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RELATIONSHIPS BETWEEN EXPOSURE AND DOSE IN AQUATIC TOXICITY TESTS FOR ORGANIC CHEMICALS

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Abstract: There is continuing debate about the merits of exposure-based toxicity metrics such as median lethal concentration (LC50) versus organism-based metrics such as critical body residue (CBR) as indicators of chemical toxicity to aquatic organisms. To demonstrate relationships and differences between these 2 metrics, the authors applied a simple one-compartment toxicokinetic mass-balance model for water-exposed fish for a series of hypothetical organic chemicals exhibiting baseline narcotic toxicity. The authors also considered the influence of several toxicity-modifying factors. The results showed that the results of standard toxicity tests, such as the LC50, are strongly influenced by several modifying factors, including chemical and organism characteristics such as hydrophobicity, body size, lipid content, metabolic biotransformation, and exposure durations. Consequently, reported LC50s may not represent consistent dose surrogates and may be inappropriate for comparing the relative toxicity of chemicals. For comparisons of toxicity between chemicals, it is preferable to employ a delivered dose metric, such as the CBR. Reproducible toxicity data for a specific combination of chemical, exposure conditions, and organism can be obtained only if the extent of approach to steady state is known. Suggestions are made for revisions in test protocols, including the use of models in advance of empirical testing, to improve the efficiency and effectiveness of tests and reduce the confounding influences of toxicity-modifying factors, especially exposure duration and metabolic biotransformation. This will assist in linking empirical measurements of LC50s and CBRs, 2 different but related indicators of aquatic toxicity, and thereby improve understanding of the large existing database of aquatic toxicity test results. *Environ Toxicol Chem* 2014;33:2038–2046. © 2014 SETAC

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INTRODUCTION

A fundamental task in ecotoxicology is measuring the toxicity of chemical substances under standard test conditions. This allows comparisons of relative toxicity to be made for both regulatory and research purposes. Although a variety of organisms—including fish, invertebrates, and algae—can be employed, fish are often used as the test organism, with exposure being by respiration from an aqueous solution. Standard protocols and interpretive guidance have been established by, for example, the Organisation for Economic Co-operation and Development (OECD) [1] and the US Environmental Protection Agency (USEPA) [2]. Results are used to assess the toxicity of individual substances as a component of aquatic risk assessment in which monitored environmental concentrations are compared with concentrations that cause acute or chronic toxic effects expressed as median lethal concentrations (LC50s) or compared with no-observable-effect concentrations (NOECs). Such data may also be used to compare and rank the toxicity of a series of chemicals as part of priority-setting exercises, for example, using persistence, bioaccumulation, and toxicity criteria. Recent publications have raised issues about the validity and utility of aquatic toxicity tests such as those giving a LC50 [3–7].

An alternative to the LC50 is to express the toxicity as a critical body residue causing 50% mortality (CBR50), which may be a whole-body or lipid-normalized organism concentration [8]. Notable among the reviews of this topic are those of Barron et al. [9], Escher and Hermens [10], and Hendriks et al. [11]. Proponents of the CBR as a metric of toxicity point out that whereas LC50 is based on an external exposure concentration, CBR50 is a measure of delivered internal dose to a whole organism. The CBR approach is more consistent with practices in mammalian toxicology, in which considerable effort is devoted to elucidating the absorption, distribution, metabolism, and excretion processes that convert external exposure to quantities of chemical in target organs. To accomplish this, physiologically-based pharmacokinetic or toxicokinetic models are routinely used to undertake the conversion from exposure to dose for both humans and organisms of environmental interest as reviewed by Krishnan and Peyret [12]. Part of the criticism of LC50 tests relates to uncertainties regarding the roles of test duration, exposure conditions, water quality, and formation of toxic metabolites or derivatives, which can confound interpretation. But perhaps most fundamental is the issue discussed in the present study, relating to the circumstances in which the exposure metric of LC50 is preferable to the dose metric of CBR50 and vice versa.

Certainly, LC50 estimation is easier and less expensive because there is no requirement to measure test concentrations in test organisms. Because there is organism-to-organism variability in adverse response (the causes of which are not fully understood) substantial numbers of test organisms are needed to obtain statistically significant results. The use of CBR50 adheres more closely to the fundamental concept articulated in the paraphrased Paracelsus adage that the "dose makes the poison." However, the CBR50 approach has an additional requirement of analysis of chemical levels in exposed organisms. For organic chemicals, measurements of the body lipid content are also

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desirable. Furthermore, the methodology for placing CBR-based metrics such as CBR50 on an equivalent footing to exposure-based metrics is less well developed with respect to chemical analysis, accuracy and precision requirements, number and nature of treatment groups, pooled versus single organism samples, organism sample numbers, and preferred statistical approaches.

Thus, the debate continues. This issue is not new. In 1939, Ferguson [13] argued that chemical concentration in the "circumenvironmental" medium (water in this case) causing immobilization or mortality was highly variable from chemical to chemical, whereas chemical activity causing the same effect—the ratio of aqueous concentration to liquid solubility—was relatively constant for narcotic substances. Inherent in this assertion is that activity in the water is a surrogate for the activity in the organism. An advantage of using activity instead of concentration is that it automatically includes the exposure-to-dose conversion as equilibrium is approached.

In the present study, the issue of exposure-based versus organism-based toxicity metrics is reviewed using LC50 and CBR50, respectively. We examine the nature of the linkage of exposures to whole-body wet weight concentrations as a function of time using a one-compartment toxicokinetic (1-CoTK) model employing conventional first-order uptake and loss equations [14,15]. We also briefly address toxicity-modifying factors, including metabolism, test duration, size, and lipid content of the test organism. It is important to clarify the purpose, scope, and limitations of the present study, especially in relation to other related studies. As Ashauer and Escher [16] have noted, the accepted approach is to treat toxicity quantification by modeling as a two-step process. First are toxicokinetic (TK) models, which characterize the time course of transport of the chemical from the external medium (water) to the organism, its elimination, and possible biotransformation. Additionally, the model may address the internal distribution of the chemical. This can be accomplished by relatively simple mass balance models such as those of Arnot and Gobas [14] and Stadnicka et al. [17], and other bioconcentration models as reviewed by Barber [18]. Second are toxicodynamic (TD) models, describing the chemical events at the target site(s) that result in adverse effects. This is a more demanding task that may describe reversible or irreversible damage. These 2 steps can be combined in TK-TD models as reviewed by Ashauer and Escher [16]. Notable are the damage assessment model of Lee et al. [19] and the general unified threshold model of survival outlined by Jager et al. [20]. A recent detailed TD-TK model is that of Nyman et al. [21], which addresses the toxicity of parent pesticides and metabolites to 3 invertebrate species and thus explains differences in species sensitivities.

