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An LC_{50} vs Time Model for the Aquatic Toxicity of Reactive and Receptor-Mediated Compounds. Consequences for Bioconcentration Kinetics and Risk Assessment

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For aquatic toxicants that act by so-called nonpolar narcosis, it is generally acknowledged that the Critical Body Residue (CBR) at death, as a surrogate dose metric for the amount of target that has interacted with the toxicant, is constant. This constancy is not only maintained across exposure times but also across different (narcosis) compounds as well as species. We present here an alternative model, applicable to reactive and receptor-mediated toxicants, that implies that for these compounds there is *no* constant CBR. The model also shows that for each single species-compound combination, the Critical Area Under the Curve (CAUC) is constant and independent of exposure time. These findings can have profound consequences for the interpretation of experimental toxicity data (such as 96 h LC_{50} values) in risk assessment. Among other things, it shows us that for compounds other than nonpolar narcotics, LC_{50} vs time values may decrease significantly even after bioconcentration steady state has been achieved. Consequently, it also shows us that the incipient LC_{50} will be severely overestimated (i.e. toxicity underestimated) when using the familiar models based on just bioaccumulation kinetics.

Introduction

Xenobiotic chemicals that are present as aqueous contaminants may present a toxicological hazard to fish. In order for that to happen, the aqueous contaminant has to be taken up by the fish, generally, but not necessarily exclusively, via (passive) diffusion over the gill membrane and subsequently has to be distributed over the animal's body, to reach certain target organs or structures, either via passive convective and/or diffusive transport or via active transport (*1*, *2*), and there attain a sufficient level for toxic impact. This results in the phenomenon that the actual concentration of a compound at the (perceived) target structure is not instantaneously related to the external concentration but usually displays a behavior that can be described as a function of the external concentration and a negative exponential of time (*3*)

$$C_{int}(t) = \mathrm{BCF} \times C_w \times (1 - e^{-k_2 t}) \quad (1)$$

with C_{int} = the internal concentration in the organism in mol kg^{-1}, C_w = the external, aqueous concentration in mol L^{-1}, assumed for the moment to be constant, BCF = the bioconcentration factor of the xenobiotic of interest for the organism in L kg^{-1}, and k_2 = the first-order elimination constant of the compound for the organism in d^{-1}. Equation 1 assumes a system that can be described by first-order/one-compartment kinetics; this suffices for the sake of the argument, since the actual comparison of the "classical" model and the alternative model that we present in this paper holds, *mutatis mutandis*, for higher order and/or multiple compartment kinetics as well.

Equation 1 implies that only at infinite time, when the exponential part of the function has acquired constancy, there is a one-to-one relationship between external exposure concentration and internal target concentration, the so-called bioconcentration factor. In real world situations, a certain time limit, smaller than infinity, can be agreed upon, at which the internal concentration is sufficiently close to the final concentration (which is an asymptote) to state that for all practical purposes a steady state has been attained. Usually T_{95}, which is the time at which C_{int} reaches 95% of $C_{int}(\infty)$, is taken for this. T_{95} can be calculated as follows (*4*):

$$T_{95} = \frac{3}{k_2} \quad (2)$$

Feijtel and co-workers (*4*) used this T_{95} criterion, together with a quantitative structure−activity relationship (QSAR), as proposed by the Organization for Economic Co-operation and Development OECD (5)

$$\log k_2 = -0.414 \times \log K_{ow} + 1.47 \quad (3)$$

where k_2 is expressed in d^{-1}, to predict that for nonmetabolized toxicants having a log K_{ow} (log-transformed octanol−water partition coefficient) of up to 3.8 a continuous exposure to a constant aquatic concentration of 4 days (96 h) should suffice to attain an equilibrium concentration in small fish (similar to e.g. *Pimephales promelas*) and smaller aquatic organisms. Of course there is a number of reasons why for individual compounds eq 2 might not be valid, such as enhanced elimination, metabolism, direct reactivity, or specific receptor action.

