Predicting environmental risk: A road map for the future

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ABSTRACT

Frameworks for environmental risk assessment (ERA) focus on comparing results from separate exposure and effect assessments. Exposure assessment generally relies on mechanistic fate models whereas the effects assessment is anchored in standard test protocols and descriptive statistics. This discrepancy prevents a useful link between these two pillars of ERA, and jeopardizes the realism and efficacy of the entire process. Similar to exposure assessment, effects assessment requires a mechanistic approach to translate the output of fate models into predictions for impacts on populations and food webs. The aim of this study was to discuss (1) the central importance of the individual level, (2) different strategies of dealing with biological complexity, and (3) the role that toxicokinetic-toxicodynamic (TKTD) models, energy budgets, and molecular biology play in a mechanistic revision of the ERA framework. Consequently, an outline for a risk assessment paradigm was developed that incorporates a mechanistic effects assessment in a consistent manner, and a 'roadmap for the future'. Such a roadmap may play a critical role to eventually arrive at a more scientific and efficient ERA process, and needs to be used to shape our long-term research agendas.

INTRODUCTION

Since its early conception, environmental risk assessment (ERA) focused on comparing results from separate exposure and effect assessments. In most ERA frameworks, exposure assessment relies on mechanistic fate models, and has done so for at least the last two decades. These models focus on the dominant fate processes in the environment such as diffusion, advection and degradation to translate a given emission pattern of a chemical into exposure concentrations in the relevant environmental compartments including soil, air and water. Fate models range from rather simplistic 'multimedia models' (MacLeod et al., 2010) to more elaborate ones, such as those used for oil-spill modelling (Reed et al., 1999). What these models share in common is that they are all simplifications of a complex system. They include a representation of the mechanisms that act in the environment, and hence make predictions for situations where there are no monitoring data available.

In contrast, the effects assessment in ERA is of an entirely different nature. Toxicity testing, usually in a lab setting, takes central stage. The results of these tests are treated statistically to arrive at summary statistics such as the no-observed effect concentration (NOEC) or estimated concentration for x% effect relative to the control (ECx). To these values, safety factors or further statistics such as species-sensitivity distributions are applied to arrive at a 'safe' concentration in the environment or predict the 'fraction of species affected'. The entire complexity of the affected biological system is thus reduced by statistical manipulation into a single number. This procedure, together with the strong focus on standardization of test protocols, is masking a range of problems (Jager, 2011). Most importantly: the value that emerges from the effects assessment bears no significant relationship whatsoever to the environment and the emission scenario that is modelled in the exposure assessment. Toxicity is not a value but a process; a process that depends upon environmental properties such as temperature, and on time and pattern of exposure. Even under constant exposure, the magnitude of the adverse effect depends upon time, and different life-history traits (endpoints) show different dynamic effect patterns (Alda Álvarez et al., 2006b). To make matters worse, these patterns are also chemical and species dependent.

The classical strategy to deal with these complexities has been standardization of the toxicity tests. It is known that an individual's response to a toxicant depends upon the exposure pattern, exposure time, environmental conditions, and choice of endpoint such as growth, reproduction or survival. Therefore, all these factors are arbitrarily fixed in test protocols: standard toxicity tests are always performed over a prescribed duration, with an (as much as possible) constant exposure concentration, constant test conditions, and focus on a single endpoint. Our fate models, in contrast, produce time-varying concentrations for the (possibly time-varying) conditions in the environment that one wants to protect; an environment that might deviate considerably from the standardized test conditions. Clearly, the results from the effects assessment do not match those of exposure assessment, as toxicity tests describe the response in a different (and usually irrelevant) exposure situation. Further, the ecological relevance of the response of a single life-history trait after an arbitrary exposure time is questionable. The protection goals in ERA are at higher levels of biological organization (population level and higher), and effects on a single trait, of a single species, under constant lab conditions are not even predictive for the impacts at the population level (Forbes and Calow, 1999; Martin et al., 2014).

These problems are indicative of a general lack of relevance of the effects assessment, which can result in both severely over- and under-protective regulatory decisions (Forbes et al., 2011). In this way, the ERA process makes inefficient use of time, resources and test animals. What is required is an effects assessment that is able to accept time-varying concentrations of fate models, as well as associated (time-varying) environmental conditions, and translate them into ecologically relevant impacts on populations and food webs that one aims to protect. This study provides a broad outline of a more efficient and scientific risk assessment paradigm, based upon this principle. Clearly, risk assessment frameworks are not easily changed; this is a process that takes considerable time and effort. Therefore, a 'road map' is proposed with the steps needed to eventually arrive there. Reshaping the effects assessment is mainly a question of how to deal with the complexity of biological systems under stress, which is discussed in general terms in the next section.

