Toward Refined Environmental Scenarios for Ecological Risk Assessment of Down-the-Drain Chemicals in Freshwater Environments<!--<query>**Please confirm or correct the names of the authors, the affiliations, and the correspondence note.</query>-->**

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ABSTRACT
Current regulatory practice for chemical risk assessment suffers from the lack of realism in conventional frameworks. Despite significant advances in exposure and ecological effect modeling, the implementation of novel approaches as high-tier options for prospective regulatory risk assessment remains limited, particularly among general chemicals such as down-the-drain ingredients. While reviewing the current state of the art in environmental exposure and ecological effect modeling, we propose a scenario-based framework that enables a better integration of exposure and effect assessments in a tiered approach. Global- to catchment-scale spatially explicit exposure models can be used to identify areas of higher exposure and to generate ecologically relevant exposure information for input into effect models. Numerous examples of mechanistic ecological effect models demonstrate that it is technically feasible to extrapolate from individual-level effects to effects at higher levels of biological organization and from laboratory to environmental conditions. However, the data required to parameterize effect models that can embrace the complexity of ecosystems are large and require a targeted approach. Experimental efforts should, therefore, focus on vulnerable species and/or traits and ecological conditions of relevance. We outline key research needs to address the challenges that currently hinder the practical application of advanced model-based approaches to risk assessment of down-the-drain chemicals. *Integr Environ Assess Manag* 2016;12:000–000. © 2016 SETAC.

**Keywords:** Down-the-drain chemicals, Ecological models, Environmental scenario, Ecological risk assessment, Spatial models

## INTRODUCTION

The lack of ecological realism is a widely recognized limitation in current regulatory practice for chemical risk assessment. The conventional risk assessment paradigm based on the ratio between predicted environmental concentrations (PECs; calculated for worst-case exposure scenarios) and predicted no effect concentrations (PNECs; generally extrapolated from individual-level laboratory toxicity data for a few standard test species) provides some evidence of ecological risks but is aimed at being protective rather than predictive. In countries where chemical regulation is established, protection goals are often vaguely defined and a precautionary approach is usually taken to translate them into conservative safety thresholds (Hommen et al. 2010). In Europe, such regulatory inadequacies have been highlighted (Scientific Committee on Emerging and Newly Identified Health Risks et al. 2013). Three key scientific challenges have been identified to achieve better informed risk management decisions from environmental risk assessments: 1) the definition of relevant protection goals matching societal needs; 2) the development of relevant, spatially explicit exposure assessment tools; and 3) the development of mechanistic effect models (Price and Thorbek 2014). These challenges are interdependent and need to be addressed using an integrated approach. The values of environmental parameters used in exposure assessments may not correspond with realistic worst-case conditions from an ecological perspective, thus resulting in a potential mismatch between the predicted exposure and the ecological scenario that is represented in a risk assessment (Rico et al. 2016). To address this, we envisage a pragmatic and flexible framework to derive environmental scenarios for risk assessments tailored for the specific chemical emission and exposure profile, the ecotoxicological modes of action, and the biological entities to be protected (e.g., individuals or populations) derived from established protection goals.

Aquatic ecosystems receiving treated or untreated domestic wastewater are typically exposed to low concentrations of a wide range of chemicals, such as ingredients of home and personal care (HPC) products or pharmaceuticals, resulting from continuous point source emissions. Although emissions are relatively constant in time, exposure is variable in space and time because of seasonal variations in river flows, the removal efficiency of sewage treatment plants, and use and disposal patterns. Other water-quality stressors associated with wastewater (e.g., BOD, ammonia, nitrate, nitrite, and suspended solids) represent an important stress to ecosystems, particularly downstream of untreated discharges. In such a scenario, the ecological consequences of exposures exceeding a PNEC value derived to protect all species may or may not be a concern because of our limited understanding of ecosystems' baseline structure and function and of multiple stressors effects. Alternative protection goals have been proposed for a generic direct discharge scenario (Finnegan et al. 2009).

Ecological effect models have been proposed to extrapolate from responses observed for individuals in laboratory toxicity tests to expected effects on populations (see Galic et al. 2010 for a review). Models have also been designed to address effects on communities and to integrate multiple stressors typically present in real ecosystems, but they have primarily been developed for pesticides (Galic et al. 2010). For most household use chemicals, significant gaps exist in the chronic ecotoxicological data sets, which is most relevant to the continuous, low levels of exposure of down-the-drain chemicals in aquatic systems. Our understanding of population- or community-level responses (including direct and indirect effects and recovery potential) to chemicals in multistressed freshwater ecosystems is also limited (Baird et al. 2015).

In Europe, research efforts are being made to incorporate aspects of ecological relevance in prospective chemical risk assessment of down-the-drain chemicals (Forbes et al. 2011; De Laender, Van den Brink et al. 2014; Lombardo et al. 2015), focusing on scenarios representative of developed regions. In developing regions, where the ecological status of freshwater bodies is often characterized by poor water quality resulting from direct discharge of untreated wastewater, the need to improve the ecological realism of chemical risk assessment is equally compelling. The lack of a systematic approach to defining environmental scenarios, and in particular the ecological component of such scenarios, hinders the application (and regulatory acceptance) of ecological effect models in risk assessment. The need to develop realistic ecological scenarios for higher tier risk assessment has been recently recognized with the development of ecological models for risk assessment and the definition of acceptance and evaluation criteria (Augusiak et al. 2014; European Food Safety Authority 2014). Realistic but generalized scenarios representative of different geographies are needed to parameterize models that are able to integrate exposure and effects in a prospective risk assessment framework. In the present study we propose a stepwise strategy to develop and implement environmental scenarios in a framework suitable for down-the-drain chemicals.

