Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment†

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Received 19th May 2010, Accepted 23rd August 2010
DOI: 10.1039/c0em00234h

Toxicokinetic-toxicodynamic (TK-TD) models simulate the processes that lead to toxicity at the level of organisms over time. These dynamic simulation models quantify toxicity, but more importantly they also provide a conceptual framework to better understand the causes for variability in different species' sensitivity to the same compound as well as causes for different toxicity of different compounds to the same species. Thus TK-TD models bring advantages for very diverse ecotoxicological questions as they can address two major challenges: the large number of species that are potentially affected and the large number of chemicals of concern. The first important benefit of TK-TD models is the role that they can play to formalize established knowledge about toxicity of compounds, sensitivity of organisms, organism recovery times and carry-over toxicity. The second important aspect of TK-TD models is their ability to simulate temporal aspects of toxicity which makes them excellent extrapolation tools for risk assessment of fluctuating or pulsed exposures to pollutants. We provide a general introduction to the concept of TK-TD modelling for environmental scientists and discuss opportunities as well as current limitations.

Introduction

Aquatic ecotoxicology provides the scientific basis for ecological risk assessment of chemicals. Ecotoxicology faces two major challenges: the large number of species that are potentially affected as well as the large number of chemicals – especially organic xenobiotics – that are used by human society, emitted into the environment[1,2] and may act together in mixtures. Another more practical issue of current concern is that of fluctuating and pulsed exposure concentrations of pollutants in the environment and how to assess toxic effects resulting from such patterns.[3–5] Taken together mixtures and time variability of exposure are even more complex to address.

We will here argue that all these current challenges may be addressed by toxicokinetic (TK) and toxicodynamic (TD) modelling. TK and TD models simulate the processes that lead to toxicity at the level of organisms over time. These dynamic simulation models quantify toxicity, but more importantly they also provide a conceptual framework to better understand the causes for variability in different species' sensitivity to the same compound as well as causes for different toxicity of different compounds to the same species.

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† Published as part of a special issue dedicated to Emerging Investigators.

Toxicokinetics and toxicodynamic models

Toxicokinetics describe the time-course of toxicant uptake, internal distribution, biotransformation and elimination in an organism, *i.e.* what the organism does with the toxicant. The toxicokinetics link the external exposure concentration to the concentration at the target site, which is the biologically effective dose[6] (Fig. 1). Toxicodynamics describe the time course of toxic action at the target site, subsequent physiological impairment of the organism as well as the influence of any compensating mechanisms and finally the emergence of toxic effects at the level of the organism such as mortality, *i.e.* they describe what the toxicant does to the organism. Fig. 1 shows a simple, illustrative example of TK-TD modelling, where internal concentration (TK) and the damage (TD) are simulated in response to exposure and lead to increased mortality. The separation of TK and TD allows identifying properties of toxicants that determine the TK, and other properties that determine the TD (Fig. 2). Similarly it can be suggested that some species traits influence TK whereas other species traits influence TD.[7,8]

Environmental impact

As toxicokinetic-toxicodynamic models bring advantages for very diverse ecotoxicological questions they deserve greater attention and a wider use. The first important benefit is the role that toxicokinetic-toxicodynamic models can play to formalize established knowledge about toxicity of compounds, sensitivity of organisms, organism recovery times and carry-over toxicity. The second important aspect of toxicokinetic-toxicodynamic models is their ability to simulate temporal aspects of toxicity which makes them excellent extrapolation tools for risk assessment of fluctuating or pulsed exposures to pollutants.

