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Analysis of the Time-Dependent Acute Aquatic Toxicity of Organophosphorus Pesticides: The Critical Target Occupation Model

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A model is presented for the acute toxicity of organophosphorus (OP) pesticides belonging to the class of phosphorothionates. The acute toxicity of these pesticides is governed by the irreversible inhibition of the enzyme acetylcholinesterase (AChE), after their metabolic activation to oxon analogues. The model is based on the idea that, for chemicals exhibiting an irreversible receptor interaction, mortality is associated with a critical amount of "covalently occupied" target sites, i.e., the "critical target occupation" (CTO). For a given compound and species, this CTO is associated with a critical time-integrated concentration of the oxon analogue in the target tissue, which can be modeled by the critical area under the curve (CAUC) that describes the time−concentration course of the phosphorothionate in the aqueous phase or in the entire aquatic organism. In contrast to the classical critical body residue (CBR) model, the CTO model successfully describes the 1−14-d $LC_{50}(t)$ data of several phosphorothionates in the pond snail and guppy. Furthermore, the time dependency of lethal body burdens (LBBs) of phosphorothionates is explained by the model. Although the CTO model is specifically derived for OP pesticides, it can be applied to analyze the acute toxicity and to estimate incipient LC_{50} values of organic chemicals that exert an irreversible receptor interaction in general.

Introduction

Narcotic chemicals are assumed to elicit their toxicity by a nonspecific reversible disturbance of the cell membrane caused by their accumulation in these hydrophobic phases within the aquatic organism (*1*). Their toxic potency, expressed as an ambient concentration, is therefore entirely dependent on the hydrophobicity of the chemical (*1*). McCarty (*2, 3*) derived that the molar whole-body concentration of narcotic chemicals at the time of death, referred to as the lethal body burden (LBB) or critical body residue (CBR), is constant. This concept is based on the idea that residue levels at the cell membrane are well correlated with whole-body concentrations. Several studies have demonstrated that LBBs of narcotic compounds are indeed fairly constant, varying from 2 to 8 mmol/kg of organism (*1, 4*−*6*). Moreover, it has been shown that organic chemicals exhibiting the same mode of action are associated with a specific range of LBBs (*7*). This finding led to the proposal of the CBR as a relevant parameter for the risk assessment of organic chemicals among mode of actions (*7*).

In contrast to the nonspecific character of narcosis, organophosphorus (OP) pesticides exert a very specific, receptor-mediated effect. The inhibition of acetylcholinesterase (AChE) in nervous tissue and other target organs is generally considered to be the critical effect leading to the acute toxicity of OP pesticides. Inhibition of AChE results in accumulation of acetylcholine in synapses, leading to an excessive stimulation of the cholinergic nerve system organs (*8, 9*). Most OP pesticides belong to the class of phosphorothionates, which are poor AChE inhibitors themselves and need to be metabolically activated by the cytochrome P450 system to yield their corresponding oxon analogues prior to the inhibition of AChE (*8, 9*). Since the oxon analogue cannot be recovered after binding to AChE, the interaction between oxon analogues and AChE can be considered irreversible (*10, 11*). This fact gives rise to the discussion of whether whole-body residues or even target tissue residues can be applied as surrogates for residue levels of the oxon analogue bound to the AChE receptor.

AChE inhibition and mortality due to OP pesticide exposure have been shown to be dependent on both exposure concentration and duration for a variety of aquatic organisms (*12*−*15*). The fact that time dependency was also observed after relatively long exposure times with respect to the time required to reach a steady-state concentration in the organism gives reason to doubt the applicability of the CBR toxicity model to explain time-dependent toxicity (*5, 16*).

In this paper, we propose a new model that is based on the irreversible receptor interaction of OP pesticides, i.e., the critical target occupation (CTO) model. The model is validated based on 14-d $LC_{50}(t)$ values and LBBs for the phosphorotionate chlorthion in the pond snail (*Lymnaea stagnalis*). Furthermore, 14-d $LC_{50}(t)$ data for five different phosphorothionates and LBB data for methidathion in the guppy are evaluated. The applicability of this CTO model is compared with the classical CBR toxicity model.

Theory

Receptor Interactions and Effects. In general, the intensity of a toxic effect exhibited by a toxicant depends on the degree of receptor "occupation". Receptor interactions can be divided in two broad classes (modified from refs *17*−*19*):

Reversible Receptor Interactions. These chemical interactions are noncovalent, i.e., electrostatic and hydrophobic interactions. Most drugs and many toxicants belong to this class. The extent of the exhibited toxic effect by these toxicants is directly related to the free toxicant *concentration* at the target (A) on one hand and to the receptor affinity of the toxic agent on the other hand, which is related to the reciprocal of the dissociation constant, K_D:

$$A + R \overset{K_D}{\rightleftarrows} A{-}R \rightarrow \text{toxic effect}$$

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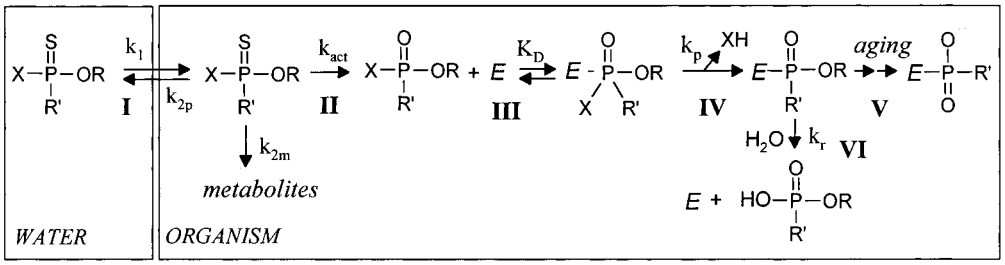

FIGURE 1. Main kinetic processes involved in the inhibition of acetylcholinesterase (*E*) in aquatic organisms exposed to organophosphorus pesticides, belonging to the class of phosphorothionates: I, Bioconcentration kinetics determined by the uptake rate constant k_1 and by the overall elimination rate constant k_2, which incorporates both elimination by passive diffusion (k_{2p}) and metabolic elimination (k_{2m}); II, Metabolic activation by the cytochrome P-450 system to yield the oxon analogue (k_{act}); III, Formation of a transient unstable intermediate complex with AChE. The dissociation constant K_D is a measure for the affinity of the substrate for the enzyme; IV, Irreversible and rapid phosphorylation of the enzyme (k_p); V, Aging by dealkylation to yield an irreversibly inhibited enzyme incapable of being dephosphorylated; and VI, Spontaneous reactivation to regenerate the active enzyme (k_r) (Modified from refs *33* and *11*).

where R stands for the receptor molecule, and A−R stands for the reversibly bound toxicant−receptor complex.

