Incorporating Mechanistic Effect Models into the Risk Assessment of Pesticides for Amphibians and Reptiles

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May 5, 2024

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1 Introduction

Amphibians and reptiles are currently not explicitly considered in the environmental risk assessment (ERA) schemes for pesticides, assuming that the most sensitive aquatic and terrestrial are protective for amphibians and reptiles. However, the development of ERA schemes for amphibians and reptiles is an ongoing process. Most ERA schemes rely heavily on standard toxicity testing, but minimizing animal testing is necessary for both practical and ethical reasons. Furthermore, environmentally relevant protection goals address higher levels of organization than standard toxicity tests do, i.e. populations, communities and ecosystems.
For these reasons, mechanistic effect models (MEMs) are an important tool for modern ERA schemes.

1.1 Incorporation of MEMs in ERA: General perspective

MEMs describe the effects of stressors on organisms and populations as dynamic processes, based on biological considerations and using measurable parameters. In practice, models lie on a continuum between amechanistic and mechanistic. MEMs can be used as research and extrapolation tools. As research tools, they enhance our understanding of observed effects *a posteriori*. As extrapolation tools, they predict effects in previously untested scenarios. In principle, MEMs can support ERA as both research and extrapolation tools. While those two functions of MEMs are not completely separable, the focus for application in ERA is tpyically on the extrapolation aspect.
Extrapolation may include extrapolation across exposure profiles (from constant to time-variable), across levels of organization (from individuals to populations), or across exposure types (from single-stressor exposure to multiple stressor exposure).
Which MEMs could be included in the ERA for amphibians and reptiles and how is currently not defined. Therefore, a workshop was held as part of the EU Cost action PERIAMAR in September of 2023, with the aim of firstly defining the current state of MEMs for amphibians and reptiles, and secondly laying out a roadmap for further development and integration into ERA.

1.2 Objectives

The objectives of this document are firstly to summarize the outcome of the PERIAMAR effect modelling workshop, including the state of the art of mechanistic effect models for incorporation into risk assessment of amphibians and reptiles and roadmap for further development. Since a number of challenges emerge in the mechanistic modelling of

toxicant effects to amphibians, we will discuss these in more detail and outline possible solutions. We do not claim completeness with respect to the discussed issues.
Secondly, we compare our conclusions with other relevant documents, specifically the EFSA scientific opinion on risk assessment of amphibians and reptiles (Ockleford, Adriaanse, Berny, Brock, Duquesne, Grilli, Hernandez-Jerez, Bennekou, Klein, Kuhl, Laskowski, Machera, Pelkonen, Pieper, Smith, et al., 2018) and pop-guide guidance on development of population models for risk assessment (Raimondo et al., 2021).

2 Development of organism-level models

Overview of models under development To our knowledge, three groups of organism-level models for amphibians and reptiles are currently under development or have been developed recently, all of which are based on Dynamic Energy Budget (DEB) theory. Firstly, DEB models for reptiles have been developed for multiple species based on the standard DEB model, for the purpose of extrapolating effects to the population-level with account for long-term accumulation of pollutants. Secondly, a DEB model based on standard DEB, but adopted to account for amphibian metamorphosis, has been under development for the purpose of predicting the effects of temperature and other environmental factors on amphibian life-history traits related to metamorphosis. Thirdly, a DEB model based on DEBkiss (Jager et al., 2013) has been under development within the EFSA-funded project AmphiDEB. The purpose of this model is to account for the effects of multiple stressors, including pesticide mixtures and pathogens, and extrapolate effects to the population-level. Further published DEB models for amphibians include those by Pfab et al., 2020 and Mueller et al., 2012.
In order to present possible modifications for amphibians, we will first briefly summarize the general functioning of DEB models. More formal introductions to DEB theory are available elsewhere (Kooijman, 2010; van der Meer, 2006).

2.1 The generic DEB model

DEB models generally describe individual life history in terms of a mass or energy balance. Energy is taken up in the form of food. Energy from food is assimilated and allocated to maintenance, somatic growth (increase in structure), maturation and reproduction. The abstract state variable maturity describes the cumulative amount of energy invested in maturation processes, and may be associated with additional maintenance costs. Life stage transitions occur at certain threshold values of maturity, which introduces the option to de-couple life stage transitionts from