DEALING WITH COMPLEXITY

Levels of biological organization

In redefining the effects assessment, the first question to address is on the level of biological organization that needs to be modelled. ERA is generally concerned with effects on populations, food webs, or even ecosystems, in contrast to human risk assessment, which focusses on health of individuals. Building predictive models at such high levels of organization is, however, hard (and perhaps even impossible) without considering lower levels, e.g., how toxicants are taken up and affect the performance of individuals. Further, these higher levels of organization are not suitable for routine experimental testing; such tests are expensive, time consuming, and results are extremely difficult to interpret due to the large number of variables at play (which also results in low statistical power to identify adverse effects). Clearly, it is not possible to test every chemical at these higher organization levels, let alone for all relevant exposure situations and combinations with other stressors. The lower levels of biological organization are more amenable to experimental testing, but the ecological relevance also decreases. A response at the molecular and cellular level is hardly interesting for ERA unless the relevance for individuals and population dynamics can be firmly established (Forbes et al., 2006; Garcia-Reyero and Perkins, 2011; Groh et al., 2015; Hendriks, 2013).

The level of the individual is particularly appealing as a central level of organization, as it is amenable to experimental testing and has a clear ecological relevance (impacts on individual traits are directly relevant for population dynamics). Kramer et al. (2011) termed it the 'keystone' cementing the sub-organism responses to population-level effects. An additional advantage is that individuals usually have clearly defined boundaries with their surroundings. This can for example be used to construct mass and energy balances; the conservation laws are among the few hard laws that can be invoked in biology and ecotoxicology. Therefore, it makes sense to put the individual on center stage in our quest for a mechanistic effects assessment. The remainder of this study focuses mostly on the individual level, although translation from individuals to higher levels of organization comes with its own issues when dealing with complexity.

Mechanistic effects models at the level of the individual already exist in ecotoxicology, and are generally referred to as toxicokinetic-toxicodynamic (TKTD) models (Ashauer and Escher, 2010). Toxicokinetic (TK) models are responsible for translating (time-varying) exposure concentrations into time-varying internal concentrations. This is a well-established field in ecotoxicology and a range of mechanistic mass-balance models were developed (Mackay and Fraser, 2000). Toxicodynamics (TD) is concerned with the translation from internal concentrations (at a target site) to effects on the individual. It needs to be stressed here that, for the purpose of ERA, the endpoints that one is interested in are those that directly influence population dynamics: life-history traits such as growth, reproduction and survival.

Time scales

Now that the focus is on the individual as the central level of biological organization, the second point to address is the matter of time scales. Life-history traits of organisms play on a timescale of hours to years. If one wants to dynamically model these traits, it is possible to take processes at a shorter timescale (such as the dynamics at the molecular level) as instantaneous, and ignore slower processes like evolutionary and climatic changes. Modelling a system in which dynamic processes at different time scales are combined is problematic and generally inefficient. Focussing on the processes with a relevant speed (i.e., time-scale separation) is thus an efficient strategy to deal with the complexity of biological systems. For the purpose of ERA, the relevant time scale is in the range of hours to years, and can usually be narrowed down for specific risk assessment questions.

General strategies for dealing with complexity

An organism is a complex biological system, so how does one model its life-history traits, and how does one deal with stress responses on these traits? Three broad strategies in modelling can be defined (Jager, 2015): a 'black-box' approach, a 'white-box' approach, and a 'simple-box' approach (a very similar categorisation, linked to art forms, was presented by Hendriks, 2013). In the black-box approach, there is no attempt to understand the system; modelling is restricted to providing a description of the relationships between input and output of the system. Classic dose-response modelling falls into this category: an organism is exposed under standardized conditions and the model describes the effect on a trait at the end of the test as a function of the exposure concentration. This approach works well for interpolation, but can not be used to extrapolate as there is no representation of the underlying mechanisms (Jager et al., 2006). Since extrapolation is key in linking effects and exposure assessments, as discussed in the introduction, the black-box approach is not useful for a science-based ERA.

A white box in software engineering is a system where all internal components are visible. In modelling, this term can be applied to a strategy where models are built from detailed knowledge about the innards of the system. In (eco)toxicology, 'systems biology' falls under this heading. In this approach to the problem, the complexity is basically taken for granted; enormous amounts of data are gathered at the molecular and cellular level, requiring advanced 'bioinformatics' to analyze, which is expected to eventually yield predictive models (Garcia-Reyero and Perkins, 2011). To quote Van Straalen (2003): "In a bioinformatics approach to ecological systems, the high degree of internal complexity is accepted as an inherent property, and consequently the state of the system must be analyzed in terms of possibly several thousand measurable variables." In other words, large quantities of experimental data at a low level of organization define the models to be used at higher levels.

