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Data quality and relevance in ecotoxicity: The undocumented influences of model assumptions and modifying factors on aquatic toxicity dose metrics

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ABSTRACT

A model-based approach using hypothetical organic chemicals examines how aquatic toxicity test results are influenced by toxicity modifying factors such as hydrophobicity, exposure duration, body size, lipid content, mode of toxic action (via Critical Body Residue differences), and metabolic degradation. Differences of up to one to three orders of magnitude were identified for modeled LC50s. Dominance of CBR by low log Kow chemicals can cause further influences. Such differences cause significant changes in the relationship between exposure- and organism-based doses and create substantial difficulties for both interpretation of test results and extrapolation to other laboratory or field exposure conditions. The resulting variability is not readily evident in toxicity testing as insufficient data are collected to validate fundamental assumptions. Consequently, results obtained with standard aquatic toxicity test protocols do not yield consistent, comparable measures of relative toxicity and are inappropriate for quantitative toxicology and risk applications. The substantial uncertainties in testing results created by such undocumented variability must also be given serious consideration in data quality and relevance assessments. Necessary improvements in aquatic toxicity testing methodology should include explicit estimation of toxicokinetics and toxicodynamics and routine validation of toxicological model assumptions.

1. Introduction

The 96 h LC50 aquatic test is a common example of widely employed environmental toxicity tests characterized by the use of dose metrics based on exposure media concentrations, various fixed length exposure durations, a variety of effect endpoints, and a diversity of test organisms. Although the methodology continues to be updated, conceptual designs have changed little since inception. The key assumption is that toxicity metrics based on exposure media concentrations are adequate surrogates for the unmeasured critical body residue (CBR) of a test substance. CBRs in turn are assumed to be adequate surrogates for the unknown concentration of test substance at organism site(s) of toxic action causing the adverse effect(s) in question. In other words, the ratio of LC50:LR50:Target Site(s) is assumed to be constant. A further simplifying assumption is that a single mode of toxic action is primarily responsible for the selected adverse effect endpoint.

The relative merits of the 96 h LC50 and LR50 (lethal CBR associated with 50% mortality) as toxicity metrics were previously evaluated using a simple toxicokinetics model to link water-based exposures to whole-body wet weight concentrations (Mackay et al., 2014). Three conclusions were reached regarding variability in external-internal dose surrogate relationships (i.e., LC50-LR50 ratios).

Firstly, increasing hydrophobicity increases elimination half-life (T½) and increases the time necessary to reach steady-state (SS). Thus, for more hydrophobic chemicals, a 96 h exposure can be inadequate to reach the effective LR50 levels and, to achieve organism residues sufficient to cause 50% mortality in 96 h exposures, water concentrations must be elevated.

Secondly, the other modifying factors examined – exposure duration, body size, lipid content, mode of toxic action (MoA), and metabolic biodegradation – can produce alterations in LC50-LR50 relationships beyond that attributed to hydrophobicity alone.

Thirdly, for chemicals in the log Kow range <~1, high concentrations in the organism water phase can dominate the whole-organism CBR. Although the toxicity target site(s) for baseline neutral narcosis are hydrophobic in character the bulk of a

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hydrophilic chemical is in the organism water phase, with lower concentrations in the lipid phase, the opposite of the case for hydrophobic chemicals.

The objective of this paper is to further examine these commonly undocumented issues by estimating influences over plausible ranges of the modifying factors. This will aid in estimating the magnitude of alterations in dose surrogate relationships and the consequences for toxicity test data quality and relevance. Typically employed toxicity testing protocols are "black boxes" models, largely eschewing organism-specific toxicological information, other than adverse organism responses, that might be used better define and validate key model assumptions. Hence, a model-based approach remains necessary.

2. Materials and methods

2.1. Model details

The same one-compartment, first-order model equations, abbreviations, and initial parameters used previously were employed in a spreadsheet-based model (see Mackay et al., 2014 for details). Two hypothetical chemicals (log Kow −2, and −1, molecular weight 100) were added to better examine the issue of hydrophilicity. Organism water phase concentrations of hydrophilic chemicals begin to dominate CBRs starting at ~log Kow 1.3 and increasingly overwhelm the influence of the body lipid contributions to the point that CBR levels become close to exposure water concentrations (McCarty et al., 1992).

Thus, when examining hydrophilic chemicals, the contribution of the organism water phase must be considered. An adjustment factor was included to approximate the whole-body concentration across the entire log Kow range as a function of both lipid and water phase content. Where C_F is organism concentration. C_W is exposure water concentration, and BCF is the bioconcentration factor, at a body lipid fraction of 0.05 the steady-state whole body residue C_f (i.e., SS-LR50) associated with the SS-LC50 is the sum of lipid and water phase concentrations which are 5% and 95% of the organism:

$$C_F = C_W \cdot (\text{Lipid Fraction} \cdot \text{Kow}) + C_W \cdot (1 - \text{Lipid Fraction})$$
$$= C_W \cdot \text{BCF} + C_W \cdot 0.95$$

Chemical bioavailability also affects bioconcentration (e.g., Arnot and Gobas, 2004). Although 100% bioavailability has been assumed, binding of chemicals to dissolved and particulate organic matter in both lab exposure media and field situations could lower bioavailability, contributing additional variability.