## ENVIRONMENTAL SCENARIOS

In the regulatory risk assessment of chemicals, an environmental scenario can be defined as the conceptual and quantitative description of the environmental context relevant to the risk assessment (European Food Safety Authority 2014). An environmental scenario is composed of 2 fundamental components: the exposure scenario and the ecological scenario (Rico et al. 2016; Figure 1). Standardized exposure scenarios have been used for many years in regulatory frameworks and are widely accepted by stakeholders (e.g., the Forum for the Coordination of Pesticide Fate Models and Their Use [FOCUS] scenarios for pesticides [FOCUS 2011] and the European Union System for the Evaluation of Substances scenarios for general chemicals [Vermeire et al. 2004]). Conceptually, an exposure scenario is defined by its spatial and temporal scale and by a qualitative description of the environmental context it represents. For example, in the European Union System for the Evaluation of Substances, the regional scale exposure scenario is a steady-state representation of a generic 200 × 200 km densely populated, industrialized European region. In quantitative terms, exposure scenarios are developed by choosing the spatiotemporal resolution and by assigning parameter values in a given mathematical modeling framework, typically including an emission and an environmental fate component. For screening risk assessment purposes, such parameterization is often based on a realistic, worst-case situation. The emission component of the exposure scenario, often referred to as the emission scenario, consists of the assumptions about chemical use, consumer habits, disposal pathways, and wastewater treatment infrastructure. The environmental fate component of the exposure scenario corresponds to the parameterization of all abiotic and biotic factors that influence the environmental fate and exposure of chemicals.

The conceptual description of the ecological context relevant to conventional risk assessment frameworks can be defined loosely as the entire pool of species potentially present in a given geographical context. Indeed, in contrast with exposure scenarios, ecological scenarios are far less well defined. In aquatic risk assessment, the number of tested species is limited in most cases to 3 species, representing different trophic levels (algae, daphnia, and fish), chosen primarily for practical reasons, such as ease of culture. The experimental laboratory conditions of standard toxicity tests (i.e., controlled medium composition, temperature, optimal food availability, no predation, among others) are poorly representative of realistic ecological conditions (Van den Brink 2008, Tannenbaum 2013). The characterization of an ecological scenario should relate to the natural factors influencing the biological integrity of the ecosystem (e.g., climate, river morphology, and water quality), as well as to the specific stress to be evaluated, in our case, chemical stress. Rico et al. (2015)<!--<query>Rico et al. 2015 does not appear in the References. Is Rico and Van den Brink 2015 meant instead?</query>--> defined ecological scenarios as the combination of biotic and abiotic parameters that influence chemical-induced effects and recovery of populations. In prospective risk assessment, a vulnerability-based ecological scenario can be defined as a realistic worst-case representation of such parameters. The biotic parameters that define the scenario should describe the taxonomic composition along with the biological characteristics or traits influencing organism-level sensitivity, recovery potential, and propagation of effects to higher levels of biological organization through indirect effects (Rico et al. 2015).<!--<query>Rico et al. 2015 does not appear in the References. Is Rico and Van den Brink 2015 meant instead?</query>--> Examples of biological traits influencing toxicant effects at the individual level include respiration type, size, life cycle duration, or degree of sclerotization (Baird and Van den Brink 2007; Rubach et al. 2012; Rico and Van den Brink 2015). Examples of biological traits influencing the resilience and the ability of populations and communities to recover include the reproductive characteristics and recolonization ability of the disturbed populations (Gergs et al. 2016; Rico and Van den Brink 2015), the trophic state of the exposed system (oligotrophic or eutrophic; Alexander et al. 2013; De Hoop et al. 2013; Gabsi et al. 2014), the strength of interspecific and intraspecific species interactions in a food-web context (e.g., predation, competition; De Laender et al. 2015), and the complexity of this food web (De Laender et al. 2015). In the context of ecological effect modeling, it has been proposed to define an ecological scenario by allocating 1 value to each variable potentially influencing population- and ecosystem-level responses to (a mixture of) chemicals (De Laender et al. 2015).

Exposure and ecological scenarios share a number of important variables that influence both exposure and effects (De Laender et al. 2015; Morselli et al. 2015). For example, temperature may influence exposure concentrations through temperature-dependent degradation kinetics, but it may also influence the population response through temperature-dependent growth kinetics (Heugens et al. 2006). Other parameters that may affect both exposure and effects include flow velocity, concentrations of suspended and dissolved solids, suspended and dissolved organic matter, nutrients, pH, as well as landscape features such as the connectivity of exposed and nonexposed habitats and the presence of refugees (e.g., Traas et al. 2004). Therefore, it has been proposed to integrate both “environmental scenarios” and to define them using a combination of biotic and abiotic parameters, which result in a realistic worst-case representation of the exposure, effects, and recovery of the biological entities that we intend to protect (Rico et al. 2016). A major challenge in the unification of exposure and ecological scenarios is the selection of the suitable spatiotemporal scales that can adequately represent realistic worst-case combinations of exposure (e.g., low-flow season) and ecological scenarios (e.g., sensitive life stages). Compared with chemicals characterized by pulse input exposure at certain points in time corresponding to specific life stages in seasonal organisms, the consideration of spatiotemporal scale for down-the-drain chemicals is somewhat facilitated by the (semi)continuous nature of environmental emissions.

## DEVELOPMENT OF ENVIRONMENTAL SCENARIOS IN A TIERED RISK ASSESSMENT FRAMEWORK

Two considerations are important in accounting for spatial and temporal variation in biotic and abiotic characteristics of ecosystems for chemical risk assessment. One is in defining specific protection goals (SPGs) for different spatial units, and the other is in developing exposure and toxicity assessment methods and models that predict safe thresholds for the ecological entities in the environmental scenarios.

The current regulatory approach of protecting all species everywhere, all of the time, is likely to be overly conservative in locations where the more sensitive taxonomic groups do not occur. As an alternative to this approach, SPGs could provide guidance for the selection of the biological entities and spatiotemporal dimensions that the scenarios should address. Defining SPGs could be achieved either by applying the top-down ecosystem services concept or by use of the bottom-up empirical characterization of scenarios with representative ecological community structures and functions derived from biomonitoring data. Both approaches are suitable for chemicals in HPC products when higher tier refinement of generic approaches is needed, that is, for high-volume chemicals with small safety margins. The advantage of using ecosystem services to set SPGs for environmental scenarios is that the approach facilitates the identification of key service-providing traits or taxonomic units (Nienstedt et al. 2012) that can be aligned to service-related water management objectives, for example, fisheries, flood protection, and amenity value.

The implementation of SPGs in prospective risk assessment requires the identification of reasonable worst-case environmental scenarios, as well as quantitative descriptions of acceptable and/or unacceptable impacts on biological entities so that toxicity testing and ecological modeling can be suitably designed. Conventional endpoints measured in standard toxicity tests (e.g., LC50 or EC50) refer to impacts defined at an individual organism level, and the safety threshold is derived via the use of default assessment factors to account for extrapolation from individual-level endpoints to higher levels of biological organization (as well as other uncertainties, e.g., differences in species intrinsic sensitivity; Hommen et al. 2010). Although this approach lacks mechanistic rationale, it is simple and easy to apply. Further research is needed to better define how to derive chemical concentration thresholds that are protective of different SPGs. Because SPGs refer to the structural and functional health of defined environmental typologies, they are better described by the integrity of species populations or, for groups of species with similar functional roles in the ecosystem (e.g., microorganisms), by the integrity of functional roles. Therefore, in the present study we assume that ecological scenarios and models will target the population level of biological organization. However, a thorough evaluation and a consensus on which SPGs should be applied in the prospective risk assessment of down-the-drain chemicals are still to be reached.