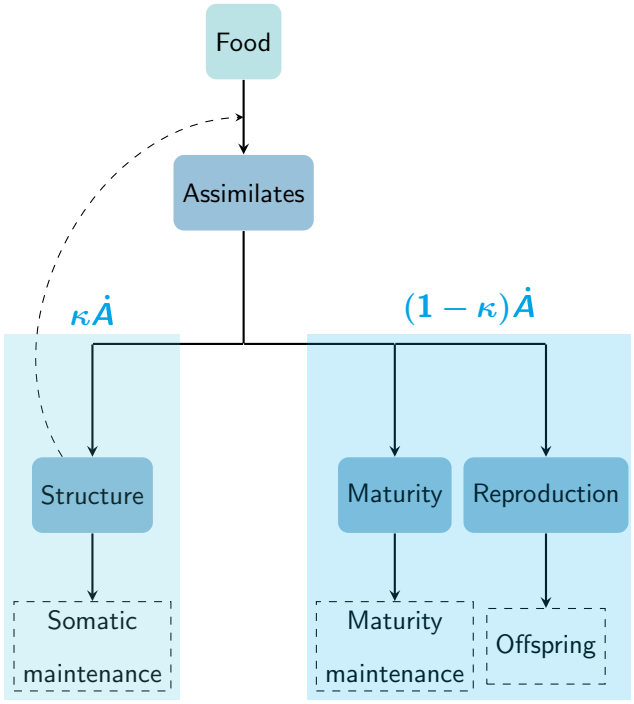

Figure 1: Conceptual diagram of energy fluxes in a reserveless DEB model with maturity. Generalizable DEB models for amphibians require the maturity component to reproduce plasticity in developmental traits, such as the body size at life stage-transitions.

body size.

The κ-rule dictates that a fixed fraction of assimilated resources, κ, is allocated to somatic growth and somatic maintenance, whereas the remainder 1-κ is allocated to maturation, maturity maintenance and reproduction. Three life stages are included in the generic model: Embryos are assumed to not feed from an external resource. Juveniles feed from external resources and invest energy in maturation, but not reproduction. Adults feed from external resources and reproduce, but do not mature any further. Variants of DEB models may be generally divided into reserveless models (e.g. DEBkiss (Jager, 2020; Jager et al., 2013)) and models including a reserve (e.g. the "standard DEB" model (Kooijman, 2010)). Reserveless models assume that assimilates are used instantaneously to fuel downstream processes. In the standard DEB model, assimilates are instantaneously converted into reserve, and reserve is mobilized according to a mobilization rate. The mobilization rate is defined so that the assumption of weak homeostasis holds (Kooijman, 2010). This means that the composition of the organism (amout of reserve relative to amount of structure, or reserve density), remain constant for constant food availability.

The main output of a DEB model are somatic growth (structure) and reproduction over time, but also the individual energy fluxes such as assimilation rates, as well as cumulative energy budgets. As long as food availabilty and other environmental factors are constant over time, generic DEB models predict organisms to grow according to von Bertalanffy growth:

$$\frac{dL(t)}{dt} = r_B(L_\infty - L(t)) \tag{1}$$

In eq. 1, L is the length of the organism, r_B is the von-Bertalanffy growth rate and L_∞ is the maximum length, which will be approached asymptotically.

2.2 DEB model modifications for amphibians

The nature of the amphibian life cycle and development elicits changes to the previously described generic model. Firstly, larval development may be subject to metabolic acceleration. That means, parameters which are assumed to be constants in the generic model now vary with developmental state. The most obvious consequence is that models with metabolic acceleration will not predict organisms to grow according to von Bertalanffy-growth (Equation 1). Metabolic acceleration for amphibians may be modelled by a change in the energy allocation κ, and this change has previously been shown to be species-specific (Mueller et al., 2012). Another rate which may vary with developmental state is the size-specific ingestion rate. It is unclear whether changes in size-specific ingestion rate during larval development (up to Gosner stage 42) are relevant for organism-level models, but a clear decrease in ingestion

rates during metamorpgic climax (Gosner stages 42 - 46), up to complete inhibition of ingestion (Pfab et al., 2020), should be taken into account: A decrease in ingestion has consequences for energy fluxes because maintenance costs still have to be paid and maturation continues during metamorphic climax, which also requires energy (Figure 1). One possibility to achieve this is to allow for the use of structure to fuel maintenance and maturation, hereafter referred to as structural mobilization models. The models presented by Pfab et al., 2020 achieve this, but also make the specific assumptions that $\kappa = 1$ and that maturation proceeds at a constant rate, thus limiting the applicability of these specific model formulations for extrapolation purposes. However, it is not generally necessary to make these assumptions, and a structural mobilization model can also be integrated into a full DEB model formulation. A second possibility is to include a separate reserve compartment, which is built up during larval development and depleted during metamorphic climax (Hansul et al., unpublished), hereafter referred to as metamorphic reserve model. Such a metamorphic reserve behaves differently from the reserve in the standard DEB model, because it violates the weak homeostasis assumption. This also has consequences for the temporal pattern of larval growth when expressed in total mass, since the metamorphic reserve contributes to total body weight.