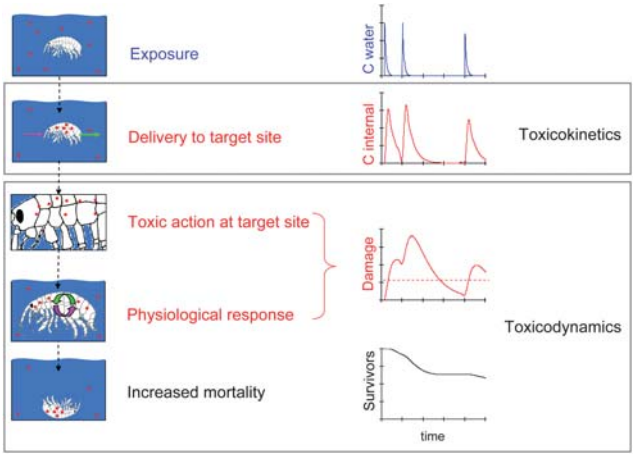

Fig. 1 Dynamic simulation of processes causing toxicity and their grouping into toxicokinetics and toxicodynamics, illustrated on the example of the aquatic invertebrate *Gammarus pulex*.

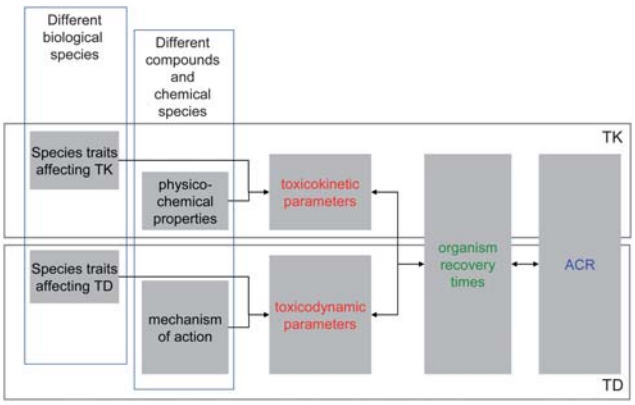

Fig. 2 Conceptual model for linking TK-TD parameters to chemical characteristics and species traits as well as organism recovery times and acute-to-chronic ratios (ACR).

A brief historical perspective on TK-TD modelling

Ecotoxicologists, especially those with a strong focus on testing for regulatory purposes; predominantly have used continuous exposures and fixed durations for testing toxic effects.[9–12] Time has rarely been explicitly considered as a variable for toxicity in a risk assessment or regulatory context (for some notable exceptions see[13–17] and those in the reviews of[3,4,18]). Nevertheless, Haber's rule is often used implicitly as this is the basis for time-weighted average approaches.[4] Haber's rule postulates that the same effects result from exposures that exhibit the same product of concentration and time ($c \times t$) and has been advocated as a vital rule in toxicology (for reviews see[14,19]). A more recent concept is that of time-to-event models (TTE).[16,20] TTE provides information on the time needed until a certain event occurs (*e.g.*, death) for each organism.

In parallel to these empirical relationships between exposure time and effect more mechanistic approaches have been developed. Most prominent is the Critical Body Residue (CBR) model,[13,21–26] which assumes that an organism dies when an internal threshold concentration is exceeded, the so-called Critical Body Residue or Lethal Body Burden. Hence there is a temporal relationship between bioaccumulation kinetics and toxicity. The CBR concept is applicable for reversibly acting compounds, such as narcotic chemicals. Narcotic compounds are all equally effective once they have reached their target site, the biological membranes, where they disturb the membrane structure and function.[27] For narcotics whole-body internal effect concentrations in fish are fairly constant for a wide variety of compounds.[25,28–30] The CBR model assumes that the time course of the internal concentrations determines the dynamics of toxicity. However, this concept is not applicable when a compound reacts irreversibly with a specific receptor. Therefore, the CBR model has been extended to the Critical Target Occupation (CTO) model,[31] where mortality is assumed to occur when a critical number of targets are irreversibly occupied. The CTO model is also applicable for reactive chemicals that form covalent bonds with target molecules like DNA[32] or compounds that elicit receptor-mediated toxicity. Further approaches related to the CBR concept are the PULSETOX model[33] and the acute toxicity model of DEBtox[34] where the effect is proportional to the concentration of the compound that exceeds an internal no-effect concentration (NEC) in the organism.