## Toward spatially explicit exposure scenarios

In the lower tiers of regulatory risk assessment of general chemicals, the exposure scenario consists of a simple unit environment. The Mackay-type steady-state multimedia box models have proved a convenient platform to reflect the multimedia nature of potential chemical emissions, transport, and removal pathways. A key reason for the widespread use of these models is their simple structure and, probably more importantly, their simple outputs (a single PEC for each environmental compartment), which facilitates easy use in risk assessment and decision making. Input data requirements correspond to the base set of physicochemical and environmental fate properties generated through chemical registration procedures in a tiered approach. Multimedia box models can also be used to identify sensitive input parameters (Figure 1). For example, sensitivity and uncertainty analysis have shown that chemical emissions and hydrological parameters are essential inputs independent of chemical properties, whereas other inputs and model parameters such as biodegradation rates, temperature, organic matter content, and pH can be important depending on the physicochemical and environmental fate properties (Ying et al. 2014). However, these models are typically limited to 1 box per region or continent and 1 set of landscape characteristics per box, and they cannot account for highly spatially differentiated or localized emissions and exposure pathways. Under other chemical regulations, bespoke local-scale scenarios have been developed to reflect the specific use settings of different product types (e.g., biocides) or regional-specific landscape and climatic properties (e.g., pesticides; FOCUS 2001). Accordingly, numerous high-resolution spatial models have been developed for agrochemicals. Large-scale spatially explicit environmental fate models can play a key role in the identification of catchments or river section of higher exposure. Many spatially explicit models have been developed to cover higher resolution assessment of rivers on a catchment or continental scale and should be considered to avoid duplication of efforts. Most are designed for agrochemicals or for pollutants prioritized under water regulation, such as the Water Framework Directive (WFD). Some are specifically designed for down-the-drain chemicals. For example, the in-STREam Exposure Model, iSTREEM, is designed to evaluate exposure of chemicals in down-the-drain products (Aronson 2012). It predicts concentrations in more than 28<td:hsp sp="0.25"/>000 river reaches representing more than 200<td:hsp sp="0.25"/>000 river miles resulting from discharges from more than 10<td:hsp sp="0.25"/>000 wastewater treatment plants across the continental United States. Aqueous concentrations are primarily determined by removal in wastewater treatment plants, dilution, and a simple constant in-stream removal rate. GREAT-ER has been developed as a georeferenced model for high-tier exposure assessment (Kehrein et al. 2015) and has been used to simulate the fate and exposure of chemicals in whole watersheds (Price et al. 2009). However, data requirements for parameterization of such models are not readily available at larger scales. Another limitation is the lack of the multimedia transport component to describe atmospheric and terrestrial pathways (e.g., volatilization, sludge application to soil, and irrigation). Recent developments in the prediction of spatial emissions over entire continents (ScenAT model; Hodges et al. 2012) enabled researchers to determine variations in emissions of chemicals in HPC products. The ScenAT model is based on demographic, economic, as well as household water use and treatment indicators. The model combines market research data on product sales with ingredient inclusion levels to estimate spatial environmental emissions down to 1-km resolution.

Projections of chemical emissions into the environment provide the input to spatially refined exposure models. Spatial multimedia fate models have been developed at a $2^{\circ}$ by $2.5^{\circ}$ (approximately $200 \times 200$ km at temperate latitude) resolution for entire continents (Humbert et al. 2009), but such a resolution is not sufficient to analyze spatial variations in down-the-drain chemicals. Developments in large-scale hydrological modeling have enabled the incorporation of high-resolution hydrological information in multimedia fate models (Lidim et al. 2016). The multiscale multimedia fate and exposure model Pangea offers the unique ability to create multiscale grids and project spatial data onto these grids at runtime (Jolliet et al. 2012). A GIS engine based on ArcGIS is used to produce 3-dimensional multiscale grids to project spatial data sets and to compute geometric and topological parameters. This multiscale, flexible parameterization can predict concentrations at the global scale, with refinement of the grids to a higher resolution for specific areas of interest. The routed hydrological component of the model is currently based on the gridded $0.5^{\circ} \times 0.5^{\circ}$ water network and annual average flows defined by the World Water Development Report II (Vörösmarty et al. 2000a, 2000b) and its adaptation by Helmes et al. (2012). On the global scale, the HydroSHEDS data set and the HydroROUT model (Lehner and Grill 2013) offer the possibility of refining the hydrological network with a subkilometer resolution. Data and attributes calculated by HydroSHEDS for each of the 12 complementary resolutions include annual discharge, flow direction, average depth, and surface area of river and lakes. Highly spatially refined exposure scenarios are meaningful only if all sensitive model inputs and environmental parameters can be refined to a similar level of resolution. For many factors that affect emissions (e.g., chemical use and wastewater infrastructure), environmental fate, and bioavailability (e.g., particulate and dissolved organic matter; Figure 1), this is feasible only on a limited number of site-specific catchment scenarios because of data availability or, more practically, to manage model complexity. Specific scenarios can be selected based on large-scale simulations to identify areas of higher exposure and/or can be based on data availability. Crucially, a robust global scale model framework enables characterization of the significance of a chosen catchment scenario in the context of risk assessment over large regions (e.g., a 90th percentile worst-case catchment scenario in a given region). High-resolution (sub)catchment-scale scenarios need to be defined to develop and evaluate models for higher tier exposure assessments. The validity of the steady-state assumption, which may be acceptable at lower to mid-tier assessment levels, given the (semi)continuous nature of down-the-drain chemical emissions, needs to be reconsidered. Changes in hydrological regimen and, for some product types, seasonality in emissions (e.g., higher use of pharmaceuticals in winter and sunscreens in summer) result in temporal variability in exposure. Seasonal low-flow conditions are associated with lower dilution and therefore higher exposure (Grill et al. 2016). Higher tier exposure models should also consider a refined parameterization of factors affecting bioavailability, such as fluxes, concentration, and organic matter content of suspended and dissolved solids, which can be highly dynamic, implying significant deviations from steady-state. Sediment transport increases dramatically during high-flow events (Dale et al. 2015). Organic matter varies with seasonal cycles of primary and secondary production (Morselli et al. 2015). At this tier, exposure models should provide suitable exposure input data for ecological effect modeling. A coherent parameterization of the abiotic and biotic factors relevant to both exposure and effects (i.e., integrated environmental scenario) is required to reduce the mismatch in the spatiotemporal scale and parameterization between exposure and effect assessments (Figure 1). Regardless of the model design, freely dissolved concentration should be the common metric at the interface between exposure and effect assessment because it reflects external exposure as seen by organisms. Examples of exposure scenarios of simple lotic systems designed for the integration of exposure and ecological models demonstrated the importance of spatiotemporal resolution in particulate and dissolved organic matter driven by seasonal dynamics in primary productivity on water-dissolved concentrations (Morselli et al. 2015).