There are currently no comparative studies on metamorphic reserve and structural mobilization models. Both have in principle the same degree of flexibilty and can be implemented with a minimum of two added parameters. Consequently, there is also no general agreement about which variant is favorable.

2.3 DEB-TKTD models: Generic approach

TKTD models describe the change in internal concentration (toxiokinetics) and consequent effect on the organism (toxicodynamics).

In the context of DEB-TKTD models, the internal concentration is often connected to a damage compartment. Damage represents an internal concentration which is specific to the Physiological Mode of Action (PMoA). Since measurements of internal concentrations are often not available, internal concentrations may scaled by the bioconcentration factor. The scaled internal concentration then has the same dimension as the external concentration (Jager et al., 2011) and the equilibrium of the scaled internal concentration (under constant exposure) will be equal to the external concentration. Effects of body size on toxiokinetics are often taken into account by correcting for structural length L, which is a quantity related to the somatic mass of the individual (conversion to measurable length requires application of an allometric model, such as the so-called shape factor). This results in the following

Figure 2: Schematic representation of the relationship between external concentration and effects on life-history in DEB-TKTD models. The damage is PMoA-specific and multiple PMoAs might be simultaneously responsible for the observed effects. The damage compartment allows to model temporal trends in the effects which cannot be explained by changes in the total internal concentration, for example because the temporal dynamics in effects is slower than toxicokinetics.

equation for the change in damage D (Jager & Zimmer, 2012):

$$\frac{dD}{dt} = k_D \frac{L_{max}}{L_t} (D_t - C_{W,t}) - D\frac{3}{L}\frac{dL}{dt} \tag{2}$$

In eq. 2, the first term decribes uptake and elimination with account for changes in the surface area to volume-ratio, where L_{max} is the physiological maximum of structural length (can be calculated analytically from DEB parameters) and $C_{W,t}$ is the external concentration at time t. This term contains the dominant rate constant k_D with dimension $\frac{1}{t}$, which has to be estimated from data. The second term accounts for diluation by growth; The PMoA is a DEB process or combination of processes which are adversely affected by the associated (scaled) damage. Commonly considered are increase in growth costs (decrease in growth efficiency, G), increase in maintenance costs (M), decrease in assimilation efficiency (A) and decrease in reproduction efficiency (R) (Ashauer & Jager, 2018). Other PMoAs are possible and sometimes necessary to describe observed effects on life-history. For aquatic invertebrates, this has been shown for such as a change in the size of eggs in *Daphnia pulex* exposed to metals (Hansul et al., 2024) or direct effects on maturation in the copepod *Nicrota spinipes* exposed to Citalopram (Koch & De Schamphelaere, 2021).

2.4 DEB-TKTD models: Considerations for amphibians and reptiles

Specific modifications for amphibians in DEB-TKTD models are currently not implemented in the mentioned models under development. A toxicokinetic model for amphibians was developed by Mingo et al., 2024. This model provides much more detail than the previously described toxicokinetic equation used in DEB-TKTD models (eq. 2) by describing the mass balance explicitly and including mechanistic details such as multipel uptake mechanisms and allometric considerations. This TK model could also help to support DEB-TKTD modelling. It has to be noted however that the (scaled) damage is in general more crucial than the total internal concentration, since it is more directly related to the temporal dynamics of observable effects.

However, some aspects can be highlighted which require attention with respect to DEB-TKTD modelling for amphibians.

Modelling toxikokinetics during metamorphosis A special case emerges when a stressor increases maintenance costs and toxikokinetics is modelled according to eq. 2. Especially when metamorphosis is modelled through a structural mobilization model, an increase in maintenance costs will lead to a further decrease in structure (L in eq. 2), which will lead to a reversion of dilution by growth and consequent further increase in damage, which further increases maintenance costs. The result is a positive feedback loop between damage and effect, and a rapid collapse of metabolism following any non-zero initial effect.
We expect that the extent and speed at which this feedback occurs in the model is likely not realistic, independent of the underlying parameter values. In this case, a metamorphic reserve model might alleviate or solve this issue, since this type of model can de-couple the coverage of maintenance costs from the change in structure.