Irreversible Receptor Interactions. In these interactions, a covalent bond between the toxicant, or its active metabolite, and the receptor is formed. Examples are the interactions between electrophiles and DNA or between OP pesticides and AChE. The magnitude of the adverse effect is related to the number of adduct molecules formed and hence to the total *amount* of covalently bound toxicants:

$$A + R \xrightarrow{k} AR \rightarrow \text{toxic effect}$$

where k is the reaction rate constant, and AR is the adduct. In this latter case, the degree of receptor occupation will increase as long as the receptor is exposed to the active substance and is proportional to the total amount of substance that has reached the receptor since the beginning of the exposure and to the reaction rate constant k.

Critical Body Residue (CBR) Model. According to the CBR concept, an aquatic organism dies at a constant molar internal threshold concentration of a toxicant (*2*−*4*, *7*, *20*). It is important to realize that this concept is solely applicable if the internal whole-body concentration can be regarded as a surrogate for the target concentration, which is in fact only the case for reversibly acting compounds that have their target located in the lipid phase (*19*). A class of chemicals that obey these conditions are narcotic chemicals, for which the CBR concept has been originally derived and successfully applied.

At constant exposure concentrations, the internal whole-body concentration of chemicals in aquatic organisms (C_{wb}, in μmol/kg) is generally modeled by a one-compartment first-order bioconcentration model (*21*):

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

where BCF (L/kg) is the bioconcentration factor, defined as the ratio between the uptake and elimination rate constants k_1 (L kg^{-1} h^{-1}) and k_2 (h^{-1}); C_w (μM) is the external aqueous concentration of the chemical; and t (h) is the exposure time. The combination of the CBR concept (LBB = constant) with eq 1 results in the following description for time-dependent toxicity (*4*, *5*, *16*):

$$LC_{50}(t) = \frac{LBB}{BCF \times (1 - e^{-k_2 t})} = \frac{LC_{50}(\infty)}{(1 - e^{-k_2 t})} \quad (2)$$

where $LC_{50}(t)$ (μM) represents the LC_{50} after t h of exposure, LBB (μmol/kg) is the internal concentration at lethality, and $LC_{50}(\infty)$ is the incipient LC_{50} value. This equation rearranges to

$$LBB = LC_{50}(\infty) \times BCF \quad (3)$$

According to this model, the LBB will be constant and thus independent of exposure concentration and time of death. The LC_{50} will reach its incipient value when the internal body concentration has reached an equilibrium with the external (constant) aqueous concentration.

Critical Target Occupation (CTO) Model. In Figure 1, the main processes involved in the acute toxicity of OP pesticides to aquatic organisms are shown. Metabolic detoxification of the oxon analogues, which mainly proceeds through hydrolysis catalyzed by oxonases (enzymes belonging to the A-esterases family), is not taken into account in the model. The knowledge that exists on the role of these enzymes in the aquatic toxicity of OP pesticides is scarce and contradictory (*22*).

First, the toxicity model that we derive for OP pesticides in this paper is founded on a direct relationship between adverse effects and the extent of AChE inhibition in the target tissue. More precisely, mortality is assumed to occur at a fixed AChE inhibition percentage. Second, this model assumes that the AChE concentration in the target tissue is constant. Due to the covalent interaction between oxon analogues and their receptor, the lethal AChE inhibition percentage is, under the above-mentioned conditions, related to a critical amount of covalently occupied target sites, which we define as the critical target occupation (CTO).

To derive a dose metric for OP toxicity, the following additional assumptions are made: (i) the metabolic activation of an OP to its oxon analogue is described by first-order kinetics, (ii) the activation rate constant (k_{act}) contributes negligibly to the overall elimination rate constant k_2 of the parent OP, and (iii) the reactivation rate constant (k_r) is very small as compared to the overall AChE inhibition rate constant (k_i), which is defined as the ratio between the phosphorylation rate constant (k_p) and the dissociation constant of the phosphorylated enzyme (K_D) (*8*, *23*). Assumption iii seems justified since aquatic organisms are only slightly or not at all capable to reactivate phosphorylated AChE (*24*−*26*). Consequently, the inhibition of AChE can be considered virtually irreversible.

As a consequence of the irreversible character of AChE inhibition by oxon analogues, the rate of AChE inhibition is determined by the AChE inhibition rate constant (k_i) and the concentration of the oxon analogue in the target tissue (C_{oxon}, in μmol/kg). The degree of receptor occupation will gradually increase in time as long as oxon analogues are present in the target tissue. Over a certain time period, the total amount of inhibited AChE molecules (or receptor-bound oxon analogues) in the target tissue equals the amount of oxon analogues that have been "removed" from the target tissue. This amount is dependent on both the inhibition rate constant k_i and the time course of the concentration or "time-integrated concentration" of the oxon analogue in the target

tissue. This time-integrated concentration can be estimated from the area under the curve (AUC), which describes the concentration of the oxon analogue in the target tissue as a function of time. To our knowledge, the time-integrated concentration and AUC have not been applied in aquatic toxicology before. In pharmacokinetic modeling, however, the area under the plasma concentration versus time curve is commonly applied to estimate the total amount of substance eliminated from the body over a certain time period, i.e., the "clearance" (*17*). The CTO, which is defined as the amount of inhibited AChE molecules (or AChE-bound oxon analogues) per mass unit of target tissue at the time of death, is determined by the k_i and the critical area under the curve, which describes the concentration of the oxon analogue in the target tissue until the time of death ($CAUC_{oxon}$):

$$CTO = k_i \int_0^t C_{oxon}\, dt = k_i \times CAUC_{oxon} \quad (4)$$

where CTO is expressed as μmol/kg, k_i is expressed as h^{-1}, and CAUC is expressed as μmol·h/kg.

Although the brain and the skeletal muscle are known to be the main target tissues for OP poisoning (*11, 27*), it is not possible to assign the precise location where AChE inhibition is critical for mortality. Furthermore, it is not exactly known which organ is responsible for the enzymatic formation of the oxon analogues that will eventually reach the critical target (*28*). Therefore, we will simplify the chemical's behavior in the organism by applying two different compartment models. First, we approach the aquatic organism as a single compartment and regard the entire aquatic organism as a "reference compartment" for the target tissue (*29*). In other words, we presume that the oxon analogue and the parent OP pesticide show a proportional distribution over the different tissues (*29*). As a consequence, the AUCs of both the OP pesticide and the oxon analogue in the target tissue are proportional to their overall AUCs in the aquatic organism (the whole-body CTO model or CTO_{wb} model). Second, we assume the organism to consist of two compartments: a lipid compartment and an aqueous compartment. The aqueous compartment, which is represented by blood plasma of fish and by the hemolymph of molluscs and crustaceans, is now considered as a reference tissue for the target tissue and for the tissue were OP pesticides are biotransformed. The choice for applying the aqueous compartment as a reference for the target tissue is obvious, taking into account that adverse effects of OP intoxication depend on a reaction taking place in the aqueous phase (*30*), i.e., in the synaptic cleft. Thus, the AUCs of the parent OP pesticide and the oxon analogue in the target tissue are assumed to be proportional their respective AUCs in the aqueous compartment (aqueous CTO model or CTO_a model).