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Advances in global-scale chemical emission and hydrological models offer an opportunity to improve spatial exposure models and to identify priority catchment scenarios.</B1>

Higher tier exposure modeling should focus on a few prioritized (sub)catchment-specific scenarios to capture the spatial and temporal variability of sensitive input parameters.</B1></BL>

Vulnerability-based ecological scenarios

Characterization of ecosystem type.

An initial step toward the development of realistic worst-case scenarios is the characterization of the type of aquatic ecosystems that may be exposed to down-the-drain chemicals. This exercise can be done a priori and does not require any chemical-specific information. In temperate and humid zones, typical freshwater bodies receiving domestic wastewater discharges mainly consist of lotic ecosystems, ranging from minor urban streams to medium and large lowland rivers. Lentic ecosystems such as lakes, ponds, or lagoons can also be an important scenario in certain regions. In regions with poor wastewater infrastructure, untreated wastewater is often discharged to artificial open drainage channels before reaching natural ecosystems. In (semi)arid regions, wastewater is often discharged to ephemeral water bodies or even reused directly or after treatment for groundwater recharge, irrigation, or urban landscaping. Large-scale data on the emission scenario, such as the type of household drainage system, local or centralized wastewater treatment infrastructure, can help characterize the typology of ecosystems to be assessed (Figure 1). Data need to be collected at scales relevant for the size of the aquatic system, including the main habitat parameters that determine the ecological status in taxonomic and functional terms such as flow velocity, hydrological regimen, depth, light intensity, temperature, geological substrate, trophic status, and chemical water quality. Continental-scale assessments of freshwater habitat typologies and pressures (EEA 2015) provide a valid data source.

Taxonomic and traits-based description of aquatic communities.

A second step would be to describe the community of each ecosystem type based on taxonomy and traits. Ecological monitoring surveys such as those used for the evaluation of the ecological status of the European water bodies as part of the WFD can be of great help to compile taxonomic descriptions of community structures. A challenge in interpreting these data will be the selection of representative ecosystems unaffected by chemical or physical anthropogenic stressors. The data sets collected for reference freshwater ecosystems for the derivation of Environmental Quality Standards in the eco-regions established as part of the WFD intercalibration exercise (Borja et al. 2007) could be used to derive taxonomic collections representative of ecosystems unaffected by major environmental stress. Because species composition is likely to vary across subcontinental scales, the description of aquatic communities in terms of their biological traits would increase the generality of such characterizations and subsequent transferability between scenarios (Van den Brink et al. 2011). The taxonomic information could be transferred into trait-based descriptions using available trait databases for aquatic organisms (e.g., Usseglio-Polatera et al. 2000, Poff et al. 2006). Traits can be constant for all individuals (e.g., basic life stages, degree of sclerotization, among others) or changing over a lifetime (i.e., those that are plastic, e.g., size or lipid content).<!--<query>Please verify wording "(i.e., those that are plastic, e.g., size or lipid content)."</query>--> Accounting for intraspecific variability of traits combinations, as often reported in existing databases, will increase the realism and relevance of the scenarios and may prevent overestimation of impacts on community composition (De Laender, Melian et al. 2014).

Habitat filtering can be applied to predict the presence of species with competitive traits under a combination of environmental factors, including natural and anthropogenic stressors (e.g., Kearney and Porter 2009; Kearney et al. 2010). Because of the co-occurrence of multiple water-quality stressors in effluent discharge areas (e.g., oxygen depletion, ammonia, nutrient, or chemical mixtures), different filters can be applied to an initial pool of all potential species to establish baseline conditions in the absence and in the presence of anthropogenic (but nonchemical) stress. In this way it will be possible to assess the impact of chemicals stress under realistic conditions. If unstressed baseline conditions cannot be established (i.e., because of widespread contamination from wastewater), ecological scenarios for impacted ecosystems may be the only feasible baseline. In such situations, however, it will be difficult to unravel the effects of chemical stress as compared with other wastewater stressors.

Selection of vulnerable taxa.

The "population vulnerability" concept developed by Van Straalen (1994) considers 3 factors that affect the vulnerability of populations: likeliness of exposure (organism level), intrinsic sensitivity (organism level), and population sustainability (population level); later, Van den Brink (2008) added indirect effects (ecosystem level) as a measure of propagation of impacts.

The susceptibility of organisms to exposure from chemical stress largely depends on the mobility of the organisms, their home range in relation to the exposed area, and their capability to actively avoid exposure.

Intrinsic sensitivity is related to the effect of chemicals at the individual level and can be explained by the toxicokinetics (TK) and toxicodynamics (TD) of a substance in the exposed organisms (Rubach et al. 2012, Nyman et al. 2014). TK are determined by traits such as lipid content, surface-to-volume ratio, breathing mode, dietary habits, and rate of metabolic degradation. Differences in metabolic rates are a key factor determining species sensitivity (Baas and Kooijman 2015), but rates are often unavailable or difficult to generate. TD, in contrast, depend on the chemical modes of action, on cellular-scale damage-repair mechanisms, and on the adverse outcome pathway from cellular to organism scale. In general, greater interspecific variations in TD are expected for specifically acting chemicals, such as biocides or pharmaceuticals, than for baseline toxicants, such as the majority of HPC ingredients (Rubach et al. 2011). Unfortunately, the information available is often inconclusive for determining the most important toxicity mechanisms. Many biocides used in HPC products affect multiple target sites and metabolic pathways in microbial cells, which may reflect in multiple toxicity mechanisms in nontarget organisms (e.g., Dann and Hontela 2011). In other cases, toxicity mechanisms of high concern, such as endocrine effects, have been observed in the laboratory (Kunz et al. 2006), but it remains unclear whether for chemicals suspected of endocrine effects these represent the major toxicity mechanism at environmentally relevant concentrations.

