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Incorporating sub-organismal processes into dynamic energy budget models for ecological risk assessment

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Abstract

A working group at the National Center for Mathematical and Biological Synthesis (NIMBioS) is exploring the feasibility of integrating two complementary approaches relevant to ecological risk

assessment. Adverse outcome pathway (AOP) models provide “bottom-up” mechanisms to predict specific toxicological effects that could impact an individual’s ability to grow, reproduce, and/or survive from a molecular initiating event. Dynamic Energy Budget (DEB) models offer a “top-down” approach that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of resources. Thus, AOP models quantify linkages between measurable molecular, cellular or organ-level events, but they do not offer an explicit route to integratively characterize stressor effects at higher levels of organization. While DEB models provide the inherent basis to link effects on individuals to those at the population and ecosystem levels, their use of abstract variables obscures mechanistic connections to suborganismal biology. To take advantage of both approaches, we developed a framework to link DEB and AOP models by interpreting AOP key events as measures of damage-inducing processes affecting DEB variables and rates. We report on the type and structure of data that are generated for AOP models that may also be useful for DEB models. We also report on case studies under development that merge information collected for AOPs with DEB models, and highlight some of the challenges. Finally, we discuss how the linkage of these two approaches can improve ecological risk assessment, with possibilities for progress in predicting population responses to toxicant exposures within realistic environments.

Introduction

Ecological risk assessment is used to support decisions regarding the risks of introduced chemicals on populations, communities and ecosystems but is often based on estimates of biological impact (or hazard) at the organismal level (Landis et al., 2003). However, if we were to require natural population or community level data on the biological impact of chemical, we would likely be estimating the impact too late - usually after the exposure and potential population decline has occurred. Thus prospective ecological risk assessment inevitably involves integrating information at multiple levels of biological organization. There is no ideal resolution of this problem – all approaches have pros and cons (Rohr et al 2016) – but to be effective and proactive, we must measure the impact of a chemical and breakdown products over a range of doses in controlled laboratory experiments. With over 80,000 chemicals and millions of species, many of which are critically endangered (Perkins et al in review), toxicity testing at the whole organism level is infeasible. Further, there are increasing concerns surrounding animal welfare and regulations surrounding animal testing. In 2007 in the US, the National Research Council recognized the limitations in the current status of toxicological testing and called for action to radically improve toxicological evaluation. The shift required much of the science that is focused on testing the harmful effects of chemicals on biota to move towards the development of techniques that are focused on *in vitro* and *in silico* testing to extrapolate molecular and cellular level effects to the whole organism and populations of organisms. This shift was followed by the passage of the bipartisan reforms to the Toxic Substance Control Act (TCSA) in 2016 that embraced alternative testing strategies that include mechanisms based in vitro assays and in silico approaches (Nel and Malloy, 2017).

To that end, a working group at the National Center for Mathematical and Biological Synthesis (NIMBioS) is developing a framework that would allow for the extrapolation of

toxicological impacts to populations from molecular level responses to chemicals. This framework will integrate two complementary modelling approaches relevant to ecological risk assessment—Adverse Outcome Pathway (AOP) and Dynamic Energy Budget (DEB) models. Adverse outcome pathway models provide “bottom-up” mechanisms to predict specific toxicological effects that could impact an individual’s ability to grow, reproduce, and/or survive from a molecular initiating event (Ankley et al 2010). Dynamic Energy Budget models offer a “top-down” approach that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of resources.