Whole-Body CTO Model (CTO_{wb} Model). If we approximate the aquatic organism as a single compartment and take into account assumptions 1 and 2, $CAUC_{oxon}$ can be directly related to a critical area under the time–concentration curve of the parent OP pesticide in the entire organism ($CAUC_{wb}$, in μmol·h/ kg). The CTO is in this case defined as the amount of receptor-bound oxon analogues (or inhibited AChE molecules) per mass unit of organism (μmol/kg) and described as follows:

$$CTO_{wb} = k_i k_{act} \times CAUC_{wb} \quad (5)$$

where $CAUC_{wb}$ can be derived from the first-order one-compartment bioconcentration model as follows:

$$CAUC_{wb} = \int_0^t BCF \times C_w \times (1 - e^{-k_2 t})\, dt = BCF \times C_w \times \left(t - \frac{1 - e^{-k_2 t}}{k_2}\right) \quad (6)$$

Both k_i, k_{act}, and $CAUC_{wb}$ are determined by species characteristics and chemical properties of the OP pesticides. CTO_{wb} may be considered constant among individual OP pesticides since a species is expected to die at certain fixed AChE percentage, regardless of the characteristics of the molecules that have caused this inhibition. Among species, however, CTO_{wb} might show some variation due to species differences in the AChE inhibition percentage nessecary to cause mortality and/or due to species differences in AChE concentrations. As follows from the combination of eq 5 with the constancy of both CTO_{wb}, k_{act}, and k_i for a given species and compound, lethality is accompanied with a constant $CAUC_{wb}$ for a given species–compound combination. Consenquently, the LC_{50} of OP pesticides may be described as a function of time, through the rearrangement of eq 6 (C_w is regarded as $LC_{50}(t)$):

$$LC_{50}(t) = \frac{CAUC_{wb}}{BCF \times \left(t - \frac{1 - e^{-k_2 t}}{k_2}\right)} \quad (7)$$

This equation implies that $LC_{50}(t)$ will reach zero at infinite exposure durations. In practice, however, the organism will put compensating mechanisms into action, such as de novo AChE synthesis. Hence, the LC_{50} is eventually expected to reach an incipient value ($LC_{50}(\infty)$):

$$LC_{50}(t) = \frac{CAUC_{wb}}{BCF \times \left(t - \frac{1 - e^{-k_2 t}}{k_2}\right)} + LC_{50}(\infty) \quad (8)$$

Substitution of C_w from eq 1 by $LC_{50}(t)$ (eq 8) leads to the following description of the internal lethal concentration as a function of time ($C_{wb}(t) = LBB(t)$):

$$LBB(t) = \frac{CAUC_{wb}}{\frac{t}{(1 - e^{-k_2 t})} - \frac{1}{k_2}} + BCF \times (1 - e^{-k_2 t}) \times LC_{50}(\infty) \quad (9)$$

Aqueous CTO Model (CTO_a Model). According to the above-mentioned underlying basic assumptions of the CTO_a model, we can describe the CTO, which is now defined as the amount of receptor-bound oxon analogues (or inhibited AChE molecules) per volume unit aqueous phase (μM) as follows:

$$CTO_a = k_i k_{act} \times CAUC_a \quad (10)$$

where $CAUC_a$ (μM·h) denotes the critical area under the time–concentration curve of the parent OP in the aqueous compartment. Next, we assume that the concentration of the OP pesticide in the aqueous compartment of the organism (C_a, in μM) instaneously reaches an equilibrium with its concentration in the aqueous exposure phase. This assumption agrees with the proposed modeling of the concentration kinetics of chemicals in aqueous phases of aquatic organisms by Barron et al. (*29*), who suggested applying the external exposure water as a reference compartment for the internal aqueous phase. The instaneous equilibrium between the external and internal aqueous compartments is kinetically represented by an infinite k_2 value ($k_2 = \infty$), where k_2 represents the total elimination rate constant for the aqueous compartment. The BCF, which is defined as C_a/C_w in the