Population sustainability is determined by demographic and reproductive traits including voltinism, dispersal capacity, swimming mode, drifting ability, and the presence of emergent life stages (Van den Brink et al. 1996; Beketov et al. 2008; Galic et al. 2012, 2014; Rico and Van den Brink 2015). Sensitivity-related traits can be used to evaluate the relative sensitivity of aquatic organisms to chemical exposure. For example, Baird and Van den Brink (2007) and, more recently, Rubach et al. (2012) and Rico and Van den Brink (2015) identified correlations between some traits and the empirical sensitivity of aquatic organisms. In the study by Rico and Van den Brink (2015), regression models were established that allow prediction of the relative sensitivity of aquatic invertebrates to some specific insecticidal modes of action. Similar correlations could be established for down-the-drain chemicals with known mode of action allowing the ranking of species according to their expected sensitivity. Several examples exist in the literature that deal with the vulnerability and recovery potential in time and space of aquatic taxa exposed to pesticides (e.g., Gergs et al. 2011, Ibrahim et al. 2014, Rico and Van den Brink 2015); comparable examples for species inhabiting larger lotic systems impacted by down-the drain chemicals remain to be developed. For this, it is important to take into consideration the exposure dynamics resulting from semicontinuous point-source emission into surface waters. Besides intrinsic sensitivity, traits related to mobility and habitat range of different taxonomic groups influence the effects on population abundances. Three conceptual spatial scenarios can be outlined for lotic systems (Figure 2). Small planktonic organisms (Figure 2a), for instance, are influenced by drift, and thus effects may be seen further downstream, depending on their population-level recovery traits (e.g., reproductive behavior). The population abundance of benthic organisms such as rooted macrophytes or benthic invertebrates downstream of effluent discharge points is likely to be characterized by their dispersal and reproductive behavior (Figure 2b). The recolonization of areas where chemical exposure causes direct toxic effects will be achieved only if the species is able to adapt physiologically or genetically. In contrast, fish species (Figure 2c), which usually have a larger home range than the area in which exposure results in toxic effects, may hardly show abundance declines in specific areas and require a larger scale spatial evaluation to observe population declines. Traits such as active avoidance, migration, and swimming behavior influence their distribution, in relation to chemical exposure or other stress factors.

Construction of food-web scenarios.

Food-web scenarios can be constructed from available quantitative and/or qualitative biomonitoring data and fundamental constraints related to the conservation of (bio)mass and energy within and across biota compartments (e.g., production of 1 group is enough to support the consumption by its consumer). The most important functional groups from the taxonomic and traits analyses need to be assembled into representative food-web structures. Interactions affecting internal exposure (e.g., biomagnification; De Laender et al. 2009 and many others), as well as responses to stress (e.g., competition for resources or predation; De Hoop et al. 2013, De Laender and Janssen 2013), need to be characterized to assess community- and ecosystem-level endpoints. In addition, the food-web structure influences the vulnerability of community assemblages at the ecosystem level (ecosystem vulnerability). Clearly, a daunting number of variables potentially influence ecological effects and therefore risk, whereas limited experimental data are available to evaluate whether and how the variables making up the environmental scenario actually influence ecosystem-level responses. De Laender et al. (2015) used mechanistic models to theoretically explore the influence of various ecological variables on the response of ecosystems to different types of chemicals. In these simulations, ecosystem-level effects were larger in mesotrophic systems than in oligotrophic systems, suggesting trophic state as an important variable. Regardless of trophic state, interaction strength (quantified using grazing rates) was suggested as a more important driver for the size and recovery from direct and indirect effects than dispersal rate.

In selecting the spatial scale of a food web, the species with the largest lifetime spatial range will define the scale of the whole ecosystem to be considered, because organisms with smaller spatial ranges will reoccur within the large system. For example, individual periphyton or macrophytes influence and are influenced by only the immediate surrounding environment, but populations colonize wider areas, so it is possible to integrate them into a fish-dominated ecosystem also in a spatially explicit sense.

Key messages include:

Taxonomic and traits analysis combined with habitat filtering can be used to derive baseline conditions in reference and impacted ecosystem scenarios exposed to down-the-drain chemicals.</B1>

Current knowledge gaps in (sub)organism- to population-level traits affecting population vulnerability constrain our current ability to target most vulnerable species.</B1>

Ecosystem-level modeling can help to identify vulnerable ecological scenarios by identifying key factors that affect responses to chemical stress in real food webs.</B1></BL>

## SCENARIO-BASED ECOLOGICAL MODELS FOR RISK ASSESMENT

Environmental scenarios developed at different scales and levels of resolution (Figure 1) can be applied at a given tier of assessment according to need for refinement and data availability. The degree of integration between exposure and effect assessment increases at higher tiers because the matching of the abiotic parameter values and the spatial-temporal scales is maximized. The spatial and temporal integration of exposure and effect models is a key challenge. Spatial exposure and effect assessments can be fully integrated if exposure and effect models have a consistent scale and resolution. This may be feasible only in specific high-tier assessments. In comparison, the implementation of temporally explicit modeled exposure data into the TK component of ecological models is relatively straightforward because most TK models are designed to simulate dynamic exposure.

In this section we outline potential approaches to introduce ecological realism in a tiered framework for prospective risk assessment of down-the-drain chemicals. Effect models can be developed for identified vulnerable species (Figure 1). Different types of ecological models, ranging from organism to ecosystem level, may be used to assess relevant endpoints according to the SPGs derived to protect structural integrity (e.g., biodiversity) or specific ecosystem services.