Adverse Outcome Pathways and Ecotoxicology

The AOP framework was adopted from mode of action approaches, which originally included *chemical-specific* mechanistic information into hazard assessment (Meek et al 2003; Seed et al 2005, Boobis et al 2006, 2008). The AOP, however, is chemical agnostic and conceptualizes the transfer of information from molecular to organismal levels of organization as a first step to inform human and ecological risk assessment (Ankley et al. 2010; Villeneuve et al, 2014). The AOP framework is an effective tool for arranging information at sub-organismal levels of organization, and may aid in interpreting data from high-throughput screening methods for the purpose of risk assessment. With these methods, the potential of thousands of chemicals to interact with molecular and cellular processes can be determined rapidly and cost efficiently. For human health risk assessments, which focus on the protection of the individual, AOPs that link lower level events to organismal level responses may be sufficient to indicate risk. For example, adverse outcomes may include cellular endpoints such as skin sensitization, or cell proliferation (Nel and Malloy 2017), but how those interactions translate into impacts on organismal performance and ecological processes remains uncertain (Margiotta-Casaluci et al. 2016). Ecological risk assessments, by contrast, are generally concerned with the protection of populations, food webs, or ecosystems, and there is a critical need to develop AOPs that inform these higher levels of biological organization (Rohr et al 2017). Cellular and molecular data may never be sufficient for prediction of ecological impacts, but they could be used to predict, for example, diverse outcomes on individuals that relate to the general processes of growth, survival and reproduction, processes that are included in population-level assessments (Kramer et al. 2011).

Thus, to effectively use AOPs in ecological risk assessment, we must develop biologically based, quantitative extrapolation tools or models that allow us to extrapolate cell or tissue-level data to organismal level endpoints by creating *quantitative* AOPs (qAOPs). A qAOP mathematically describes the processes within an AOP from a molecular initiating event (MIE) to an adverse outcome (AO). Quantitative AOPs can be a series of quantitative response-response relationships that describe transitions between key events (KEs) (Conolly et al 2017; Perkins et al, in review); ideally, these response-response relationships are mechanistic and can be constructed using a variety of modeling techniques, but correlative information can also be useful (Conolly et al 2017; Perkins et al, in review).

Quantifying the detailed mechanistic information necessary to develop qAOPs on a single species is challenging, expensive and labor and time intensive (Perkins et al, in review; Margiotta-Casaluci et al. 2016), and it is infeasible to repeat this exercise for thousands of species. Also, existing AOPs generally converge on single biological endpoints as if independent (e.g., growth *or* reproduction), and thus ignore the trade-offs implied by resource limitation, e.g. through the competition for energy among physiological processes. Furthermore, important feedbacks exist at each level of organization. Exposure to toxicants commonly triggers protective physiological responses such as enhanced synthesis of antioxidant compounds in response to oxidative stress (Klanjscek et al 2016). Also, individual organisms may induce protective (or other) changes in their physico-chemical environment (Stevenson et al 2013) or their food environment (Martin et al 2013, 2014). Furthermore, some species may be capable of relatively rapid adaptation to chronic chemical stress (Di Guilio and Clark, 2016, Nacci et al 2016; Reid et al 2016; Du et al 2015). In short, linking a molecular chain of events to effects on growth and reproduction requires the use of a generalizable systems theory at the organismal level of organization. This theory should be able to explain the effects of toxicants on an organism's acquisition of resources from the environment and the consequences for energy demanding traits such as growth and reproduction (Jager et al. 2016). Because of these limitations (and others), the AOP framework has not been applied to regulatory policy, and is not likely to be applied in the next decade because of the uncertainty surrounding the quantitative linkages and application to ecological risk assessment. However, where it can make an impact is in "win-win" situations, such as screening of potential new chemicals and prioritization of chemicals for further testing (Elliott et al, 2017). It is also likely to gain traction when development and acceptance of AOPs are done with full transparency and engagement with stakeholders (Elliott et al, 2017).

Dynamic Energy Budgets and Ecotoxicology

In contrast with the "bottom up" AOP approach, Dynamic Energy Budget (DEB) theory (Jusup et al 2017, Kooijman 1986, 2010, Nisbet et al. 2000) offers a "top down" mechanistic conceptual framework for connecting suborganismal to organismal processes. The starting point is a dynamical systems model of the performance (growth, development, reproduction, mortality risk) in arbitrary environments. The DEB approach employs unifying metabolic theory that can be applied to any species using a small number of parameters (Kearney et al 2015); this approach can potentially be used to model effects of chemicals on individual organisms (e.g. Jager et al. 2011, 2006). Moreover, the approach offers the possibility to extrapolate population and higher- level dynamics from individual level energy budget by the means of individual based modelling (Martin et al. 2013, 2014, Gergs et al. 2014, 2016). This suggests that there is potential to provide a connection from an AOP to ecologically important levels of organization, but only if we have some quantitative approach for relating qAOP and DEB models.

