THE CHEMICAL EXPOSURE TOXICITY SPACE (CETS) MODEL: DISPLAYING EXPOSURE TIME, AQUEOUS AND ORGANIC CONCENTRATION, ACTIVITY, AND ONSET OF TOXICITY

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(Submitted 14 July 2016; Returned for Revision 2 September 2016; Accepted 27 October 2016)

Abstract: A 1-compartment toxicokinetic model is used to characterize the chemical exposure toxicity space (CETS), providing a novel graphic tool that can aid in the design of aquatic toxicity tests for fish and for interpreting their results. The graph depicts the solution to the differential equation describing the uptake kinetics of a chemical by a modeled fish under conventional bioassay conditions. The model relates the exposure concentration in the water to a dimensionless time and the onset of toxicity as determined by an estimated or assumed critical body residue or incipient lethal aqueous concentration. These concentration graphs are specific to each chemical and exposure and organism parameters and clearly demonstrate differences in toxicity between chemicals and how factors such as hydrophobicity influence the toxic endpoint. The CETS plots can also be used to assess bioconcentration test conditions to ensure that concentrations are well below toxic levels. Illustrative applications are presented using a recent set of high-quality toxicity data. Conversion of concentrations to chemical activities in the plots enables results for different baseline toxicants to be superimposed. For chemicals that have different modes of toxic action, the increased toxicity then becomes apparent. Implications for design and interpretation of aquatic toxicity tests are discussed. The model, and pictorial visualization of the time-course of aquatic toxicity tests, may contribute to improvements in test design, implementation, and interpretation, and to reduced animal usage. Environ Toxicol Chem 2017;36:1389–1396. © 2016 The Authors. Environmental Toxicology and Chemistry Published by Wiley Periodicals, Inc. on behalf of SETAC.

Keywords: Aquatic toxicity Bioconcentration Toxicokinetics

INTRODUCTION

In conventional acute aquatic toxicity tests such as those promulgated by the Organisation for Economic Co-operation and Development (OECD) test guideline 203 [1], test organisms (fish) are exposed to defined concentrations of the subject chemical in aqueous solution for a specified time that is typically 96h. The objective of the experiment is to establish the concentration that causes death of a defined percentage (usually 50%) of the fish at the conclusion of the test, namely the LC50. Alternatively, a potentially lethal concentration may be prescribed, and the time-to-death of 50% of the fish is measured. Such tests have demanding requirements, especially if the results are to be used for regulatory purposes. Test conditions are normally tightly controlled in terms of concentration, temperature, oxygen saturation, and number and condition of the test organisms, resulting in considerable expense. There is thus an incentive to avoid excessive and unproductive testing by careful experimental design and execution. Such design may be assisted by employing a predictive model of the uptake process, thus relating planned aqueous concentration to time and to a body burden or incipient lethal concentration that is postulated to result in lethality. The model need not be highly accurate, but even an approximate model can suggest whether conditions are likely to be successful. The model can also contribute to the design of bioconcentration factor (BCF) tests in which the aim is to measure the uptake and accumulation of chemical from water, but under sublethal conditions. If there is insufficient time to achieve steady state, a correction can be applied employing an estimate of the loss rate constant (i.e., using an uptake rate constant \[k_1\]–loss rate constant \[k_2\] approach such as that described by Hendriks et al. [2]).

In a series of reviews of aquatic toxicity testing, McCarty et al. [3] pointed out that regulatory demands have increased, while the quality of many data in well-accepted databases is often inadequate. This is because, in the translation from conceptual models to operational testing models, not all important toxicity modifying factors influencing linkages between fundamental thermodynamics and kinetic processes and adverse toxic effects associated with conventional dose metrics are considered.

We present a predictive model that employs equations and a graphic display to describe the dynamic uptake kinetics of a chemical by a fish under conventional bioassay conditions, thus relating exposure concentration in the water to time and onset of

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Published online 16 November 2016 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/etc.3668

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toxicity. Furthermore, the display and equations can be presented in terms of chemical activity, which has been shown to provide predictive capability for baseline nonpolar narcotic substances, often referred to as having a toxic mode of action (MOA1) or, in the case of polar substances, MOA2 [4]. One appealing feature of the model is that the results may be graphically presented to visualize the approximate relationship between the parameters and the outcomes. The corresponding set of equations can describe these relationships more accurately. Essentially, the graph and model satisfy the need for an a priori assessment of the likely success of the proposed test design.