### Linking exposure to individual-level effects

A requisite for the accurate integration of exposure and effect assessments is the use of consistent exposure data (i.e., total, bioaccessible, or bioavailable concentrations). The bioavailable exposure concentration depends on environmental factors (e.g., sorption to organic matter), which is why the free aqueous concentration is more representative of the exposure experienced by aquatic organisms and, therefore, is the most appropriate metric for linking exposure and effects. However, it is not the external concentration that causes the effect, but rather the concentration at the target site. Using internal dose as a metric can begin to account for the species sensitivity differences caused by TK (Escher and Hermens 2004, Hendriks et al. 2005, Nyman et al. 2014). TK-TD models can explicitly separate TK from TD processes (Ashauer et al. 2015). Thus, it is possible to model the influence of physical-chemical properties, some species traits (Buchwalter et al. 2008, Rubach et al. 2012, Poteat and Buchwalter 2014), and environmental factors (Ruotsalainen et al. 2010) on TK, as well as the influence of toxicity pathways (Gunnarsson et al. 2008, Lalone et al. 2013), species traits (Rubach et al. 2012), and environmental factors (Heugens et al. 2003) on TD (Rubach et al. 2011, Jager 2013, Ashauer et al. 2015). A single parameter, such as temperature, can influence TK, by changing uptake, elimination, and biotransformation rates (Buchwalter et al. 2003, Heugens et al. 2003, Harwood et al. 2009), as well as TD, by changing physiology and intrinsic sensitivity (Harwood et al. 2009).

The physiological and ecological parameterization of effect models can, to a large extent, be based on species traits information or on collections of model parameters for specific modeling approaches, for example, the add-my-pet database for dynamic energy budget (DEB) models (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/; Lika et al. 2011). Such parameterizations will set the baseline for any selected taxonomic aggregation. Conversely, parameterization of chemical effects requires significant experimental efforts. In some cases, detailed toxicity test results for vulnerable species will be available and can be used to parameterize the TD component of effect models, but such cases are the exception rather than the rule. Chronic experimental tests are required, ideally using most vulnerable species, and need to include measurements of reproduction and growth over time (Lika et al. 2011).

The integration of chemical stress with other environmental and anthropogenic stress variables is an essential element of ecological realism. Although the impact of environmental factors such as temperature, food availability, competition, and predation on organisms' responses to chemical stress has been observed experimentally (e.g., Heugens et al. 2003, Stampfli et al. 2011, Del Arco et al. 2015), the ability of ecological models to predict interactions between such factors and chemical stress remains largely untested. Environmental and chemical stressors impact survival, growth, and reproduction at the organism scale; therefore, models at this scale are required. Environmental stress, such as starvation, has been integrated with toxic effects on survival in a straightforward model by treating in a similar way environmental and chemical stress (Nyman et al. 2013). Integrating environmental stressors with sublethal chemical effects is more challenging because growth and reproduction are interrelated via an organisms' energy allocation (Sousa et al. 2010, Jager 2013). However, DEB models offer a platform to simulate sublethal, organism-level toxicity and integrate environmental stressors because effects on growth and reproduction by environmental factors also act via changes to the organisms' energy allocation (Jager 2013). For example, food limitation can be modeled by lower energy intake, and competition or physiological stress by higher energy requirements for maintenance (e.g., because of wider foraging ranges). Future research needs to define the relationships between the effect model parameters and the main environmental factors that influence survival, growth, and reproduction. Temperature, food availability, and water-quality stressors associated with domestic wastewater (e.g., oxygen deficit or ammonia) are sensitive stress factors and need to be included in forthcoming research. Of course, other, non–energy-related interactions are also conceivable (e.g., photosensitivity), which would require additional modeling.

Key messages include:

External and internal free aqueous concentrations are the correct exposure metrics to link environmental exposure with TK-TD models.</B1>

Environmental stressors need to be considered in organism-level effect models along with chemicals stressors to introduce ecological relevance in higher tier assessments.</B1></BL>

Population-level effect models

Population models can be applied at the higher tiers of the proposed framework (Figure 3). They can link individual-level effects to relevant processes at the population level such as reproduction, density-dependent regulation mechanisms, or dispersal. The consideration of sublethal effects requires an appropriate integration of individual-level models into population-level models to capture long-term effects. Further, population models can function as building blocks to analyze species interactions and hence build the interface to community-level modeling. Population models for combinations of species groups (defined by key traits) and endpoints need to be developed from the existing portfolio of modeling approaches. The physiological-ecological parameterization of population models can, to a large extent, be based on collections of species traits that exist for fish, benthic invertebrates (e.g., Usseglio-Polatera et al. 2000, Poff et al. 2006), and aquatic macrophytes.

For fish, relevant traits such as avoidance, dispersal capacity, and migration have an explicit spatial dimension (Figure 2). Therefore, population-level models for fish require individual-level exposure history data in a spatially explicit context as input of TK-TD model components (Beaudouin et al. 2015). The time frame required to integrate individual-level sublethal effects with population-level processes needs to be sufficiently long to cover multiple life cycles, which may involve simulation periods of several years for fish.

In the case of benthic invertebrates and rooted macrophytes, which disperse over smaller spatial scales and generally occur in higher numbers, individual-based models (IBMs) or compartment-based ordinary differential equation models are suitable modeling approaches. IBMs have been combined with TK-TD components (Baveco et al. 2014), including DEB models, which can account for sublethal effects (Martin et al. 2012). Population models still need to account for site-specific exposure while including population-level density regulation mechanisms. For example, an IBM population model for the water louse *Asellus aquaticus* has been integrated with spatially explicit landscape-level dynamic fate models for pesticides in an agricultural environmental scenario (Focks et al. 2014). Analogous modeling approaches for down-the-drain chemicals may need a different spatial resolution because variability in exposure is probably more significant at larger catchment scales.

Planktonic organisms that passively move with the water flow require the integration of population models with appropriate hydrological information (Figure 2). One conceptually straightforward method is to integrate population-level dynamics with hydrology-based catchment scale fate models with a mass balanced approach using ordinary differential equations.

The key message is:

Differences in life history and mobility traits in fish, benthic invertebrates, and planktonic organisms determine the optimal choice of population models.</BL></B1>

Community-level effect models

Community ecology deals with how abiotic variables and interactions between and within species determine coexistence, community composition, and biodiversity (Chesson 2000). Two-species IBMs have been developed to examine the role of species interactions on pesticide effects and subsequent recovery (Viaene et al. 2015). Most communities, however, consist of many more species, especially at lower trophic levels. For example, the site-specific macroinvertebrate species richness in temperate European lotic ecosystems may vary between less than 10 in small agricultural ditches to more than 50 in larger rivers (Davies et al. 2008). Recently, a model has been developed to predict community composition and biodiversity along gradients of chemical stress (De Laender, Melian et al. 2014). This approach can be considered a stochastic formulation of an IBM (Black and McKane, 2012) and works by calculating the probabilities of reproduction and death per species at each time step, based on exposure and on the interspecific and intraspecific variability in sensitivity. The model correctly predicted algal diversity along herbicide and metal toxicity gradients in lentic systems. It only needs a distribution of algal ECxs<!--<query>Please spell out ECxs at only mention in text here if an abbreviation.</query>--> that represents interspecific variability and an estimation of the long-range passive dispersal rate (the number of immigrants per period of time). A disadvantage is that it does not account for large niche differences between species and that its validity has not been proven for communities other than algae. Overall, the high number of species in algal communities and the smaller niche differences compared with heterotrophs justifies this methodology.