The simplest TD endpoint is to assume that mortality occurs to a defined fraction of the organisms when the whole-body or the lipid-normalized concentration reaches a critical value, the CBR. This CBR is then largely independent of the time required to reach it. This applies to baseline narcotics and essentially assumes that transport to and from the target is reversible, and no irreversible damage occurs. If damage does occur as a result of chemical interactions at the target, more complex situations can be treated, including the effects of prolonged or chronic exposure, pulsed exposures, carryover, and delayed toxicity and recovery times. An additional issue is that of explaining and expressing mathematically why organisms display different sensitivities. The most common and robust empirical approach is to determine the dose-response relationship and the water concentration that causes 50% mortality after a defined exposure time (i.e., the LC50 and the associated CBR50). This sensitivity distribution may result from differences in individual uptake rates or tolerances, or it may be simply stochastic in nature [22]. Jager et al. [20] have comprehensively reviewed this extensive literature.

In the present study, we apply a relatively simple TK model, and we assume that the TD effect is reversible baseline toxicity characterized by a CBR50. Our purpose is to examine the LC50 to achieve this CBR50 in the specified test duration, regardless of the resulting fractional approach to steady-state conditions between the water and the organism. Sensitivity differences or chemicals that exhibit specific modes of toxic action causing irreversible damage at the target are not addressed. Our primary purpose is to demonstrate that empirical data obtained from time-limited tests can be profoundly influenced by the TK processes, especially when there is a relatively low fractional approach to steady-state or equilibrium conditions. We demonstrate and stress the importance of quantifying this fractional approach when designing tests and interpreting test results and when determining the "toxicity" of a chemical for regulatory purposes. In extreme cases, we show that LC50 data can be virtually meaningless as a metric of toxicity. As a result of the assumptions outlined above we stress that the conclusions reached are limited to conditions in which the simple TK model applies and the TD is expressible as a CBR50.

We believe that it is useful to take a pedagogical approach to illustrate the implications of the model assertions versus actual implementation practices and to clarify the fundamental issues surrounding the CBR versus LC50 debate as a basis for further discussion. The objective is not to present new data or parameterize a new model; rather, it is to apply a well-accepted model to explore the implications of the assumptions inherent in the model as they affect implementation practices, constrain data interpretation, and enable scientifically sound comparisons of relative toxicity between chemicals.

METHODS

Toxicokinetic model

The conventional 1-CoTK model is based on the differential equation [11,14,15]

$$dC_F/dt = k_1 \times C_W - C_F(k_2 + k_M) \quad (1)$$

where C_F is the concentration in the exposed aquatic organism (mol m^{-3} wet wt); C_W is the dissolved concentration in the water (mol m^{-3}); and k_1 is the chemical uptake rate constant, which is a function of the respiration rate, expressed in units of m^3 of water per m^3 of fish per hour or liters of water per kilogram fish per hour or numerically per hour when organism density is assumed to be 1.0 kg L^{-1}. The respiratory loss rate constant is k_2 (h^{-1}), k_M is the whole-body metabolic biotransformation first-order rate constant (h^{-1}), and t is time (h). We assume no intake of food, no growth dilution, and no egestion losses in the model simulations, as is typical of short-term toxicity tests. In the present simple model, metabolic biotransformation relates to the primary reaction (loss) of the parent chemical from the mass balance and may include various pathways of transformation. Specific metabolite toxicity and specific elimination processes, such as biliary excretion, are not explicitly considered.

The solution to this differential equation gives C_F as a function of C_W and time as

$$C_F = C_W \times (k_1/[k_2 + k_M]) \times (1 - \exp[-\{k_2 + k_M\}t]) \quad (2)$$

The steady-state fish concentration (C_{FSS}) is given as

$$C_{FSS} = C_W \times k_1/(k_2 + k_M) \quad (3)$$

The bioconcentration factor (BCF), C_{FSS}/C_W, is expressed in units of m^3 of water per m^3 of fish or liters per kilogram of fish and is dimensionless when the fish density is assumed to be 1.0 kg L^{-1}. When k_M is 0 (no metabolism), C_{FSS} is $C_W \times (k_1/k_2)$ or $C_W \times BCF$. For many nonpolar, neutral, nonmetabolized organic chemicals the steady state and equilibrium, BCF or fish–water partition coefficient can be estimated as $L \times K_{OW}$, where L is the actual or effective fractional lipid content of the fish. This simple BCF equation fails for very hydrophilic chemicals that partition appreciably into the water phases within the organism and for chemicals for which octanol is not a suitable surrogate for lipids or other sorbing phases such as proteins [14].

Rearranging Equation 2 to express the concentration in the water (C_W) or LC50 that yields C_F or CBR50, with both quantities in units of moles per m^3 at a defined exposure time, t, gives

$$C_W = \text{CBR50}/([1 - \exp\{-(k_2 + k_M)t\}] \times k_1/[k_2 + k_M]) = \text{LC50} \quad (4)$$

If k_M is 0, this simplifies to

$$C_W = \text{CBR50}/([1 - \exp\{-k_1 t/L \times K_{OW}\}] \times L \times K_{OW}) = \text{LC50} \quad (5)$$

where $k_1/(L \times K_{OW})$ is k_2. Equation 5 relates LC50 directly to CBR50, expressed here on a whole-body basis, but the dependence is a function of uptake rate constant, lipid content, K_{OW}, and test duration. A key underlying assumption for a time-independent LC50–CBR50 relationship is that steady state or equilibrium occurs or is closely approximated. The time to approach steady state is determined by the total elimination rate constant k_T, which in the present case is $(k_2 + k_M)$. Specifically, the half-times for uptake and clearance are $0.693/(k_2 + k_M)$ h. It is instructive to calculate the fractional approach to steady state, F_{SS}, for the calculated LC50 at the conclusion of the test at time h. This is simply

$$F_{SS} = (1 - \exp[-\{k_2 + k_M\}t]) \quad (6)$$

It is noteworthy that when $k_2 t$ is relatively small ($<< 1.0$), as applies to very hydrophobic chemicals, and the duration of exposure is short, the unitless F_{SS} is approximately equal to $(k_2 + k_M)t$. When F_{SS} is 1.0 and steady state applies, the concentration in water is CBR50/BCF and is the "incipient" LC50 as defined by Sprague [23] or the "threshold" LC50 as recommended in 1952 by Wuhrmann [24], who stated that "If only one quantity may serve to characterize toxicity of a substance we consider the threshold concentration as the most appropriate quantity."

