A review of DEB theory in assessing toxic effects of mixtures

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Abstract

In this manuscript we review the use of mechanistic models to interpret effects of mixtures of compounds within the framework of the Dynamic Energy Budget (DEB) theory. Within this approach the effect of a mixture is built up from the effects of the individual components making up the mixture. Understanding effects of mixtures is essential as it is impossible to assess effects of all possible mixtures experimentally.

In contrast to the more classical way of interpreting effects of mixtures with concentration addition or effect addition models, DEB theory offers a single consistent framework to understand effects of mixtures on growth, reproduction and survival in an integrated, way. It systematically incorporates exposure time and biology of the organisms, including the natural links between the processes of feeding, maintenance, growth, development and reproduction. We also give directions for an experimental setup to interpret the results within the DEB framework.

The DEB framework was successfully applied to assess effects of complex mixtures on survival and binary mixtures on sub-lethal endpoints. It gives the possibility to explain observed interactions by the underlying biological mechanisms or pinpoint interactions. We expect this approach to help in identifying key mechanisms and enable to focus further research in cooperation with modelers and experimentalists to improve our understanding of the mechanisms underlying mixture toxicity.

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that compounds can exert effects in mixtures in concentration ranges in which the single toxicants do not show effects (e.g. Baas et al., 2009; Silva et al., 2002; Walter et al., 2002). Because it is impossible to experimentally assess the effect of all possible mixtures for all species and exposure conditions we need tools to make extrapolations and to understand effects of mixtures. For example, a mixture of 20 pesticides, contains 190 pairs and more than a million possible combinations involving pairs, triples, etc. (Lydell et al., 2004). Consequently, there is a need for (preferably simple) models that can predict the toxicity of complex mixtures.

The tools most often used to assess effects of mixtures are the concentration addition (CA) and effect addition (EA, also known as Independent Action) models (Loewe and Muischnek, 1926; Loewe, 1927; Bliss, 1939). CA is generally used for mixtures of compounds with similar modes of action and EA is generally used for mixtures of compounds with different modes of action. Both are descriptive and use effect data of single compounds to predict effects of mixtures or to find interactions for binary mixtures at a single point in time (Jonker et al., 2005). Interactions are defined as statistically significant deviations from the CA or EA models.

The main drawback of this approach is that these approaches regard the exposed organism as a black box. Effects on different endpoints are interpreted separately and the cause of the interactions is not understood, which hampers the possibility to extrapolate experimental results to e.g. different points in time, different or more compounds, time-varying exposure, food limitation, different organisms or different endpoints. Consequently finding some statistically significant interaction at some point in time for some (binary) mixture for some endpoint without further perspective should not be the aim of one’s research.

A viable alternative should provide a process-based description of the population relevant endpoints mortality, growth and reproduction within a single consistent framework. Endpoints in general must not be viewed separately as they reflect toxic effects in the same organism. A focus on the level of resource allocation and energy budgets offers a natural entrance to interpret toxic effects of multiple organisms. A focus on the level of resource allocation and energy budgets must be considered as closely interlinked processes of hazard modeling. For effects on growth and/or reproduction, the approach is the assumption that toxicants must be taken up by the organism before they can exert an effect. The internal concentration determines the magnitude of effects and once it is built up above a certain threshold level effects start to show. The process of uptake and elimination, in its simplest form described by a one compartment model, plays a vital role on how effects build up in time. Therefore the first step should be a toxicokinetics model. Once the toxicant is inside the organism the internal concentration affects one or more parameters in the DEB model. Affected parameters can be the feeding rate, the maintenance costs, the costs for an egg, the probability to die, etc (see e.g. Jager et al., 2004). Toxic effects on the different processes have specific patterns in time, which implies that the affected processes can generally be identified from the data and translated to an impact on parameter values. This approach has the advantage that the same modeling framework can be applied to a large variety of different species without modifications. This in contrast to the physiology based pharmacokinetic pharmacodynamic (PBPK-PD) approaches, which are very species specific.