MODEL AND PARAMETERS

To develop the model and the graphic display, we employ the conventional 1-compartment first-order toxicokinetic model as described by Arnott and Gobas [5] in their AQUAWEB model. This gives the equation for uptake from water by respiration as it applies to standard short-term bioconcentration and toxicity tests such as OECD guidelines 305 [6] and 203 [1], which are described by Newman [7].

\[ C_F = C_W \times (k_1/[k_2 + k_M]) \times (1 - \exp(-[k_2 + k_M]t)) \]  

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\(^{-3}\)), \( k_M \) is the first-order rate constant for metabolism or biotransformation (h\(^{-1}\)), and \( t \) is time. We estimate the respiratory \( k_1 \) is used to model metabolism or biotransformation (h\(^{-1}\)) as \( G \times E \), where \( G \) is the respiration rate (m\(^3\) water/m\(^3\) fish h) and \( E \) is the chemical transfer efficiency estimated from the chemical's octanol–water partition coefficient (\( K_{OW} \)) [5,8]. Conventionally \( k_1 \) is expressed in units such as mmol kg\(^{-1}\) h\(^{-1}\), but the unit of h\(^{-1}\) gives identical numerical values assuming the fish density is equal to that of water, 1000 kg m\(^{-3}\).

The respiratory rate loss constant \( k_2 \) (h\(^{-1}\)) is estimated as \( k_1/(L \times K_{OW}) \) where \( L \) is the fractional lipid or effective octanol content of the fish. For a more rigorous treatment of biotransformation, it may be necessary to apply Michaelis–Menten kinetics. Applying Equation 1 implies that the fish are not fed, and thus there is no chemical intake in food, growth dilution is negligible, and there are no losses by fecal egestion. When \( t \gg 1/(k_2 + k_M) \), a steady-state concentration \( C_F \) corresponding to a BCF (m\(^3\) m\(^{-3}\)) is approached

\[ C_F/C_W = BCF = k_1/(k_2 + k_M) \]  

When \( k_M \) approaches 0, \( C_F \) approaches \( C_W \times (k_1/k_2) \) or \( C_W \times BCF_E \), where \( BCF_E \) is a steady-state and equilibrium BCF that can be estimated as \( L \times K_{OW} \) for many nonpolar, neutral chemicals. If metabolism occurs, the lower steady-state but nonequilibrium bioconcentration factor \( BCF_M \) is \( k_1/(k_2 + k_M) \). Alternatively, estimates can be made of \( k_1 \) and \( k_2 \) and their ratio equated to the BCF. The LC50 is assumed to occur when \( C_F \) reaches a critical body residue corresponding to an effect on 50% of the organisms (CBR50), usually in the range of 2 mol m\(^{-3}\) to 8 mol m\(^{-3}\) or, equivalently, mmol kg\(^{-1}\) by assuming a fish density equaling that of water, 1000 kg m\(^{-3}\). This range applies to CBRs for acute baseline narcosis of hydrophobic chemicals in small aquatic organisms with approximately 5% lipid content [9] and is consistent with the Redman et al. [10] or Di Toro et al. [11] analyses employing the target lipid model. Biochemically reactive chemicals may exhibit specific modes of toxic action and have lower CBRs [4].

Rearranging Equation 1 to estimate the LC50 concentration \( C_W \) that yields a \( C_F \) or CBR50 at a defined exposure time \( t \) gives

\[ C_W = LC50(\text{mol m}^{-3}) = \frac{CBR50/[(1 - \exp\{ -\{k_2 + k_M\}t\} \times k_1/[k_2 + k_M])]}{(1 - \exp\{-k_1/(L \times K_{OW})\})} \times L \times K_{OW} \]  

where \( k_1/(L \times K_{OW}) \) is equal to \( k_2 \), and the BCF is equal to \( L \times K_{OW} \). If \( k_M = 0 \), Equation 3 simplifies to

\[ C_W = LC50(\text{mol m}^{-3}) = \frac{CBR50/[(1 - \exp\{-k_1/(L \times K_{OW})\})]}{L \times K_{OW}} \]  

