to humans (*68*). A TR-based indicator, however, makes it possible to classify such a chemical as specifically toxic on the basis of acute toxicity results. (This does not imply that mutagenicity or carcinogenicity can be recognized from acute toxicity tests. The point is that, if a chemical's reactivity and related toxicity leads to LC_{50} below $LC_{50,bl}$, this information should be reflected by the T indicator.) Altogether, around 30% of the chemicals investigated change their T classification if TR is used as toxicity indicator (fields B and F).

Threshold Value for Intrinsic Toxicity. The threshold value separating baseline toxicants from specifically acting chemicals was set at TR = 10, a value proposed and further confirmed by Verhaar et al. (*42*, *69*) and Russom et al. (*56*). A factor of 1 order of magnitude accounts for the variability and uncertainty of the experimentally determined LC_{50} data. The TR values for baseline toxicants are log-normally distributed (*42*, *70*), and it was found that this distribution is centered around zero and spans about 1 log unit in each direction (*70*).

In combination with the value of $ILC_{50,bl} = 5$ mmol/kg for the toxicity baseline, the threshold of TR = 10 corresponds to $ILC_{50} = 0.5$ mmol/kg. This value is close to the threshold of 1 mmol/kg proposed by Gobas et al. in their plea for a toxicity indicator reflecting the intrinsic toxicity of chemicals (*15*). The reference point of the TR scale, $ILC_{50,bl} = 5$ mmol/kg, is in the range of 2−8 mmol/kg previously reported for baseline toxicity in fathead minnow (*22*). (All data on wet weight basis.)

The calculated $ILC_{50,bl}$ value is also in good agreement with published experimental ILC_{50} values for other organisms (data from a compilation in ref *46*). For a variety of fish species, the range is 0.2−30.5 $mmol/kg_{ww}$, and the average is 4.9 $mmol/kg_{ww}$. Daphnia have measured values ranging from 0.6 to 5.3 $mmol/kg_{ww}$, with an average of 2.0 $mmol/kg_{ww}$. For algae, measured data range from 0.6 to 16 $mmol/kg_{ww}$, with an average of 8.6 $mmol/kg_{ww}$.

Finally, one can ask what ILC_{50} corresponds to the currently used criteria of $LC_{50} = 1$ mg/L and BCF = 500 or BCF = 5000. If the LC_{50} threshold of 1 mg/L is converted to mM with an average molecular weight of 100 g/mol and multiplied by a BCF threshold of 500 ($f_{fat} = 0.05$, $\log K_{ow} = 4$), an ILC_{50} of 5 mmol/kg is obtained. With a BCF threshold of 5000 ($\log K_{ow} = 5$), the implicit ILC_{50} threshold is 50 mmol/kg, which clearly exceeds typical values for $ILC_{50,bl}$ and is not a useful ILC_{50} criterion. This conversion of currently used LC_{50} and BCF criteria into ILC_{50} values makes it possible to check the implicit meaning of these criteria. If the TR-based approach is used, the implicit ILC_{50} criterion, which is always contained in LC_{50} and BCF criteria, is replaced by a more mechanistically based threshold for ILC_{50}.

Reliability of the Assessment Concept. Classifying substances according to their TR and interpretation of TR as a measure of ILC_{50} as defined in this paper, is only possible if the expression $\rho^{-1} f_{fat} K_{ow} LC_{50}$ (eqs 2 and 5) is a reasonable approximation of $ILC_{50,exp}$. For example, this approach fails for substances metabolized by fish. For such chemicals, measured BCF values are lower than estimates based on K_{ow} (eqs 4 and 5) and so are also the corresponding $ILC_{50,exp}$. In such cases, the ILC_{50} derived from the K_{ow} is too high and therefore underestimates the intrinsic toxicity. Also for chemicals that have not reached steady state, measured BCF values are lower than estimates based on K_{ow}.