A schematic view of the modeling framework is shown in Fig. 1. If a toxicant affects the growth pattern of an organism, it automatically also affects the toxicokinetics of all other components in the mixture. So different compounds can have largely independent toxicokinetics, but an effect on body size of one of the constituents in a mixture influences toxicokinetics for all components in a mixture in the same way. This can lead to interactions in mixtures, that can be readily explained by the toxicokinetics.

Effects on survival can be interpreted with simplifications from DEB theory. In acute survival test, the organisms are generally not fed during exposure, and the test duration is short enough to avoid major starvation effects. Therefore we may ignore all considerations about metabolic processes and focus solely on the stochastic aspects of hazard modeling. For effects on growth and/or reproduction, the energy budgets must be considered as closely interlinked processes requiring a more elaborate modeling framework. In the next sections we will first give a description of how effects on survival are interpreted and then we exceed to the more elaborate interpretation of combined survival and sub-lethal effects.

2.2. Survival

Survival is assumed to follow the hazard model with a threshold concentration, the no effect concentration (NEC) (Bedaux and Kooijman, 1994). The toxicant, once inside the organism and at the target site, may increase the probability of death. If the organism changes size during

![Fig. 1. Schematic view of the modeling system. The first step is a toxicokinetic module, followed by a description of how processes are affected by a toxicant with a feed back on the kinetics, resulting in an observed effect.](image-url)
exposure the kinetics change and corrections have to be made (Kooijman and Bedaux, 1996). The corrections however, do not change the conceptual framework.

Combining the one compartment model with the hazard model gives a link between external concentrations and effects, with few parameters. For each compound in a mixture three toxicity parameters are needed to describe its effect on survival at any point in time: the no effect concentration (expressed as an environmental concentration), the killing rate (1/(environmental concentration · time)) and the elimination rate (1/time).

One extra parameter is needed to correct for the mortality in the controls, this parameter does not depend on the effects of the mixture. The no effect concentration (NEC) is the time independent threshold concentration to which an organism can be exposed for a prolonged time without an effect, basically the LC₅₀ for an infinite exposure time. Note that the NEC might be zero, in that case any exposure, however small will ultimately have an effect. We showed that compounds in a mixture can all contribute to the NEC (Baas et al., 2009), which implies that in mixtures effects can be expected in concentration ranges where the individual compounds making up the mixture do not show effects. Compounds with a different mode of action have their own individual NEC. Once the NEC is exceeded there will be an effect. The “strength” of the effect is described by the killing rate, the more toxic the compound, the higher the killing rate. The last parameter is the elimination rate. This parameter describes how fast the equilibrium between internal and external concentrations is achieved.

If exposure is to a mixture of components the number of parameters increases rapidly if all possible interactions are taken into account. For a mixture of $n$ components we need $1 + 3n + n(n - 1)/2$ parameters to describe the effect and all interaction parameters. For binary mixtures, estimating interaction parameters is feasible, but for mixtures containing more compounds this rapidly becomes impossible. However, strong interactions appear to be an exception, especially in mixtures with increasing numbers of components (Lydy et al., 2004; Warne and Hawker, 1995).

2.3. Growth, reproduction and survival for single compounds

If a toxicant reduces reproduction, there must be energetic consequences; apparently, less energy is devoted to offspring formation, which could mean that less resources are taken up (e.g., a reduction in feeding rate) or that resources are allocated to other metabolic processes (e.g., defense against toxic damage). Another possibility is that the toxicant affects the growth process, which has indirect consequences for reproduction as body size determines feeding rates, and affects the start of reproduction. Ecotoxicological studies usually do not report data about the toxicokinetics or energy budgets; at best we have the time course of effects. Therefore, the physiological modes of action generally have to be inferred from the time course of toxicity alone (Alda Alvarez et al., 2006). However, the different modes of action have distinct consequences for the growth and reproduction effects in time (Kooijman and Bedaux, 1996; Jager et al., 2004; Alda Alvarez et al., 2006). An example of how the different modes of action lead to different growth trajectories is shown in Fig. 2 (more examples can be found in the paper on effects in time by Baas et al. elsewhere in this special issue).