It is convenient to define the group \((1 - \exp\{-\{k_2 + k_M\}t\})\) as \( \Phi \), the fractional approach to steady state or equilibrium. This group is routinely used to correct measured fish concentrations at near-equilibrium conditions \( (\Phi_{eq}) \) to estimates of steady-state concentrations \( (t_{eq}) \); that is, \( t_{eq} = \Phi_{eq}/\Phi \) [1,2]. At equilibrium and steady state, \( \Phi = 1 \). When \( t = 0 \), \( \Phi = 0 \) and thus \( \Phi \) is essentially a dimensionless time that is dependent on both the organism and chemical. This gives an equation for LC50 as a function of CBR50, \( \Phi \), BCF\(_E\) (for nonmetabolizing chemicals) or with BCF\(_M\) for metabolizing chemicals, and an equation for the ratio BCR50/BCF, which is designated in the present study as the incipient lethal concentration (ILC50) as defined by Sprague [12,13] or the threshold LC50 as defined by Wurhan [14]. Newman [7] describes this approach in more detail. Rearranging to express LC50 and \( \Phi \) as a function of ILC50 gives

\[ BCR50/BCF = ILC50 = LC50 \times \Phi \]  

Equation 5 suggests that the combined toxicity and bioconcentration of the substance can be conveniently expressed as BCR50/BCF, or ILC50. The ILC50 is the concentration in water (mol m\(^{-3}\)) that results in the organism reaching its critical body residue at steady state, as would apply after long exposure times when \( \Phi \) equals 1.0. It is thus the minimum aqueous concentration necessary to cause toxicity. The time to reach steady state is determined by the chemical half-life in the fish, and is longer for more hydrophobic and more persistent chemicals because total elimination rates become slower (i.e., \( k_2 \) is reduced). When \( k_M > k_2 \), the BCF, and hence the relationship between the external exposure concentration and the internal exposure concentration nearer the site of toxic action, can be lower than the thermodynamic equilibrium BCF\(_E\).

These equations capture the expectation that the LC50 depends on the aqueous exposure concentration, time of exposure, and toxicity of the substance expressed in the CBR. There is a hyperbolic relationship between exposure concentration (LC50) and time expressed by \( \Phi \), their product being the ILC50. These algebraic concepts are relatively simple and transparent but can be difficult to visualize their relationships and significance. Accordingly, there is an incentive to present them in graphic form in what we call a chemical exposure toxicity space (CETS) plot analogous to the chemical partitioning space diagrams of the air–water partition coefficient (\( K_{AW} \)), the \( K_{OW} \), and the octanol–air partition coefficient (\( K_{OA} \)) used to identify...
the likely multimedia partitioning tendencies of chemicals [15]. When the concepts are presented visually, the diagrams may convey the relationships among exposure concentration, time, and toxicity more clearly. In the present study, we display the concepts graphically, providing a method by which the problems associated with long uptake half-times of chemicals and chemicals of various hydrophobicity can be identified. The basis of a CETS plot is Equation 5, in which the quantities determining toxicity, CBR, and BCF or their ratio ILC50 are related to the product of \( C_W \) or the LC50 and exposure time expressed as \( \Phi \). This is graphically depicted by plotting LC50 as a function of \( \Phi \) (x-axis) and \( C_W \) (y-axis), as illustrated in Figure 1; \( \Phi \) ranges from 0 to 1.0, and the corresponding times from 0 to \( \infty \). The half-time for uptake occurs when \( k_2 t \) is 0.693 and \( \Phi \) is 0.5. The locus of the intersections of the horizontal \( C_W \) and vertical \( \Phi \) values is a hyperbolic curve or boundary condition of constant LC50 and represents the combinations of water concentrations and times to death that result in 50% or another defined percentage of mortality. Increasing the toxicity has the effect of reducing the LC50 and moving the curve closer to the origin, resulting in a reduction in LC50 values or shorter times-to-death, or both.

To construct a CETS plot, the ILC50 is defined as the aqueous concentration at \( \Phi = 1 \), and the line corresponding to the hyperbolic boundary condition is established for CBR50/BCF by defining individual water concentrations (or LC50s) as ILC50/\( \Phi \). For a specified time of exposure (\( \Phi \)), the LC50 is equal to \( C_W \) at the intersection of \( \Phi \) and the CBR50/BCF or ILC50 boundary condition. Conversely, the time to death for a specified LC50 is equal to \( \Phi \) at the intersection of \( C_W \) and the ILC50 boundary condition. If both exposure time and LC50 are known, the intersection of \( C_W \) and \( \Phi \) on the ILC50 boundary condition defines the ILC50. It may be desirable to add a secondary scale giving time directly under the \( \Phi \) axis. If the test yields time-to-death data for a single or specific fish, the same relationships hold but the LC50 becomes an LC. The CBR and ILC can then be measured directly.

