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Effects of fluctuating contaminant concentrations

Is it true that "the dose makes the poison"? How long does it take for exposed organisms to recover? What are the effects of sequential exposure to contaminants? These are some of the questions we are trying to answer with the aid of toxicokinetic/toxicodynamic models and experiments.

Traditionally, toxic effects of contaminants have mainly been measured and assessed on the basis of concentrations in the environment or in the test medium. Toxicity is described in terms of the concentrations at which 50 % of test organisms (e. g. fish or freshwater amphipods) show a response (effective concentration/EC_{50}) or die (lethal concentration/LC_{50}). However, little attention is paid to the period of exposure producing these effects. The duration of ecotoxicology tests is often determined in an arbitrary way: for example, one of the most important tests – the acute fish toxicity test – runs for four days, so it can be conveniently carried out in the course of a working week. For these historical reasons, there are hardly any instruments or methods in ecotoxicology and chemical risk assessment that take temporal aspects (dynamics) into account.

Nonetheless, advances in environmental monitoring, chemical analysis and modelling of the environmental behaviour of chemicals mean that, increasingly, exposure to contaminants is no longer simply described by a single concentration, but by concentration time series. Concentrations are of course not constant over time but can fluctuate widely, with repeated peak exposures occurring, for example, at wastewater treatment plant outlets and following heavy rainfall in agricultural areas (e. g. after the application of pesticides) or on urban surfaces (e. g. leaching and runoff from facades and roads). Eawag therefore aims to develop theoretical concepts, experimental approaches and mathematical models allowing explicit characterization of the time course of toxic effects. One particular priority is the development of toxicokinetic/toxicodynamic (TKTD) models [1–4].

Eawag technician Anita Hintermeister monitoring an experiment that involved exposure of *Gammarus pulex*.

Toxicokinetics: what the organism does with the substance – toxicodynamics: what the substance does to the organism. TKTD models comprise mathematical equations that describe two key aspects of how a toxic substance interacts with a test organism: firstly, the toxicokinetic component, which deals with various processes from uptake through metabolism to elimination and, secondly, the toxicodynamic component, which is concerned with when toxic effects occur and how potent they are.

Thus, in contrast to classical EC_{50} or LC_{50} values, which merely provide a snapshot, TKTD models cover the entire time course of the processes associated with toxic action. To do so, however, the model must first be parameterized – i. e. a series of model parameters need to be determined experimentally in advance. In the case of our Threshold Damage Model (TDM), these are: the uptake and elimination rate constants, the damage and recovery rate constants, and the threshold above which damage to the organism is sufficient for toxic effects to become visible. These parameters depend on physicochemical properties and modes of action, and they are specific to each compound and test organism. At Eawag, we use the freshwater amphipod *Gammarus pulex* as a model organism. The TDM is, however, applicable for different modes of action with different rates of recovery, while most other models designed for this purpose are special cases of the TDM which are only applicable with certain restrictions, i. e. only for particular modes of action [4]. A universal model of this kind represents a major advance, providing an improved framework for carrying out mechanistic ecotoxicology on a quantitative basis.

Significance of timing, as well as dose, for toxicity of multiple exposures. With pesticide contamination in particular, recur-