Modelling temporally disparate effects Exposure during larval development and metamorphosis can have consequences for later life stages, such as reproductive output or survival probability to maturity, even if exposure is discontinued after metamorphosis.
This poses a challenge to the modelling of whole life-cycle effects, since the effect at time t is typically calculated from the damage at the same time, D_t. This issue deserves further investigation, as there is currently no clear solution. A possible solution might present itself if the exposure during early life-stages has effects on development which are linked to the effects on later life stages, since this link can (hypothetically) be provided through DEB state variables such as maturity.

Life stage-specificity of PMoAs and TKTD parameters For other organism groups like aquatic invertebrates, the PMoA is often inferred by fitting alternative models to growth and reproduction data simultaneously. This is likely not an option for amphibians because a) reproduction data is scarce and b) it has to be considered that the dominant PMoAs for aquatic life stages differ from those for terrestrial life stages, which means that the PMoA for aquatic (i.e. non-reproducing) life stages has to be inferred from growth data and possibly other observations. To this end, it is worth to investigate whether observations on development (e.g. weight change during metamorphosis metamorphosis) carry information on the PMoA. If this is not the case, the use of Bayesian techniques might become essential in the use of DEB-TKTD models for amphibians, as this would allow for the propagation of uncertainties about the PMoA during extrapolation.

3 Development of population models

Population models which are relevant in the context of ERA for amphibians and reptiles can be roughly divided into matrix population models, used for simple projections of population growth rate, and agent-based models (ABMs), which can provide theoretically unlimited flexibility, but require considerable effort for development, computational power and expertise. The development of matrix population models for amphibians (Awkerman et al., 2020; Ockleford, Adriaanse, Berny, Brock, Duquesne, Grilli, Hernandez-Jerez, Bennekou, Klein, Kuhl, Laskowski, Machera, Pelkonen, Pieper, Stemmer, et al., 2018) and the incorporation with DEB models (Klanjscek et al., 2006) has been discussed elsewhere. In the following, we focus on aspects which mostly regard the development and use of ABMs for amphibians and reptiles.

3.1 ABMs for amphibians: Challenges and open questions

Since the organism-level model forms the basis of the ABM, challenges related to the development of organism-level models (section 2) also apply to the development of ABMs. As the development of ABMs for amphibians to support ERA is currently in an early state, it is more difficult to point out the most important questions. However, some additional aspects certainly have to be addressed. These include, but are likely not limited to:

1. The incorporation of space and movement
2. The incorporation of seasonality effects (e.g. movement, timing of spawning), including the interaction between chemical stressors and seasonally varying factors (e.g. temperature)
3. The incorporation of sexual differences, sexual reproduction and associated behaviour
4. The incorporation of additional stressors

The incorporation of these aspects requires a careful consderiation of the required level of detail in each of these aspects (Ockleford, Adriaanse, Berny, Brock, Duquesne, Grilli, Hernandez-Jerez, Bennekou, Klein, Kuhl, Laskowski, Machera, Pelkonen, Pieper, Stemmer, et al., 2018), which in turn depends on how exactly the model is finally intended to be used in the ERA process. This highlights the importance of the scenario definition for the incorporation of MEMs in ERA. One crucial question in this context is the extent to which other stressors, such as pathogens or habitat fragmentation, should be included in the baseline scenario.

3.2 Validation of ABMs

Validation of MEMs is as a crucial prerequisite for their succesful integration into ERA. Especially in the context of ABMs, the availability of suitable is an issue that needs to be addressed. ABMs often integrate a mutlitude of processes grouped into submodels.

In the ideal case, a validation dataset reflects the outcome of all submodels and their interactions. Obtaining such datasets is often unrealistic for complex population models. Therefore, it should be considered that models have to be validated with focus on specific combinations of submodels and processes, hereafter referred to as piecewise validation. For example, a model which incorporates the effects of mixtures of contaminants along with other environmental factors (e.g. habitat fragmentation) would ideally have to be validated based on field data including exposure data on all mixture components, as well as abundance and distribution data over time. In a piecewise validation process, this validation would be broken down by first validating that the model can predict mixture toxicity from single-substance toxicity, and then validating that the model can predict population dynamics from the calibration data (e.g. life-history data). This would require multiple separate datasets for validation, but each dataset might be more realistic to obtain.

This piecewise validation approach ties in with the notion that the outcome of model validation is non-binary (Hansul, Vermeiren et al., unpublished [1]). That is, a model is assigned a degree of validity with respect to an application, rather than the label "valid" or "not valid". While the operationalization of the degree of validaty still has to be carried out, it can take into account how many submodels and combinations of submodels have been validated with which kind of data.

