Exploring community-based environmental hazard assessment of mixtures using mode-of-action based approaches

Technical Report No. 135

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

Exploring community-based environmental hazard assessment of mixtures using mode-of-action based approaches

Technical Report No. 135

Brussels, May 2020

ISSN-2079-1526-135 (online)

ECETOC Technical Report No. 135

European Centre for Ecotoxicology and Toxicology of Chemicals
Rue Belliard 40, B-1040 Brussels, Belgium.

Exploring community-based environmental hazard assessment of mixtures using mode-of-action based approaches

Contents

SUMMARY

The assessment of the effects of chemicals on aquatic environments generally focuses on individual chemical substances or products and mainly relies upon ecotoxicological datasets comprised of a limited number of single species tested separately in the laboratory. In the real environment, the situation is much more complex where aquatic communities composed of many different species are exposed to mixtures of chemicals.

Assessing the potential effects of mixtures of chemicals on aquatic communities introduces elements of both chemical and ecological complexity. Considering the different communities potentially exposed to chemicals and the mixture of chemicals to which they are potentially exposed, the number of combinations is practically infinite.

It has been recognised that there needs to be some way of prioritising and/or simplifying assessment methodologies. This ECETOC Task Force has considered the approaches and tools currently available which could help achieve prioritisation and simplification with a focus on these chemical and ecological complexities.

Chemical complexities. Initial consideration was given to how different chemical properties will affect how chemicals will be distributed between environmental compartments. The Task Force considered methods for assessing mixture toxicity, both whole mixture toxicity testing and the component-based approach using concentration addition and response addition (independent action). Mode-of-action information is important here for assessing mixture effects, as it helps to group chemicals with assumed additive effects and to separate different mode-of-action groups which would be assumed to act independently from each other. This component-based approach is well established for chemical mixtures of known composition with concentration addition being accepted as a worst-case approach.

Ecological complexities. Chemicals differ in their toxicity to particular taxa. Species interact within communities in various ways and thus effects from chemicals can be through both direct toxicity and or a consequence of these interaction. The Task Force has reviewed experimental data found in the open literature for studies investigating community effects from chemical mixtures. Such community studies can give some insight into whether risk assessment of single compounds is protective for the mixture and can also to some extent serve as the basis for testing community model simulations with empirical data. Despite the use of community level studies in aquatic ecotoxicology being well established for more than 30 years, it seems that there are still only a limited number looking at chemical mixtures and these are mostly mixtures from within chemical sectors e.g. plant protection products, pharmaceuticals or hydrocarbons, rather than mixtures across different sectors. Furthermore, the application of the available studies to community model validation is limited by the level of detail assessed in the studies.

Solutions. Given the number of potential combinations, modelling approaches are seen as key to evaluating potential mixture effects on communities. The Task Force presents an overview of the different modelling approaches from effects at the individual level, evaluating their advantages and disadvantages, through to populations, communities and ecosystems. Having the data to parameterise these models is key to their application for both single and mixtures of chemicals. Lastly, the Task Force proposes a strategy for studying mixture effects at higher levels of biological organisation that is based on (1) classification; (2) the ecological scenarios paradigm; and (3) model-aided synthesis and design of informative experimenting.

1. INTRODUCTION

The assessment of the risk of chemicals to the environment is generally concerned with the assessment of single chemicals or products. This assessment is usually based on exposure assessment, i.e. data evaluating the physicochemical properties, fate and transport of chemicals, and effects assessment, i.e. data evaluating the toxicity of the chemical or product to groups of individuals of a number of representative species, generally tested in the laboratory in acute and chronic single-species bioassays, using maintained concentrations of a single test item. However, there is an increased awareness of the real-world situation, where rather than just individuals it is populations, communities and ecosystems that are exposed and to a mixture of chemicals with varying concentrations in time, rather than single chemicals. In chemical monitoring of aquatic systems, large numbers of chemicals are found in detectable concentrations in environmental samples (e.g. Moschet et al., 2014, Dulio et al., 2018). It is clear, that chemicals in the environment do not occur in isolation, and an open and often discussed question is about the effects of such complex mixtures of chemicals. There is a question as to whether current risk assessments are protective for these mixtures. This question clearly needs to be addressed, but the way forward is not simple. To address mixtures and their potential for effects, introduces elements of both chemical and ecological complexity into the assessment. Considering both the communities potentially exposed to chemicals and the combinations of chemicals to which they are exposed, the number of combinations is practically infinite. It is clearly not feasible, nor is it necessary, to consider all possible combinations and it has been recognised that there needs to be some way of prioritising and/or simplifying assessment methodologies.

One way of dealing with chemical complexity is to group chemicals with similar properties. The behaviour of a chemical in the environment and hence the potential for exposure of organisms in and through different environmental compartments is influenced, in part, by its physico-chemical properties. Assuming exposure, the potential for effects is driven by the mode-of-action (MoA) of the chemical, that is the interaction between the chemical and the organisms at a cellular and sub-cellular level. Chemicals can be grouped together by their mode-of-action and for mixtures of chemicals with the same mode-of-action, as for single chemicals, it should be possible to derive environmental thresholds, i.e. concentrations below which no impact on the environment is expected. Given the potential for different communities to be exposed, as with chemical complexity, there may be ways to simplify, classify and link different levels of biological complexity. For the assessment, it will be necessary to combine chemical and ecological complexity, again simplifying as much as possible the potential interactions, perhaps through scenario-based, ecological modelling approaches combining both exposure and effect models.

An ECETOC Task Force was put together to look at community level ecological assessment with a view to possibly incorporating these approaches, with the following Terms of Reference.