As described by McCarty (*6*, *7*) and by van Hoogen and Opperhuizen (*8*), for so-called narcosis-type toxicants (*9*, *10*), the internal concentration at death (also called Lethal Body Burden, or Critical Body Residue) is assumed to be constant. Kooijman (*11*) implicitly used this concept to describe LC_{50} as a function of time and incipient LC_{50}, or $LC_{50}(\infty)$

$$LC_{50}(t) = \frac{LC_{50}(\infty)}{1 - e^{-k_2 t}} \quad (4)$$

with LC_{50} in mol L^{-1} and k_2 in d^{-1}. It can easily be seen that this implies that for compounds for which this relationship is valid, the LC_{50} at T_{95} is 1.0524 times the incipient LC_{50}. Equation 3 then tells us that for appropriate compounds with a log K_{ow} < 3.8 (T_{95} < 91 h), a 96-h exposure should result in an LC_{50} determination that for all practical purposes

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represents the incipient LC_{50} value. More generally, eq 4 implies that whenever a stationary LC_{50} is attained, the organism under study has also reached a steady state in bioconcentration kinetics. It should be noted here that McCarty and Mackay have previously mentioned that compounds with nonreversible mechanisms of action may not adhere to the Critical Body Residue concept, hence the internal concentration corresponding to a toxic effect level will not be constant, nor will it serve as a good dose metric (*3*).

Recently Tyle and co-workers (*12*) suggested that the reciprocal relationship, namely that a nonstationary LC_{50} value implies that a steady-state bioconcentration has not yet been attained, should also hold. To use this reciprocal relationship for testing the relationship between a compound's log K_{ow} and its T_{95}, they screened the so-called Fathead Minnow Database, as compiled by Geiger and coworkers (*13*) at the U.S.-EPA Environmental Research Laboratory (currently U.S.-EPA Mid-Continent division) in Duluth, MN. They specifically targeted compounds having an LC_{50} (72 h) that was significantly higher than the LC_{50} (96 h). Out of a total of 647 substances, 46 compounds were identified as having a nonstationary LC_{50} value after 96 h. Of these 46, 40 were found to have a log K_{ow} of less than 3.8, as predicted by the KOWWIN computer program (*14*). More importantly, this finding suggested that the concept of steady-state bioconcentration and resultant effects is not universal, thereby supporting the need for development of a theoretical framework that can account for non-steady-state based effects.

Our paper presents such a framework by clearly demonstrating that there is no generally valid reverse relationship between a stationary LC_{50} value and the attainment of bioconcentration steady state.

Methods

Theoretical calculations were done with the assistance of Theorist for Macintosh software version 1.51 (*15*). Calculation of LC_{50} values for selected compounds as well as nonlinear regression fitting of these LC_{50} values vs time was performed with Prism software for Macintosh, version 2.0, GraphPad Software, San Diego, CA.

Theory

Verhaar and co-workers (*10*, see also refs *16* or *17*) provisionally classified the organic aquatic toxicants into four basic classes, namely nonspecific narcotics, polar narcotics, reactive chemicals, and specifically-acting (or receptor-mediated) toxicants. As already mentioned above, for narcosis-type, or class 1 chemicals, it is assumed that the Critical Body Residue (CBR) for a toxic effect such as lethality or growth inhibition is a constant, independent of either compound or exposure time. This corresponds to a pharmacological model where C_{int}, or the instantaneous (peak) concentration at the target site, is the dose metric (see e.g. ref *11*). If we rewrite eq 1 in terms of CBR, we get

$$\mathrm{CBR} = \mathrm{BCF} \times C_w \times (1 - e^{-k_2 t}) \equiv \text{constant} \qquad (5)$$

and, realizing that in situations where the external exposure concentration is kept constant throughout the exposure it holds that $C_w = LC_{50}(t)$ or more generally $EC_{50}(t)$

$$EC_{50}(t) = \frac{\mathrm{CBR}}{\mathrm{BCF}} \frac{1}{(1 - e^{-k_2 t})} \qquad (6)$$

which basically is a rewrite of eq 4.