As previously mentioned, the degree of validity is assigned in the context of the application. Effective model development for the support of ERA could therefore benefit from the clear definition of the specific risk assessment questions to be answered using MEMs. This may appear in contrast to the use of population models proposed in the EFSA SO on the state of the science on pesticide risk assessment for amphibians and reptiles (Ockleford, Adriaanse, Berny, Brock, Duquesne, Grilli, Hernandez-Jerez, Bennekou, Klein, Kuhl, Laskowski, Machera, Pelkonen, Pieper, Stemmer, et al., 2018), where it is suggested that population models are used to define specific protection goals (SPGs). This contradiction is resolvable though, by considering the option that the broader risk assessment question may be defined *a priori* to guide population model development, and is then specified using population models.

[1] reference to MAD Book Chapter 6 to be inserted here

4 Aspects of good modelling practice

The importannce of good modelling practice for the incorporation of MEMs into amphibian and reptile ERA cannot be understated. The corresponding EFSA SO (EFSA Panel on Plant Protection Products and their Residues (PPR), 2014) remains the most important reference in this context, but we identified some specific aspects to highlight in the context of modelling species with poor data coverage with complex models.

Documentation and open access The use of a detailed documentation format like TRACE (Grimm et al., 2014) should be a prerequisiste for the application of MEMs in ERA.
In addition, open access to code and data used for modelling will greatly facilitate the future development of models. Ideally, this should not only include the model code itself, but also the code to reproduce published results, i.e. demonstrating how to effectively use the model.
Long-term reproducibility is a related issue which needs to be addressed. Since the use of external packages is common in programming languages like R, Python and Julia, developers need to make use of mechansims such as virtual environments to ensure that updates to those dependencies do not make the models unusable later.

Collaboration Tight collaboration between modellers and empirical researches is crucial to a) make sure that modellers are aware and have access to relevant available data b) promote experimental designs which lead to useful datasets for modeling (e.g. data over time, at least 4 concentrations for effect data, measurements which are comparable to model outputs). Modellers need to formulate data needs precisely and with realistic experimental setups in mind. A great deal of the succesful collaboration will rely on finding workable compromises, as the ideal case from a modelling perspective is often impossible to achieve from a practical perspective.
Finally, practices of data recording and storage can have an enormous impact on the partical usability of data. The effort it takes to tidy datasets can make it impossible to incorporate useful data within a given time frame. Data tidying and preprocessing either needs to be accounted for in the planning of modelling projects, unless the data was initially recorded in a format useable for modelling.
In practice, following the simple principles of *tidy data* (Wickham, 2014) will in most cases lead to datasets which are readily usable be modellers, irrespective of the complexity of the dataset.

5 Conclusions

5.1 Short-term applicability

Oragnism-level models The development of organism-level models for amphibians and reptiles is in a relatively advanced state. We conclude that for specific applications, they could support ERA on the short term, pending validation with respect to those applications.

Possible applications include the extrapolation across exposure profiles as done for sublethal effects using the General Unified Threshold Model of Surival (GUTS, Jager et al., 2011; Ockleford, Adriaanse, Berny, Brock, Duquesne, Grilli, Hernandez-Jerez, Bennekou, Klein, Kuhl, Laskowski, Machera, Pelkonen, Pieper, Smith, et al., 2018), but might also include more amphibian-specific aspects like the modelling of temporally disparate effets (section 2.4). It should be noted however that with respect to such amphibian-specific aspects, more developmental effort is still necessary than for the uses of MEMs which are already established.

5.2 Long-term applicability

ABMs for amphibians fall within the area of possible long-term applicability. The development of the models itself and the validation with data requires considerable developmental effort, which needs to be accounted for in the prioritization of research in order to advance the use of ABMs in ERA.

From a developer perspective, it is worthwile to integrate the development of organism-level and population models to some extent, since models which produce plausible dynamics and predictions on the organism-level can fail when incorporated into an ABM. Identifying such blind spots early in the development of organism-level models can also enhance the development and applicability of ABMs

5.3 Roadmap

Based on the outcome of the PERIAMAR modelling workshop and additional considerations made in this document, we propose that the definition of risk assessment questions to be addressed with MEMs is the most central point to advancing the incorporation of MEMs in ERA for amphibians and reptiles.

These will determine the level of detail biological detail required for specific submodels and processes. When risk assessment questions are broadly defined, the formulation of SPGs can be supported by MEMs as a research tool.

In parallel, strategies for model validation need to be worked out. This includes the systematic evaluation of available relevant data and an operationalization for the degree of validity in the context of a piecewise validation

approach. These processes can also be influenced by more advanced ERA schemes, e.g. for birds and mammals.

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