We emphasize that this is a simple 1-compartment model that does not treat the time-dependent distribution of the chemical within the fish, nor in its present form does it address differences between partitioning to octanol and various lipid/hydrophobic phases, especially membranes that are the probable target site of neutral narcosis toxicity (MOA1 and MOA2). The assumption that BCF is \( L \times K_{OW} \) also becomes invalid for hydrophilic chemicals that partition appreciably into aqueous phases in the fish. Our aim in the present study is only to present the concept of the CETS in the expectation that future versions may include these refinements.

**Constraints**

A first obvious constraint for an aquatic bioassay is that the LC50 or \( C_W \) must be less than the solubility of the test chemical. The area on the CETS plot where \( C_W \) is greater than the solubility may thus be identified as being out of bounds, as depicted by the red zone in Figure 1. The minimum feasible LC50 for a test chemical may be estimated as the ILC50. For a test to be feasible, \( C_W \) is thus limited to an upper limit of the solubility and a lower limit of the ILC50 under typical conditions of exposure.

A second constraint for an aquatic bioassay is that the maximum test time must be reasonable (because of feeding constraints, etc.). The area on the CETS plot where \( \Phi \) is greater than the maximum test time may be identified as being not experimentally feasible because of excessive test duration, as depicted by the blue zone in Figure 1. Only the remaining green region in Figure 1 represents a feasible combination of \( C_W \) and test duration as specified by the 2 constraints and the ILC50 boundary condition. The time (h) corresponding to each \( \Phi \) is \( -\ln(1-\Phi)/k_2 \).

In addition to these aquatic bioassay constraints, we emphasize that the domain of applicability is a 1-compartment approximation of multicompartiment organisms where all kinetics are assumed to be first order, all compartments achieve a reasonable approximation of steady state within the test duration, there is no significant biotransformation, and only 1 mode of toxic action dominates the adverse effect associated with the response endpoint being employed.

**Interpretations and implications of the CETS plot**

In the interests of simplicity, we first discuss a series of conditions assuming \( k_2 t \) to be negligible. Increasing chemical hydrophobicity then has 2 effects and results in the shrinking of the green zone for feasible test conditions (Figure 1). Specifically, if the hydrophobicity (i.e., \( K_{OW} \)) of the test chemical doubles, it is likely that solubility of the test chemical is reduced by a factor of 2 and the horizontal solubility limit line falls (i.e., the area of the red zone increases). If a test duration such as 96 h is prescribed, then for a given \( k_1 \) for the test organism, \( k_2 \) falls by a factor of 2 and \( \Phi \) is reduced by a similar factor and the vertical maximum test time decreases (i.e., the area of the blue zone increases). The ILC line also falls. The net effect is that the green zone of feasible test conditions shrinks and can approach a single point or even vanish when ILC50/\( \Phi \) is equal to the chemical’s solubility. Figure 2 illustrates the condition that corresponds to an aquatic bioassay being conducted at the solubility of the solid or liquid state chemical as appropriate, and toxicity occurs at the prescribed test time.

For some chemicals, the green zone of feasible test conditions may not include the ILC50 boundary condition, and thus it is impossible to demonstrate a toxic effect because both constraints (i.e., low solubility and excessive exposure duration) apply, as shown by the purple zone in Figure 3. The locations of the solubility and the ILC50 boundary condition are fixed by toxicity and BCF, as determined by \( K_{OW} \) and lipid content \( L \), respectively. Thus, the only option for conducting a
successful bioassay is to create a green zone by employing a smaller organism with larger values of $k_1$ and $k_2$, the ratio of which (BCF) is constant and dictated by $L$ and $K_{OW}$. This effectively moves the ILC50 boundary condition to the left and thus closer to the origin.

An obvious strategy when designing an aquatic toxicity test is to compile a CETS diagram similar to Figure 1 but for the intended organism and chemical of interest and determine the extent of the green zone, especially if it includes the ILC50 line, assuming first that the chemical is only a baseline toxicant (MOA1 or MOA2). If a chemical is believed to have an additional MOA, then the CBR and ILC50 can be reduced by the toxic ratio.

Chemicals with no green zone are likely to be hydrophobic, with low solubilities in water and low values of $k_2$, necessitating long exposure times to achieve appreciable body burdens. Chemicals of this type include highly hydrophobic substances such as long-chain alkanes, chlorinated alkanes or paraffins, cyclic and linear permethy siloxanes, and dyes. These constraints may also apply to certain highly hydrophobic aromatics such as PCBs, dioxins, and brominated fire retardants, but these substances may display other modes of toxic action. Experimental determination of the aquatic toxicity of these hydrophobic substances is thus fraught with difficulties relating to the necessarily low aqueous concentrations, possible losses by evaporation, decreased bioavailability in the water phase caused by the inevitable presence of organic matter, and excessively long exposure times, which prevent the organisms from being maintained in reasonable condition. To achieve a toxic endpoint, it may be necessary to feed the organisms with a diet of contaminated food similar in principle to that suggested in the modified OECD aqueous and dietary exposure bioaccumulation test (OECD guideline 305 [6]). There are obvious economic, scientific, animal usage, and regulatory incentives to determine in advance the existence and area of the green zone, thus avoiding nonproductive tests.