The last strategy in my categorization is the simple-box approach, named after the multimedia fate model 'SimpleBox' (Brandes et al., 1996). This strategy rests on making a simplification of the system to represent the dominant processes, and ignore the details. The models are not built from analyzing experimental data, but derive from a creative process in which modellers think about the logical components of a system, and how to represent them. Multimedia fate models are clear examples of this strategy: the environment is divided into several compartments, which are assumed to be homogeneous and well-mixed, and the focus is on the mass fluxes that transport the compound to another compartment, or degrade it (MacLeod et al., 2010). Although a range of more realistic models are available, the multimedia models have the advantage of low data and computational requirements, and a high-level of transparency (which is essential for ERA purposes). In ecotoxicology, TK and TKTD models follow this strategy (Ashauer and Escher, 2010; Jager et al., 2006; Mackay and Fraser, 2000), but in general, application of this approach for dealing with complexity is rare in eco(toxico)logy. As Van Straalen (2003) stated: "Ecologists tend to ask very complicated questions; unlike physicists, they do not simplify their object of study and analyze an idealized part of reality." What ecologists tend to overlook is that the questions in physics are not necessarily less complex as those in ecology. Physicists, however, have learned that the best way to deal with complexity in their domain is to simplify and idealize reality, whereas ecologists traditionally tend to focus on describing the details and the exceptions.

The black-box strategy to effects assessment fails to support science-based ERA. However, to move ERA forward, should one focus on a white-box or on a simple-box strategy? The molecular focus in ecotoxicology is rapidly gaining popularity, a clear sign of the white-box approach. Therefore, two questions regarding the necessity and sufficiency of this approach need to be addressed first: should one include details at the molecular level into effects models for ERA, and if so, would responses at the molecular level be sufficient to explain and predict effects on life-history traits?

Should TD models include molecular detail?

Organisms are made up of molecules, and chemicals elicit responses in the network of molecules that makes up the organism. Thus, it might make sense to answer this question approvingly, and the rapid evolution in molecular analysis and computing power is making the white-box strategy more and more alluring. Focussing on the molecular level in ERA represents a form of methodological reductionism (Forbes and Calow, 2012); the conviction that a system is best understood using a detailed representation of the smallest possible entities that make up the system. Interestingly, Sauer et al. (2007) actually contrast the systems-biology approach against reductionism, as the experimental data are integrated by mathematical models (and hence, the rules of the system are induced from the data). However, it is conceivable that reducing a biological system to interactions at the molecular level, and computational treatment of large datasets, is not a productive starting point when facing the problem of predicting toxicant effects on life-history traits. The art of modelling lies in finding the simplest possible representation of a system for the purpose at hand. Depending on the purpose, models with a different level of detail might be superior. To build useful models, it makes sense to start with the logic of a system, rather than the substrate in which this logic is implemented. A computer, for example, can be understood as a series of interconnected functional blocks (memory, central processor, input and output device, etc.), which describe the logic of the system, irrespective of the technology used for these blocks such as vacuum tubes versus silicon chips or tape drive versus hard disk.

Here, it is interesting to look at fate models again, as these models are a mainstay for ERA, and have held that position for decades. Fate modelling deals with molecules of a toxicant and how they are transported and degraded in the environment. The environment is of course made up of an enormous diversity of molecules, but fate models do not include this level of detail. Instead, fate models focus on process descriptions of the toxicant mass flows (often using first-order kinetics), and a simplification of the environment. Clearly, these models are not built from the molecular level up but from the largest scale down: starting with the logic of the system that needs to be modelled, and adding more detail at smaller scales when the purpose of the model demands it.

In ecotoxicology, TK models have a long history, and also follow a top-down strategy. Often, all of the complexity of the organism can be sufficiently represented by a single well-mixed compartment with first-order kinetics. If needed, additional well-mixed compartments are added (e.g., to represent a metabolite), or a different form of kinetics is used (e.g., saturating kinetics). Of course, organisms are not well mixed, and it is even difficult to speak of 'the concentration' in the organism, as that term is more appropriate for chemicals in homogeneous liquids or gases. The purpose of these models is to predict the body burden in an organism over time, as a function of the external concentration, with the least amount of parameters, and simple TK models are expertly suited for that purpose.

For effects models (TD), one similarly starts with the logic of an individual organism that needs to be represented, and includes more detail when required for the purpose of the model. In an ERA context, a model is required that is able to translate environmental concentrations (that might be time-varying, and/or mixtures) to effects on life-history traits under a given set of environmental conditions (that might be time-varying, and differ from the conditions in the toxicity test). As these models are to be used in a decision-making process, a further constraint is that TD models need to be as simple and transparent as possible, and not specific for one chemical or species; there are too many (mixtures of) chemicals and species in the environment to build dedicated models for each of them. It is not so apparent that details at the molecular level are needed to build models for the purpose of ERA, especially given the fact that fate and TK modelling in ERA may apparently function without such a level of detail.

Can we go from molecules to traits directly?

One of the major problems of systems biology has been, and still is, linkage between responses of the molecular level and those at the individual level or higher (Garcia-Reyero and Perkins, 2011; Ankley et al., 2006; Groh et al., 2015). Will collecting more data at the molecular (or cellular) level be enough to bridge the gap to the effects on the life-history traits that one is interested in? For some mechanisms of action, it will, especially for those were there is a relatively straightforward chain of events from the molecular level to traits of population-level interest (e.g., linking endocrine disruption to reproductive success, Ankley et al., 2010). However, in general, one should not expect that systems biology suffices to close the gap to the individual-level traits. The life-history traits that one is interested in are emergent properties of the living system, i.e., it is behavior that results from the complexity of the interconnected system of molecules, interacting with its environment, but it is not part of the behavior of the molecules themselves. Capturing behavior of a living organism by following processes at the molecular level is just as daunting as describing behavior of a computer from the individual electronic components of which it is made.