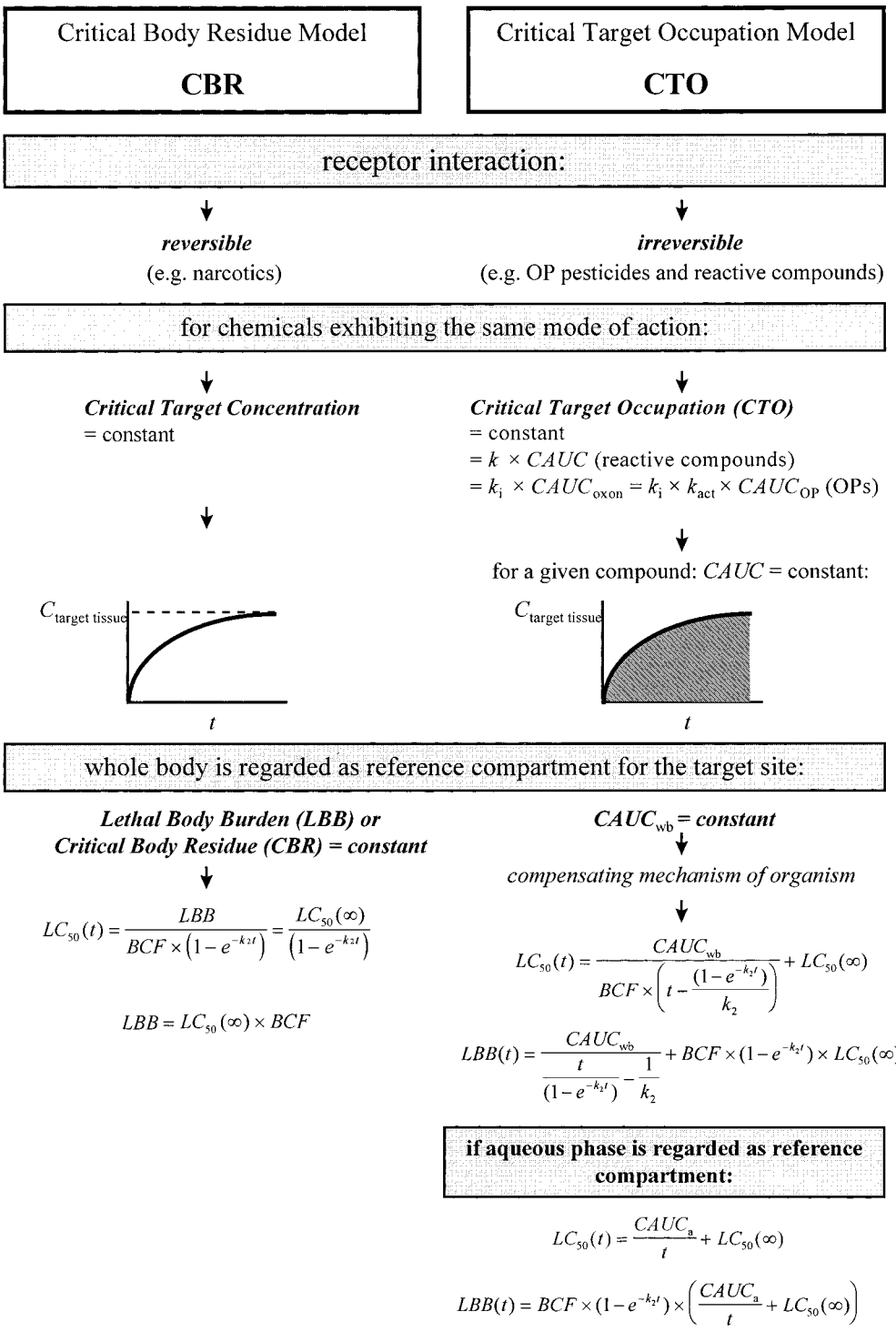

FIGURE 2. Schematic overview of the basic principles of the critical body residue (CBR) toxicity model and the critical target occupation (CTO) model. CAUC denotes the critical area under the curve, k is the rate constant for the irreversible reaction between reactive compounds and their target, k_i is the rate constant for the inhibition of AChE by the oxon analogues of organophosphorus compounds (OPs), LC_{50} is the median lethal external concentration, LBB is the lethal internal concentration, BCF is the bioconcentration factor, and k_2 is the one-compartment elimination rate constant.

CTO_a model, is assumed to have a value of 1. This seems legitimate since the concentrations in the external and internal aqueous phases are not expected to deviate significantly, according to the principle of partitioning. As a consequence of the values for k_2 and BCF, eq 6 rearranges to

$$\mathrm{CAUC_a} = (C_w t)_\dagger \qquad (11)$$

where $(C_w t)_\dagger$ denotes the product of the aqueous exposure concentration and duration at death (†). Additionally, eq 8 rearranges to

$$\mathrm{LC_{50}}(t) = \frac{\mathrm{CAUC_a}}{t} + \mathrm{LC_{50}}(\infty) \qquad (12)$$

As can be seen, eq 12 supplies a very simple model describing time-dependent LC_{50} values of OP pesticides. In contrast to

TABLE 1. Input Parameters and Parameter Estimates for the CBR, CTO_{wb}, and CTO_a Toxicity Models, Applied to the $LC_{50}(t)$ Data of the Pond Snail and the Guppy[a]

			parameter estimates				
			CBR model	CTO_{wb} model		CTO_a model	
compound	log K_{ow}[b]	input parameter[c]	$LC_{50}(\infty)$	$CAUC_{wb}$/BCF	$LC_{50}(\infty)$	$CAUC_a$	$LC_{50}(\infty)$
			Pond Snail				
chlorthion	3.63	$k_2 = 0.013$[d]	6.5 ± 0.5	188 ± 11	4.3 ± 0.3	825 ± 25	1.6 ± 0.2
			Guppy				
methidathion	2.45	$k_2 = 0.148$[e]	0.34 ± 0.06	12 ± 3	0.20 ± 0.06	18 ± 4	0.16 ± 0.05
azinophos-methyl	2.76	$k_2 = 0.101$[e]	0.8 ± 0.2	42 ± 4	0.26 ± 0.09	70 ± 5	0.09 ± 0.07
phosmet	2.81	$k_2 = 0.095$[e]	2.2 ± 0.5	118 ± 10	0.7 ± 0.2	201 ± 10	0.2 ± 0.1
malathion	2.94	$k_2 = 0.081$[e]	3.9 ± 0.6	122 ± 2	2.27 ± 0.04	218 ± 10	1.8 ± 0.1
phenthoate	3.96	$k_2 = 0.023$[e]	0.30 ± 0.06	17 ± 3	0.09 ± 0.05	47 ± 5	−0.04 ± 0.05

[a] Elimination rate constants (k_2) are expressed in h^{-1}, $LC_{50}(\infty)$ values are in μM, $CAUC_{wb}$/BCF values are in nmol·h/mL, and $CAUC_a$ values are in μM·h. Values of the estimated parameters are presented ± SE. [b] Ref 31. [c] Used as input parameter in the CBR and CTO_b toxicity models only. [d] Ref 32. [e] Estimated based on the following QSAR for chlorobenzenes in the guppy: log k_2 (d^{-1}) = −0.539 log K_{ow} +1.872, $n = 5$, $r = 0.996$ (*31*).

TABLE 2. Input Parameters for Prediction of Lethal Body Burdens (LBB, in mmol/kg Wet Weight) of Chlorthion in Pond Snail and of Methidathion in Guppy, According to the CBR, CTO_{wb}, and CTO_a Models[a]

		CBR model		CTO_{wb} model		CTO_a model	
input parameter	unit	chlorthion	methidathion	chlorthion	methidathion	chlorthion	methidathion
BCF	L/kg	31[b]	12.6[c]	31[b]	12.6[c]	31[b]	12.6[c]
k_2	h^{-1}			0.013	0.148	0.013	0.148
$LC_{50(\infty)}$	μM	6.5	0.34	4.3	0.20	1.6	0.16
$CAUC_a$	μM·h					825	18
$CAUC_{wb}$	μmol·h/kg			5813[d]	157[d]		

[a] Elimination rate constants (k_2) and values for $LC_{50}(\infty)$ and $CAUC_a$ are obtained from Table 1. [b] Ref 32. [c] Estimated as the average ratio between the reported average lethal internal concentration of methidathion in the guppy and the mean exposure concentrations in the low and high exposure groups, respectively (*35*). [d] Calculated as the product of the estimated value for $CAUC_{wb}$/BCF (Table 1) and the BCF (this table).

the whole-body CTO model, the aqueous LC_{50} model does not require kinetic input parameters.