Model simulations

In the following equations, we calculate k_1 as a function of fish mass, chemical uptake efficiency (E), and dissolved oxygen concentration as suggested by Arnot and Gobas [14]. Values of the effective respiration rate, G (L d^{-1}), are derived from an allometric relationship for fish mass, W (kg), and oxygen consumption, (mg O_2 d^{-1}), and require the oxygen saturation concentration in water, C_{OX} (mg L^{-1}), at the defined temperature, T (°C), and fraction of oxygen saturation, S, to give k_1 as

$$G = 1400 \times W^{0.65}/C_{OX} \quad (7)$$

$$C_{OX} = S \times (14.04 - 0.24 \times T) \quad (8)$$

$$1/E = 1.85 + 155/K_{OW} \quad (9)$$

$$k_1 = E \times G/W \quad (10)$$

For the present purposes, we express the rate constants in terms of hours; therefore, $k_1 = E \times G/(24 \times W)$ h^{-1}, where E (unitless) is a respiratory chemical assimilation efficiency that depends on K_{OW} and characterizes chemical transport resistances in a series in organic (lipid) and aqueous phases; k_2 is estimated as $k_1/(L \times K_{OW})$.

We assume that 50% lethality occurs when C_F reaches a critical body residue designated as CBR50 and selected here as 5 mmol kg^{-1}, or equivalently 5 mol m^{-3}, assuming an organism density of 1 kg L^{-1} (1000 kg m^{-3}). This is approximately the CBR associated with acute baseline neutral narcosis in small aquatic organisms with about 5% lipid content [8]. The use of this narcosis endpoint is adequate for the present purposes. Obviously, when chemicals exhibit specific modes of toxic action, especially when irreversible receptor binding or irreversible damage occurs, the assumption of this 5 mmol kg^{-1} CBR parameter becomes invalid.

We initially parameterize the model for a 3-g fish with a lipid content, L, of 0.05 in water at a temperature of 20 °C, and an oxygen saturation of 80%. For illustrative purposes, we address a series of 9 hypothetical organic chemicals, designated A to I, varying in log K_{OW}s from 0 to 8 (Table 1). The more polar chemicals of log K_{OW} from 0 to 3 are assigned a molar mass of 150 g mol^{-1}. Those of log K_{OW} from 4 to 8 are assigned an increasing molar mass from 210 g mol^{-1} to 450 g mol^{-1} in increments of 60 g mol^{-1}, reflecting the increasing hydrophobicity with molecular volume, as is observed for chlorinated benzenes and biphenyls. The LC50 is the concentration in exposure water that will produce 50% mortality at the specified exposure time and will result in achieving the CBR50, which is expressed here on a whole-body wet weight basis, namely 5 mmol kg^{-1}, and the exposure period is 96 h. We examine first the effect of chemical and fish properties and time on the fractional approach to steady state (Fss) and the fish concentrations and the LC50s. Subsequently, we conduct an examination of several key model parameters, including some well-known toxicity-modifying factors presented in Table 2.

RESULTS

Our primary objective is to clarify the roles of chemical hydrophobicity and some toxicity-modifying factors that influence the approach to steady state and chemical equilibrium between the exposure water and the organism and thus key determinants of toxicity as expressed by CBR50 and LC50. The nomenclature employed here is C_W mol m^{-3} or equivalently mmol L^{-1} for concentrations in water and C_F mmol kg^{-1} for concentrations in fish. For steady-state conditions, the additional subscript SS is added; for defined test exposure times, the additional subscripts 96 and 48 are added.

Table 1. Results of applying the model to a series of hypothetical chemicals of varying hydrophobicity for a 3-g fish of lipid fraction 5%, for a 96-h test at 20 °C, with a critical body residue (CBR50) of 5 mmol kg^{-1} wet weight or 100 mmol kg^{-1} lipid weight and no metabolic biotransformation

	Chemical								
	A	B	C	D	E	F	G	H	I
Log K_{OW}	0	1	2	3	4	5	6	7	8
Molar mass (g mol^{-1})	150	150	150	150	210	270	330	390	450
E (unitless)	0.006	0.058	0.29	0.50	0.54	0.54	0.54	0.54	0.54
k_1 (h^{-1})	0.38	3.5	18	30	32	33	33	33	33
k_2 (h^{-1})	7.7	6.9	3.5	0.60	6.5×10^{-2}	6.5×10^{-3}	6.5×10^{-4}	6.5×10^{-5}	6.5×10^{-6}
BCF (i.e., $k_1/[k_2 + k_M]$)	0.05	0.5	5	50	500	5.0×10^{3}	5.0×10^{4}	5.0×10^{5}	5.0×10^{6}
F_{SS} (unitless)	1.0	1.0	1.0	1.0	1.0	0.46	0.061	6.2×10^{-3}	6.3×10^{-4}
$T_{½}$, (h)	0.09	0.10	0.20	1.2	11	106	1.06×10^{3}	1.06×10^{4}	1.06×10^{5}
C_{WSS} (mol m^{-3})	100	10	1.0	0.10	0.010	1.0×10^{-3}	1.0×10^{-4}	1.0×10^{-5}	1.0×10^{-6}
96-h LC50 (mol m^{-3})	100	10	1.0	0.10	0.010	2.1×10^{-3}	1.6×10^{-3}	1.6×10^{-3}	1.6×10^{-3}

K_{OW} = octanol–water partition coefficient; E = chemical uptake efficiency; k_1 = chemical uptake rate constant; k_2 = respiratory loss rate constant; k_M = whole body metabolic biotransformation first-order rate constant; BCF = bioconcentration factor; F_{SS} = fractional approach to steady state; $T_{½}$ = half-time; C_{WSS} = exposure water concentration required to reach the CBR50 at conditions of steady state; LC50 = median lethal concentration in water.