There are however many toxicants that act by other modes of action. When confining ourselves to the field of aquatic toxicology, this includes all reactive (class 3) and specific (class 4) toxicants. These modes of action are either receptor mediated (class 4) or involve a direct chemical reaction with a biological substrate (macromolecule). It is a well-known concept in pharmacology that for compounds (both toxicants and drugs) that act through a reaction with a substrate, the Area Under the Curve (AUC; the integral over time of the internal (target) concentration) is a more appropriate dose metric (*18*). This can be understood by realizing that from a pharmacokinetics point of view, the irreversible reaction of a compound with a (any) target can be described as a clearance. Simply put, this means that it is not the concentration of the agent at the target site that is directly related to the magnitude of the effect but the "concentration" of the affected target structure. The concentration of the affected target can be modeled as follows

$$\frac{dC_{\text{affected target}}}{dt} = k_a \times C_{\text{target}} \times C_{\text{agent}} - k_d \times C_{\text{affected target}} \qquad (7)$$

or, assuming irreversible interaction

$$C_{\text{affected target}} = k_a \times C_{\text{target}} \times \int_0^t C_{\text{agent}} dt \qquad (8)$$

If we, analogous to the CBR rationale, assume that there is a certain $C_{\text{affected target}}$ at which a certain effect manifests itself, we can easily see from eq 8 that it is *not* the internal concentration (C_{agent}) that is constant, but the time integral of C_{agent}, or Critical Area Under the Curve (CAUC). Since we know from eq 1 how to relate the internal concentration to the external (aqueous) concentration C_w and time, we can see that CAUC can be written as

$$\mathrm{CAUC} = \int_0^t \mathrm{BCF} \times C_w \times (1 - e^{-k_2 t}) dt \equiv \text{constant} \qquad (9)$$

with CAUC in mol d kg^{-1}. In this case, the Critical Body Residue, or the "threshold" concentration of compound at the toxicological target, is dependent on the exposure time. The dependence of EC_{50} on time is then described by the following function:

$$EC_{50}(t) = \frac{\mathrm{CAUC}}{\mathrm{BCF}} \times \frac{1}{t - (1 - e^{-k_2 t})/k_2} \qquad (10)$$

Interpretation. It is appropriate to note that both descriptions of the dependence of LC_{50} on time (C_{int} and CAUC) are limiting cases. The situation where C_{int} is the dose metric describes what happens when the interaction of a compound with its target is instantaneous and completely and instantaneously reversible. Since nonpolar narcosis is hypothesized to be due to the physical "interaction" of a compound with a (nerve) cell membrane, i.e., the partitioning of the compound between the surrounding aqueous phase and the cell membrane (*19*), it is easy to see that the assumptions for C_{int} as dose metric will hold well enough.

The other extreme, where CAUC, or $\int C_{int} dt$ is the dose metric, applies when the interaction of the compound with its target, be it a chemical reaction with a macromolecule or a receptor interaction, is instantaneous and completely *irreversible*. This can be interpreted as a compound interacting with either a receptor molecule or a macromolecule, thereby depleting a percentage of the available receptors. If (a) this reaction is completely irreversible and (b) there is no *de novo* receptor synthesis, or other replenishment mech-