To evaluate the approximation of $ILC_{50,exp}$ in terms of K_{ow} and LC_{50}, three different types of ILC_{50} values were compared: measured, BCF-based, and calculated ILC_{50} values. "Measured" refers to ILC_{50} values experimentally determined from fish; "BCF-based" means that the BCF is a measured value that is multiplied by LC_{50} to yield an ILC_{50}, and "calculated" refers to values obtained with the expression $ILC_{50} = \rho^{-1} f_{fat} K_{ow} LC_{50}$. Because for baseline toxicants ILC_{50} is approximately the same independent of the fish species, data from other fish species were included for chemicals without measured or BCF-based ILC_{50} for fathead minnow. For baseline toxicants and specifically acting toxicants together, 41 measured ILC_{50} for 34 chemicals and 8 different fish were collected (*23*, *24*, *28*, *30*, *34*, *71*−*79*). Thirty BCF-based ILC_{50} for fathead minnow were obtained with eq 2 from measured $BCF_{fathead\ minnow}$ from EHS. Ten BCF-based ILC_{50} were obtained for other fish species than fathead minnow. When several BCF values were available for the same fish species, the geometric mean was calculated. Fat-based BCF values were not included.

As shown in Figure 4, most of the BCF-based and the measured ILC_{50} values fall into the area predicted by the corresponding calculated values in the $\log ILC_{50}$-$\log K_{ow}$ space, i.e., the baseline toxicants have ILC_{50} values above the line for $ILC_{50} = 0.5$ mmol/kg (Figure 4A) and the specifically toxic substances lie below this line (Figure 4B). In Figure 4A (TR < 10), 5 BCF-based values and 1 measured ILC_{50} value lie below the line. As the same LC_{50} values were used for calculated and BCF-based ILC_{50} values, the different classification of the BCF-based values stems from an overestimation of BCF by $\rho^{-1} f_{fat} K_{ow}$. The measured value is for diazinon, an organophosphate pesticide that is an AChE inhibitor in guppy but a baseline toxicant in zebra fish. In this case, the discrepancy between the values below (guppy)