Hydrophobicity

The parameters and model results for a series of 9 hypothetical organic chemicals are presented in Table 1, which illustrates the well-established dependence of BCF, k_1, and k_2 on hydrophobicity expressed as log K_{OW} when k_M is assumed to equal 0. The k_1 value is small when K_{OW} is small because of the high organic phase transport resistance (E is low), but E and thus k_1 become larger and fairly constant beyond a log K_{OW} of 3 as the transport resistance is increasingly controlled by the aqueous phase [25]. In contrast, k_2 falls to lower values as hydrophobicity increases. The corresponding uptake half-times ($t_{½} = 0.693\ k_2^{-1}$) are also given in Figure 1 and, for the model parameters specified, exceed the test time of 96 h when log K_{OW} exceeds approximately 4.5. As a result, the fractional approach to steady state (F_{SS}) falls to low values as K_{OW} continues to increase.

Model outputs for 3 exposure scenarios are also presented in Table 1. The first are the exposure water concentrations, C_{WSS}, required to reach the CBR50 at conditions of steady state or infinite time. This equals CBR50/BCF, and is the "incipient" LC50. The second is the exposure water concentration, C_{W96},

Table 2. Dependence of median lethal concentration (LC50), fractional approach to steady state (F_{SS}), and uptake half-time ($T_{1/2}$) of 5 toxicity modifying factors[a]

	Chemical								
	A	B	C	D	E	F	G	H	I
Log K_{OW}	0	1	2	3	4	5	6	7	8
I. Shorter test of 48 h									
C_{WSS} (mol m^{-3})	100	10	1	0.1	0.01	0.001	1×10^{-4}	1×10^{-5}	1×10^{-6}
48-h LC50 (mol m^{-3})	100	10	1.0	0.10	0.01	3.7×10^{-3}	3.3×10^{-3}	3.2×10^{-3}	3.2×10^{-3}
F_{SS} (unitless)	1.0	1.0	1.0	1.0	0.96	0.27	0.031	3.1×10^{-3}	3.1×10^{-4}
$T_{½}$ (h)	0.09	0.10	0.20	1.2	11	106	1.1×10^{3}	1.1×10^{4}	1.1×10^{5}
BCF (i.e., $k_1/[k_2 + k_M]$)	0.05	0.50	5.0	50	500	5000	50 000	500 000	5×10^{6}
II. Smaller organism mass of 0.3 g, C_{WSS}, and BCF as in I. above									
96-h LC50 (mol m^{-3})	100	10	1.0	0.10	0.01	1.3×10^{-3}	7.7×10^{-4}	7.2×10^{-4}	7.1×10^{-4}
F_{SS} (unitless)	1.0	1.0	1.0	1.0	1.0	0.75	0.13	0.014	0.0014
$T_{½}$ (h)	0.04	0.04	0.09	0.51	4.8	47.5	475	4750	47 500
III. Higher lipid content of 10%, $CBR50_{WW}$ of 10 mmol/kg^{-1}, C_{WSS} as in I. above									
96-h LC50 (mol m^{-3})	100	10	1	0.1	0.011	3.7×10^{-3}	3.3×10^{-3}	3.2×10^{-3}	3.2×10^{-3}
F_{SS} (unitless)	1.0	1.0	1.0	1.0	0.96	0.27	0.031	3.1×10^{-3}	3.1×10^{-4}
$T_{½}$ (h)	0.18	0.20	0.39	2.3	21	213	2.1×10^{3}	2.1×10^{4}	2.1×10^{5}
BCF (i.e., $k_1/[k_2 + k_M]$)	0.1	1.0	10.0	100	1000	10 000	1×10^{5}	1×10^{6}	1×10^{7}
IV. More toxic chemicals with $CBR50_{WW}$ of 0.5 mmol/kg^{-1} BCF as in I. above[b]									
96-h LC50 (mol m^{-3})	10	1	0.1	0.01	0.001	1×10^{-4}	1×10^{-5}	1×10^{-6}	1×10^{-7}
F_{SS} (unitless)	10	1	0.1	0.01	0.001	2×10^{-4}	1.7×10^{-4}	1.6×10^{-4}	1.6×10^{-4}
$T_{½}$ (h)	1.0	1.0	1.0	1.0	1.0	0.46	0.061	6.2×10^{-3}	6.3×10^{-4}
96-h LC50 (mol m^{-3})	0.09	0.10	0.20	1.2	11	106	1064	1.1×10^{4}	1.1×10^{5}
V. Chemicals with a metabolic rate constant (k_M) of 0.01 h^{-1}									
96-h LC50 (mol m^{-3})	100	10	1.0	0.102	0.012	2.5×10^{-3}	1.6×10^{-3}	1.5×10^{-3}	1.5×10^{-3}
BCF (i.e., $k_1/[k_2 + k_M]$)	0.05	0.5	5.0	49.2	433	1971	3059	3237	3256
F_{SS} (unitless)	1	1	1	1	1	0.79	0.64	0.62	0.62
$T_{½}$ (h)	0.09	0.10	0.19	1.13	9.28	42.0	65.1	68.9	69.3

[a]The critical body residue is (CBR50) of 5 mmol kg^{-1} wet weight or 100 mmol kg^{-1} lipid weight (except for C and D) and no metabolic biotransformation.
[b]Reduced critical body residue (CBR50).
K_{OW} = octanol–water partition coefficient; C_{WSS} = exposure water concentration required to reach the CBR50 at conditions of steady state; k_1 = chemical uptake rate constant; k_2 = respiratory loss rate constant; k_M = whole body metabolic biotransformation first-order rate constant; BCF = bioconcentration factor.

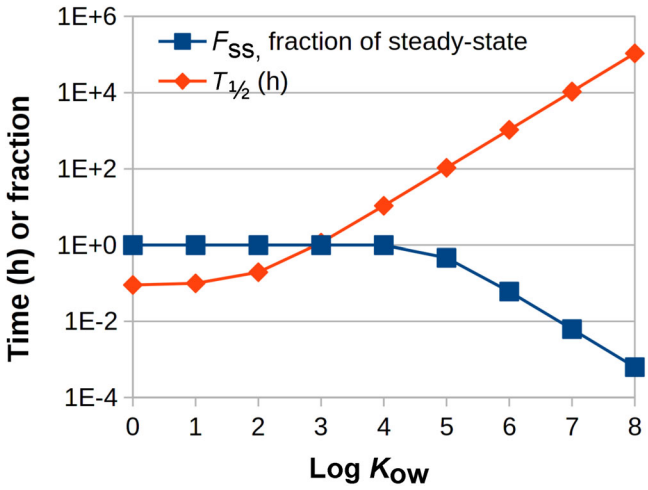

Figure 1. Dependence of fractional approach to steady state (*Fss*) and half-time ($T_{1/2}$, h) on log K_{OW} for 9 hypothetical chemicals showing the increasing failure to reach steady state when log K_{OW} exceeds 4.5 and when the half-time for uptake exceeds the test duration of 96 h. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

needed to achieve the CBR50, but after only 96-h exposure. This is necessarily higher than C_{WSS}, especially when log K_{OW} exceeds 4.5. Third is the fish concentration after 96 h exposure to C_{WSS}. This is also CBR50/F_{SS} and shows the relatively slow approach to steady state for more hydrophobic chemicals. In the latter two 96-h exposure cases, the system is not at steady state for the more hydrophobic chemicals, and LC50 depends on exposure time.