In general the DEB-modes of action are rather non specific, for example, the DEB- mode of action “effect on assimilation” may result from a decrease in feeding rate, a decrease in assimilation efficiency in the gut, or perhaps even from changes in the cellular machinery responsible for synthesizing reserve compounds.

In mixtures toxicants can show synergistic or antagonistic effects resulting in complex behavior in time. If two compounds affect different energy allocation processes (e.g. maintenance and assimilation) an interaction between these processes has become inevitable because of the allocation rules in the DEB model. Some compounds, each with a specific DEB-mode of action, can show synergistic effects. If one compound reduces the uptake of food and other compound reduces maintenance we expect a synergistic response. Because when less food is taken up maintenance will make up a larger part of the total energy budget, therefore the same factor of increase in maintenance produces a stronger effect on growth and reproduction, similar to a synergistic effect of food limitation.

3. Application of the modeling framework

3.1. Survival

The hazard modeling approach was used to analyze the effect of 6 binary mixtures of heavy metals in the springtail Folsomia candida (Baas et al., 2007). Survival was scored daily over a period of 21 days and the complete time series of the effect was fitted simultaneously with only 8 parameters. The effect of 5 out of 6 mixtures could be fitted without any additional interaction parameter.

A further application was in the effect of a mixture of 4 poly aromatic hydrocarbons (PAHs) on the survival of the flour beetle Tribolium castaneum. For PAHs or narcotic compounds in general the hazard model predicts that the toxicity parameters are a function of their resp. $K_{\text{mic}}$ values, which was supported by experimental work (Jager and Kooijman, 2009). This means that for a mixture of PAHs one only needs experimental data on one of the PAHs in the mixture and then the whole mixture effect can be calculated for any point in time.

![Fig. 2](image-url) Left, example of the impact of an increase for the cost for structure and reproduction on the body length of Caenorhabditis elegans exposed to pentachlorobenzene, concentrations in mg/l agar (data taken from Alda Alvarez et al., 2006). Right, example of the impact of an increase for the cost for maintenance on the body length of Folsomia candida exposed to triphenyltin, concentrations in mg/kg food. Data taken from Jager et al. (2004).
using the $K_{ow}$ values to obtain the other toxicological parameters (Baas et al., 2009).

The DEB approach also proved to be very successful in predicting effects of mixtures as can be found in Dutch surface waters. In these waters over 80 different toxic components occur in varying concentrations (PAH, pesticides, heavy metals, salts, nutrients, PAHs). In addition to the chemical contamination the survival of in situ exposed Daphnia magna is measured (Baas et al., 2009). In 92% of the cases we investigated we made a correct prediction on the occurrence of survival or mortality of the exposed daphnids given the chemical contamination in the surface waters. In case of mortality it was possible to identify the compound or group of compounds causing the observed mortality. Knowing which compounds cause the observed effect in such a complicated system is of course of great importance to water management. This approach also removes the need to use time consuming toxic identification evaluations (e.g. Bailey et al., 1996; Norberg-King et al., 1991).

3.2. Growth, reproduction, survival

The DEB approach for sub-lethal endpoints has been applied to a range of single chemicals and organisms. Also combinations of chemicals with another stressor (especially food limitation) have been presented (e.g. Heugens et al., 2003; Pieters et al., 2006). Only recently appropriate datasets have become available to test the DEB-sub-lethal approach to (binary) mixtures of toxicants. The main achievement is that effects on growth, reproduction and survival can be interpreted simultaneously within one single consistent theoretical framework and that interactions can be understood from a biological point of view.