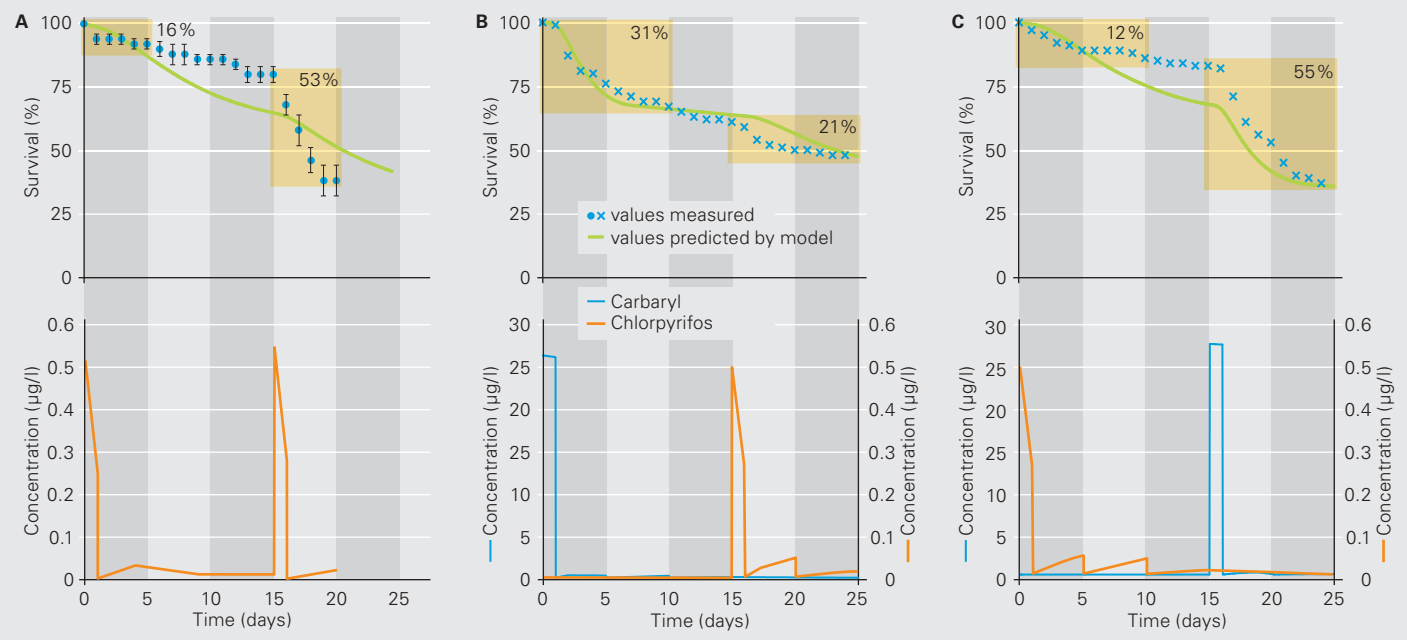


Fig. 1: Survival of *Gammarus pulex* (top) exposed to fluctuating concentrations of contaminants (bottom). A: Repeated exposure to the pesticide chlorpyrifos, with an interval of 14 days between pulses. B and C: Exposure to the pesticides chlorpyrifos and carbaryl in different sequences, with intervals of 14 days between pulses. Yellow boxes = Percentage of organisms dying.

rent peaks are to be expected in surface waters. For this reason, we chose this group of substances for our first experiments. In Switzerland, specific concentration limits are currently being elaborated for each pesticide – the acute (AQC) and the chronic quality criterion (CQC) (bottom of Fig. 2) [5]. Unlike the AQC, it is permissible for the CQC to be exceeded in natural waters. However, it has yet to be established scientifically how often such exceedences may occur, and at what intervals. The values to be specified for intervals between exposures and transgression of the CQC will depend on how rapidly an organism can recover from a previous exposure to a contaminant – and our TKTD models can be used to answer such concrete questions.

Our experiments show, for example, that delayed toxicity may occur if the recovery period between two equally intense pesticide exposures is not sufficiently long (Fig. 1A). Even if the *Gammarus* had enough time – here, 14 days – to eliminate the pesticide from the first exposure, their physiological condition may not yet have returned to the normal range. The second exposure is then more toxic than the first. In the case of chlorpyrifos, about 16 % of the organisms died after the first contact with the pesticide, and another 53 % after the second exposure. This means that the toxicity is determined not only by the dose, but also by the timing of the application in relation to previous exposures to the same [3] or different stressors [2].

Sensitivity affected by the sequence of exposures. An important role is also played by the sequence of exposures to different contaminants. This is illustrated by the example of the pesticides chlorpyrifos and carbaryl, as shown in Fig. 1B and C. When the *Gammarus* were exposed first to carbaryl and then, 14 days later, to chlorpyrifos, the death rates were 31 % and 21 % respectively (Fig. 1B). When the order was reversed, 12 % of the organisms died after exposure to chlorpyrifos and 55 % after exposure to carbaryl (Fig. 1C). Thus, the mortality associated with carbaryl is 31 % in one case and 55 % in the other, although the organisms were exposed to the same dose in both cases. The increased toxicity is caused by the previous exposure to chlorpyrifos, even though this occurred 14 days earlier. This suggests that the organisms had not recovered sufficiently. The difference between the death rates associated with chlorpyrifos (12 % or 21 %) is less marked, indicating that the organisms were able to recover more rapidly from the previous exposure to carbaryl.

The toxicity of a substance thus also depends on the situation with regard to earlier exposures. Overall, this means that the toxic potential of a substance will be greater if organisms have been damaged by previous exposure to a contaminant (either the same or a different one).