Figure 2 illustrates these relationships between exposure water and organism concentrations expected for nonmetabolizing chemicals. The solid horizontal line is the assumed constant CBR50 for all chemicals of 5 mmol kg^{-1}. The solid lower diagonal line is the water concentration, C_{WSS}, required to achieve this steady-state CBR50, CBR50/BCF, and shows that

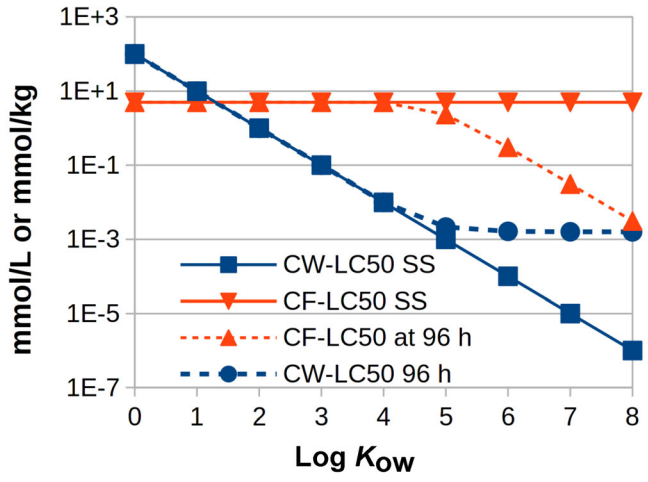

Figure 2. Estimated median lethal concentration (LC50) in water (mol m^{-3}) corresponding to a critical body residue causing 50% mortality (CBR50) of 5 mmol kg^{-1} at steady state (SS) or infinite time exposure (square) and after 96-h exposure (circle), showing the higher, near constant LC50 when log K_{OW} exceeds 4.5. Also shown is the fish concentration C_F (downward-pointing triangle) mmol kg^{-1} (i.e., the CBR50) when exposed to the steady-state LC50 concentration in water and after 96-h exposure to that concentration (upward-pointing arrow), showing the slow approach to steady state when log K_{OW} exceeds 4.5. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

increasing chemical hydrophobicity results in the lower water concentrations necessary to cause mortality.

The dashed line rising above the solid diagonal C_{WSS} line starting at log K_{OW} of 4.5 is the water concentration required to achieve the target CBR50 in 96 h expressed by Equation 4 (C_{W96}). When F_{SS} decreases because of increasing hydrophobicity, the water concentration must be increased for the CBR50 target to be reached. These higher water concentrations level off, and C_{W96} becomes relatively constant because the 2 terms in the denominator of Equation 5 compensate for each other: F_{SS} falls by a factor approaching 10 per unit increase in log K_{OW}, and BCF increases by the same factor. The dashed line dropping below the solid horizontal steady-state C_{FSS} line starting at log K_{OW} of 4.5 is C_{F96} in which the organism is exposed to the water concentration C_{WSS}, but only for 96 h. The exposure is then less than that necessary to achieve the CBR50 and little or no toxicity is expected to occur at 96 h. At concentrations below the C_{WSS} 50% mortality is not expected regardless of exposure time.

There is intersection of the water and organism concentrations when log K_{OW} is 1.3. This is an artifact of the simplifying assumption used in the present simulations that neglect chemical partitioning into the water phase of the organism as noted earlier.

To illustrate the results in more detail, we discuss the estimates from a 96-h test of a nonmetabolizable chemical (F) with a log K_{OW} of 5 and molar mass 270 (Table 1). The uptake rate constant, k_1, is 33 h^{-1}, and k_2 is 0.0065 h^{-1}, so their ratio is the BCF of 5000. This BCF value is also equal to the product of the lipid content of 0.05 and K_{OW} of 100 000 because, as can be seen in Equations 4 and 5, the BCF term ($k_1/[k_2+k_m]$) simplifies to $L \times K_{OW}$ when there is no metabolism. The half-time ($T_{½}$) for uptake and clearance is 106 h; so at 96 h, there is only a 0.46 approach to steady state, F_{SS}. To reach the toxic CBR of 5 mmol kg^{-1} at steady state (F_{SS} =1.0), an exposure concentration in water (C_{WSS}) of 5/BCF or 0.001 mol m^3 is needed. To reach this CBR50 by 96 h requires a higher water concentration of 0.0022 mol m^{-3}, which is greater than C_{WSS} by a factor of 2.2 or 1/F_{SS}.

For less hydrophobic chemicals (A–E), F_{SS} is larger and approaches 1.0 because of the apparently more rapid fish-water exchange caused by the larger k_2. The more rapid approach to steady state of less hydrophobic substances is the result not of faster uptake but of the lower chemical mass that is required to be transferred to the fish to approach steady state. For more hydrophobic chemicals (F–I), the approach to steady-state ranges from 0.46 to 0.00063; thus, conditions become far from steady state. Despite all chemicals having equal CBR50s, the values of dissolved concentrations in water for chemicals F to I vary considerably. Under steady-state exposure conditions the water concentrations (C_{WSS}) range from 10^{-3} mol m^{-3} to 10^{-6} mol m^{-3}, a factor of 1000. On the other hand, to achieve lethality for these chemicals in 96 h requires exposures to dissolved concentrations in water ranging from 0.0022 mol m^{-3} to 0.0016 mol m^{-3}, a factor of only 1.4. Clearly, LC50 and CBR are giving widely different quantitative metrics of toxicity for these chemicals, attributable to the substantial effects of varying hydrophobicity and uptake kinetics.