Although it is not considered in any detail in the present study, the domain of applicability of this model is not limited to hydrophobic chemicals. An examination of hydrophilic chemicals (log $K_{OW} < 1$) is likely to suggest that standard fixed-duration exposure testing such as 96 h or 24 h may be excessively long for some chemicals and organisms. This is consistent with the long-standing advice of Sprague [12,13] to conduct and report aquatic toxicity tests to incipient, threshold, or steady state rather than at fixed exposure durations.

When biotransformation occurs and $k_M$ contributes to the overall chemical elimination rate constant and the metabolites are relatively nontoxic, this increases the total elimination rate constant ($k_2 + k_M$), reducing the BCF, increasing the ILC50, and reducing $\Phi$ and the half-time for uptake. If biotransformation rate constant estimates are available (e.g., from in vivo, in vitro, or in silico methods), the estimates should be included to better characterize the range of experimental conditions.

**Logarithmic version**

The curvature of the LC50 boundary condition in Figures 1–3 can be eliminated by taking logarithms of the $x$ and $y$ parameters in Equation 5, yielding Equation 6:

$$\log(CBR50/BCF) = \log(ILC50) = \log(LC50) + \log(\Phi) \quad (6)$$

This linearizes the LC50 boundary condition on the CETS plot, as shown in Figure 4. An attractive option is to draw this diagram with a series of parallel LC50 lines of slope −1 representing different CBRs or BCFs. The ILC50 can be determined by extrapolating the boundary condition to $\Phi$ of 1.0 or correspondingly to $\log(\Phi)$ of 0.

**ILLUSTRATION**

To demonstrate the use and value of the CETS plot, we reproduce the bioassay conditions described by van der Heijden et al. [16], who have reported a set of high-quality toxicity tests for 6 organic chemicals conducted under carefully controlled conditions by using passive dosing from a polymer phase to establish and maintain constant water concentrations. In the present study we focus on their results for the guppy (Poecilia reticulata), with approximately 10 mg to 110 mg weight range, approximately 5% whole body lipid, and approximate temperature of 20 °C. The chemicals, properties and selected test results are given in Table 1, namely, 1,2,4-trichlorobenzene, 1,2,3,4-
tetrachlorobenzene, pentylenzene (QCB), 2,3,4-trichloroaniline (2,3,4-TCA), 2,3,5,6-tetrachloroaniline (2,3,5,6-TeCA), and 4-chloro-3-methylphenol (4-Cl-3-MP). Biotransformation rates are assumed to be negligible in these calculations. The solubility and $K_{OW}$ values used in the present study were obtained from a handbook by Mackay et al. [17], except for 2,3,4-TCA and 2,3,5,6-TeCA, which were obtained from EPI SuiteTM [18]. They are slightly different from the solubility values reported by van der Heijden et al. [16], which were calculated using SPARC software [19]. Table 1 also contains chemical properties including estimated incipient lethal concentrations (ILCs) and incipient lethal activities (ILAs).

Three water concentrations were prescribed (low, medium, and high) at levels corresponding to chemical activities (ratios of concentration to water solubility of the liquid state chemical) of approximately 10% to 50%, and thus toxicity was inevitable. The experimental technique was to measure the time-to-death of the fish at the 3 exposure levels at 20°C. The exposure regimes used by van der Heijden et al. [16] corresponded to moving the test condition point along the horizontal concentration line of a CETS plot until it reached the ILC boundary condition, at which time death occurred. These times-to-death and resulting CBRs for individual fish were measured [16].