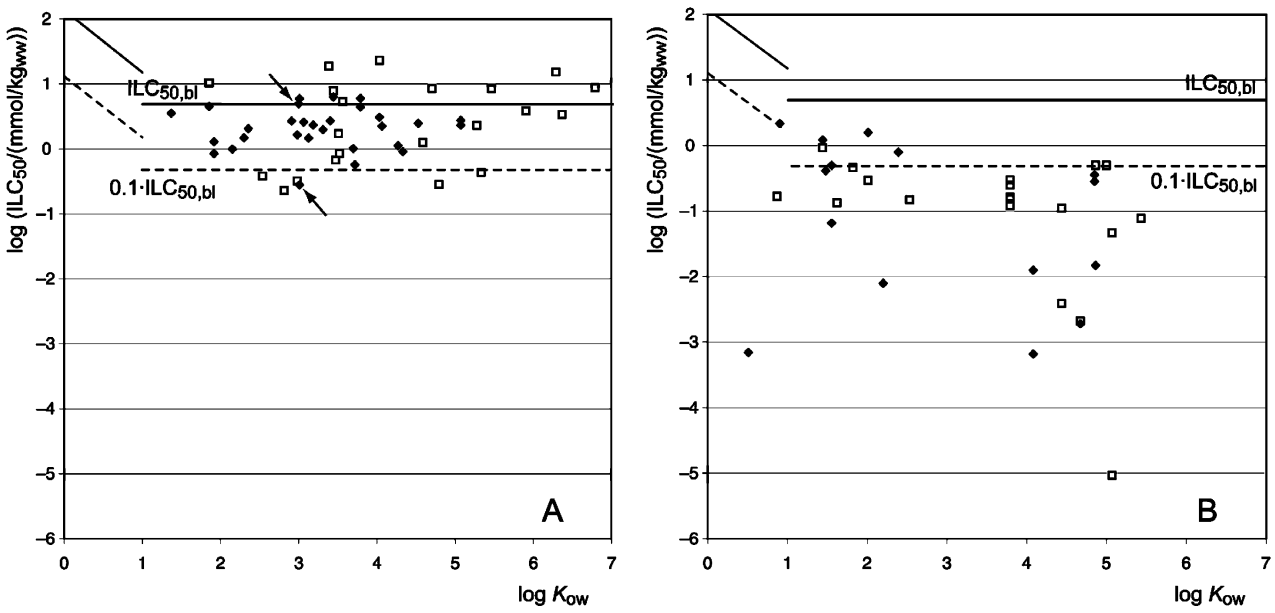

FIGURE 4. BCF-based (open squares) and measured (full diamonds) ILC_{50} values of selected chemicals with TR < 10 (A) and TR ≥ 10 (B). BCF-based values from eq 2 with measured BCF values; measured ILC_{50} values from refs *23*, *24*, *28*, *30*, *34*, and *71*−*79*. $ILC_{50,bl}$ is 5 mmol/kg. Arrows in panel A indicate measured ILC_{50} values of diazinon for guppy (bottom, specific toxicity) and zebra fish (top, baseline toxicity).

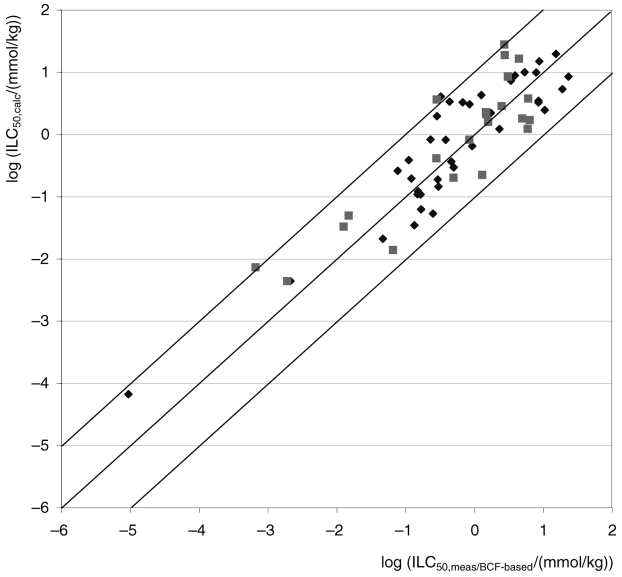

FIGURE 5. Calculated ILC_{50} values vs BCF-based and measured ILC_{50} values. Due to the scarcity of measured ILC_{50} values, data for additional species were included. See Supporting Information.

and above the line (zebra fish) can be related to different rates of bioactivation and metabolism in different fish species (*79*−*81*).

The four chemicals (5 points) above the line for TR = 10 in Figure 4B (TR ≥ 10) are known narcosis II compounds (aniline, phenol, 4-chlorophenol, quinoline). The first three belong to the 7.5% of the 281 baseline toxicants in FHM that have TR > 10, which is caused by the fact that K_{ow} is not an ideal surrogate for cell membranes, especially for polar compounds (*21*). Quinoline is not included in the FHM set of baseline toxicants but is identified as a narcosis II compound in ref. (*24*).

For 47 chemicals, it was possible to calculate 62 differences between calculated and BCF-based and/or measured log ILC_{50} values (Figure 5). The number of data is higher than the number of chemicals because one chemical can have measured or BCF-based values for several species. In 58 of the 62 cases, these differences are smaller than 1 order of magnitude.

In general, the results in Figures 4 and 5 support the approach of using TR = 10 as a threshold for specific toxicity and of approximating ILC_{50} by K_{ow} and LC_{50}. However, relevant differences are observed for hydrophobic chemicals for which the calculated ILC_{50} values seem to be overestimated (Supporting Information). Several reasons may explain these discrepancies. First, the K_{ow} of hydrophobic chemicals is difficult to measure and is therefore uncertain (*82*).

Second, a direct comparison between measured and calculated BCF values reveals that for chemicals with a log K_{ow} > 5, measured BCF values are often lower than predicted by the expression $\rho^{-1}f_{fat}K_{ow}$ (eq 5). As discussed in the literature (*39*, *83*−*85*), different causes contribute to this effect: the duration of the test might be too short, and the chemical does not reach its steady-state concentration in fish (*39*), or the sorption of highly hydrophobic compounds to dissolved organic matter reduces the bioavailable fraction of the chemical (*84*, *85*). With BCF values lower than derived from K_{ow}, the points in a plot like Figure 3 are shifted to the left (i.e., from field F to field E) and a greater distance from the toxicity baseline (i.e., a higher TR) is obtained.