Table 2 gives results for changes in model parameters that reflect the influence of 5 toxicity-modifying factors (designated I–V in Table 2). Estimates for F_{SS} and $T_{1/2}$ are presented, along with C_{WSS} and concentrations in water, such as C_{W96}. Concentrations in fish exposed to C_{WSS} but at the defined exposure time are readily calculated as CBR50/F_{SS}.

Test duration

The first toxicity-modifying factor examined is exposure duration. The values for exposure water concentrations causing 50% lethality at steady state (C_{WSS}) are the same as in Table 1. In this case, the duration of the test is reduced by half to 48 h, resulting in smaller values of F_{SS} (an increased departure from steady state) for the more hydrophobic chemicals. The factor reduction in F_{SS} is approximately 2 for more hydrophobic chemicals—G, H, I—because F_{SS} approaches k_2t. For exposure to C_{WSS} the fish concentrations after 48 h (C_{F48}), which can be estimated as CBR50 $\times$ F_{SS} are less than that at 96 h given in Table 1. Consequently, to achieve the target CBR50 requires a higher water concentration for 48-h exposure versus 96-h exposure, especially for the more hydrophobic chemicals. For example, for chemical I the C_{W48} and C_{W96} estimates are 0.0032 and 0.0016 mol m^{-3}, respectively, a factor of 2 increase. For less hydrophobic chemicals, this increase is smaller and is only 4% for chemical *E* with a log K_{OW} of 4. It is emphasized that the necessary increases in the water concentration to achieve the CBR50 do not imply that the system has approximated steady state because the time to approach steady state is controlled by k_2. The simple and often assumed relationships between steady-state values; CBR50, C_{WSS}(LC50), and BCF_{SS} are no longer applicable. The implication is that one can achieve non–steady-state LC50s at shorter exposure durations with higher water concentrations and these values are different from steady-state LC50s obtained using longer exposures at lower water concentrations.

Organism size

Table 2 shows that for a smaller organism (0.3 g) the steady-state LC50 exposure water concentrations (C_{WSS}) are again the same as in Table 1 for the 3-g fish. Because the CBR50 is the same, the changes in LC50 and fish concentrations are attributable to the larger k_1. The respiration rate G of the smaller fish is lower, but because k_1 and k_2 are normalized to body weight, both increase by a factor of $10^{0.35}$ or 2.24 [14,26]. When F_{SS} increases, it causes higher fish concentrations at shorter exposures. In essence, smaller organisms approach steady state faster. Lower exposure water concentrations (C_{W96}) are required to achieve the CBR50 for the 0.3-g fish versus 3-g fish; for example, for chemical I, these concentrations are 7.1×10^{-4} mol m^{-3} versus 16.0×10^{-4} mol m^{-3}, respectively, again a factor of 2.24 difference. A convenient rule of thumb is that a factor of 10 decrease in fish size causes a factor of 5 increase in the specific respiration rate, given equivalent temperatures and oxygen concentrations.

Effect of lipid content

Doubling the lipid content is equivalent to increasing K_{OW} by a factor of approximately 2 or log K_{OW} by approximately 0.3 units. Mathematically, this is because K_{OW} and *L* appear in Equation 5 as a product. Equation 9, however, defining *E*, includes a term where K_{OW} appears alone. The BCFs also double. The issue arises that CBR50 expressed on a whole-body mass basis will likely vary with lipid content—survival of the fattest [27]—whereas CBR50 on a lipid basis is expected to be relatively constant for hydrophobic narcotic chemicals when the toxicity depends on the concentration in the target lipids [10]. We examine 2 options.

If we assume, as in Table 2, that the lipid-based CBR50 is unchanged and both the lipid content and the whole-body CBR50 double, then C_{WSS} is unaffected. The BCF doubles as does $T_{1/2}$ and k_2 is halved. The F_{SS} and C_{W96} values are largely unaffected for the fast uptake for the less hydrophobic chemicals, but F_{SS} decreases to half and C_{W96} doubles for the more hydrophobic chemicals that are under kinetic control.

If the lipid content increases, but the same whole-body CBR50 applies, then the CBR50 on a lipid basis falls. Bioconcentration factor and $T_{1/2}$ increase as above, but C_{WSS} falls, as does k_2. In this case, F_{SS} is similar for the less hydrophobic chemicals and falls for the less hydrophobic chemicals because of the increase in $T_{1/2}$. The net effect is that C_{W96} is reduced for the less hydrophobic chemicals but remains similar for more hydrophobic chemicals. The interactions between lipid content and CBR50 metrics are thus not immediately obvious, especially at low lipid contents, when much of the chemical is in aqueous phases in the fish. For hydrophobic chemicals, the lipid-normalized CBR50 is preferable.

Among lipid-related issues not addressed by this simple model are whether the ratio of target to nontarget lipid changes, whether the character and components of the whole-body lipid vary [10], and whether the target sites for the mode of toxic action in question can be reasonably approximated by octanol as a hydrophobic phase. In general, as body lipid content increases, the time to reach steady state increases because more chemical must be absorbed. For more hydrophobic chemicals that are under kinetic control, the LC50 must be increased to compensate for this greater quantity of absorbed chemical.

Chemicals of greater toxicity

For the results presented in Table 1, the CBR50 of 5 mmol kg^{-1} is approximately that associated with baseline neutral narcosis. As more specific modes of toxic action typically require lower fish concentrations to elicit their effects, the influence of lower CBRs is examined. Table 2 illustrates the effect of reducing the whole-body CBR50 by a factor of 10 mmol kg^{-1} to 0.5 mmol kg^{-1}, increasing the chemical toxicity or potency by a factor of 10. All values of steady state and 96-h LC50s and fish concentrations fall by a factor of 10, as expected. It is interesting to compare chemical D from Table 2 with chemical E from Table 1. In both cases, steady state is approached during the test and F_{SS} exceeds 99%, so the LC50s are not influenced by the approach to steady state. The 96-h LC50 of the more toxic chemical D of 0.01 mol m^{-3} in Table 2 is the same as that of the less toxic chemical E of 0.01 mol m^{-3} in Table 1. Thus, from an LC50 viewpoint, both chemicals appear to be of equivalent toxicity. The interaction of hydrophobicity and CBR50 has masked the true toxicity difference between these 2 chemicals. It is possible to give examples of pairs of chemicals in which the more toxic chemical (lower CBR) has a higher LC50 than the less toxic chemical because of differences in K_{OW}. This illustrates that the use of LC50 data to rank chemicals for toxicity can be suspect if there are also differences in chemical hydrophobicity and CBR.