For a specific chemical with a known $K_{OW}$, the BCF can be estimated as the product of lipid content (5%) and $K_{OW}$ which is also $k_1/k_2$. At the time of death ($t$), the fish is undersaturated with chemical; that is, $C_B < C_W \times BCF$ and the fraction of equilibrium $\Phi$ is $CBR/(C_W \times BCF)$. The ILC is, by definition, equal to both CBR/BCF and $C_W/\Phi$. The loss rate constant $k_2$ can then be calculated as $-\ln(1-\Phi)/t$ and $k_1$ as $k_2 \times BCF$. A $k_1$ can then be calculated for each data point and an average deduced for each of the low, medium, and high concentration data sets. Average values of CBR, $k_1$, $k_2$, and ILC are calculated and are given in Table 1. An ILA is also calculated as $ILC/S_W$ where $S_W$ is the chemical’s (liquid or subcooled) solubility in water (mol m$^{-3}$). In practice, it is preferable to give greatest weight to points for which $\Phi$ is relatively small (i.e., <0.2). Under these conditions, most distant from equilibrium, $k_1$ can also be estimated as approximately $CBR/(C_W \times t)$. It is expected that the chemical-to-chemical variation in ILA is smaller than that of the ILC. The reason is that ILCs are CBR/BCF and can vary greatly because of differences in BCF as influenced by $K_{OW}$. The ILC is thus not a good metric for comparing relative toxicity of different chemicals, the ILA being a better metric. This is because ILA is $ILC/S_W$ and is thus CBR/BCF or $CBR/(C_B \times S_W)$. The group $K_{OW} \times S_W$ is an estimate of the solubility of the chemical in octanol, which, according to the general solubility equation of Ran and Yalkowsky [20], is fairly constant and approximately 3000 mol m$^{-3}$. Because $K_{OW} \times S_W$ is fairly constant, ILA is proportional to the lipid normalized CBR.

As CBR is also expected to be fairly constant for a group of chemicals with similar modes of toxic action; therefore, ILA should also be fairly constant. This constancy in CBR is evident for the entire group, but not for the ILA of 4-Cl-3-MP, which should also be fairly constant. This constancy in CBR is evident from 1900 mol m$^{-3}$ to 2800 mol m$^{-3}$ for the first 5 chemicals, but a much larger value of 91,700 mol m$^{-3}$ apparently applies to 4-Cl-3-MP because of the high solubility in water. van der Heijden et al. [16] give a lower value of $S_W$ by a factor of 2, which still gives a very high value of $S_O$. This high solubility in water is reflected in a very low ILA. Ionization is not the cause

due to chemical exposure toxicity space (CETS) model

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**Table 1.** Chemicals tested by van der Heijden et al. [16]: 1,2,4-TCB, 1,2,3,4-TeCB, QCB, 2,3,4-TCA, 2,3,5,6-TeCA, and 4-Cl-3-MP$^a$

| Property                  | 1,2,4-TCB | 1,2,3,4-TeCB | QCB | 2,3,4-TCA | 2,3,5,6-TeCA | 4-Cl-3-MP |
|--------------------------|-----------|--------------|-----|-----------|--------------|-----------|
| $\log K_{OW}$            | 4.1       | 4.5          | 4.9 | 3.33      | 4.10         | 3.10      |
| $S_W$ (liquid state, mol m$^{-3}$) | 0.22       | 0.06         | 0.026 | 0.95      | 0.17         | 72.83     |
| $S_O = K_{OW} \times S_W$ | 2775      | 1897         | 2065 | 2031      | 2140         | 91 700    |
| BCF = $L \times K_{OW}$  | 629.5     | 1 581        | 3972 | 106.9     | 629.45       | 62.95     |
| CBR (mmol/kg)            | 8.76 (4.9–18.3) | 8.36 (7.0–31.9) | 16.1 (9.8–22.9) | 5.59 (1.7–8.2) | 2.14 (0.6–2.8) | 4.96 (1.4–5.8) |
| $k_1 = k_2 \times BCF$ (h$^{-1}$) | 36.37 (24.1–57.9) | 62.72 (40.9–132.1) | 73.04 (39.2–97.7) | 31.19 (3.9–80.7) | 34.23 (10.0–70.2) | 7.47 (1.0–17.2) |
| $k_2 = \ln(1-\Phi)/t$ (h$^{-1}$) | 0.0587 | 0.0397 | 0.0184 | 0.291 | 0.0544 | 0.119 |
| $I_1$ = $k_1 \times BCF$ (mol m$^{-3}$) | (0.04–0.09) | (0.03–0.08) | (0.01–0.02) | (0.04–0.76) | (0.02–0.11) | (0.02–0.27) |
| ILC = $CBR/BCF$           | 0.014     | 0.0055       | 0.0042 | 0.049 | 0.0332 | 0.066 |
| IIA = $ILC/S_W$ (unitless) | (0.008–0.03) | (0.004–0.007) | (0.002–0.006) | (0.016–0.077) | (0.001–0.004) | (0.022–0.093) |

$^a$Values for $\log K_{OW}$ and $S_W$ were taken from Mackay et al. [17] if available; otherwise EPISuiteTM [18] was used. Values for lipid content (L) and CBR are from van der Heijden et al. [16]. Values for $k_1$, $k_2$, ILC, and ILA were calculated for each guppy trial reported by van der Heijden et al. [16], and then an average value was calculated for each chemical (including low, medium, and high concentrations) and reported in this table. Ranges for values are in parentheses.