The internal OP concentration in the entire organism at time of death can subsequently be described as follows, according to eq 1:

$$\mathrm{LBB}(t) = \mathrm{BCF} \times (1 - \mathrm{e}^{-k_2 t}) \times \left(\frac{\mathrm{CAUC_a}}{t} + \mathrm{LC_{50}}(\infty)\right) \quad (13)$$

In Figure 2, the basic principles of the CBR model and the two CTO models are schematically presented.

Experimental Section

Toxicity Test with Pond Snails. For the determination of LC_{50} values and LBBs of chlorthion in the pond snail, six groups of 10 pond snails each were exposed semi-statically (24 h renewal) to six different chlorthion (3-chloro-4-nitrophenyldimethylphosphorothionate, 98% pure, Riedel-de Häen AG, Seelze, Germany) concentrations in a 14-d LC_{50} test. Every 24 h, snails were monitored for mortality. A detailed description of the test is given in the Supporting Information.

Extraction, Cleanup, and Chemical Analysis of Chlorthion from Water and Pond Snails. See Supporting Information.

Lipid Determination Pond Snails. See Supporting Information.

LC_{50} Experiments Guppy. The 14-d LC_{50} tests for the phosphorothionates methidathion, azinophos-methyl, malathion, phenthoate, and phosmet were previously performed by De Bruijn and Hermens (*33*). Since only 14-d LC_{50} values were reported, the original mortality data for the individual days were retrieved for analysis (J. H. M. De Bruijn, personal communication).

Estimation $LC_{50}(t)$ Values from Mortality Data. See Supporting Information.

Estimation of Bioconcentration Factors of Chlorthion in Pond Snails of Toxicity Test. See Supporting Information.

Fitting of CBR and CTO Models to the $LC_{50}(t)$ Data. To evaluate the CBR model, $LC_{50}(t)$ values were fitted according to eq 2. Additionally, the $LC_{50}(t)$ data were fitted based on eq 8 (CTO_{wb}) and eq 12 (CTO_a), respectively. In Table 1, the input parameters for the equations are presented. The elimination rate constant k_2 for chlorthion in the pond snail was obtained from a previous study (*32*). Elimination rate constants for the OP pesticides in the guppy were estimated based on their octanol−water partition coefficients (K_{ow}), applying a quantitative structure−activity relationship (QSAR) for chlorobenzenes in the guppy (Table 1). All curve fittings were performed using the "non-linear regression" option of Graphpad Prism software (Graphpad Software Inc., Version 2.0).

Prediction of Lethal Body Burdens by the CBR and CTO Models. The LBB values of chlorthion in pond snail and of methidathion in the guppy were predicted for different exposure times according to eq 3 (CBR model), eq 9 (CTO_{wb} model), and eq 13 (CTO_a model). The applied input parameters in the equations are presented in Table 2. Model estimations of the LBB values of methidathion in the guppy were compared with measured LBBs, as reported by De Bruijn et al. (*35*).

Statistical Evaluation of the Models. The correlation coefficients (r^2) and the sum of squares of the residuals (SS) of the optimal fits of the $LC_{50}(t)$ data were calculated by the Graphpad Prism Software.

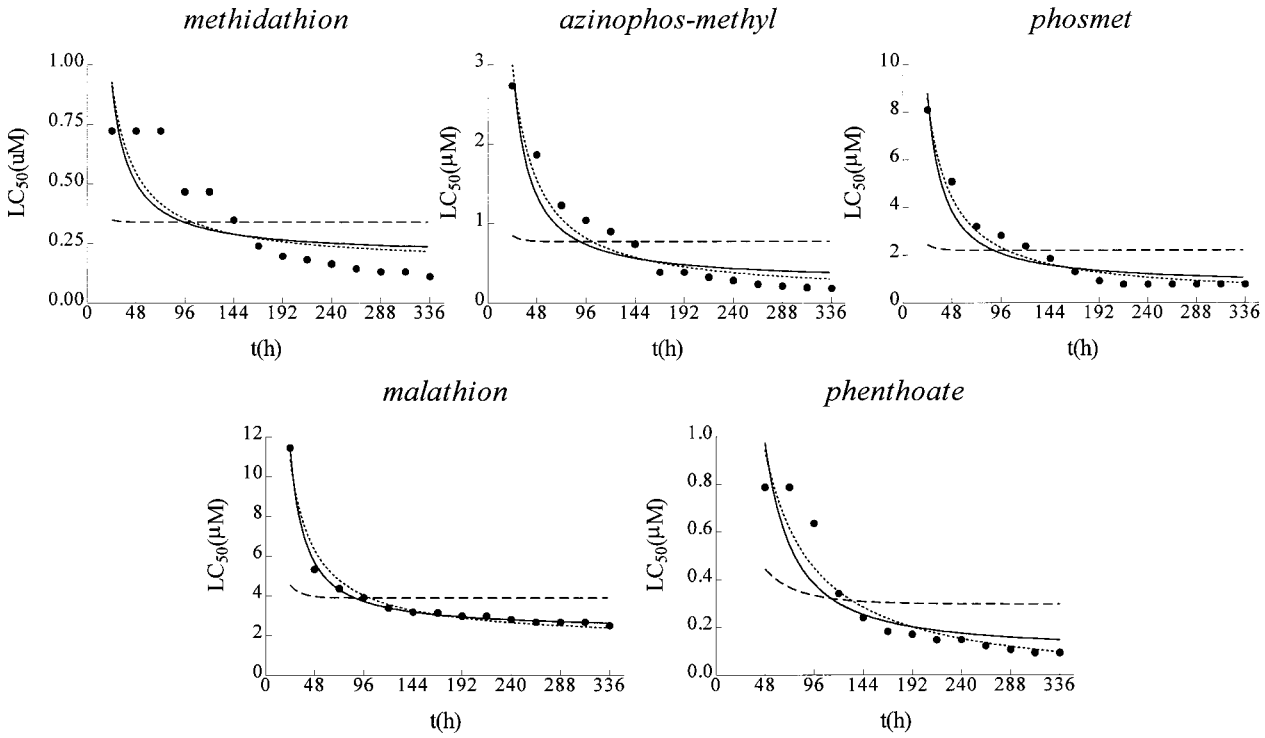

FIGURE 3. Fits of the critical body residue model (CBR, — —), the aqueous critical target occupation model (CTO_a, - - -), and the whole-body critical target occupation model (CTO_{wb}, ——) to the $LC_{50}(t)$ data of five OP pesticides in the guppy.