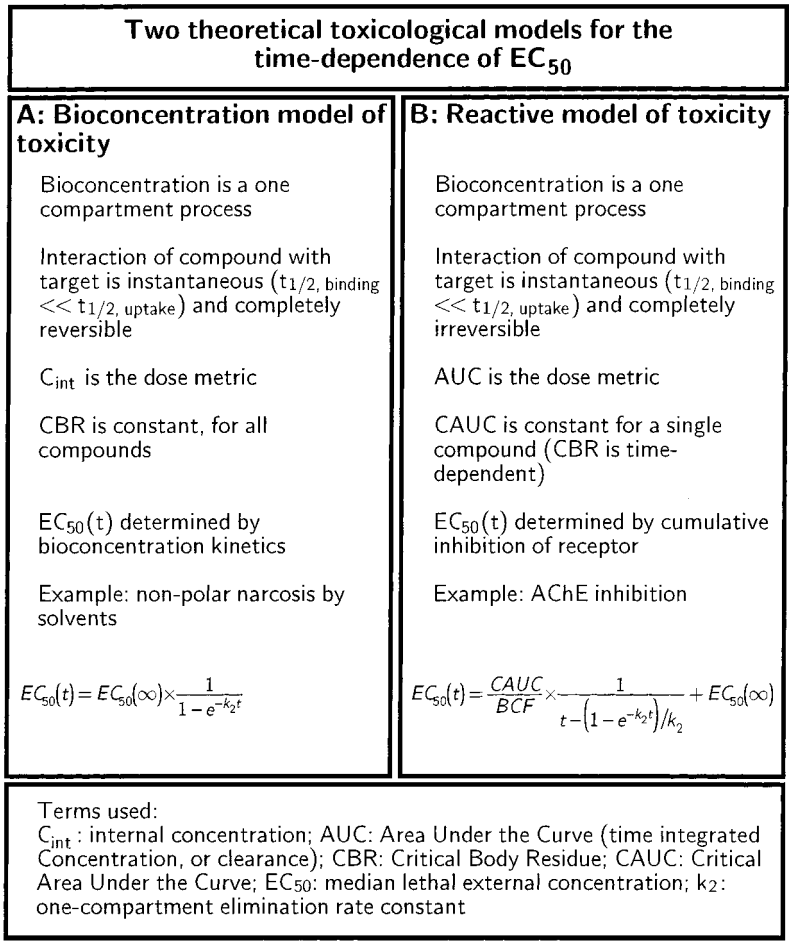

FIGURE 1. Summary of toxicological mechanisms.

anism, the percentage of receptor depletion is a function of $C_{int} \times t$, according to

$$\frac{dC_{receptor}}{dt} = -k_{interaction} \times C_{receptor} \times C_{int} \qquad (11)$$

This means that the percentage of receptor depletion is a function of $C_{int} \times t$ or, more accurately, of the time integral of C_{int}. The toxicological effect is still coupled to a threshold occupation of a "receptor" (just as in the narcosis example), but the threshold occupation now is a function of $C_{int} \times t$, instead of a (peak) C_{int} as in the narcosis example; see Figure 1 for a summary of the two mechanisms. A prime example of compounds acting in such an irreversible way are the organophosphate acetylcholinesterase (AChE) inhibitors (*20*, *21*).

A consequence of using CAUC as a (surrogate) dose metric for the amount of occupied (inhibited) receptor molecules is the fact that even though we postulate the toxicant-receptor interaction to be fast in comparison with bioconcentration kinetics, the actual reactivity of the compound towards the target (especially its thermodynamics), still is a determinant of CAUC. In other words, unlike the CBR for narcosis toxicants, which is theorized to be similar, or the same, for all narcosis compounds, the CAUC is compound dependent. It only describes the constancy of the AUC for a single compound-species combination under different exposure regimes.

In actual situations involving reactive or specifically-acting aquatic toxicants, compound-target interactions will not be completely irreversible, and some replenishment mechanisms will likely be active, so eq 10 will likely give an overestimation of the actual toxicity. To illustrate this, consider that eq 10 always predicts the incipient EC_{50} to be 0. However, it can be seen that in most instances the reversibility of interaction and/or the effect of replenishment mechanisms will result in a simple elevation of the incipient EC_{50} to a value > 0, according to

$$EC_{50}(t) = \frac{CAUC/BCF}{t - (1 - e^{-k_2 t})/k_2} + EC_{50}(\infty)$$

$$\text{for } t \to \infty \qquad (12)$$

$$EC_{50}(t) \cong \frac{CAUC/BCF}{t - 1/k_2} + EC_{50}(\infty)$$

Implications. What we have now is two aquatic toxicity EC_{50} vs time relationships, one, eq 6, for compounds that act through a narcosis mechanism (class 1 and possibly class 2 compounds as per (*10*)) and another, eq 10 for compounds that act by less or nonreversible mechanisms. For class 1 compounds, eq 6 tells us that $EC_{50}(t)$ follows an exponential function that basically is the inverse of the uptake, or bioaccumulation, curve for the compound. It can easily be shown that $EC_{50}(t)$ and $C_{int}(t)$ are inversely related, so that there is a direct relationship between the attainment of bioconcentration steady state and the incipient EC_{50}. In other words, for narcosis compounds, the attainment of bioconcentration equilibrium implies a stationary EC_{50} and vice versa.