A significant step in the development of TK-TD modelling was the introduction of a state variable (a variable that changes over time and describes a property of the system) for damage by Ankley *et al.* in 1995.[35] Damage represents the reduced health of the organism very generally and is supposed to be applicable for different mechanisms of action.[36] The use of damage as an explicit representation of toxicodynamics was further developed by Lee *et al.*[37,38] into the Damage Assessment Model (DAM), which is based on the assumption of an individual tolerance distribution (see[39] for explanation). The DAM has been widely used to fit LC50 data *vs.* time.[40–43] The Threshold Damage Model (TDM) also uses damage to model toxicodynamics, but is based on the assumption of stochastic death and has been applied to simulate survival following fluctuating or repeated pulsed exposures.[36,44–46] One advantage of using damage as a toxicodynamic state-variable is, that those toxicants that act reversibly with their targets, for example with enzymes or other specific targets, can be modelled appropriately using intermediate recovery rates, *i.e.* without assuming the extreme cases of instantaneous toxicodynamic recovery (CBR concept) or irreversible interaction (CTO concept).[47] For a review of models for time dependent effects and their interrelationships see Ashauer *et al.* 2006[4] and Ashauer & Brown 2008.[47] A conceptual approach for integrating TK-TD models based on the assumption of individual tolerance with those based on the assumption of stochastic death has also been put forward.[48]

Perhaps not surprisingly there are only few TK-TD concepts that model the temporal aspects of sub-lethal toxicity endpoints mechanistically. Most prominent is the DEBtox approach[49–51] which relates internal concentrations to sub-lethal effects based on the idea that toxicants modify the allocation of energy in an organism. TK-TD models for mechanisms of sub-lethal toxicity that do not act *via* modification of energy allocation are urgently needed.

TK-TD modelling as a two step procedure

In order to use a TK-TD model one would first need to obtain parameter values for TK processes such as uptake,

biotransformation and elimination rate constants. Values for these parameters can be obtained by measuring the time-course of the toxicant of interest in the species of interest and fitting the TK sub-model to the internal concentration data (see for example[46,52–54]). The best fit parameter values are then used to simulate the time-course of internal toxicant concentrations for any other exposure pattern, including other toxicity tests or measured exposure time series. If measured internal concentrations are not available then these rate constants can also be predicted using the hydrophobicity of the compound together with the species' weight, trophic level and lipid content.[55] Further predictive approaches for TK parameters are based on in vitro methods.[56] The TK part of any TK-TD model can be refined by including more compartments and partitioning processes. Hence TK are also described as processes of adsorption, distribution, metabolism, elimination (ADME)[56] or with physiologically-based pharmaco-kinetic (PBPK) models.[57–59] Such more detailed models may provide a closer prediction of the concentration at the target sites[56–58,60] and could facilitate extrapolations between species by using different physiological parameters.

In a second step the toxicodynamic parameters need to be estimated. To do so, the TD part of the TK-TD model is fitted to experimental data, whilst the TK part of the model is run with the previously estimated TK parameters to simulate internal concentrations.[45,46] The TD model structure and method of parameter estimation can differ widely, depending on the endpoint that the model predicts (*e.g.* survival[31,36,38,46,61] or sub-lethal endpoints[51,62,63]), the model assumptions about the reversibility of effects[47] and the question addressed (*e.g.*[46,50,51,59,64,65]).

Once all parameter values of a TK-TD model are established, it can be used to predict toxic effects for different exposures, including fluctuating exposure patterns, to calculate organism recovery times or to establish model based extrapolation of toxicity to untested compounds and species (see below).