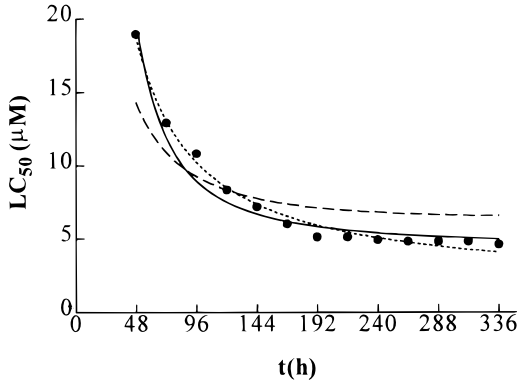

FIGURE 4. Fits of the critical body residues model (CBR, — —), the aqueous critical target occupation model (CTO_a, - - -), and the whole-body critical target occupation model (CTO_{wb}, ——) to the $LC_{50}(t)$ data of chlorthion in the pond snail.

Results

Exposure and Observations in Toxicity Test with Pond Snails. During exposure, the water temperature was 18.4 ± 0.4 °C. The pH was 7.4 ± 0.1, and the DO content was 8.4 ± 0.3 ppm. Average aqueous chlorthion concentrations during exposure were 0.9 ± 0.1 μM (n = 29), 1.8 ± 0.3 μM (n = 33), 4.3 ± 1.6 μM (n = 32), 6.2 ± 1.0 μM (n = 26), 10.4 ± 1.6 μM (n = 19), and 17.7 ± 3.6 μM (n = 11) for the different exposure groups. All values are expressed as mean values of the individual water samples ± standard deviation; the number of samples is given in parentheses. Time trends in the exposure concentrations were not observed.

$LC_{50}(t)$ Values for OP Pesticides in the Pond Snail and Guppy. $LC_{50}(t)$ values for chlorthion in the pond snail are presented in Table A1 (see Supporting Information) and plotted in Figure 4. As can be seen, estimated LC_{50} values decrease until t = 264 h, after which they tend to stabilize. The estimated $LC_{50}(t)$ values for methidathion, azinophos-methyl, phosmet, malathion, and phenthoate in the guppy are given in Table A2 (see Supporting Information) and are plotted versus exposure times in Figure 3. The estimated LC_{50} values at t = 336 h were in good agreement with the previously reported 14-d LC_{50} values (*33*). As can be seen in Figure 3 and Table A2 (see Supporting Information), the toxicity of the OP pesticides in the guppy increases until t = 216 h (phosmet), t = 312 h (phenthoate), or t = 336 h (methidathion, azinphos-methyl and malathion).

TABLE 3. Correlation Coefficients (r^2) and Sum of Squares of the Residuals (SS) of the Optimal Fits of CBR, CTO_{wb}, and CTO_a Models to the $LC_{50}(t)$ Data for Pond Snail and Guppy (see Figures 4 and 5)

	CBR model		CTO_{wb} model		CTO_a model	
compound	r^2	SS	r^2	SS	r^2	SS
		Pond Snail				
chlorthion	0.75	54.4	0.96	8.10	0.99	2.15
		Guppy				
methidathion	0.01	0.73	0.56	0.31	0.65	0.26
azinophos-methyl	0.04	7.05	0.88	0.86	0.94	0.42
phosmet	0.05	56.1	0.92	4.52	0.97	1.74
malathion	0.14	60.2	1.00	0.21	0.98	1.73
phenthoate	0.25	0.62	0.79	0.18	0.88	0.10

Quality and Parameter Estimates of the $LC_{50}(t)$ Fits. The optimal fits of the CBR, CTO_{wb}, and CTO_a models to the $LC_{50}(t)$ data for chlorthion in the pond snail are visualized in Figure 4. In Figure 3, the optimal $LC_{50}(t)$ fits for the five OP pesticides in the guppy are presented. Estimated values for $LC_{50}(\infty)$, $CAUC_{wb}$/BCF, and $CAUC_a$ are presented in Table 1 for the different chemical−species combinations. Statistics associated with the fits of the various models are given in Table 3.

As can be seen from Figures 3 and 4, neither the $LC_{50}(t)$ data for chlorthion in the pond snail nor the data for the five OP pesticides in the guppy are fitted accurately by the CBR model. The CBR model consistently overestimates toxicity at short exposure times of about 24−96 h and substantially

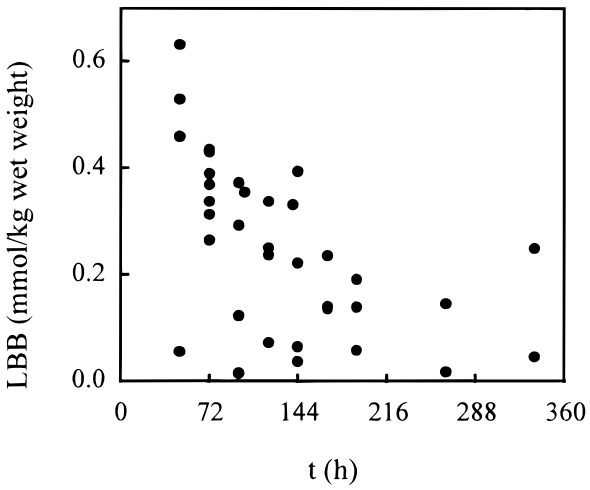

FIGURE 5. Measured lethal body burdens (LBB) of chlorthion in the pond snail for the different times of death.

underestimates toxicity at exposure times of longer than 168 h. The low qualities of the $LC_{50}(t)$ fits of the CBR model are further expressed by the low correlation coefficients (r^2), by the relatively high residual sum of squares (SS), and by the model estimates of $LC_{50}(\infty)$, which are inaccurate since they are higher than the LC_{50} values at $t = 336$ h for the respective OP pesticides (see Tables 1, 3, and A2).

Both the aqueous and whole-body CTO models describe the data in a much more accurate way and are in correspondence with the observed increasing toxicity until $t =$ 216−336 h. Although both the r^2 values and the residual sum of squares indicate the quality of the fits for the CTO_a model to be of a slightly higher degree for six of the seven OP pesticides, quality differences between the fits of the CTO_a and CTO_{wb} models are small (see Table 3 and Figure 3). The estimated incipient LC_{50} values by the CTO_a and CTO_{wb} models seem accurate since they are reasonably in agreement or lower than the observed LC_{50} values at $t = 336$ h. Although differences in incipient LC_{50} estimates between the aqueous and the whole-body CTO model are significant for some of the compounds ($p < 0.05$), the differences are small.