Metabolic biotransformation

Metabolic biotransformation of chemicals is a largely ignored modifying factor in most standard aquatic toxicity testing data. Rather than carrying out the demanding analytical work necessary to assess its influence, it is routinely assumed that for the relatively short duration of typical tests that there is little or no metabolic degradation. In the present study, the effect of a modest rate of metabolic biotransformation of 0.01 h^{-1}, corresponding to a metabolic biotransformation half-life of 69.3 h, is illustrated. The rates of chemical loss increase, and the net uptake half-times are reduced, as can be seen in Table 2. The BCFs decrease especially for the more hydrophobic chemicals. For chemical F, the overall $T_{½}$ (based on $k_2 + k_M$) falls from 106 h to 42 h, whereas the approach to steady state, F_{SS},

increases from 0.46 to 0.80, and the BCF falls from 5000 to 1970 compared with the case where k_M is 0 (Table 1). The LC50s are affected differently depending on K_{OW}; for example, for chemicals A to E, the LC50s are insensitive to metabolism because of the fast water to fish exchange. For chemical F, the LC50 rises from $0.0022\,mol\,m^{-3}$ with no metabolism to $0.0032\,mol\,m^{-3}$ with metabolism; metabolism reduces the apparent toxicity. For other more hydrophobic chemicals, LC50 plateaus at $0.0025\,mol\,m^{-3}$.

For the more hydrophobic chemicals, most of the elimination of chemical is by metabolism, and the continuing decrease in k_2 with increasing hydrophobicity is of little consequence because the sum of k_2 and k_M becomes insensitive to the small value of k_2. Even very low rates of metabolism can thus have a profound effect on the LC50, especially for very hydrophobic chemicals. The test is actually measuring the concentration in water that is required to generate a concentration in the fish such that the product $CBR50 \times k_M$ approaches the rate of uptake $k_1 \times C_W$, and $T_{1/2}$ approaches the metabolic biotransformation half-life.

It is thus insightful to consider the relative magnitudes of k_2 and k_M or the corresponding half-lives. When $k_2 >> k_M$, metabolism is relatively unimportant, as applies to persistent organic chemicals. When $k_2 << k_M$, metabolism dominates the loss process. In the intermediate region, when k_2 and k_M are similar in magnitude, both processes are significant. It is erroneous to allege that the LC50s in fish are unaffected by metabolism when values of k_M are below a certain threshold, because any threshold depends on K_{OW} and k_2. Knowledge of both k_2 and k_M [28,29] is thus desirable for both test design and interpretation.

Additionally, when interpreting test results, another problem may arise concerning the nature of the metabolites. At least 1 metabolic degradation product, and possibly multiple metabolic degradation products, may be generated. Metabolites may be of a similar toxicity to the parent chemical (a default assumption of baseline neutral narcosis) or may have 1 or more specific modes of toxic action. Metabolites may be rapidly excreted and not reach the target sites of toxic action, whereas others may be excreted more slowly and may reach target sites. It is clear that even modest rates of metabolic biotransformation may introduce confusion and uncertainty in standard toxicity test interpretations, which cannot be resolved with current testing methodologies that rely exclusively on exposure-based metrics.

Other modifying factors

Other modifying factors may influence the test results. These include the effect of differences in species sensitivities, chemical bioavailability in the water (especially for hydrophobic substances), dissolved oxygen concentration, possible effects of water solubility limitations, and differences between organism–water and target site–water partitioning, which are not adequately characterized by K_{OW}. More thorough consideration of their influences with appropriate methods is warranted.

DISCUSSION

LC50 versus CBR as complementary but different metrics of toxicity

The chemicals addressed in Tables 1 and 2 with a CBR of $5\,mol\,m^{-3}$ can be regarded as having equal toxicities, because they cause 50% mortality at identical internal concentrations CBR50. Their LC50s vary considerably; thus, they can appear to have widely different toxicities. This is essentially a restatement of Ferguson's assertion that the use of "circumenvironmental" concentration such as LC50 as an indicator of toxicity can be misleading [13]. If this concept is accepted, a compelling case can be made that when comparing toxicities of different chemicals, an organism-based metric such as a CBR50 is a better surrogate dose for levels at the effective target sites that are eliciting the toxic effects than indirect metrics such as LC50, which are based on exposure media concentrations.

For example, in simple terms, if 2 chemicals are suspected of causing toxic effects, 1 at a water concentration of $10\,mol\,m^{-3}$ and the second at a water concentration $1\,mol\,m^{-3}$, it can be argued that the second is more toxic. This is true only if they have comparable partitioning properties (and possibly similar values of k_M) and thus different CBRs. The second chemical may have a much higher fish–water partition coefficient (BCF) and thus appears to be more toxic. The extent to which substances exert toxicity depends on the nature and potency for the mode of toxic action under examination and the extent to which the toxic moiety reaches the target sites—the water-to-target tissue partitioning characteristics. It is not surprising that baseline toxicity of organic chemicals characterized by LC50 correlates well with log K_{OW}, as pointed out 30 yr ago by Konemann [30] and Veith et al. [31]. This is because the incipient LC50 is CBR50/BCF, and BCF is proportional to K_{OW}. Outliers are found in most LC50 quantitative structure-activity relationships (QSARs), which may be due to differences in toxicity, but CBR-based approaches are better suited to identifying chemicals and metabolites that exhibit different toxicities.

The relationship between CBR50 and LC50 as expressed by Equation 5 also contains other organism-specific and chemical-specific parameters. Clearly, 3 factors (partitioning, approach to steady-state [F_{SS}], and metabolism) play important roles in determining the empirical LC50 and thus the apparent toxicity, but they do not influence the CBR to the same extent. It can thus be argued that CBR is therefore a more meaningful and robust indicator of relative chemical toxicity than LC50. The LC50 has the advantage that it can be used directly to assess if a given water concentration will result in toxicity, provided that respired water is the dominant source of exposure to the receptor and the receptor and exposure conditions in the environment are similar to those in the toxicity test. The LC50 is a meaningful descriptor of toxicity only if the final exposure condition is at steady state. However, if the chemical is subject to trophic magnification or trophic dilution (different relationships between the water concentration and the organism concentration than occurs in a laboratory toxicity test), or the ecological receptor or environmental conditions have different properties from the test conditions (body size, lipid contents, metabolism rates), then the CBR is the preferred metric for comparing effect concentrations with exposure concentrations for risk assessment purposes. When the organism is not at steady state with respect to its environment, an LC50 can be very suspect, especially for hydrophobic chemicals and measured body burdens and CBRs can also be suspect.