The key message is:

Stochastic formulations of individual-level models are a pragmatic approach to asses effects on communities made of many species, that is, at lower trophic levels.</B1></BL>

## Ecosystem-level effect models

Ecosystem-level studies analyze fluxes of matter and energy between functional groups and the abiotic environment, mostly using food-web theory to describe the direction and magnitude of these fluxes. Thus, ecosystem-level effect models in chemical risk assessment are used to simulate effects on such fluxes (ecosystem functioning) and on the size of functional groups (ecosystem structure). In general, these models are able to realistically reproduce seasonal fluctuations of biomass and nutrients observed in the field (e.g., Sommer et al. 1986). They are an ideal platform for integrating exposure and ecological scenarios because they can simulate seasonal dynamics of biotic and abiotic variables (e.g., biogeochemical cycles) with which the functional groups interact and on which the exposure of certain chemicals may depend. By integrating chemical stress with general chemical water-quality stressors associated with wastewater, ecosystem-level effect models can provide a more realistic representation of the Impact Zone concept, which has been suggested for risk assessment of down-the-drain chemicals in untreated discharge scenarios (Finnegan et al. 2009). Ecosystem-level models are also suitable for studying indirect chemical effects (Fleeger et al. 2003), which is most important when transient or local scale effects are acceptable or if indirect effects are greater than direct effects. In their simplest form, they are composed of a limited set of ordinary differential equations that are coupled according to food-web interactions and extended with concentration–response relationships in a nonspatially explicit environment (e.g., De Laender et al. 2008b, 2015; Everaert et al. 2015). Nutrient dynamics can be either explicitly modeled (e.g., De Laender et al. 2008b) or considered as external forcing functions (e.g., De Laender et al. 2015). Examples of intermediate complexity include integrated models of aquatic systems, such as Aquatox (Park et al. 2008) and CASM<!--
<query>Please spell out CASM at only mention in text if an abbreviation.</query>-->

(Nair et al. 2015), which combine (inorganic and organic) nutrient dynamics, food-web interactions, chemical fate, and ecotoxicological processes in site-specific environmental scenarios. Recently, Aquatox has been used to simulate potential ecosystem-level effects of 2 ingredients found in HPC products in a lowland river ecosystem (Lombardo et al. 2015). The present study showed that indirect effects can be of similar magnitude as direct effects and can both exacerbate and compensate for direct toxicity. To our knowledge, the highest level of ecosystem model complexity seen to date is currently being developed, where networks of IBMs are constructed that simulate ecosystem dynamics, starting from individual-level processes (De Laender, Van den Brink et al. 2014).

A major challenge to community and ecosystem effect models is calibration and external validation. Because of the level of biological organization considered, model calibration and validation are cumbersome in practice (but see De Laender et al. 2008a, Sourisseau et al. 2008). Indeed, mesocosm studies are rarely available for down-the-drain chemicals, let alone cosm studies that encompass ecological responses for different environmental scenarios. An alternative is to conduct laboratory-scale studies for a selection of stress scenarios that examine how processes key to community composition or ecosystem functioning (e.g., competition or predation) combine with chemicals in affecting simplified study systems consisting of few species (Liess and Foit 2010, De Hoop et al. 2013, Viaene et al. 2015).

The key message is:

</B1>Ecosystem-level models provide the most comprehensive platform to integrate exposure and ecological scenarios, but calibration and validation are an almost daunting challenge. Their utility in risk assessment remains to be demonstrated.</B1></BL>

Uncertainty analysis and probabilistic approaches to decision making

The seemingly overwhelming challenge of incorporating the complexity of stress ecology into a pragmatic risk assessment framework calls for a holistic consideration of uncertainty. Uncertainty, broadly defined as the combination of epistemic uncertainty and variability, needs to be assessed at different levels, from scenario (e.g., representativeness and variability of scenarios) to model and parameters uncertainty.

Quantitative sensitivity and uncertainty analysis of model input data and parameters has been addressed in exposure models used in regulatory frameworks (Matthies et al. 2004, Hollander et al. 2009), although less attention has been paid to higher levels of uncertainty, those associated with the definition of the scenario (Hollander et al. 2009) or with the mathematical representation of that scenario (model uncertainty). We envision the use of iterative model simulations at increasing resolution combined with sensitivity and uncertainty analysis to refine sensitive parameters in prioritized scenarios. Global- to catchment-scale exposure scenarios will be compared and evaluated for their ability to identify areas of higher exposure and for their accuracy in estimating measured concentrations. Specific enhancements, such as the refined parameterization of compartment phases (e.g., the distinction between dissolved and suspended organic matter), transport processes (e.g., dynamic solids transport), or the addition of transport processes not usually included in multimedia fate models (e.g., wastewater reuse and irrigation) could be implemented at higher tiers, if statistically relevant.