For all other compounds however, no such simple relationship exists. If we examine eq 12, we can deduce that

TABLE 1. Model (Eq 6 vs Eq 12) Parameters for Two Simulated Toxicants[a]

	narcosis toxicant	receptor toxicant
k_2 (d^{-1})	0.0417	0.0417
BCF (L kg^{-1}) wet	178	178
CBR (mmol kg^{-1}) wet	25	
CAUC (mmol kg^{-1} d)		1.85×10^3

[a] Completely reversible vs completely irreversible.

TABLE 2. Some LC_{50} Model Results for Simulated Toxicants[a]

	narcosis toxicant	receptor toxicant
72 h LC_{50}	0.148	0.211
96 h LC_{50}	0.144	0.144
14 d LC_{50}	0.140	0.033
incipient LC_{50}	0.140	0.000
72 h/96 h ratio	0.97	0.68

[a] See eq 6, eq 12, and Table 1.

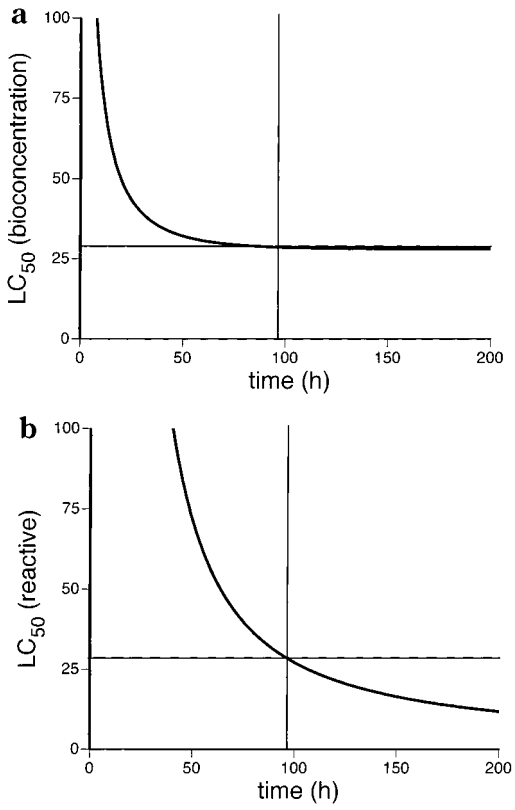

FIGURE 2. (a) $LC_{50}(t)$ for narcosis toxicant. Thick black line represents the course of LC_{50} relative to t. Thin black lines (cross) indicate the 96 h LC_{50} (horizontal line LC_{50}, vertical line the 96 h point). (b) $LC_{50}(t)$ for reactive toxicant. Thick black line represents the course of LC_{50} relative to t. Thin black lines (cross) indicate the 96 h LC_{50} (horizontal line LC_{50}, vertical line the 96 h point).

$EC_{50}(t)$ becomes a function of $1/t$, or a hyperbolic function, for sufficiently large values of t, subject to the following inequality

$$t > \frac{a}{k_2}$$

with a being an arbitrary constant. The constant a should be chosen such that $t \gg 1/k_2$, so that the deviation is less than a certain percentage.