Kooijman's DEB theory captures the metabolic dynamics of an individual organism through its entire life-cycle, be it ectothermic or endothermic, autotrophic or heterotrophic, and is explicitly tied to food/substrate availability and temperature. The life cycle of an individual is the primary focus, from which sub- and supra-organismic levels are considered. Thus,

DEB theory can serve as a pivotal framework for building process-based models that link molecular, cellular, and tissue level responses to apical endpoints, such as survival, growth, and reproduction (Murphy et al, 2017), and subsequently to those at higher levels of ecological organization (Martin et al 2013a,2013b; Forbes et al 2017; Gergs et al. 2014, 2016).

The first systematic body of work using DEB models to interpreting toxicity data involved a suite of models (Kooijman and Bedaux 1996) that established methodology for using information from standardized toxicity tests to obtain *biology-based* measures of no-effect concentrations and other metrics that were independent of experimental protocols (see Baas et al 2010). Wider applications of the approach, now generally called DEBtox (for a readable introduction, see https://leanpub.com/debtox_book) followed, as well as an OECD Guidance document (OECD, 2006). However, this guidance document appears to be the extent of the incorporation of DEB into regulatory practices to date. DEBtox is more established in the EU than elsewhere, but incorporation into risk assessment in EU is the responsibility of the companies applying to register their chemicals through Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), and on companies that are interested in DEB modeling as a risk assessment tool.

The key to DEBtox is the concept of “physiological mode of action” (PMoA) that summarizes how a stressor impacts parameters associated with processes involving energy acquisition and utilization. Modeling toxicity requires coupling the DEB representation of physiological processes to toxicokinetic (TK) and toxicodynamic (TD) sub-models (Ashauer et al, 2011; Ashauer and Escher, 2010). TK models describe the dynamics of bioaccumulation, elimination, and chemical transformations of chemical contaminants within an organism. Toxicodynamic (TD), sometimes call “toxic effect”, models describe processes leading from toxicant interaction with a biological target to effects.

DEBtox models have been used to analyze chronic toxicity data under (assumed) constant exposure conditions (e.g. Jager et al. 2006, Jager and Selck 2011, Goussen et al. 2015), or time-varying exposure (Pieters et al. 2006) and effects resulting from chemical mixtures (Jager et al. 2010). These models have usually been applied to organismal growth and reproduction data to identify the likeliest physiological mode of action. However, in many cases, the analyzed data could be well described by any of the physiological modes of action and additional data on, for example, respiration and feeding are needed for identification (Muller et al. 2010, Jager et al. 2016). Furthermore, sublethal chemical effects might not be adequately described by a single mode of action, rather it is conceivable that organisms respond in multiple ways to the same toxicant.

In parallel with advances relating to chronic toxicity using DEBtox, there have been advances in modeling *survival* that use toxicokinetic-toxicodynamic (TK-TD) approaches, but do not consider detailed physiological processes that cause mortality. These have, to a great extent, been reconciled within the General Unified Threshold model for Survival (GUTS, Jager et al. 2011), which invokes a dose metric, assumed proportional to the hazard (i.e. per capita mortality) rate, and may include processes such as bioaccumulation, distribution within the organism, biotransformation and elimination, damage accrual and

recovery, and physiological compensation processes. Of particular importance for this paper is the abstract dynamic variable *damage*, described by Kooijman (2010) and related to the physiologically determined component of mortality. The complexity of the dynamic equations for *damage* differs among studies, depending on data availability and the processes considered in the approach. One approach is to use a one parameter scaled damage model (Jager et al. 2011), while other approaches may include damage accrual and recovery (Ashauer et al. 2007, Klanjscek et al 2016).

Previous work suggests that the damage concept may have value for linking qAOP and DEB. An early study of receptor kinetics by Jager and Kooijman (2005) analyzed survival of organisms exposed to organophosphorus pesticides. This remarkably simple model assumed that functional receptors are knocked out by the chemical, and functional receptors are turned into non-functional ones. Veltman et al. (2014) extended this approach to predict sodium loss and acute mortality in several aquatic species. Enzyme (acetylcholinesterase) inhibition was also considered in the time-dependent accrual of damage on the molecular level to explain differential sensitivity at the organism level (Kretschmann et al. 2011, 2012).