Our experiments also showed that the measured values are in close agreement with the results predicted by the model (Fig. 1). In future, it will therefore be possible not only to predict realistic toxicities for fluctuating contaminant concentrations using the model alone, but also to include directly in the simulation the safety factors (e. g. 100-fold factor) that are required in risk assessment (Fig. 2).

Ongoing experimental studies and future potential. After our work on pesticides, we are currently seeking to extend the TKTD model to a larger number of substances with different properties

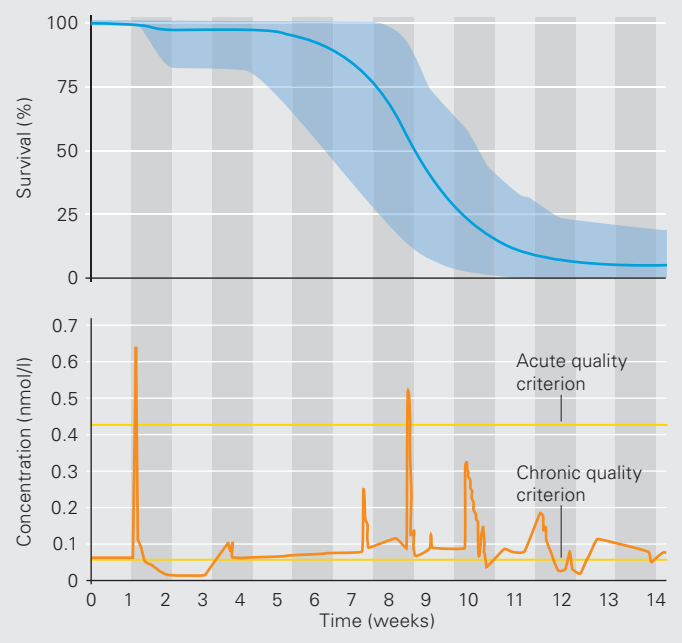
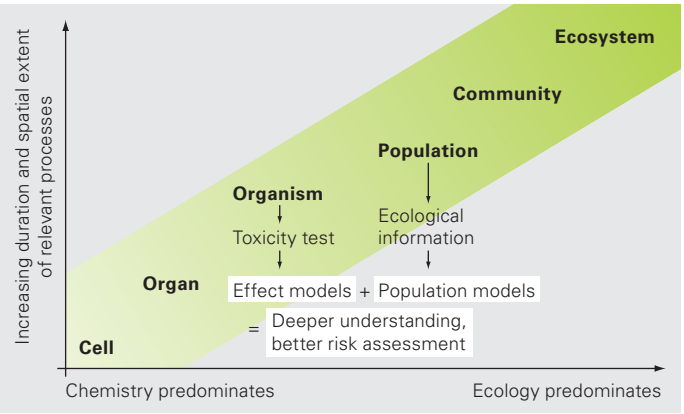


Fig. 2: Typical contaminant concentration profile in a stream (bottom) and the associated survival probabilities (blue band, top) simulated using the TKTD model. To obtain the model input, the concentrations are multiplied by a safety factor.

and modes of action. We are studying the hypothesis that certain toxicodynamic parameters, such as the recovery rate, depend on the mode of action. If this were the case, these parameters could be derived from the modes of action, or conversely, the mode of action from the parameters. As well as broadening the foundations of ecotoxicology, such relationships would also be helpful in estimating the environmental toxicity of numerous chemicals without the need for additional experiments. This would be very useful, e.g. in connection with REACH, the European Union's new chemicals regulation. As the TKTD models explicitly simulate time courses and processes, we also hope to gain a better understanding of how short-term and long-term toxicity values are related – so-called acute-to-chronic ratios (ACRs). These ratios are an important element in the determination of safety factors for risk assessment. As well as improving risk assessment, a better understanding of ACRs would reveal how they relate to the recovery time of organisms. In cooperation with Wageningen University (the Netherlands), we intend to use TKTD models to explain how observed differences in sensitivity between different aquatic organisms are attributable to species-specific characteristics.

In order to include in chemical risk assessment not only processes occurring at the individual level but also the ecology of aquatic organisms, we are also seeking to integrate TKTD models into population models (Fig. 3). This should make it possible to analyse the relative contributions of the recovery of aquatic organisms at various levels of biological organization and, on this basis, to formulate appropriate protection measures.

Fig. 3: Integration of mechanistic effect models (e.g. TKTD models) and ecological information described by population models. As different processes (biochemical vs ecological) predominate at different scales, a combination of different models provides a more comprehensive understanding and an improved risk assessment of chemicals.



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