From a more abstract perspective, species can be classified by their position in the demand-supply spectrum (Lika et al., 2014). In demand systems, growth and reproduction rates are largely pre-programmed and the organism has to work hard to obtain enough resources to meet this demand. Birds and mammals, including humans, are clearly at the demand end of the spectrum. Reproduction is not so much constrained by energetics (feeding is upregulated during egg production or pregnancy) but by the demands of parental care. In such species, it may be feasible to link exposure to effects with a molecular chain of events. Human toxicology only needs to deal with one species, where a lot of research effort is already directed, and endpoints of interest are related to the health and well-being of the individual, rather than population-relevant traits such as survival and reproduction. For these reasons, a useful bottom-up TD model in the form of a 'virtual human' may be feasible in the near future (Yang et al., 2004).

The majority of species on the planet is, however, close to the supply end of the spectrum. In supply systems, growth and reproduction heavily depend on the environment, especially on food availability and temperature. Invertebrates and ray-finned fish are almost pure supply systems (Lika et al., 2014), and hence show a large flexibility in terms of growth rate, maximum size, and reproduction rates depending upon environmental conditions that they experience. For supply systems, energetics is key to understanding and predicting their life-history traits, and the interaction of stressors with these traits. For example, if a stressor decreases the reproduction rate, there may be a range of mechanisms underlying this response. Firstly, the stressor may directly influence the reproduction process (e.g., endocrine disruption), and it might be possible to link the effect to a molecular chain of events. However, the stressor may also affect reproduction indirectly, making it impossible to create such a direct causality chain. The stressor may decrease feeding or assimilation, so less resources are available for production of offspring. It might affect the growth process; smaller organisms eat less, and hence reproduce less, and slower growth generally implies a delay in the start of reproduction. Alternatively, the stressor might increase the allocation to other processes that compete with reproduction, such as maintenance costs, or costs of combatting the stressor's effects (Jager et al., 2006).

The affected energetic process was dubbed the physiological mode of action or pMoA by Alda Álvarez et al. (2006a). Knowing which process is inducing the effect on the traits is not only of scientific interest; it is essential to understand and predict the interactions between the toxicant stress and the environmental conditions (Jager et al., 2006; Alda Álvarez et al., 2006a), as well as interactions in mixtures of chemicals (Jager et al., 2010). Martin et al. (2014) recently demonstrated that identification of the correct pMoA turns out to be crucial to predict the population consequences of chemical stress, due to the feedback mechanisms between the species of interest and its food. It may be possible to link a molecular chain of events to the feeding process, but the translation of an effect on feeding to the effects on growth and reproduction requires a model at a different level of organization (and logical abstraction): an energy-budget model, which is detailed in the next section.

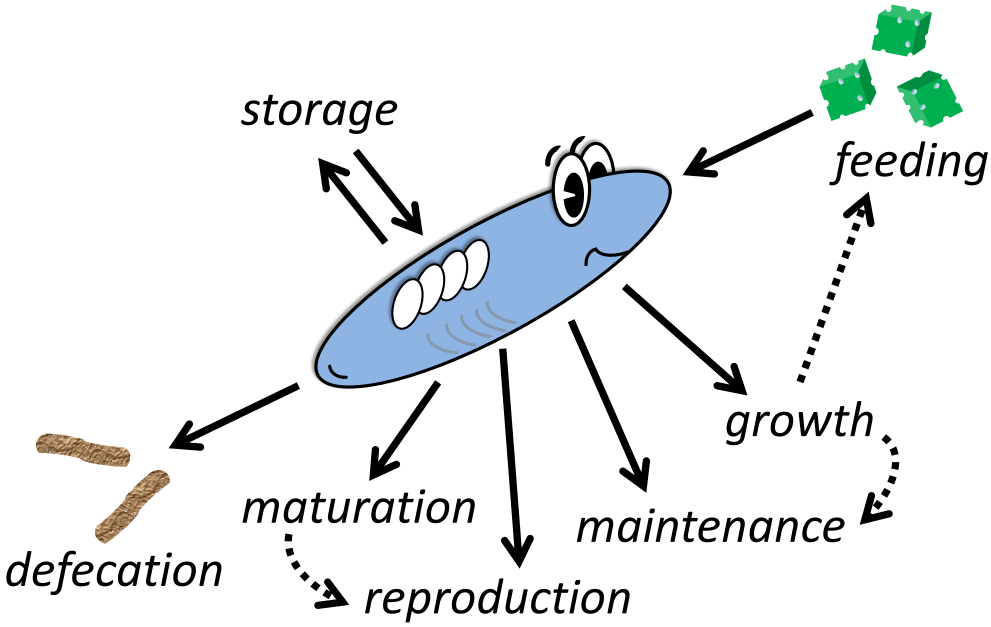

FIGURE 1. Major components of the energy budget for an animal at the supply end of the supply-demand spectrum. Solid arrows indicate mass flows, while dashed arrows indicate logical links considered in DEB theory.