The appealing simplicity and generality of DEB theory comes with a price; model quantities and processes have a relatively high level of abstraction. Auxiliary assumptions (that may be organism specific) link abstract variables to quantities that can be measured directly such as length, wet or dry weight, respiration, time to/ length at first brood, egg output, and so on (Lika et al. 2011). Yet there is a large body of literature on methods for estimating DEB model parameters, including routine multivariate, nonlinear regression (or analogous likelihood) methods (Kooijman et al. 2008), a computer-intensive state-space method (Fujiwara, et al. 2005), a Bayesian approach (Johnson et al. 2013) and an innovative, heuristic “pseudo-Bayesian” approach (Lika et al. 2011) that is currently, the most widely used approach to the estimation of DEB parameters (called “add-my-pet” http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html).

Linking AOP to DEB

qAOP and DEB models have contrasting strength and weaknesses. We now outline an approach to linking them in the hope that the integrated insight can give added value to the insight from the approaches considered separately. Both the AOP and the DEB approaches have the ultimate goal of informing predictions on ecologically important process, most of which occur at population, community or ecosystem levels (Fig. 1). The DEB modeling framework has the potential to integrate suborganismal processes from the AOP framework, to fine tune the mechanism by which a stressor operates and to potentially incorporate high throughput testing, potentially from previously developed assays in ToxCast (<https://www.epa.gov/chemical-research/toxcast-dashboard>). The importance of our overarching goal of using information on suborganismal toxicity for population dynamic projections was highlighted by Martin et al. (2014) who constructed an individual-based population model for *Daphnia* interacting with their algal food. They found that the outcome commonly depends strongly on the suborganismal mode of action. Indeed, when consumer-resource interactions are considered, toxicity mediated by different physiological processes that lead to the same outcome in a standard reproduction test may cause drastically different effects at

the population level. These ranged from almost no effect to extinction, with contrasting impacts on total population and biomass emerging from substantial changes in size structure.

Essentially an AOP represents a pathway that is integral to a DEB, and we propose that translations from one to the other are possible by utilizing the damage variable introduced in the preceding section. Within the AOP, KEs and adverse outcomes that occur at the molecular, cellular, organ and whole organism level can influence DEB parameters, whose values may in turn depend on the damage variable(s). A theory that offers semi-mechanistic submodels for connecting damage to fluxes was recently developed (Muller et al, submitted) and provides a flexible framework. This potentially integrates the AOP into a more holistic DEB model that uses a small number of variables to describe the integrated effect of all metabolic processes, allows for tradeoffs of energy between growth, reproduction and survival, which are population relevant outputs.

We propose a conceptual model to initiate exploration into mechanistically linking AOP to DEB (Table 1; Fig 2).

A model of organism-level dynamics should not simply allow everything to be connected to everything else and we describe a mathematical structure that recognizes 5 types of variables (summarized in Table 1) and their causal connections. *KEs* are measureable endpoints in an AOP (eg. Fig 2). These key events either represent, or are themselves, measures of *damage* that is manifest at the level of cells, organs or of the entire organism. Damage is caused, directly or indirectly, by *internal toxicant concentrations*– the accumulation of damage being described by some TK/TD representation. Damage impacts the processes in a *bioenergetic* model. This flow of causal connections restricts the form of dynamic equations we can use (see Table 1 for a list of variables and functional dependencies).

In applications that we currently envision, the model of the complete organism will use dynamic (differential or difference) equations to describe “performance” – growth, development, reproduction, risk of mortality etc. These are described by the DEB model which is tightly coupled to the body burden dynamics (growth impacts internal toxicant concentration and vice versa), and also to the damage dynamics. Changes in internal concentrations are described by a TK submodel. The damage variables will commonly be decoupled from the higher level processes, but connection is possible, e.g. oxidative stress involves production or reactive oxygen species (ROS) from both “routine” metabolism and from toxicity.

