Making sense of sub-lethal mixture effects

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1. Introduction

Typical approaches for analyzing mixture ecotoxicity data only provide a description of the data; they cannot explain observed statistical interactions, nor explain why mixture effects can change in time and differ between endpoints. To improve our understanding of mixture toxicity, and ultimately make accurate predictions for untested mixture and exposure situations, we need to explore the use of biology-based models. These models allow us to implement our knowledge on biological and toxicological mechanisms, and test whether this is sufficient to explain the observed response of organisms. When experimental data deviate from the model predictions, the nature of the deviation provides excellent information for designing additional research. In this contribution, we present an extension of the biology-based method DEBtox to deal with sub-lethal effects of mixtures on growth and reproduction. The mixture approach for survival was presented earlier by Baas *et al*. [1]. DEBtox explicitly accounts for the toxicokinetics of all components in the mixture. The toxicodynamic component of the method is now formed by a non-simplified implementation of dynamic energy budget (DEB) theory [2], which provides a natural framework for the interactions between different metabolic processes in organisms (and toxic effects on these processes).

2. Theory and model development

Any biology-based approach for the analysis of toxicity data should consider toxicokinetics (going from external concentration to target site) and toxicodynamics (going from target site to effects on specific endpoints). To understand life-history responses such as growth and reproduction, it is essential to have a theoretical framework explaining the links between feeding, growth, development and reproduction over the life cycle. For this purpose, we focus on dynamic energy budget (DEB) theory (Fig. 1). This theory explains how individuals acquire and use resources over their life cycle, based on a set of simple rules for metabolic organization. Within this theory, organisms are treated as dynamic systems with explicit mass and energy balances. DEB theory formed the basis of the DEBtox approach for sub-lethal effects [3], as recently included in OECD guidance [4]. Toxicant effects are treated as a change in a parameter of the metabolic machinery, for example, as an increase in the maintenance costs or a decrease in the assimilation of energy from food. The DEB rules subsequently establish how a change (over time) in such a parameter affects growth, development and reproduction over the life cycle.

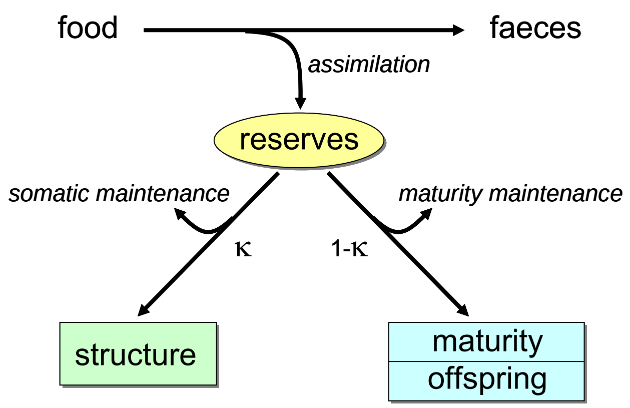

Figure 1: Conceptual basis of Dynamic Energy Budget (DEB) theory for animals.

The DEBtox approach that has been used so far applies a reduced implementation of DEB theory to simplify the calculations; the reserves were assumed to be in steady state at all times, reproduction was assumed to start at a fixed body length, and the costs for offspring were taken constant. When toxicants change metabolic parameters, however, these assumptions turn out to be inconsistent in some cases. For example, a change in the costs for making new structure has logical consequences for the length at first reproduction and the costs for the production of an egg. The quantitative consequences of violating these assumptions are usually minor, but the current speed of computer processing does not limit the model formulation to the extent that it did a decade ago. Therefore, we decided to depart from the extended DEB equations as recently presented [2], including the explicit calculation of reserves and maturity as state variables. Apart from full consistency in the energy budget, the full implementation also means that alternative modes of action (such as effects on the allocation fraction, κ, see Fig. 1) can now be calculated.

3. Implementation of mixture effects

Accounting for mixture exposure in a DEB context is conceptually straightforward (Fig. 2). All chemicals have their own toxicokinetics module; exposure to a constant mixture composition leads to a time-varying mixture inside the organism. Once inside, the toxicants can interact with targets that in turn affect the physiological processes in the DEB model (i.e., metabolic parameters). Two chemicals in a mixture may affect the same physiological process (through the same or different target sites), or different processes (necessarily through different target sites). The integrated effect of the physiological processes on growth and reproduction is determined by the DEB allocation rules. We do not *a priori* assume interactions between the mixture components. However, the different metabolic processes (Fig.1) interact in their effect on growth and reproduction, and therefore, interactions on the life-cycle response have in fact become unavoidable.

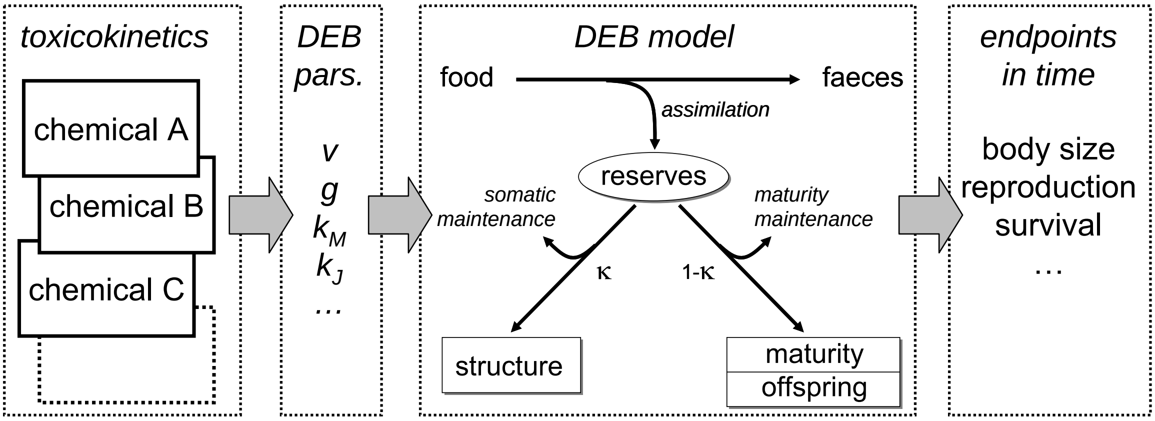

Figure 2: Schematic representation of the biology-based approach towards mixture toxicity.

In the scheme of Figure 2, there is (theoretically) no limit to the number of components in the mixture. Furthermore, toxicant concentrations and mixture composition may change in time, and it is also straightforward to include non-toxicant stressors (e.g., food limitation and temperature). Furthermore, DEB theory also accommodates effects on other endpoints such as respiration and product formation.

4. Outlook

We present a biology-based approach to analyse mixture effects on sub-lethal endpoints. The approach is based on an implementation of DEB theory for a generic animal. Because this method departs from physiological processes, it explicitly predicts toxic effects on all endpoints in time over the entire life cycle. Furthermore, it provides a natural framework for extrapolation to environmentally relevant exposure situations. This approach is still awaiting rigorous testing, but we will illustrate the procedure with an analysis of experimental data for two PAHs on growth, reproduction and survival in *Daphnia magna*. This analysis is a "proof of concept", and clarifies what type of experimental setup is required for a biology-based analysis.

5. References

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