THE ROLE OF THE ENERGY BUDGET

Introducing the energy budget

To explain the effect of stressors and environmental conditions on the life-history traits of supply systems, a quantitative energy budget is required. Such a budget explains how individuals obtain resources from their environment and use them to fuel their life histories (Figure 1). If toxicants interfere with the acquisition or use of energy, there will be consequences for energy-demanding traits such as growth and reproduction. Dynamic Energy Budget (DEB) theory (Kooijman, 2001; Nisbet et al., 2000) is the best-tested and most comprehensive theory for the energy budget of organisms. This theory follows the simple-box strategy outlined in the previous section: make an abstraction of the system of interest, focussing on the logic of the system and not on the substrate in which the logic is implemented. The DEB abstraction of individual organisms is based upon major flows of resources in the organism: feeding, assimilation, maintenance, growth, maturation and reproduction. These processes are common to all organisms, even though their genetic make-up and biochemistry may vary. Effects of stressors are interpreted as an increase or decrease in the parameters that govern these physiological processes (Jager et al., 2006). The rationale underlying this idea is simple: when a change in growth or reproduction is observed, there *must* be a change in one or more of these parameters. Growth and reproduction are energy-requiring processes, so a change in them implies a corresponding change in the energy budget due to the conservation laws. If less energy is invested in offspring, less energy is taken up, or reproductive energy is diverted to other energy-demanding processes.

The DEB approach has proven its value in ecotoxicology in a wide range of case studies with single chemicals as well as mixtures (list of publications on www.debtox.info/papers_debtox.html). It has also been included under the denominator 'biology-based methods' into ISO and OECD guidance (OECD, 2006), although its application in ERA is still rare. Several, even more simplified, models have been derived from this theory, which might be better suited for environmental risk assessment and incorporation into population models (Jager et al., 2014). An important advantage of DEB-based models is that one does not need to construct a model for each species from scratch: species differ mainly in parameters values and less in structure of their energy budget, which is largely conserved in evolution (Kooijman, 2001). The parameter values, further, tend to co-vary in a specific manner between species, which aids inter-species extrapolation of the basic parameters of the energy budget (Lika et al., 2011). This makes DEB theory an interesting option to efficiently standardize the individual-level processes in population models (Grimm and Martin, 2013).

Linking TK to the energy budget

TK models provide internal concentrations (at a target site), which generally vary over time. How does one link these internal concentrations to the parameters of the energy budget? In the future, it may be possible to model an entire chain of molecular events from the internal concentrations, via target sites and 'interaction networks' to physiological processes such as assimilation and maintenance (Figure 2). Considering the energy budget provides an excellent general approach for 'phenotypic anchoring': it is far more promising to try to logically connect molecular-level responses to energy flows like maintenance or assimilation (main pathway in Figure 2), rather than to traits such as growth or reproduction (the shortcut B in Figure 2). The scheme in Figure 2 is conceptually similar to the framework of Adverse Outcome Pathways (AOP) that is gaining interest in ecotoxicology (Groh et al., 2015; Ankley et al., 2010). Full-scale AOP models, however, would be highly species specific, and one can not hope to build them for more than a few charismatic species. Further, their complexity and lack of transparency might well make them unsuitable for ERA purposes (Forbes and Calow, 2012). The integration of AOP with DEB-based models, as advocated by Groh et al. (2015) and Kramer et al. (2011), is a promising avenue to address these problems, and fits well with the view outlined here.

In the current applications of DEB models in ecotoxicology, a more descriptive linkage is used (shortcut A in Figure 2): the value of a physiological model parameter is a linear-with-threshold function of the internal concentration (at a target site). Below the threshold (the no-effect concentration), a chemical (or other stressor) exerts no effect on the physiological model parameter, and above that threshold, every molecule has the same contribution to the effect. In principle, every physiological parameter may be affected by a stressor, but one may assume that (at least at low concentrations) only a single process is affected (the pMoA, Alda Álvarez et al., 2006a). Each pMoA has a recognizable set of effect patterns on growth and reproduction over the life cycle (Jager, 2015), but also on other energy-related endpoints such as respiration and embryonic development. In practice, the most likely pMoA, and the model parameters, are inferred from the effects patterns on growth and reproduction over time, and hence this shortcut requires (partial) life-cycle testing with regular observation over time. As the model parameters relate to underlying mechanisms, they form a solid basis for constructing predictive relationships for extrapolations between chemicals (Jager and Kooijman, 2009), between life stages (Gerritsen et al., 1998), and between species (Baas and Kooijman, 2015; Lika et al., 2011). In the future, a closer link to systems biology may help to estimate model parameters of the energy budget (see next section). In this area, however, a far more concerted research effort is needed before one can use such predictive methods to reduce (and perhaps ultimately even obviate) the need for life-cycle toxicity testing.