Fluctuating and pulsed exposure and resulting effect patterns

Influx of pollutants into aquatic environments is typically non-continuous, with pulses of high peaks and fluctuating concentrations and loads.[3,66–71] Very little is known about the effects of short but high peaks of toxicants and how intermittent phases with low or negligible concentrations allow recovery. Consequences of time-varying or repeated exposure may include cumulative effects of pulses,[36,44,46,72–75] post-exposure effects,[76–79] recovery between pulses,[80–83] and other patterns,[3,18,84] some of which depend on the mode of action.[85] These phenomena can in principle be explained and simulated by TK-TD models. As the underlying processes of the time course of toxicity and organism recovery are simulated in TK-TD models these can be used to simulate effects resulting from time-varying or repeated pulsed exposures.[5,36,44,46] The validity of toxicity predictions beyond the range of empirical data (exposure patterns, test duration) is inherently difficult - or even impossible - to assess,[86] but when such predictions are required, as for example for the unlimited number of exposure patterns produced by pesticide fate modelling for regulatory purposes,[5,87] then simulations with mechanistic TK-TD models may be more trustworthy than empirical relationships.

Carry-over toxicity

Delayed toxicity can occur after pulsed exposure to toxicants, but is usually investigated with only one exposure event. Carry-over toxicity describes the phenomenon that organisms, which have been pre-exposed to a toxicant, suffer greater toxic effects from a subsequent later exposure to the same dose of that toxicant, even if there was some time in unstressed conditions in between the two events.[46] In a sense carry-over toxicity is the logical consequence of delayed effects, although carry-over toxicity can also be observed after delayed effects have ceased because the organism may still carry some damage. Damage that does not cause delayed effects anymore may still contribute to carry-over toxicity. Carry-over toxicity can be caused by TK (accumulated toxicant) or TD (accumulated damage) or both and TK-TD models are ideal for explaining and simulating carry-over toxicity as well as delayed effects.

Delayed effects and carry-over toxicity are highly relevant for the assessment of fluctuating and pulsed exposures to toxicants. The lengths of pulses, intervals and recovery periods need to be accounted for and recovery needs to be explicitly factored in when setting up models describing delayed toxicity. As TK-TD models can simulate toxic effects over time and also account for delayed effects as well as carry-over toxicity they are ideally suited to quantify risk of adverse effects from fluctuating or pulsed exposure patterns.

Organism recovery times

The phenomenon of carry-over toxicity directly triggers the question how long it takes for an organism to recover enough so that carry-over toxicity does not occur any more. In other words: organisms have recovered from a dose, when a subsequent exposure to the same dose does not cause more toxicity than the previous exposure. Again a TK-TD model parameterised for a given combination of compound and species can also be used to calculate this organism recovery time.[45,46] Organism recovery times are specific for each combination of species and compound and depend on two processes contributing to organism recovery: the time-course of TK (elimination of toxicant) and TD (recovery of damage). For example the freshwater amphipod *Gammarus pulex* needs 3, 4, 15, 25 and 28 days to recover from exposure to pentachlorophenol, 4,6-dinitro-o-cresol, carbaryl, chlorpyrifos and diazinon respectively[45,46] (and unpublished data). With TK-TD models it is possible to calculate the corresponding effect level for any duration, hence it is also possible to calculate the fraction of a given acute-to-chronic ratio that is caused by the difference in test duration. The remaining part of the acute-to-chronic ratio must then be due to the different endpoints used (Fig. 2).

Sequence effect in mixtures

Damage is defined without reference to a specific target, biochemical marker or organ within the organism. Rather it is inferred from toxic effects observed on the organism level.

Consequently damage from different toxicants may add up and cause mixture toxicity on a temporal scale.[44] Different organism recovery times for different compounds mean that sequential exposure to these compounds may, under some conditions, result in different toxic effects depending on the order of exposure to the different compounds. Such a sequence effect has been observed for compounds acting on different targets[72] and was predicted and observed for compounds that act on the same target, although with different organism recovery times.[44] The sequence effect should be investigated for more combinations of compounds and it needs to be tested how well the sequence effect can be predicted by TK-TD modelling in other combinations of species and compounds.