Toxicity criteria

A regulatory implication is that setting a toxicity criterion when assigning regulatory priorities of an LC50 of less than 0.1 or $1.0\,mg\,L^{-1}$ as is used in some persistence, bioaccumulation, and toxicity evaluations is scientifically unsound [3–5]. Such regulatory programs may be forced to employ readily available information, and large amounts of CBR are not readily available, likely because of high costs and analytical challenges. Nevertheless, interim regulatory policies should be replaced when more appropriate methods become available. Also, despite

detailed testing methods, results from existing standardized acute toxicity testing protocols (e.g., OECD, USEPA) can be confounded by toxicity-modifying factors. As discussed in the *Results* section, this is because LC50 is an external dose surrogate that is controlled by uptake kinetics, partitioning, and metabolic biotransformation, although the magnitude of such influences is lesser for organic chemicals of log K_{OW} less than approximately 4.

Compensating for low values of F_{SS}

One option for addressing the issue of varying approach to steady state (F_{SS}) is to calculate the product of the measured LC50 and F_{SS} as a more reliable indicator of the real toxicity. This was suggested by Hendriks et al. [11], who multiplied the fixed exposure duration LC50 by (1–exp[–k_2t]), which is equivalent to F_{SS} as defined in the present study. For example, for chemical F with a log K_{OW} of 5 discussed earlier (Table 1), the 96-h LC50 is 0.0022 mol m^{-3}, and F_{SS} is 0.46. The product of 0.001 mol m^{-3} is the concentration in the exposure water that will give a body residue of 5 mol m^{-3}, the steady-state CBR50, at long exposures. It is, however, a more meaningful metric of toxicity independent of exposure duration. The value of F_{SS} is best estimated by conducting a clearance test to determine k_T (which may include metabolic losses). It can then be estimated using Equation 5, where t is the test duration at which the LC50 is determined. An alternative, less-accurate method is to determine k_1 from the uptake rate at short times and deduce k_2 as $k_1/(L \times K_{OW})$ where L is the fractional lipid content and $L \times K_{OW}$ is the BCF. A question then arises as to the reliability of test results when F_{SS} is relatively low (e.g., less than 0.5). An attractive approach is to include in the test protocol a requirement to determine and report F_{SS}. A low-percentage approach, F_{SS} of less than, for example, 75%, $2 \times T_{½}$, can serve to caution users of test results that they may lack quantitative significance.

Use of models for designing test conditions

The present study suggests that toxicity tests could be more efficient, be less expensive, and perhaps use fewer animals if a model was used in advance of actual testing. It is relatively easy to use the model to explore the nature of the expected mortality if estimates can be made of the concentrations in the organism at various times and compared with CBRs for similarly structured chemicals. Effects of possible biotransformation can be explored. One obvious simple step is to determine the proximity of the test concentration to the saturation solubility. Obviously, planned or inadvertent exposure to concentrations that exceed the water solubility limit of the test chemical are inappropriate as the organisms may be exposed to both "neat" chemical and a saturated solution. Clearly, metabolic biotransformation can significantly affect the LC50, especially for more hydrophobic substances, and it can strongly influence the time to approach steady state. When designing a test, it is useful to have at least an order of magnitude estimate of the metabolic biotransformation rate constant. Recently, a database [28] and QSAR [29] for predicting the metabolic biotransformation rate from chemical structure have been developed. Although the estimates are approximate, it is useful to use the model in advance of testing to explore the effects of the estimated range of metabolic half-lives.

Use of chemical activity for designing and interpreting tests

The present discussion has been entirely based on concentrations. An attractive complementary approach is to view the results in terms of the equilibrium criterion of chemical activity, which is readily calculated by dividing each concentration by the corresponding solubility of the liquid state chemical. For biotic phases this "solubility" can be estimated as the product of the solubility in water and $L \times K_{OW}$ or the BCF. As uptake proceeds, the activity in the fish increases and approaches that of the water. If metabolic biotransformation rates are high enough to lower the BCF below simple equilibrium partitioning (i.e., BCF $< L \times K_{OW}$), the activity in the fish cannot reach the activity in water. This explains the increase in LC50 caused by metabolism. It is generally accepted that baseline narcosis occurs at an internal activity of 0.01 to 0.1. Recently, Smith et al. [32] measured activities using a precise passive dosing technique for *Daphnia magna* and *Folsomia candida* and obtained lethality for polycyclic aromatic hydrocarbons (PAH) and PAH mixtures over a narrow range of activities of 0.02 to 0.05. This suggests that accurate measurements of activity causing mortality may yield reproducible estimates of CBR. Test results yielding activities of 0.01 to 0.1 at lethality suggest that the substance is a baseline narcotic. If lethality occurs at an activity less than 0.01, the implication is that there is a reactive toxic mechanism or mode of action. The use of activities also automatically checks that conditions are subsaturated: activities in water are < 1.0. As Reichenberg and Mayer [33] have shown, solid chemicals of relatively high melting points with low fugacity ratios may be incapable of achieving the activity necessary to cause narcotic lethality. If this is known or suspected in advance it may save unnecessary testing.

CONCLUSIONS

We conclude that the current internationally recognized standard toxicity testing methods (e.g., OCED, USEPA) as currently conducted are essential components of aquatic risk assessment and can yield valuable information on toxicity. Interpretation can be flawed, however, especially for hydrophobic chemicals with slow rates of accumulation in relatively large aquatic organism (>1 g). These LC50 metrics usually reflect exposures necessary to cause mortality from fixed duration exposures, but they do not routinely reflect steady-state toxicity estimates, as has long been recommended [6,23,24]. They are not comparable metrics of relative toxicity resulting from the delivered dose. If the aim is to compare chemicals for their toxicity, it is preferable to compare the delivered dose as reflected in the CBR. Improved understanding of the toxicological significance of the LC50 results could be obtained by calculating F_{SS}, the approach to steady-state, and applying it to estimate the incipient LC50 at longer exposure times that approximate steady state. Consideration must also be given to the complex confounding effects of metabolic biotransformation and details of the test protocol. Applying simple models such as the one described in the present study can greatly assist in the design and interpretation of such tests and their experimental results.

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