We see potential in using statistical methodologies to identify appropriate connections in Fig. 2, recognizing that increasingly, information on pathways impacted by toxicants comes from ‘omics (e.g., transcriptomics, proteomics, lipidomics, metabolomics)-based data (Murphy et al. 2017). Lists of features such as genes, metabolites or proteins that are significantly changed as a result of a given perturbation can be used to identify biological processes that are potentially impacted in response to exposure. Combinations of these biological processes are represented by DEB rates and fluxes. With small model organisms such as *Daphnia*, this may be the only approach available, as data on the effects of contaminants is measured at either the molecular level, or the whole organism level, and to

link AOP to DEB the molecular information would have to be directly translated to DEB rates.

For larger organisms such as fish, organ level data are available and in our NIMBioS working group, we are exploring mechanistic approaches to link AOPs to DEBs using endocrine disruption as the stressor, and rainbow trout (*Oncorhynchus mykiss*) as the target species. We were fortunate to have access to a rich data set on rainbow trout hormones, egg production, weights, as well as a well-developed physiologically-based model (Gillies et al 2016). We found two ways to link qAOP to DEB, and discuss them briefly here.

One method is to alter the core dynamics of the DEB framework to create demand-driven feedback mechanisms that can be exerted by the gonads on the allocation of resources to production of reproductive matter (Muller et al submitted). Using this model, species and sex specific characteristics of endocrine regulation can be modified, while keeping the remainder of the DEB core intact. This modeling approach successfully describes the time-resolved measurement of body weight, ovaries and liver, as well as the egg diameter of spawned eggs and plasma levels of estradiol and vitellogenin in rainbow trout (Muller et al submitted). The model is flexible and can be adjusted for different reproductive strategies such as iteroparity, semelparity, and batch-mode reproduction, depending on species and available data. The next step in this approach is to add toxicokinetics and simulate endocrine disruption, and is dependent on finding an appropriate data set.

Another approach is to add an egg module to the standard DEB model, allowing for hormone dynamics to control conversion of material in the DEB “reproduction buffer” into eggs (Fig 3). In this approach, well developed physiologically-based models (eg. Conolly et al 2017; Gillies et al, 2016; Li et al 2011, Murphy et al 2009; Watanabe et al 2009) are collapsed into simple forcing functions, regulators, activators and active-regulator dynamics, and incorporated into the standard DEB through synthesizing units (SUs; Lika et al in prep). Although in this example it is restricted to reproduction, forcing functions may be useful for other stressors and physiological modes of action in DEB, possibly by utilizing the functions from Muller et al., submitted).

Discussion

In this paper we described a conceptual model for linking AOP to DEB. Our working group is currently developing two case studies based on *Daphnia* and rainbow trout that merge information collected for AOPs with DEB. In this paper we have described our work on rainbow trout that focused on endocrine disruption for which there are existing quantitative AOPs that integrate molecular, cellular and organ level responses to predict effects on reproduction. Connecting with a DEB representation required either modifying the “standard” DEB model to include feedbacks that characterize the integrated effects of hormonal control mechanisms (Muller et al. submitted), or adding a module to standard DEB (Lika et al in prep). With *Daphnia*, there is little organ level data, so we are seeking correlative connections with transcriptomic data (work in progress). In both studies, our goal is to identify a set of key events, or a key event network leading to measures of “damage” that impact specified DEB parameters and rates. Eventually we envision a system where

AOPs link to DEB rates, and the DEB is then used within the construct of a whole organism where energetic tradeoffs between physiological processes are considered. Such a system would improve the predictive power of suborganismal key events, by placing such KEs into a framework that would allow for extrapolation to population (via IBMS) and up to population, community, and ecosystem effects (Fig 4)

Ashauer and Jager (2017) hypothesized that “chemicals of the same class”, i.e. triggering the same MIE and hence having the same adverse outcome, should exhibit the same pMoA when toxicity data are analyzed using DEBtox methods. They found very limited evidence to support this hypothesis; indeed “baseline toxicants” apparently exhibit different dominant pMoAs for *Daphnia magna*. They cautioned that unambiguous identification of pMoA is remarkably challenging, and discussed data requirements to resolve this. A more fundamental issue is that all DEBtox analyses known to us assume that there are one, or occasionally two, dominant pMoAs, but the energy fluxes in a DEB model represent flows of *abstractly defined generalized compounds*. As recognized earlier in this paper, one AOP might correspond to a *combination* of several DEB fluxes, and apparent differences in dominant pMoA may simply reflect different relative weights for each DEB flux. We anticipate that our approach will help resolve the apparent dichotomy identified by Ashauer and Jager.