Mechanism based extrapolation to untested compounds

The challenge of assessing a large number of chemicals can be addressed by systematically explaining how differences in toxicity emerge and by grouping chemicals with similar features (*i.e.* similar model parameters; as explained in the following). TK-TD modelling offers a conceptual framework for such understanding (*e.g.*[65]). Chemical characteristics that influence TK are for example physicochemical properties of compounds and susceptibility to biotransformation. They can be distinguished from those that influence TD such as for example the intrinsic potency of the compound (*i.e.* without the influence of its bioaccumulation potential) and the mechanism of toxicity (see Fig. 2). Reactivity of a chemical would be a parameter that both influences TK (as reactive chemicals are less persistent) and TD (as toxic potency of reactive chemicals is often correlated to their reactivity towards biological nucleophiles[88]). If quantitative relationships could be established between TK and TD model parameters on the one side and descriptors for chemicals then these could be used to predict toxicity of untested compounds. Quantitative relationships could be look-up tables for model parameters or regressions between model parameters and chemical properties. For TK, more specifically for bioaccumulation assessment, research is quite advanced[89] and such correlations have been established already,[55,90–92] but for TD these relationships have not been found yet. We suggest that a TK-TD framework would help to establish this missing link, especially because TK-TD modelling allows using mechanistic insight, for example about the mechanism of action.

Mechanism based extrapolation to untested species

The challenge of protecting a large number of organisms is hindered by an incomplete understanding of the causes of differences in species sensitivity. TK-TD modelling provides the conceptual framework for systematically understanding which species traits (properties of species) affect TK and which species traits affect TD (see Fig. 2 and Rubach *et al.* 2010[8]). It can be hypothesised that there are different species traits affecting these two groups of toxicity processes.[7] If quantitative relationships between species traits and TK or TD model parameters can be established such relationships can then be used to predict the sensitivity of untested species.[8] Again there is already a better understanding how TK relate to species characteristics (*e.g.*[26,55,93]) than TD. Investigating differences in species sensitivity within a TK-TD modelling framework is as much a systematic approach towards understanding fundamental ecotoxicological phenomena (*i.e.* why species differ in their sensitivity), as it may be the basis for extrapolating toxicity to untested species.

Models and ecotoxicological theory

Models also structure and preserve the scientific knowledge about the processes they describe. Toxicokinetic models (*e.g.*[26,55,91,92]) are a distillation of many years of research of different groups of scientists and transform this knowledge into applicable, useful tools. Furthermore they facilitate scientific process as they can be challenged by new findings which are not explained by the models. This is only possible because these models provide the most concise and rigorous representation of current assumptions in a field and make quantitative predictions. Similar benefits can be expected from using TK-TD models to formalise our understanding of toxicity. Currently used dose-response models are of limited use for investigating temporal aspects of toxicity because they do not include the temporal dimension. TK-TD models are more powerful than dose-response models because they include both, the chemical concentration as well as the temporal dimension.

An interesting example of an unresolved ecotoxicological questions is the problem of stochastic death *vs.* individual tolerance concept.[39,94] The hypothesis of stochastic death assumes that stressed individual organisms of a population have the same probability of dying whereas the hypothesis of an individual tolerance distribution assumes that each individual organism has a different tolerance for stress and dies when it is exceeded. Both theories can explain traditional dose-response curves which may have contributed to the fact that this fundamental ecotoxicological question is still unresolved today. The predictions of both theories for effects from repeated pulsed exposures differ.[39,48,94] Including TK-TD modelling in individual-based population models context may be able to connect