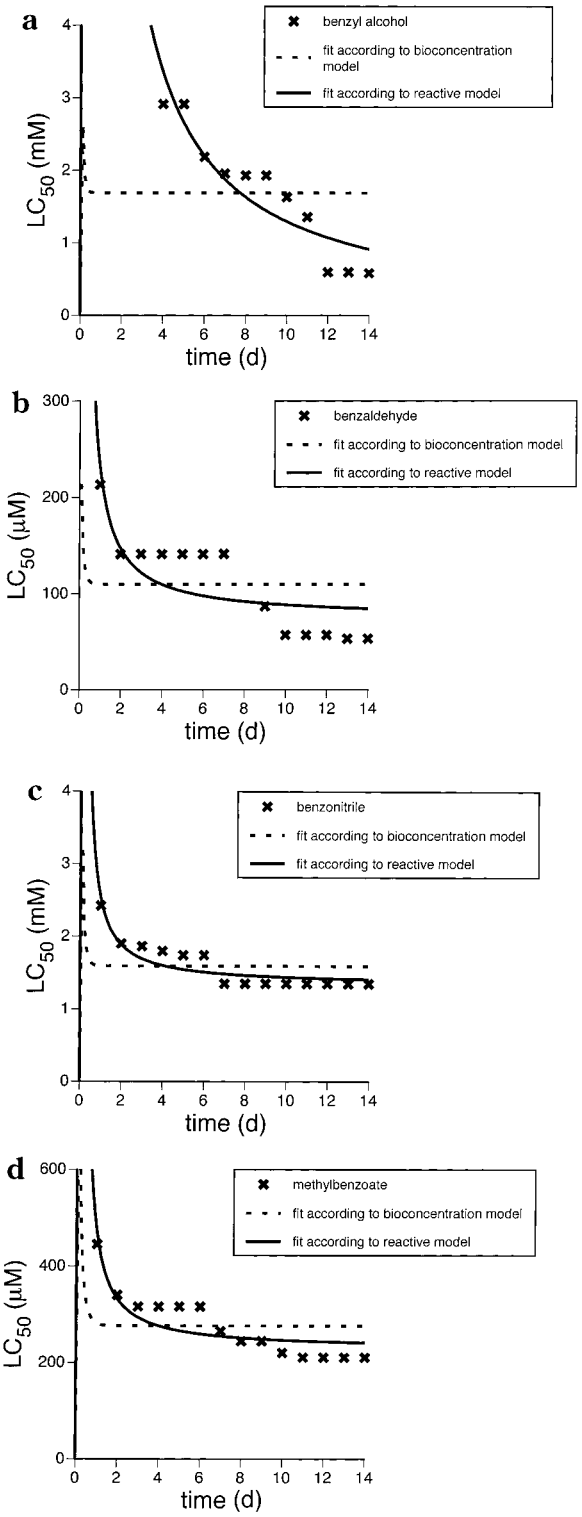

FIGURE 3. (a) LC_{50} vs time for benzyl alcohol toxicity to guppy. Unpublished data from Hemens (×); fit of bioconcentration model (dashed line); fit of reactive model (solid line). (b) LC_{50} vs time for benzaldehyde toxicity to guppy. Unpublished data from Hermens (×); fit of bioconcentration model (dashed line); fit of reactive model (solid line). (c) LC_{50} vs time for benzonitrile toxicity to guppy. Unpublished data from Hermens (×); fit of bioconcentration model (dashed line); fit of reactive model (solid line). (d) LC_{50} vs time for methyl benzoate toxicity to guppy. Unpublished data from Hermens (×); fit of bioconcentration model (dashed line); fit of reactive model (solid line).

Kooijman (1981) mentioned that hyperbolic $EC_{50}(t)$ relations only have an empirical basis. We have shown here that

TABLE 3. $LC_{50}(t)$ Data for Four Benzylic Compounds for 1−14 Days for the Guppy (*P. reticulata*; Hermens, Unpublished Results)

time (d)	benzyl alcohol LC_{50} (mM)	benzaldehyde LC_{50} (μM)	benzonitrile LC_{50} (mM)	methylbenzoate LC_{50} (μM)
1	>3	213.5	2.42	445
2	>3	141.3	1.90	340
3	>3	141.3	1.87	316
4	2.91	141.3	1.80	316
5	2.91	141.3	1.74	316
6	2.19	141.3	1.74	316
7	1.96	141.5	1.35	265
8	1.93		1.35	244
9	1.93	87.2	1.35	244
10	1.63	57.3	1.35	220
11	1.36	57.3	1.35	210
12	.59	57.3	1.35	210
13	.59	53.4	1.35	210
14	.58	53.4	1.35	210

for toxicants acting through receptor-based mechanisms, there *does* exist a theoretical basis for a hyperbolic $EC_{50}(t)$ relationship.