LBBs and BCFs of Chlorthion in the Pond Snail. The average shell lengths of the dead snails were 2.5 ± 0.2 cm, the wet weights were 0.6 ± 0.2 g, and the lipid percentages were 0.5 ± 0.2%. Neither the shell lengths and the wet weights nor the lipid percentages changed significantly during exposure. The mean recovery of the extraction and cleanup procedure was 102 ± 3%. Since no correlation between lipid content and the LBB values (mmol/kg wet weight) was found, LBBs were expressed on a wet weight basis. Individual LBBs, which ranged from 0.015 to 0.632 mmol/kg, are plotted versus their times of death in Figure 5. Average LBB(t) values are given in Table A1 (see Supporting Information) and are plotted in Figure 6. As can be seen in Figure 6, the average LBB(t) values show a decreasing trend in time. The estimated average BCF value of chlorthion in the pond snails was 35 ± 19 mL/g (wet weight). This value is in good agreement with the reported value of 31 mL/g (wet weight) (*32*), which was used to predict LBBs according to the CBR and CTO models (Table 2).

Lethal Body Burden Predictions for Chlorthion (Pond Snail) and Methidathion (Guppy). In Figure 6, the predicted LBBs by the CBR, CTO_a, and CTO_{wb} models for chlorthion in the pond snail and for methidathion in the guppy are presented. As can be seen from Figure 6, the CBR model fails to describe the apparent time dependency of the LBBs of chlorthion in the pond snail. Both CTO models, however, predict the LBBs to decrease slightly in time during the time

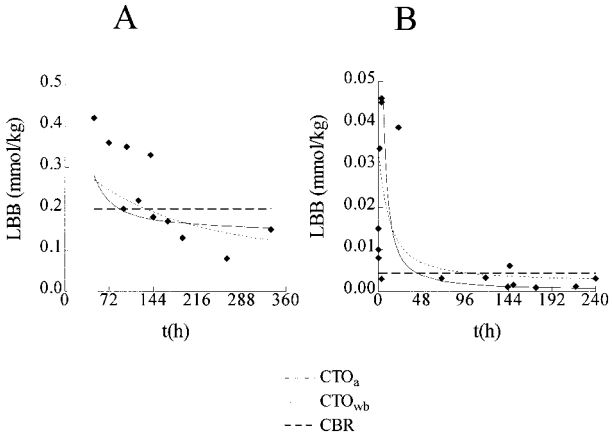

FIGURE 6. Predictions of the LBB(t) data for chlorthion in the pond snail (A) and for methidathion in the guppy (B) by the critical body residue model (CBR, − −), the whole-body critical target occupation model (CTO_{wb}, —) and the aqueous critical target occupation model (CTO_a, - - -).

scope of the sampled dead snails. The most accurate prediction of the LBBs in the ponds snail seems to be given by the CTO_a model. Nevertheless, due to the large residuals for both fits, it is not possible to give preference to either the aqueous or the whole-body CTO model on a statistical basis.

De Bruijn et al. (*35*) determined the LBBs of methidathion at two different (constant) aqueous exposure concentrations of methidathion, i.e., 0.15 μM (low exposure group) and 2.9 μM (high exposure group). Guppies in the low exposure group died between $t = 72$ and 240 h, whereas the high exposed guppies died before $t = 24$ h. The average wet weight-based LBB of the guppies were 0.0025 ± 0.0017 mmol/kg ($n = 8$) and 0.025 ± 0.018 mmol/kg ($n = 8$) for the guppies from the low and high exposure groups, respectively. These values are in good agreement with the predicted LBBs according to both the aqueous and whole-body CTO model (Figure 6). Although the predictions of the CBR model are in reasonable agreement with the LBBs of the low exposure group, they do not explain the time dependency of the LBBs.

Discussion

Validation of the CBR, CTO_a, and CTO_{wb} Models Based on $LC_{50}(t)$ Data. In contrast to the CBR model, the CTO_a and CTO_{wb} models accurately describe the time-dependent LC_{50} data of OP pesticides in the pond snail and guppy. Although the CTO_a model seems to fit the $LC_{50}(t)$ data slightly better for five of the six compounds (Table 3), the differences in quality between the two CTO models are very small.

It is important to realize that the fit of a model might be strongly determined by its input parameters. Therefore, uncertainties in the elimination rate constant k_2, which is an input parameter in both the CBR and CTO_{wb} models (Table 1), and possible consequences for the validation of the models will be discussed. The k_2 values for the five OP pesticides in the guppy were estimated by applying a QSAR for chlorobenzenes, which are generally considered as inert chemicals in fish (Table 1). However, k_2 values of OP pesticides are often higher than QSAR predictions due to the contribution of biotransformation to the total elimination (*31*). Thus, the applied k_2 values in the $LC_{50}(t)$ equations of the CBR and CTO_{wb} models might be underestimations of the actual elimination rate constants. Applying the CBR model, incipient LC_{50} values will be reached faster when higher k_2 values are applied. Consequently, this will give even more reason to reject the CBR model. The application of slightly higher k_2 values in the CTO_{wb} model (a factor 2−5) results in the coincidence of its $LC_{50}(t)$ fit with the fit of the CTO_a model.

Thus, the data will be accurately described by the CTO_{wb} model, even if slightly higher k_2 values are applied. The coincidence of the fits at slightly higher k_2 values may be explained by the fact that the CTO_{wb} and CTO_a models become approximately proportional if the internal concentration of the OP pesticide has reached a steady state before the first mortality observation time, i.e., $t = 24$ or 48 h. This steady state is indeed reached before this time in the guppy if actual k_2 values are slightly higher than the values applied in this study.

Furthermore, it may be questionned if the QSAR-based k_2 estimates for the guppy, which are determined based on sublethal exposure experiments (*31*), are representative for elimination rate constants under lethal conditions. It has been suggested that toxic stress may result in lower k_2 values under lethal conditions as compared to values under sublethal conditions (*36*). However, Van Den Heuvel et al. (*37*) and Smith et al. (*38*) demonstrated that elimination rate constants of chlorinated phenols and chlorinated benzenes in fish were not different under lethal and sublethal conditions. On the basis of these latter studies, we consider an effect of toxic stress on the elimination rate constants in the guppy not likely.