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*The chemical exposure toxicity space (CETS) model*.
of this high solubility because the dissociation constant exceeds the experimental pH. Because 4-Cl-3-MP is more polar than the other chemicals in Table 1, this and physical and biological degradation issues may influence solubility estimation. It is believed that the chemical activity coefficient in water estimated from the solubility is not equal to the activity coefficient at dilute conditions as exist during the test and during measurements of $K_{OW}$. A full discussion of the reasons for this apparently anomalous behavior is beyond the scope of the present study; but for less hydrophobic substances such as 4-Cl-3-MP, care must then be taken when interpreting CBR, ILC, and ILA as predictive metrics of toxicity.

**Single-chemical CETS plot**

Figure 5 gives the CETS plot for 1,2,4-trichlorobenzene. The solubility limit is depicted, and the 3 horizontal dashed lines correspond to the low, medium, and high exposure concentrations. As the test exposure time increases, the point corresponding to the exposure conditions moves along the horizontal line and approaches the blue ILC/Φ line (in this case, ILC is calculated to be 0.014 mmol/L), and death occurs close to the corresponding time (Φ). Because of natural variability in fish sensitivity and uptake rates, the times-to-death vary as designated by the data points. The short vertical lines represent the average values of Φ for each test. To illustrate the sensitivity of the ILC line to Φ, the gray and orange hyperbolic lines are estimates corresponding to approximate factors of 0.5 and 1.5 in the range of measured Φ. Figure 6 gives the same data in a logarithmic plot. Clearly, the model and diagram are successfully describing the general onset of toxicity as a function of time and concentration. The logarithmic plot has the advantage that the data can be extrapolated to a value of Φ of 1.0 to give an estimate of the log ILC, namely, 1.82 mmol/L in this case.

**Multiple-chemical CETS plots**

It is instructive to include in Figure 7 data for all the chemicals tested in a common CETS plot of the type shown in Figure 5. Clearly the chemicals differ considerably in lethal concentrations and times-to-death. The question then arises as to the causes of these differences. The ILC/Φ lines differ because the BCFs differ, being dependent on $K_{OW}$. Clearly, the 2 most polar chemicals corresponding to the upper lines, 2,3,4-trichloroaniline and 4-chloro-5-methylphenol, are behaving differently from the more hydrophobic chemicals because of the high solubility and different affinities for the assumed target site; that is, $K_{OW}$ may not equal the membrane–water partition coefficient, and thus different CBRs and modes of toxic action may apply. These illustrations also ignore any influence of biotransformation.

**Interpretation using chemical activity**

Insight into differences in chemical toxicity can be obtained by converting the concentrations to chemical activity, as discussed recently by Thomas et al. [21]. Concentrations of organic chemicals in water are readily converted to chemical activities by dividing by the appropriate liquid state water solubility, rendering the absolute values dimensionless. This conversion affects the y-axis concentrations on CETS plots, and the ILC becomes an ILA. The x-axis times and values of Φ are unaffected. The data from the CETS plot in Figure 7 are shown in a CETS activity plot in Figure 8 on a linear activity basis and in Figure 9 on a logarithmic activity basis.

Figure 9 provides a convenient method of obtaining the log ILA for each chemical (which has a common CBR) as the extrapolated value of activity at Φ equal to 1.0 or log Φ equal to 0. The upper lines suggest a log ILA ranging from –0.71 to –1.03 (ILA range, 0.195–0.093) for all chemicals but lower values of –1.72 (ILA, 0.019) for the chloroaniline and –3.04 (ILA, 0.00091) for the chlorophenol. These activities are within the expected range for baseline narcotics. The chloroaniline and the chlorophenol have lower lethal activities of 0.019 and 0.0009, respectively, because of the high solubilities. van der Heijden et al. [16] have discussed possible reasons for ambiguity in the relationship between CBR and time-to-death in cases of high exposure concentrations. The higher log ILA for QC8 of approximately 0.2 may be suspect because it is the most hydrophobic, and has the longest uptake half-life and time-to-death requiring considerable extrapolation of Φ.
DISCUSSION

The pictorial CETS plot clearly defines the concentration and exposure times required to achieve a toxic endpoint. Unrealistic conditions such as concentrations exceeding the water solubility and excessive test times become immediately apparent. The use of activity also shows the solubility limit directly. The plots can help identify cases in which it is difficult or even impossible to achieve a toxic endpoint. This is most likely for very hydrophobic substances that have low solubility limits and long uptake half-times. Particularly difficult are symmetrical hydrophobic substances such as hexachlorobenzene and anthracene that have low solid-state solubilities because of their high melting points. A useful feature of the CETS plots is that they clearly identify potential experimental difficulties in testing hydrophobic chemicals by aqueous respiratory exposure.