4. Discussion

4.1. General aspects

The strong point of the DEB approach in evaluating effects of mixtures is that it provides a single framework to interpret concomitantly different endpoints regardless of exposure time. Dealing with sub-lethal effects, it is essential to have a model capable of showing the relations between feeding, maintenance, growth, development and reproduction. But more importantly, the framework of DEB theory allows for extrapolation of observed toxic effects where this is much more difficult with the standard approaches. Extrapolation between species (e.g. from laboratory species to related field species of interest) is possible, because many metabolic parameters vary with body size in a predictable way (Kooijman, 2001). Analogously, test data for different species may be combined to yield a coherent set of information on a chemical. Process-based methods also aid in extrapolating observed effects for one chemical to another chemical, because the model parameters often have relationships with chemical properties such as hydrophobicity (e.g. Kooijman et al., 2007; Jager and Kooijman, 2009). This predictability of effects, as long as the mechanism of action remains the same, is especially valuable in that it allows predictions for untested compounds (Baas et al., 2009). Furthermore, process-based modeling facilitates an educated extrapolation from single-species test results to population consequences, which is impossible using the standard summary statistics.

Because processes are modeled explicitly in time, the results of short-term test can be extrapolated to chronic timescales (and vice versa), provided that model parameters can be estimated sufficiently accurately and the impact of compounds on model parameters does not change over time. It is also possible to make predictions of effects resulting from time- varying concentrations, even when the test is performed under constant exposure (Jager et al., 2006).

4.2. Comparing DEB modeling to CA/EA modeling

The most important drawback of the CA/EA approaches for assessing effects of mixtures is that these approaches are purely descriptive, with the concentrations as the starting point. Every endpoint is considered as being unrelated to other endpoints. In addition these models cannot integrate observations in time and different (statistical) interaction parameters are derived for each specific endpoint at each different point in time.

This was demonstrated in Baas et al. (2007), here survival was monitored daily over a period of 21 days for 6 binary mixtures of metals. It showed that with the hazard modeling approach most of the mixtures could be fitted without any additional interaction parameter, in contrast to the CA/EA approaches where each time point had to be treated independently and showed a specific interaction. It showed that interactions varied hugely over time. This appears to indicate that the identification of interactions with the classical models may be an artifact, following from random variation in the results (Baas et al., 2007). This view is underlined by the lack of reproducibility of specific interactions, as was shown by Cedergreen et al. (2007). For sub-lethal endpoints, there are very few studies that follow endpoints in time. An example is the study by Van Gestel and Hensbergen (1997). These authors did not use mechanistic models to analyze their data, but simply treated the data at each time step as a new data set. Also here it was shown that the apparent mixture interactions changed over endpoints and over time.

The DEB approach takes the organism as a starting point instead of the concentrations, which allows to interpret effects on growth, reproduction and survival in a single framework, using the same parameters. For sub-lethal effects the DEB approach implies certain interactions between mixture constituents (see Section 2.1). Therefore, it is to be expected that a model analysis based on energy budgets shows deviations from classical CA and EA even without imposing statistical interactions between the compounds. In addition the nature of the deviations from model expectations will provide more information on the underlying mechanisms than descriptive statistical interaction terms, and thus delivers directions for further mechanistic research.

4.3. Other process-based approaches

For survival there are other approaches to assess effects of mixtures. For a combination of survival and sub-lethal endpoints so far the DEB approach is the only approach where effects of mixtures are interpreted in one single consistent theoretical framework.

For survival the best known approaches apart from DEB are those by Lee and Landrum (2006) and Ashauer et al. (2006). The different approaches and underlying assumptions were recently compared by Ashauer and Brown (2008). The most important difference is that the damage assessment model by Lee and coworkers mortality is deterministic at the level of the individual, whereas the model by Ashauer and coworkers and the DEB model for survival are based on the hazard model, implying that mortality is a chance process. Instead of instant death when exceeding a threshold, it is assumed that death is an inherently stochastic process. The exact mechanism is probably somewhere between these two views, although dedicated fish studies showed that the stochastic component dominated for mortality (Newman and McCloskey, 2000).

Both the method by Ashauer and Lee use an extra kinetic step in the form of a repair mechanism to assess damage inflicted by a chemical in the recent past, and therefore require an extra parameter (per component in a mixture) compared to the DEB approach for survival. This extra parameter is estimated from measured or estimated internal concentrations. Jager and Kooijman (2005) applied a method that is very similar to that of Ashauer et al. (2006), also involving an extra kinetic step, to raw survival data for exposure to single components but...
the extra parameter hardly improved the fits of the model to the survival data. It did give different values for the elimination rate however. But the data available did not include internal concentrations, so the elimination rates could not be compared with experimental data. Especially for complex mixtures data on internal concentrations are usually not available and would require an elaborate experimental and modeling effort.