To model the toxicity of chlorthion in the pond snail, a measured k_2 value of 0.013 h^{-1}, which was determined at a sublethal exposure concentration, was applied in the CBR and CTO_{wb} models (see Table 1). While the fit of the CBR model deviates substantially from the $LC_{50}(t)$ data when this k_2 value is applied (Figure 4), the data are reasonably well predicted if a k_2 value of 0.0034 h^{-1} is applied. Since a large standard deviation was reported for the k_2 value of chlorthion in the pond snail, i.e., $k_2 = 0.013 \pm 0.013$ h^{-1} (*32*), it must be concluded that the relatively bad fit of the chlorthion $LC_{50}(t)$ data by the CBR model may at least be partly due to uncertainties in the input parameter k_2. Nevertheless, there are sufficient reasons that plead in favor of the CTO model for describing the toxicity of chlorthion in the pond snail. In the first place, both the CTO_a and CTO_{wb} models describe the $LC_{50}(t)$ data in an accurate way (Figure 4). In the second place, it was shown in a previous study that in vivo AChE inhibition by chlorthion in the pond snail is accurately described by a sigmoidal function of the logarithm of $C_w t$ (*15*). This study strongly supports the applicability of the CTO_a model since it demonstrates that the toxicity of chlorthion in the pond snail is indeed dependent on the time-integrated concentration of chlorthion in the aqueous phase. Based on the reported sigmoidal function, the estimated $CAUC_a$ or $(C_w t)_\dagger$ for chlorthion in this study (i.e., 825 μM·h) is accompanied with a whole-body AChE inhibition percentage of 97.4%. This percentage is in excellent agreement with the experimentally determined lethal inhibition percentages for chlorthion in this species, which range from 96 to 99% (*15*).

In conclusion, the experimental LC_{50} data for the pond snail and the guppy support the validity of the CTO_a and CTO_{wb} models, despite uncertainties in the input parameter k_2. It is not possible to give preference to either the aqueous or whole-body CTO model, based on the LC_{50} data of this study. Although the pond snail LC_{50} data for chlorthion provide unsatisfactory evidence to reject the CBR model, the LC_{50} data for the four phosphorothionates in the guppy are evidently in disagreement with the CBR model.

Time and Concentration Dependency of LBBs. Internal effect concentrations of OP pesticides in fish vary from 0.0025 mmol/kg, indicative for a highly specific intrinsic toxicity, to several mmol/kg, which correspond with a narcotic mode of action (*7, 30, 35*). LBBs of several OP pesticides in fish have been shown to decrease with increasing exposure duration (*30, 35*). This was attributed to a gradual shift in time from a narcotic to a more specific mode of action caused by a slow internal distribution in fish that died after very short exposure to high concentrations of OP pesticides.

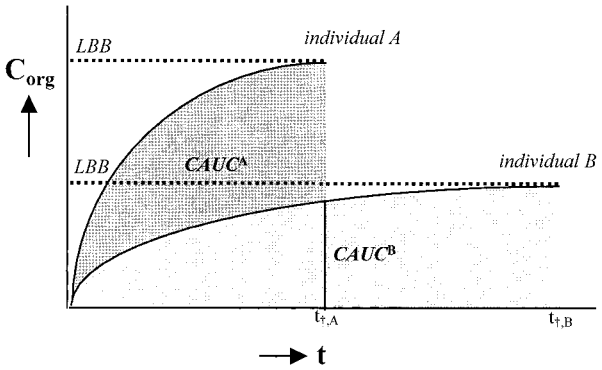

FIGURE 7. Individual A is exposed to a high (constant) external concentration of a certain OP pesticide and individual B to a low concentration. According to the critical target occupation model, the critical area under the curve at death is constant ($CAUC^A = CAUC^B$) but will be reached faster for individual A ($t_{\dagger,A}$) than for individual B ($t_{\dagger,B}$). Additionally, the accompanied internal concentrations at death (LBB) will depend on the exposure concentration.

The individual wet weight-based LBBs of methidathion in the guppy were all lower than 0.05 mmol/kg (*35*) and thus significantly lower than narcotic LBBs in fish, which are in the range of 2−8 mmol/kg (*7*). Thus, a shift in time from a narcotic to a specific mode of action seems not a plausible explanation for the time-dependent LBBs of this OP pesticide in the guppy.

According to the CTO model, mortality occurs at a critical time-integrated concentration (CAUC*)* of the toxic agent in the target tissue. Implicitly, the "time to death" of an organism is determined by the aqueous exposure concentration of the chemical (eq 6). As is illustrated in Figure 7, different exposure concentrations (and thus different times to deaths) are, for a given CAUC, theoretically associated with different internal lethal concentrations (LBBs). Thus, the dependency of LBBs on both exposure concentration and time is inherent to the CTO model. A shift in mode of action is thus not at all necessary to explain the time dependency of LBBs. Nevertheless, if internal concentrations approach narcotic levels before or at the same time that a critical AChE occupation is accomplished, toxicity will be (partly) governed by narcosis.

Implications of CTO_a Model for Risk Assessment. Whereas the CTO may be considered constant among different OP pesticides, and maybe even among species, the accompanied CAUC is dependent on both the AChE inhibition rate constant k_i and the metabolic activation rate constant k_{act} of a chemical (eq 5). Consequently, LBBs depend on the chemical characteristics of the OP pesticide, on species, and on exposure concentration and duration (eqs 9 and 13). This is obviously in contradiction with the general idea on the constancy of the LBB among time and species for chemicals exhibiting the same mode of action (*6, 7*). Hermens (*19*) and McCarty and Mackay (*7*) already mentioned that the CBR concept may not hold for chemicals exhibiting an irreversible adverse effect or a specific mode of action. The current study evidently demonstrates that the CBR concept is not applicable to chemicals that interact irreversibly with their receptor, like organophosphorus compounds. As a consequence, the use of fixed CBR for each individual mode of action as an interpretive and regulatory tool in the environmental risk assessment of chemicals (*7*) is limited to mode of actions that entail a completely reversible receptor interaction.

Fixed CTOs for groups of chemicals that act irreversibly with a specific receptor may have future potential as a tool in environmental risk assessment under the condition that

the receptor occupation can be estimated in aquatic organisms in the field. For OP pesticides, the receptor occupation may be estimated by the comparison of actual AChE activities in exposed aquatic organisms to background AChE activities in reference organisms.

The results of this study show that (acute) incipient LC_{50} values for some OP pesticides might be even a factor 10 lower than the respective 4-d LC_{50} values (Tables 1 and A2). This clearly demonstrates that it is essential to incorporate incipient LC_{50} values instead of the generally used acute $LC_{50}(t)$ values ($t \leq 4$ d) in the aquatic risk assessment for compounds that exert an irreversibly receptor interaction. The presented CTO_a toxicity model (eq 12), which does not require kinetic input parameters, supplies a simple model to estimate incipient LC_{50} values for these chemicals. Nevertheless, the model should be applied with care to very hydrophobic compounds since the CTO_a model might be restrictively applicable to situations where an internal steady-state concentration of the chemical has been (nearly) reached (which is the case for the OP pesticides in the guppy). If the CTO_a model does not succeed in the prediction of $LC_{50}(t)$ data, the more complex CTO_{wb} model should be applied instead.

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Supporting Information Available

Several pages of the Experimental Section and two tables detailing the $LC_{50}(t)$ and $LBB(t)$ values. This material is available free of charge via the Internet at http://pubs.acs.org.

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