The fact that $EC_{50}(t)$ for non-narcosis compounds follows a hyperbolic relationship also means that there is no longer a correspondence between the attainment of bioconcentration equilibrium and a stationary EC_{50}. It can be shown that for most common non-narcosis toxicants bioconcentration steady state is reached before the incipient EC_{50} is reached. To that end we will consider two hypothetical toxicants, one a narcosis compound and the other a reactive toxicant, which exhibit the same bioconcentration properties (i.e., they have the same BCF and k_2). We will furthermore assume that there is no large difference in toxicity; to that end we will assume that both compounds have the same 96 h LC_{50}. The pertinent model parameters can be found in Table 1. BCF and k_2 are chosen thus that they both correspond to a hypothetical compound with a log K_{ow} of 3.55, based on eq 3 and the simplistic but not altogether unreasonable log K_{ow} = log BCF_{lipid} approximation (lipid content set at 5%) (*22*). At this k_2, T_{95} is 72 h or 3 days. The CBR for the narcosis compound is a fairly arbitrary value, within the range commonly reported for narcosis compounds (*23*), and the CAUC for the reactive compound was chosen so that both compounds have the same 96 h LC_{50} value.

Substitution of these values in the appropriate $EC_{50}(t)$ models (eqs 6 and 12) gives the following results, summarized in Table 2 and depicted more completely in Figure 2. All LC_{50} values are in μmol L^{-1}. We see that for the narcosis toxicant the 96 h LC_{50} is almost the incipient LC_{50}, and the 72 and 96 h LC_{50}s are within 3% of each other. As we argued before, for narcosis toxicants this also means that bioconcentration steady state has been attained. Bear in mind that this also means that for our hypothetical reactive toxicant, which has the same bioconcentration behavior as our hypothetical narcosis toxicant, bioconcentration *steady state has also been attained*. However, $EC_{50}(t)$ is seen to decrease even when an organism is at bioconcentration steady state. In fact, the 72 h/96 h LC_{50} ratio is only 68%. This then clearly indicates that the 72 h/96 h LC_{50} ratio is *not* a good measure for bioconcentration behavior of class 3 and 4 compounds, unlike what Tyle *et al.* (*12*) say.

Results

$LC_{50}(t)$ Data for Selected Electrophiles. Although no widespread attention has been given to toxicokinetics and especially toxicodynamics of non-narcosis compounds within the field of aquatic toxicology, there are some reports of LC_{50} vs time behavior for such compounds. Hermens and coworkers (*24*) found that for small electrophiles (reactive allylic chlorine compounds with a log K_{ow} generally lower than 3; classified as class 3), LC_{50} did not reach a stationary value during the 14-day semichronic exposure experiments; whole body internal concentrations on the other hand generally reached steady-state values well within 96 h. De Bruijn and Hermens (*25*−*28*) observed that for organophosphorothionates (specific toxicants, class 4) a marked decrease in LC_{50} occurs from LC_{50}(48 h) to LC_{50}(96 h), which is essentially independent of log K_{ow}.

In order to show that for class 3 compounds the LC_{50} vs time profile can indeed better be described by the reactive model presented here than by the commonly used bioconcentration model, we modeled the $LC_{50}(t)$ data for several non-narcosis compounds that were previously determined in our laboratory (Hermens, unpublished results). The LC_{50} data for these four benzylic compounds are shown in Table 3.

These LC_{50} vs time data were fitted to both the bioconcentration (eq 6) and the reactive (eq 12) model. See Table 4 for an overview of the fit results and statistics. Each fit was performed twice, once with all model parameters (k_2 and $LC_{50}(\infty)$ in the case of the bioconcentration model and k_2, CAUC/BCF, and $LC_{50}(\infty)$ in the case of the reactive model) free and once with k_2's constrained to externally determined values, using eq 3, which is generally considered to be valid for small fish. Figure 3a−d show the result of the fits with fixed (realistic) k_2 values to the LC_{50} data graphically. From