Consideration of scenario and model uncertainty in effect assessments is an essential part of the development of ecological scenarios. The validity of the ecological component of environmental scenarios largely depends on the uncertainty associated with the identification of most sensitive taxa or traits and of worst-case ecosystem conditions. Admittedly, our current ability to predict population vulnerability and intrinsic sensitivity in the first place is limited. The level of detail in individual- to ecosystem-level processes together with the selected spatial scale define the model complexity and computational demands. Obviously, not all aspects mentioned in the present study can be maximized simultaneously. Models of varying complexity should be compared by balancing accuracy in predictions with uncertainty introduced by additional parameters to identify the optimal level of complexity (Baveco et al. 2014, De Laender et al. 2014).<!--<query>Please clarify which De Laender et al. 2014 reference is meant here.</query>--> Finding the optimal number of processes driving the dynamics of species or functional groups is most challenging at the community and ecosystem level because each single species may have distinct environmental response, sensitivity, and specific interactions with the rest of the community. Clearly, incorporating all this complexity would no longer be technically feasible, results would be difficult to interpret, and parameters poorly identifiable. In practice, modelers have to decide what mechanisms to include and where to simplify. Methods such as approximate Bayesian computation are excellent tools to identify what mechanisms contribute most to observed patterns and thus to optimize model complexity (Hartig et al. 2011). Finally, we envision that models should be run through an ecological sensitivity analysis using realistic ranges of physiological parameters and environmental stress variables for a given scenario to identify an optimal model complexity and to refine sensitive parameters (Figure 4). Once established, a probabilistic parameterization can describe environmental variability and uncertainty in that scenario. Defining the values of environmental parameters under baseline and stress scenarios is part of the development of environmental scenarios. In organism-level effect models this can be achieved by reviewing existing knowledge or using model simulations under different stress scenarios (e.g., in a DEB model environment). A probabilistic, scenario-based approach lends itself to the creation of effect prevalence plots for selected endpoints. Figure 4 illustrates an example of an effect prevalence plot for an organism-level endpoint (e.g., reduced number of offspring or delayed time to maturity) in a hypothetical environmental scenario. The lines in such plots can be generated from the Monte-Carlo analysis of the coupled models, representing the different environmental variables and stress scenarios. The same concept can be applied to address population- and community-level endpoints (e.g., reduction in population abundance or reduction in biodiversity indicators). For any given exposure or ecological scenario, an effect prevalence plot can be generated to form the evidence base for decision making.

Key messages include:

Iterative model simulations and uncertainty analysis can guide the construction of models of optimal complexity for prioritized scenarios.</B1>

Scenario-based probabilistic assessments lend themselves to the creation of effect prevalence plots as a basis for risk assessment.</B1></BL>

SUMMARY OF RESEARCH PRIORITIES

Our analysis departs from the awareness that embracing ecological realism and spatial variation on community structure and function in future risk assessment requires a new framework rather than incremental changes to the existing framework. We believe that a scenario-based approach that integrates spatially explicit exposure models with ecological effect models for vulnerable taxa is needed to address the challenge. This is a long-term proposition. Examples cited in the present study demonstrate the technical feasibility of model-based approaches to refine exposure and ecological effects assessment. However, challenges remain in application to prospective regulatory risk assessment. We propose the following research priorities to enable the implementation of scenario-based ecological risk assessments for down-the-drain chemicals:

Develop a spatially and possibly temporally explicit exposure modeling framework that allows tiered exposure assessment of down-the-drain chemicals from global to catchment scale. Evaluation against monitoring data combined with sensitivity and uncertainty analysis will inform needs for model refinements (e.g., environmental parameters) and data generation (e.g., biodegradation rates) for simulations at higher resolution.</B1>

Collect taxonomic and traits data to extract representative ecological scenarios starting from well-studied river catchments exposed to discharges of wastewater effluents. The combination of biological data sets, such as those collected as part of the WFD program in Europe, with available traits data sets offers an opportunity in this direction.</B1>

Implement a new paradigm in toxicity testing based on a tiered risk assessment that moves from standard test species and protocols toward a targeted approach informed by spatially explicit protection goals. This is likely to require studies on long-term effects on most sensitive species/traits, including nonstandard species. Tests need to be designed to facilitate the development, parameterization, and evaluation of effect models and to enable the consideration of key environmental variables and stressors. Among these, food availability, temperature, as well as wastewater-related stressors such as oxygen depletion and ammonia are most relevant to down-the-drain chemicals.</B1>

Develop effect models for focal species and compare modeling options to identify the optimal complexity for different ecological scenarios. The optimal model structure balances: 1) taxonomic resolution with generalizations or read-across options, and 2) mechanistic detail with model complexity and associated data requirements. A need exists to cross-apply data and learnings generated from ecological modeling of agrochemicals and to harmonize efforts across chemical types.</B1>

Develop proof-of-principle examples of integrated exposure and effect model-based assessments that use ecologically relevant effect endpoints as a basis for decision making in chemical risk assessment.</B1></BL>

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<abstract type=“short”>Key Points

A scenario-based approach that integrates spatially explicit exposure models with ecological effect models is needed to embrace ecological realism in risk assessment.</B1>

Global- to catchment-scale spatially explicit models can be used to identify areas of higher exposure hotspots and to generate exposure inputs into effect models.</B1>

Mechanistic effect models demonstrate that it is feasible to extrapolate from individual-level effects to effects at higher levels of biological organization and from laboratory to environmental conditions.</B1>

Experimental efforts should focus on vulnerable species and/or traits and ecological conditions of relevance.</B1></BL>



**Figure 1.** Development of environmental scenarios from lower to higher tier risk assessment. Key factors are incorporated at increasing spatiotemporal resolution (exposure scenario) and taxonomic resolution (ecological scenario) toward integrated exposure and ecological scenarios (environmental scenarios) for specific combinations of realistic worst-case catchment and vulnerable taxa.

**Figure 2.** Conceptual spatial illustration of population-level toxic effects expected after point-source chemical discharges for different taxonomic groups. The main traits characterizing vulnerability potential (left) and the most suitable modeling approach for assessing the ecotoxicological risks (right) are presented. TD = toxicodynamics; TK = toxicokinetics.<!--<query>Please make specific mention of panels a, b, and c in legend for Figure 2.</query>-->

**Figure 3.** Conceptual framework illustrating options to combine scenario-based exposure and ecological effect models. Box models representing simplistic scenarios (**a**) can be used in combination with simple effect assessments, that is, predicted no effect

concentrations derived from standard single-species laboratory tests (**d**) for screening assessment. Large-scale to regional exposure scenarios (**b**) modeled by coarse spatial models can be used to identify chemical areas of higher exposure and to generate exposure and risk maps. Exposure data from coarse exposure models (**b**) can be used as inputs for individual- and/or population-level models (**e**). Site-specific (sub)catchment-scale exposure scenarios (**c**) then can be parameterized for selected areas of higher exposure. Site-specific exposure data can feed into individual- and/or population-level scenarios for focal taxa (**e**) or for vulnerable ecosystems scenarios (**f**). PEC = predicted environmental concentrations; PNEC = predicted no effect concentrations.

**Figure 4.** Application of a probabilistic risk assessment for a generalized environmental scenario. Example of individual-level effect prevalence plots for a given species in unexposed (dashed line) and exposed (solid line) scenarios introducing ecological stress variables. The *x*-axis can represent different types of effects (e.g., reduction in offspring). PEC = predicted environmental concentrations.

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