2.2. Modeling scenarios

Although 96 h exposures are common, other durations may be employed. To examine the influence of exposure time 24, 48, 96, 168 h exposures were modeled. The range of organism sizes employed — 0.003, 0.3, 3 and 300 g — approximates that found for life stages (larva/fry, juveniles, and adults) of various freshwater fish species used in testing. Organism density is assumed to be 1.0 and differing sizes were allometrically scaled (fish mass$^{0.65}$ in the respiration component of k_1 (uptake rate coefficient) estimation from the 3 g fish (Mackay et al., 2014) without specific adjustments for anatomy, physiology, or metabolism. The range of whole body lipid content selected — 0.01, 0.05, 0.1, and 0.15 — is within that seen in small fish (e.g., Lassiter and Hallam, 1990). Details of the composition of neutral lipid and phospholipid components, the ratio of storage lipids to target site hydrophobic phase, and whether the site(s) of toxic action are other than hydrophobic, narcotic-like in character, were not considered.

The whole-body CBR differs with mode of toxic action, varying by ~10^5 times between acute baseline neutral narcosis and TCDD-dioxin toxicity in small aquatic organisms (McCarty and Mackay, 1993). The LR50 values chosen — 5, 0.5, 0.005, 0.00005 mmol/kg — provide a broad range for consideration. Toxicity target site differences and other mode-specific differences have not been considered.

Metabolic degradation studies for aquatic organisms continue to increase in extent and sophistication. Metabolic degradation rates (k_M) of 0, 0.001, 0.01, 0.1 h^{-1} were summed with the basic elimination rate coefficient, k_2, to obtain a total elimination estimate, k_T. The nonzero k_M values correspond to estimates of low, medium and high metabolic degradation rates (Arnot et al., 2008a,b). Complications, such as non-first-order elimination and multiple degradation products with varying elimination rates and/or differing modes of toxic action/toxicodynamics, were not modeled.

Finally, although a number of parameters have been defined to allow modeling, the objective is to illustrate the general nature and extent of modifying factors in the context of the conceptual model underlying standard toxicity testing, rather than precise quantification or exact model parameterization.

3. Results

Basic toxicokinetic information is presented in the A graph in the figures. Approach to steady-state is controlled by the half-life of elimination, T½ (h), with four half-lives being a common approximation of time to steady-state. The fraction of steady-state achieved, Fss, allows quantification of deviations from steady-state. Exposure water and organism concentrations for fixed duration exposures, usually 96 h LC50s, are presented in the B graphs, along with steady-state results. For the MoA CBR and Metabolism figures SS-LC50 data are plotted on the C graphs as combining them with the 96 h LC50 results compromises readability. The base modeling case is an 96 h LC50 for a 3 g fish of 5% body lipid content exposed to a chemical with a baseline neutral narcosis MoA and negligible metabolic biodegradation. Base case results are presented first in the figure legends and, when results overlap in a graph, the base case dominates.

3.1. Exposure duration

For 24 through 168 h exposures T½ values for the 11 chemicals examined range from 0.089 to 106,000 h (Fig. 1A). Fss is 1.0 until a log Kow of ~4 when fixed duration exposures become increasingly insufficient to achieve steady-state. Lower hydrophobicity chemicals achieve the expected SS-LR50 of 5 mmol/kg for 24 through 168 h exposures. However, starting at log Kow ~4, increasingly lower organism levels occur with shorter exposures and toxicity is unlikely as CBRs are increasingly <5 mmol/kg.

Fig. 1B shows the exposure water concentrations necessary to achieve an LR50 of 5 mmol/kg for various exposure durations. For the base modeling case (3 g, 0.05 lipid, k_M = 0, 96 h exposure) hydrophobicity alone strongly influences 96 h LC50s. For chemicals G, H, I, J, K (log Kow 4, 5, 6, 7, 8) the differences between 96 h and SS-LC50s are 1.0, 2.2, 17, 160, 1600 times, respectively. Starting at log Kow ~4, the LC50 estimates for 24 and 48 h exposures are higher than that for 96 h, while results for 168 h are lower. Thus, the extent of the influence of the exposure durations examined, from 24 to 168 h, for chemicals G, H, I, J. K (log Kow 4, 5, 6, 7, 8, respectively), is 1.3, 4.6, 6.7, 7.0, 7.0 times, respectively. The differences between fixed and steady-state exposures seen in Fig. 1B are due to a combination of hydrophobicity and exposure duration, with the latter having a lesser influence. For longer exposure times, such as 21 day survival tests (i.e., 504 h LC50), the difference between 12 h and 504 h results is 18 times.