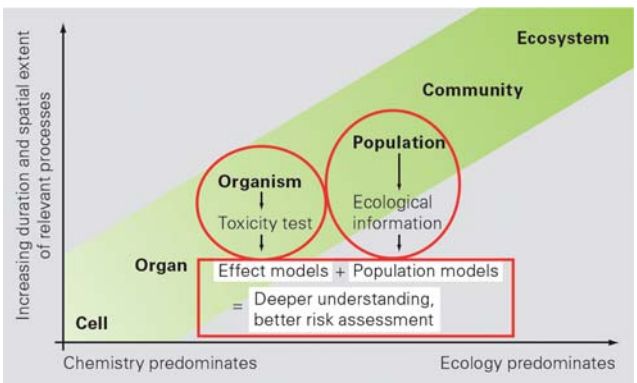

Fig. 3 Organism level toxicity models such as TK-TD models ('effect models') may be coupled to population models such as IBMs. Ecological processes dominate system at larger temporal and spatial scales whereas systems at smaller temporal and spatial scales are dominated by (bio-)chemical processes. The level of the organism is just in the middle and thus of particular interest.

both theories[48] as it appears that the whole set of TK-TD parameters of an individual may constitute its "individual tolerance".[48] The latter finding is an example of improved understanding through TK-TD modelling.

Finally there is a demand for integrating more ecology into environmental risk assessment. One way of incorporating ecological knowledge into ecological risk assessment of chemicals are individual based models (IBMs).[95,96] It appears straightforward and promising to link TK-TD models with IBMs to improve risk assessment of chemicals with dynamic models which appropriately simulate temporal aspects of toxicology as well as reflect ecological aspects of relevance (Fig. 3).

Conclusions

TK-TD modelling is currently receiving increasing attention as it has been recognised that this technique may help to extrapolate toxic effects between different exposure regimes, including fluctuating exposures encountered in, for example, risk assessment of pesticides.[5] Nevertheless there are several important challenges currently limiting the application of TK-TD modelling to risk assessment of chemicals.

• First, more work needs to be done to establish relationships between mechanisms of the processes leading to toxicity and the TK and TD model parameters. The toxicokinetics are mainly determined by the hydrophobicity and metabolic activity and the toxicodynamics should be influenced by the mode of toxic action, the reversibility of effect and the threshold of effect. Systematic research is required to explore this hypothesis and to establish mechanism based TK-TD models.

• Second, the development of more mechanistic approaches for modelling sub-lethal toxicity is needed. DEBtox models are the first choice when the toxicant affects sub-lethal endpoints by modification of energy allocation within the organism. The parameterisation of these models usually requires full life cycle testing which is difficult for many species. Furthermore there is a lack of toxicodynamic models for sub-lethal effects that are caused by mechanisms unrelated to energy allocation (*e.g.* reduced emergence of caddis fly larvae weeks after brief pulse exposure,[79] activation of estrogen receptors or other adverse outcome pathways[97]).

• Third, the variability of TK-TD parameters amongst individuals can currently not be quantified although this would be highly desirable for the link to IBMs.

• Finally there are only very few studies which systematically investigate the relationship between species traits and TK-TD parameters (*e.g.*[93]), although such relationships are potentially very useful.[8]

As TK-TD models bring advantages for very diverse ecotoxicological questions they deserve greater attention and a wider use. The most important benefits in our view are the role that TK-TD models can play to formalize established knowledge about toxicity of compounds and sensitivity of organisms, generate new hypotheses (*e.g.* carry-over toxicity) and their ability to simulate temporal aspects of toxicity which makes them excellent extrapolation tools for risk assessment of fluctuating or pulsed exposures.

Acknowledgements

We thank Mascha Rubach for suggesting the link between TK-TD modelling and species traits. We also thank two anonymous reviewers for constructive comments. This study was funded by the Swiss National Science Foundation (grant 200021-119795), the Bundesamt für Umwelt (grants 09.033.PJ/I362-1602 and 09.0012.PJ) and the SETAC-CEFIC-LRI Innovative Science Award.

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