The CETS approach is particularly appropriate for assessing time-to-death data obtained at constant water concentrations, such as those obtained in the tests designed by van der Heijden et al. [16]. Tests of this type are regarded as models for future bioassays. The nature of the increasing exposure with time is readily visualized from the plots, as is the onset of time-to-death. In the van der Heijden study [16], 2 other organisms were tested (Lumbriculus variegatus and Hyalella azteca), and similar CETS plots can be obtained but are not addressed in the present study.

The CETS plot example used in the present study employed experimental data for organism properties including CBRs, organism size, time-to-death, and lipid contents; in the absence of these data, however, approximate values can be used, such as typical CBR ranges for a given MOA. This also applies to chemicals that have less well-documented physical–chemical properties, in which case approximate or estimated values can be used for $K_{OW}$ or $S_{W}$. In such cases, when using approximate values to construct the CETS plot, the results may be less accurate; however, even an approximate model can help in developing successful test conditions.

Converting the aqueous concentrations to chemical activities can provide additional insights into the test results. Chemicals with similar modes of toxic action will group together. However, general confounding influences, including bioavailability, metabolic degradation, and different modes of toxic action, must be considered. The plots thus have the potential to discriminate differences in mode of action that are not apparent from LC50 data. The magnitude of the differences between modes of action can be regarded as a toxic ratio, as discussed by Meader et al. [22]. These ratios can be read directly from the logarithmic activity plots analogous to Figure 9. Toxicity tests are feasible only if $ILA50/\Phi < 1.0$ or $ILA50 < \Phi$. The likely value of $ILA50$ can be estimated for baseline narcotics as 0.01 to 0.03 [21]. The implication is that $\Phi$ must exceed this range for a successful test. The corresponding range of exposure times can be estimated and compared with feasible values.

The general conclusion is that as hydrophobicity increases, testing aquatic toxicity by the respiratory route becomes more difficult until it ultimately becomes impossible, especially if it is not biotransformed. Other routes of exposure by diet are then necessary. More rigorous evaluation of the differences in toxicity of these and other chemicals requires careful consideration of solubilities, lipid contents, biotransformation rate constants, fish-specific values of $k_1$ and $k_2$, $K_{OW}$, and partition coefficients that are only approximately related to $K_{OW}$. The most promising and rigorous approach for detailed interpretation of toxicity is to follow the approach of van der Heijden et al. [16] and seek data on the partition coefficients of the chemical to the various sites of accumulation including the target site of the toxic action using experimental data or

**Figure 7.** Log chemical exposure toxicity space (CETS) concentration plots for the guppy and all chemicals tested by van der Heijden et al. [16]. $C_w =$ dissolved concentration in the water.

**Figure 8.** Linear chemical exposure toxicity space (CETS) activity plot for the guppy and all chemicals tested by van der Heijden et al. [16].

**Figure 9.** Logarithmic chemical exposure toxicity space (CETS) activity plot for the van der Heijden et al. [16] data for the guppy and all chemicals. The 2 most polar chemical are now the 2 lower lines.
predictive methods such as Abraham-type polyparameter linear free energy relationship or by fundamental quantum chemical-based estimation using approaches such as COSMO-RS. Our primary purpose in the present study has been to present the concept of the CETS plot as a step toward evaluation of chemical-specific toxicity. The implications for effective and economic testing and minimizing animal usage are obvious.

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3668.

**Acknowledgment**—Financial support was kindly provided by Dow Corning, DMER, and a Natural Sciences and Engineering Research Council (NSERC) Discovery Grant to J.M. Parnis. J.A. Arnot acknowledges financial support from the European Chemical Industry Council (CEFIC) LRI ECO16 Project: Critical Body Residue Validation for Aquatic Organisms Exposed to Chemicals Causing Toxicity by Baseline Narcosis. Open access publication of this paper was provided by the Silicones and Class 2 (polar narcosis) type compounds based on chemical descriptors. J Chemometrics 10:149–162.

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