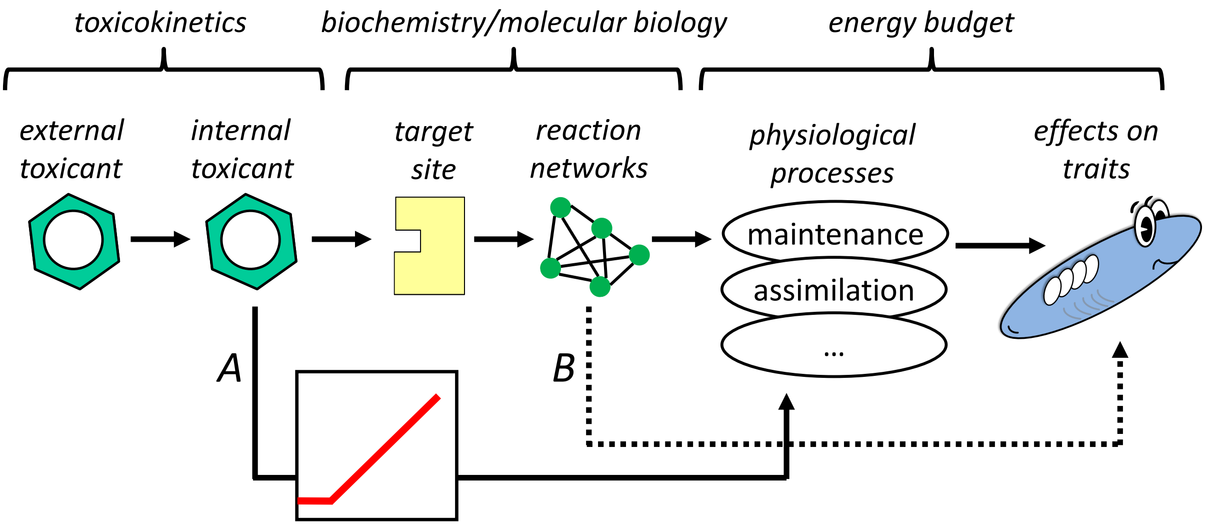

FIGURE 2. Causality chain from the external toxicant to life-history traits of the individual. Shortcut A shows how internal concentrations can be directly linked to physiological processes of the energy budget (as is common practice in DEB-based modelling). Shortcut B illustrates how some traits may be directly linked to the molecular level (e.g., endocrine disruption affecting reproductive traits). For the sake of readability, feedback loops are not depicted in this diagram (e.g, the effect of the individual's growth pattern on the toxicokinetics).

What role can systems biology play in ERA?

Systems biology currently seems to focus foremost at shortcut B in Figure 2: attempting to link molecular-level processes directly to traits of the individual (generally using statistical correlations). As indicated previously, this applies for some mechanisms of action, especially those that have a direct and exclusive effect on reproduction (e.g., endocrine disruption), and better for some species (demand systems) than for others (supply systems). Energy-budget models are particularly needed when toxicants interfere with energy flows within an organism (Groh et al., 2015), which is usually the case in supply systems when there is an effect on growth and reproduction. Understanding toxicant responses at the molecular and cellular level is interesting enough in its own right, but unravelling the entire chain of molecular events is not always necessary, and also not always sufficient, to link internal concentration to effects on life-history traits.

That being said, systems biology and AOP display a range of other benefits in the effects assessment of ERA, as discussed in detail elsewhere (Ankley et al., 2006; Garcia-Reyero and Perkins, 2011; Groh et al., 2015). Even for the same chemical, different species may show a different pMoA, and thus a different affected energetic process (Alda Álvarez et al., 2006a). Short-term tests with molecular techniques may support identification of the pMoA, and thus prioritize chemicals and reduce the need for life-cycle testing (Ankley et al., 2006; Groh et al., 2015). These techniques may thereby also help to understand why and how pMoA varies between species. Further, there needs to be a connection between effects at the molecular level and values of the model parameters that govern the toxic response in the energy budget, i.e., the quantitative link between internal concentration and effect on a physiological process in the energy budget. In the future, *in vitro* (or even *in silico*) methods may allow one to establish model parameters without toxicity testing, or act as prior information in a Bayesian context, such that the test duration and/or the number of test animals can be reduced.

At this moment, there is little research effort dedicated to elucidating the link between TKTD models and molecular events. Some attempts were made to link affected processes in a DEB model with gene expression (Swain et al., 2010), and to simultaneously model survival and biomarker responses over time (Jager and Hansen, 2013). A promising avenue for further research is an integration of AOP with DEB-based (and other TKTD) models (Groh et al., 2015; Kramer et al., 2011). The focus, however, needs to lie on biological functions (rather than selecting useful biomarkers) that can be linked to the individual's energy budget. An example is provided by Bundy et al. (2008), where changes in energy metabolism were identified from a combined transcriptomics/metabolomics approach. It must be possible to link such metabolic changes to the processes included in DEB models (Figure 1).