In recent years, with the advent of ‘omics technologies and their ever-decreasing cost, it has become feasible to more comprehensively integrate subcellular effects with higher levels of biological organization. While the computational approaches for true integration of multi-level data are still scarce, a simpler integration across these levels has been approached in a number of publications (Rohart et al. 2017; Antczak et al. in prep; Van Aggelen et al. 2010; Williams et al 2011; Joyce and Palsson, 2006). Looking forward, AOPs in particular would benefit greatly from multi-level analysis approaches as an AOP inherently represents the combination of many biological levels. Metaboanalyst 3.0 (<http://www.metaboanalyst.ca/faces/home.xhtml>) is an excellent example of the current state of multi-level integration, allowing for metabolite and gene expression level integration to understand the possible affected pathways within a given organism. With the increase of available metabolic models for more and more species, a more integrated methodology could be developed for understanding effects across multiple levels. The approach described in this paper represents a first cut at such integration.

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Figure 1:

A conceptual framework to link AOPs to DEB for a particular stressor/contaminant scenario will first require an inventory of the key events (KEs) affected in different organs and at different levels of biological organization, - such as molecular, cellular, organ level responses. C_e refers to concentration of toxicant external to the organism, and C_i is the concentration inside the organism. Dynamic Energy Budget integrate the key events act as a pivot to population level outcomes.

Figure 2.

Key event network (mode of action) translates to some measure of damage that would have to be related to key rates or allocation “decisions” (indicated by red arrows in dynamic energy budget models). Here, two different AOPs are represented, one related to reproduction, and the second related to growth. The estrogen receptor assay from toxcast (<https://www.epa.gov/chemical-research/toxcast-dashboard>) can inform vitellogenesis, egg production, and population trajectories (<https://aopwiki.org/aops/30>) and could potentially link to the partitioning rule in DEB, but also to the utilization of material in the reproductive buffer. PPARα assays: Toxcast lists 3 assays two are human liver cell based (for CIS and TRANS config); the third is cell-free. PPARα antagonism leads to reduced ability to obtain energy from fatty acids and reduces the production of ketone bodies and ultimately results in increased muscle protein catabolism that reduces body weight in an individual (<https://aopwiki.org/aops/6>). Again, one AOP may actually link to more than one flux in DEB.

Figure 3.

One approach to link qAOPs to DEB is to create a simplified submodel of the physiological processes (in this case the hormones involved in the fish hypothalamic-pituitary-gonadal (HPGaxis) and this submodel can regulate fluxes within the standard DEB by interfacing with synthesizing units (SU, blue dot). The model of the HPG axis is reduced into forcing function, regulator, activator and activate- regulator.

Fig. 4:
Schematic relating parallel descriptions of sub-organismal processes (AOP and DEB) and how they can interact to improve predictions of how whole organisms respond to stressors.

Table 1:

Proposed variables and equations used to characterize response to toxicity within an organism

Variables
$\mathbf{K} = \{K_1, K_2, \dots\}$ = set of sub-organismal key events from AOP
$\mathbf{R} = \{R_1, R_2, \dots\}$ = set of damage-related variables – may overlap with $\mathbf{K}$.
$\mathbf{Q} = \{Q_1, Q_2, \dots\}$ = set of internal toxicant-related concentrations
$\mathbf{B} = \{B_1, B_2, \dots\}$ = set of DEB model variables
$\mathbf{E} = \{E_1, E_2, \dots\}$ = set of environment variables
Dynamics:
$\frac{d\mathbf{B}}{dt}$ = functions of $\mathbf{B}, \mathbf{R}, \mathbf{E}, \mathbf{Q}$
$\frac{d\mathbf{R}}{dt}$ = functions of $\mathbf{Q}, \mathbf{R}, \mathbf{K}$ (only occasionally $\mathbf{B}$)
$\frac{d\mathbf{Q}}{dt}$ = functions of $\mathbf{B}, \mathbf{Q}$ – TK model