4.4. Experimental effort

When the DEB approach is used to interpret effects of mixtures, it is important to realize that toxic effects are interpreted as effects on energy budgets of the organism. Therefore it is important to have a constant food level (preferably two food levels, but it might be very difficult to maintain a constant food level over an experiment unless the organisms are fed ad libitum). The main drains of energy are maintenance, growth and reproduction, therefore body size (or weight) and reproduction have to be followed experimentally over time. Preferably also survival, as effects on survival can be interpreted within the same theoretical framework.

The most important consideration in the experimental setup is start simple. Avoid interactions in the medium (or understand them), use chemicals with a single, known, molecular mechanism. There is no need to measure body residues, though they might be helpful. If one is only interested in survival, avoid growth of the organisms and continue the test long enough to obtain the maximum size of the organisms.

The level of detail of the experimental data determines the acceptable level of model complexity. Energy-budget approaches are generally most informative when data on body size and reproductive output are available over time for a considerable part of the life cycle. Toxic effects are informative when data on body size and reproductive output are available over time. Preferably also survival, as effects on survival can be interpreted within the same theoretical framework.

If we want to learn more about toxic mechanisms operating in mixtures for sub-lethal endpoints we need more elaborate experimental data than are needed for the standard models CA/EA, which in turn require more experimental effort than the assessment of the effect of single compounds. One of the requirements is the availability of data on effects at intermediate points in time. This might not be a big problem (though the experimental effort is required) for most aquatic organisms (and often effects on intermediate points in time are already measured), but for soil dwelling organisms this can be more difficult. In Baas et al. (2007) normally soil dwelling organisms (F. candida) were forced to live on top of the soil by compacting the soil, which allowed to follow effects in time. Another possibility is to start the experiment with additional jars for some concentration levels and sacrifice these at intermediate points in time. It is important that the effect surface should be covered by the experimental design. Whether a full factorial design or a ray design is chosen is not very important for the interpretation of the experimental data.

In summary, for a full scale mechanistic interpretation of the data the most important features that have to be caught by the measurements are: maximum length, compared to the control, growth curve compared to the control, time of first reproduction compared to the control, nr of juveniles compared to the control, and survival in time.

5. Outlook

Looking into the future, one of the most challenging areas in chemical mixture research is answering the question on how to deal with the infinite number of combinations of chemicals and other stressors. Tools that can mechanistically interpret experimental data and allow extrapolations are a necessity to try to answer this problem. DEB theory describes simple rules for how organisms acquire and use resources for growth, development and reproduction. Toxic effects in combination with (some) natural stressors can then be viewed as a disruption of allocation processes. This means that we have to treat growth and reproduction as tightly interlinked processes in time, and make assumptions on how toxicants interfere with these processes.

To fully understand the effects of (chemical) stress on life-history responses there is a need to link the effects of chemicals with changes in resource allocation and trait performance. Process-based models, such as DEB, can provide a framework to understand the physiological basis of life history in terms of energy allocation. Mode of action prediction derived from DEB can provide a useful indication of the physiological basis of the toxic effect. However, these modes of action still represent changes in very broad metabolic processes. Studies of detailed molecular mechanisms on the other hand may provide detailed understanding of the molecular target site of a chemical, but not how interactions at this site may result in different patterns of change for the life history of the whole organism. Combined, however, the two can meet the objective of understanding both the detailed basis of chemical effect and how these translate to biologically meaningful effects on key traits. The first tentative steps in this direction are set and data are being gathered that are suitable for this kind of interpretation.

As mixture models develop we expect a more tight interaction between the modelers and the experimentalists, to arrive at natural quantifiers for effects and solve more specific questions in unraveling the mechanisms behind effects of mixtures and further rationalize experimental design for mixtures.

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