OUTLOOK

A new paradigm for ERA

It might sound trivial, but risk assessment needs to be based upon risk. Risk includes two components: the impact of an adverse event and the probability of that event occurring. At this moment, ERA deals with neither of these two factors explicitly, as impacts are not quantified and there is generally no estimation of probabilities. A comparison between an averaged environmental concentration and some predicted 'safe level' has little to do with risk (Jager et al., 2001; Forbes et al., 2011). The use of mechanistic effect models, however, opens up avenues to make ERA live up to its name. This does however require a radical change in thinking regarding environmental risk; a general outline of such a new paradigm is shown in Figure 3. Mechanistic fate models produce time-varying exposure concentrations in a given environment, which can be used by TKTD models to predict the consequences for the individual's life-history traits over its life cycle. These traits can subsequently be translated to impacts at the population level (or higher) by another suite of mechanistic models (Forbes et al., 2011; Grimm and Martin, 2013), involving additional issues in dealing with complexity. A similar coupling of models, from emissions to effects at the food-web level, was proposed by De Laender et al. (2014) in their ChimERA project.

The predicted impacts at these higher levels of organization, as a consequence of a specific release scenario in a specific environment, is exactly what ERA needs to be concerned with. Uncertainty analysis over this chain of models may be used to produce predictions of impacts with associated confidence bounds, which ties in with a strict definition of 'risk' (for elements of such an approach, see Jager et al., 2006; Ashauer et al., 2011). Quantitative uncertainty analysis further offers a reliable solution for risk refinement: the uncertainties that contribute most to the overall uncertainty in the risk estimate are the first candidates to address (Jager et al., 2001). In some cases, this may result in refinement of the estimated releases into the environment, whereas in others, the uncertainties in the effects assessment may dominate. Such a targeted refinement might seriously increase the efficiency of the ERA process, thereby reducing time, effort and costs.

The same set of environmental characteristics (such as temperature) needs to be included in every model in this chain to ensure consistency. Apart from the information on the environment, the model chain also requires estimates for model parameters. A combination of testing at various levels of biological organization (individual, cellular, or molecular) with *in silico* approaches might well be optimal to obtain the most information from the least amount of animals and budget.

At first glance, this new paradigm might look like an enormous increase in the burden placed upon the risk assessors, who now also have to deal with effects models. However, just as with the fate models, the effects models presented here are largely chemical-independent. Initially one needs to decide upon the most relevant species, food web or system to serve as our protection goal. Subsequently, one requires selection of the most applicable model(s) for these goals, and parameterize them with the basic biology of these systems. These steps might take quite a bit of time and effort, but it only needs to be done once; when the model system is in place, there is only need to worry about the limited set of model parameters that are specific to the chemical(s) under scrutiny. As with the fate models, running effects models is cheap, although it usually requires experts to judge the applicability of the model and to interpret the output.

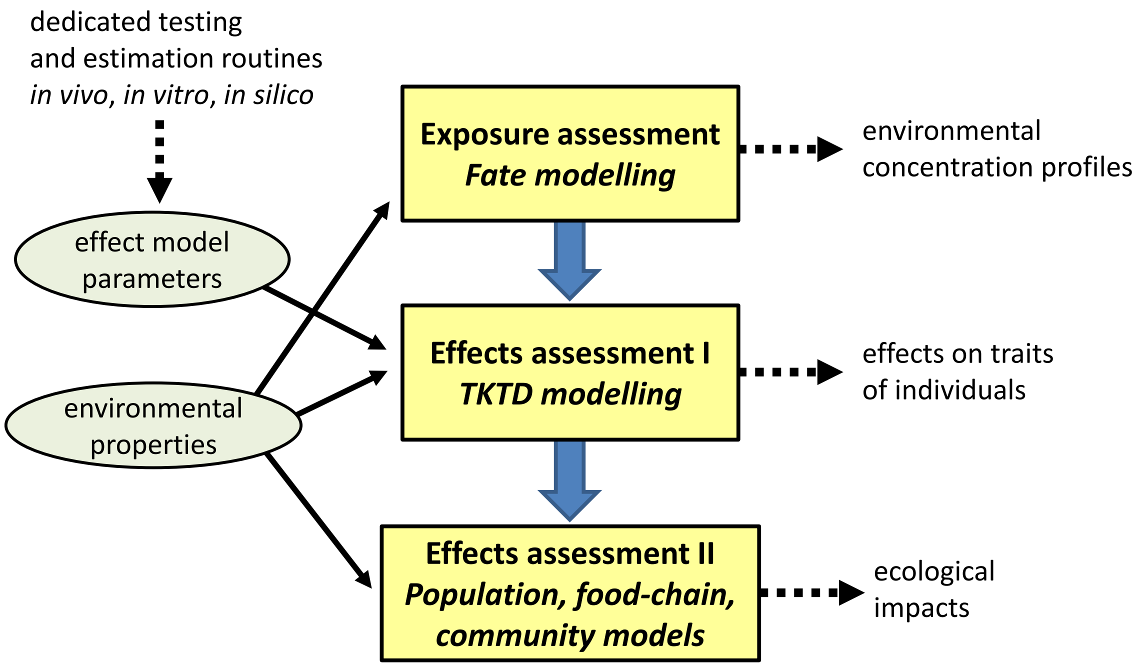

FIGURE 3. A new paradigm for environmental risk assessment. Exposure assessment feeds into two stages of effects assessment to ultimately predict environmental impacts for a certain set of environmental conditions. The focus in this scheme lies on the effects assessment; the inputs for the parameters of the fate models are not specified.