TABLE 4. Results of the Fitting of the Bioconcentration Model (Eq 6) and the Reactive Model (Eq 12) to the LC_{50} Values from Table 3

		bioconcn. k_2 free	reactive k_2 free	bioconcn. k_2 fixed	reactive k_2 fixed
benzyl alcohol	r^2	0.83	0.83	0.0	0.83
	k_2 (d^{-1})	−0.009	1.3e7	10.3	10.3
	$LC_{50}(\infty)$ (mM)	−0.127	0.91	1.69	0.0
	CAUC/BCF (mmol L^{-1} d)		13.12		13.21
benzaldehyde	r^2	0.0	0.64	0.0	0.62
	k_2 (d^{-1})	0.01	2.95e7	7.2	7.2
	$LC_{50}(\infty)$ (μM)	2.92	21.8	109.8	74.9
	CAUC/BCF (μmol L^{-1} d)		147.5		134.5
benzonitrile	r^2	0.70	0.85	0.0	0.83
	k_2 (d^{-1})	0.85	18990	6.7	6.7
	$LC_{50}(\infty)$ (mM)	1.47	1.31	1.59	1.34
	CAUC/BCF (mmol L^{-1} d)		1.21		1.02
methyl benzoate	r^2	0.67	0.81	0.03	0.78
	k_2 (d^{-1})	0.75	1.2e8	3.9	3.9
	$LC_{50}(\infty)$ (μM)	250.4	20.21	276.3	228.1
	CAUC/BCF (μmol L^{-1} d)		139.4		180.4

the results in Table 4 it can be seen that in each case the reactive model fits significantly better than the bioconcentration model. If the k_2 value is let free, both models return with nonsensical k_2 values: too small (slow) in the case of the bioconcentration model and too large (fast) in the case of the reactive model. If we fix the k_2 values at a meaningful value, we see that the bioconcentration model fails to fit any of the datasets, while the reactive model is still able to adequately describe the data, with no or only a slight deterioration in r^2 value, and meaningful $LC_{50}(\infty)$ values. Note that with the limited amount of data available from these LC_{50} studies, no assessment as to the significance of the fitted CAUC/BCF values can be given here, meaning that the CAUC/BCF values resulting from these model fits should not be used as *the* CAUC/BCF values for these compounds. What these results show us is that for at least some class 3 electrophiles, LC_{50} values for small fish decrease with time long after the bioconcentration model predicts they should have reached their incipient value. Accordingly, the reactive model for $LC_{50}(t)$ is much more successful in describing the data.

More recently Legierse et al. (*29*) explicitly showed that for the toxicity of chlorothion toward the pond snail, the time-dependence of LC_{50} followed a hyperbolic function

$$LC_{50}(t) = \frac{A}{t} + B$$

or eq 12 for $t \gg 1/k_2$. They also showed that, in accordance with this LC_{50}-vs-time dependency, the chlorothion Lethal Body Burden (or CBR) is time dependent.

Discussion

It has been shown that for mechanisms that include irreversible, or only partially reversible interactions of aquatic toxicants with target structures, there is no temporal correlation between the attainment of bioconcentration steady state and attainment of a stationary LC_{50} value. Contrary to the situation for reversible interaction toxicants (as in nonpolar narcosis), where $EC_{50}(t)$ is a function of $\exp(-k_2t)$, for reactive toxicants, $EC_{50}(t)$ is basically a function of $1/t$. Some examples for both reactive toxicants and AChE-inhibiting pesticides were given that show the validity of this EC_{50}-time dependence.

This could have two consequences for the interpretation of standard toxicity test data and subsequent risk analyses. Firstly, if only 96 h LC_{50} values are taken into account, this will lead to an underestimation of the (sub)acute toxicity (overestimation of $LC_{50}(t)$) of such compounds. This indicates that for certain compounds, 96 h toxicity studies, while they may accurately reflect bioconcentration behavior, are not sufficient to yield adequate EC_{50} values. Secondly, if LC_{50} vs time data are analyzed based on $\exp(-k_2t)$ model, a gross underestimation of the bioconcentration k_2 value will result.

Clearly, more research into under what circumstances, exposure regimes, and for what compounds $EC_{50}(t)$ varies with $1/t$, rather than with $(1 - e^{-t})^{-1}$, is needed in order to ensure adequate application of both experimental and predicted EC_{50} values in risk assessment.

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