Significance of the Toxic Ratio for the PBT Assessment. With TR as toxicity indicator, the two assessment dimensions B and T are made independent, and therefore, also the interpretation of the B and T dimensions is different from that in current PBT assessment schemes.

In current PBT classification methods, the B and T dimensions are correlated. For typical PBT chemicals (persistent compounds with high BCF), the T dimension adds additional weight to the high B scores of these chemicals because it often indicates a high baseline toxicity that is directly caused by the high BCF. If T is measured in terms of TR, this parallelity of B and T disappears and many chemicals currently classified as PBTs receive high B scores but low T scores because their actual toxicity is mainly narcosis. It is most important to the approach presented here that this different classification does not imply that hydrophobic baseline toxicants are of less concern than currently expressed by their high B and T scores. However, because their high toxicity is mainly caused by their high BCF, it should for reasons of consistency be indicated by the

high B score. Thus, one main implication is that the B dimension needs to be given a weight that fully reflects the problems of high baseline toxicity *and* biomagnification later in the food chain. In other words, there should be political concern already about chemicals with high B (and possibly P) scores. This is in accordance with the proposal of the EU (*4*, *52*) that very persistent and very bioaccumulative chemicals (BCF > 5000, log K_{ow} > 4.5) should be scrutinized because of these properties. To make the connection of the baseline toxicity with the bioconcentration tendency explicit, criteria for the B dimension can even be defined in terms of a threshold for baseline toxicity; for example, the current LC_{50} threshold of 1 mg/L leads to a threshold of log $K_{ow} = 4$.

Second, the T dimension then has a different meaning that adds information to the information contained in the B assessment. It indicates specific toxicity, and for chemicals that have already a high B score, this even increases the level of concern. So the group of PBT chemicals is smaller than in current PBT concepts, but this group now represents chemicals with high scores for three independent properties.

On the other hand, the T dimension places higher emphasis on relatively hydrophilic chemicals with TR > 10. These chemicals are not bioaccumulative (and often not persistent) but exhibit a specific toxicity that indicates a potential for causing significant adverse effects. On one hand, these chemicals are not in the focus of PBT assessments; on the other hand, they will be checked for P, B, and T in the course of PBT screenings of large sets of chemicals. Even if B and P are not problematic here, it is helpful that the T dimension is emphasized by the TR indicator so that these chemicals can be selected for more detailed toxicity tests.

For all chemicals, one has to keep in mind that the classification in terms of TR presented here is based on LC_{50} data for acute toxicity and that a chemical's full potential for adverse effects is not reflected by this quantity. The objective of using TR is to obtain first indications of a chemical's specific toxicity if this information is already contained in the acute LC_{50} value. Using TR as toxicity indicator does not replace a more detailed investigation of the toxic potential of chemicals, including additional endpoints beyond acute mortality.

Although TR was introduced several years ago (*41*, *42*), its advantages for chemicals assessment have not yet been fully acknowledged by ecotoxicologists and policy makers. TR makes it possible to fully use the information that is available in PBT assessments. Without separation of baseline toxicity and specific toxicity by calculating TR or ILC_{50} from the LC_{50} and K_{ow} data, the information contained in these data is not exhausted, which would be an undesirable and avoidable inefficiency of the assessment procedure.

Here, another clarification is necessary. The reasoning for using TR is valid only in the case of PBT assessments because then both LC_{50} and BCF (or K_{ow}) are available. It does not apply to a situation in which the toxicity of chemicals is evaluated without consideration of the B dimension. If only the T dimension is regarded, it is even helpful that LC_{50} data contain information on both the transfer from the aqueous solution into the organism and the specific toxicity. In PBT assessments, however, information is lost if the specific toxicity is not addressed by a suitable indicator such as TR.

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Supporting Information Available

Selection rules for the chemicals investigated, determinations of toxicity baseline and $ILC_{50,bl}$ values, results for toxicity baseline with slope −0.87, details on measured and BCF-based ILC_{50} values, and CAS Registry Numbers of the 845 chemicals investigated. This material is available free of charge via the Internet at http://pubs.acs.org.

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