A road map for the future

Clearly, a new paradigm for ERA is not something that can be established overnight. There is, however, no reason for complacency; the current procedures for effect assessment are scientifically flawed, and steps need to be taken to start improving them. A 'roadmap for the future' is required to face the challenges that pollution and ecosystem deterioration are continuing to pose. In my opinion, such a roadmap needs to include the following elements:

- There is a need to create awareness among the stakeholders in the ERA process that the current framework for effects assessment is flawed and inefficient, and needs to be improved. ERA requires a long-term perspective: where do we want to be in 10-20 years from now, and how can one get there?
- TKTD models are an indispensable part of a mechanistic effects assessment. These models are, however, under-utilized in ecotoxicology and therefore also barely play a role in ERA at this moment. Unlike environmental chemistry, ecotoxicology does not have a strong tradition in modelling. Much work is therefore needed to increase awareness and acceptance of these models, both in science and in regulatory settings, but also in education.
- It must be realized that a detailed knowledge at the molecular and cellular level, by itself, is not sufficient to predict the effects of chemical (and other) stress on the life-history traits. This insufficiency cannot be remedied by more data or better computational tools as it is a matter of systems logic. Research at the lower levels of biological organization has an important role to play in the future of ERA, but it needs

to be functionally connected to individual-level models (e.g., energy budgets) to realize that potential.

- An energy-budget approach is an indispensable element to explain and predict toxicant effects on life-history traits of supply systems, and the interaction with other chemicals and environmental stress. Energy-budget models can be operated without knowledge on molecular/cellular processes. However, clarifying the link between the rather abstract large-scale processes of the energy budget and the underlying molecular processes has a lot to offer. Such a link might reduce, and perhaps ultimately even obviate, the need for animal testing, and strongly shape further development of TKTD models. This step requires a closer collaboration between molecular biologists, experimental ecotoxicologists, and modellers.
- Experimental tests need to be designed in such a way that they provide information on the model parameters of mechanistic effects models. There is a need to move away from standard test protocols that are designed to generate summary statistics of little relevance (like NOEC and EC*x*). The test design needs to be driven by requirements of the effects models, and not vice versa. TKTD models place specific requirements on the protocols, and at the same time, some existing requirements can be loosened (Barsi et al., 2014).
- For the endpoint survival, there is an established database of some 600 chemicals, all tested in a 4-day acute test with fathead minnow, under similar test conditions (Russom et al., 1997). This is not only a goldmine for deriving descriptive relationships, but because the raw data are available, also for calibrating TKTD models, and looking for patterns in the model parameters (Jager and Kooijman, 2009). For sub-lethal effects, there is nothing that even comes close to this dataset, and such basic work is desperately needed. A structured testing effort with several species, several compounds from each major chemical group, and a useful test design, will provide a wealth of information on the patterns in the model parameters among chemicals and among species.
- ERA needs to start moving away from statically comparing results of exposure and effects assessment, and aim to link them coherently. This means that regulatory agencies already need to start thinking about inclusion of TKTD and (simple) population models into their ERA schemes, and redesigning test protocols to suit these model's needs.
- Stakeholders in the ERA process need to explicitly define the protection goals, and link them to potential outputs of effects models (Forbes and Calow, 2012; Forbes et al., 2011). At this moment, protection goals are vaguely defined, and, in practice, shift when moving from one tier to the next. Ideally, the protection goals (and thereby the models in Figure 3) should not change when moving to a subsequent tier of ERA. Instead, data requirements increase with each tier, and uncertainty in risk estimates decreases.

In conclusion, ecotoxicology requires a far more ambitious, and a more focussed, research agenda. First and foremost, more effort needs to be made in development and application of 'simple-box' models: relatively simple models that are based on general principles rather than detailed mechanisms (Hendriks, 2013). In my opinion, it is possible to learn from chemical fate modellers, and design mechanistic effects models as simplifications of biological systems, and then worry about the most efficient means to parameterize these models. This is not only essential to support the ERA process, but also to improve our understanding of the mechanisms by which stressors affect life-history traits. Further, there is an urgent need for more structural collaboration between various disciplines. In particular, a concerted effort is required to link systems biology to TKTD and energy-budget modelling which has an enormous potential for furthering both the science of ecotoxicology as well as the ERA process.

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