- Review available literature on extrapolation of effects from single aquatic species to the community level.
- Review available literature on the use of MoA-based approaches for approximating mixture toxicity effects with particular focus on aquatic communities.
- Address effects both direct and indirect, on communities.
- Identify knowledge gaps and research needs to close the gaps.

2. CHEMICAL COMPLEXITY AND LINKS TO EFFECTS

The chemicals that occur in the aquatic environment come from a wide range of sources, including both natural and anthropogenic. The chemicals from anthropogenic sources may come from wastewater treatment plants (including chemicals from the pharmaceutical and household and personal care products industries), wash-off from cities and agricultural landscapes, and from other sources. As such the final mixtures of chemicals in the environment will be very diverse and composed of hundreds of chemicals with vastly different properties, present at different concentrations. Their distribution in the environment may vary over time, with changing season or even time of day. The fate of the chemicals is determined by the characteristics of the receiving water bodies, as well as the physical and chemical properties of the compounds themselves. Due to the vast complexity of these mixtures the task of unravelling their effects on communities and ecosystems is a significant challenge. A first step towards addressing the challenge is understanding how chemicals partition in the environment.

2.1 Chemical Space Analysis

2.1.1 Chemical Space Analysis: Fate in the environment

We considered a small subset of chemicals covering a range of both baseline toxicants such as PAHs and specifically acting chemicals including pharmaceuticals and pesticides (fungicides, insecticides and herbicides). For these chemicals we collected a suite of the characteristics that determine their fate in the environment.

Figure 1 below shows a simple mass balance equilibrium model indicating in which environmental compartment a chemical is likely to be found following entry into water, based on its physico-chemical characteristics. The underlying calculations are based on the principles of Mackay's Unit world (Mackay, 2004). Based on this very limited sample, we see that fairly hydrophilic chemicals such as herbicides and pharmaceuticals have high bioavailability in the water phase, whereas chemicals that are more hydrophobic, such as PAHs and insecticides sampled for this exercise are predominantly predicted to partition into the sediment phase, with very little bioavailability through the water phase.

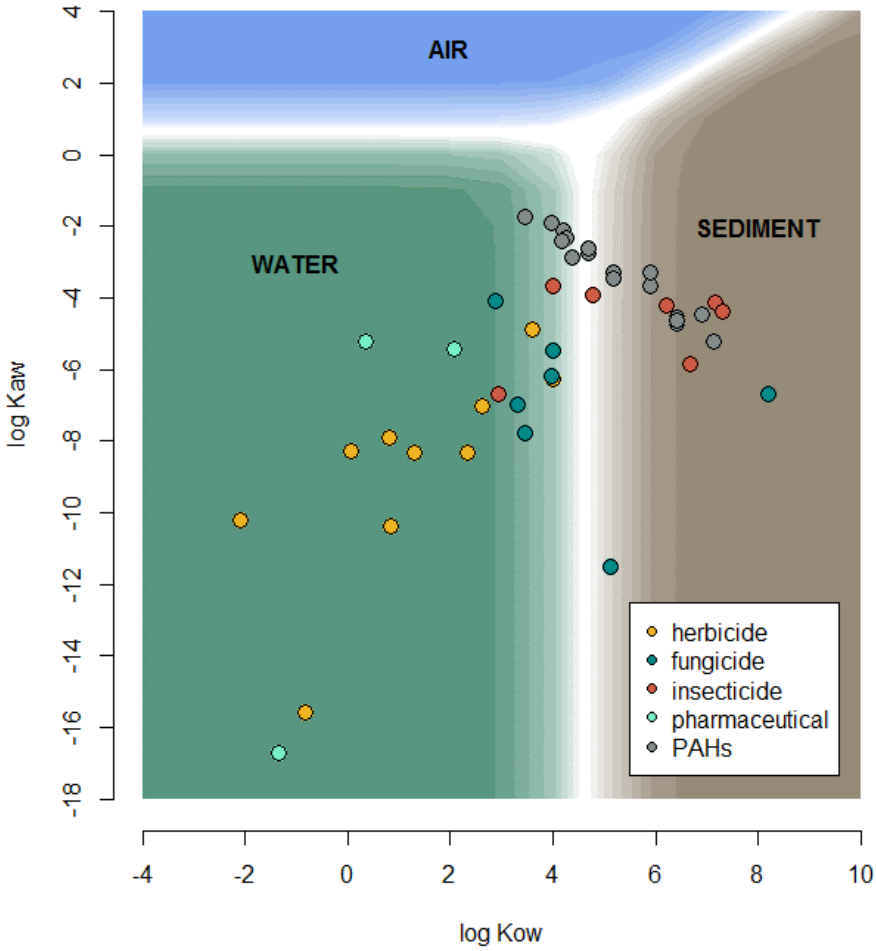

Figure 1: Partitioning of a small subset of chemicals between environmental compartments

The movement of a chemical through environmental compartments can be a determining factor when considering the effects of a mixture in the environment, as the combinations of chemicals may be very different moving from one phase to another based on their physico-chemical properties. This is an important factor to be considered when planning (higher tier) experiments to determine mixture effects.

The modelling approach illustrated here is extremely simplistic and does not consider any dynamic or spatial aspects. However, it does demonstrate how we can start to predict which chemicals are likely to be available in the environment for organisms to take up, and which chemicals are likely to coincide in specific environmental compartments (sediment, aquatic, soil, air). More sophisticated models are available that provide spatial and temporal information on how chemicals partition in the environment. These are rarely applied for chemical mixtures, however, due to the computational limitations and the complexity of the problem.

2.1.2 UVCBs

UVCBs are substances of Unknown, Variable composition, Complex reaction products and Biological materials. Given the complex and variable nature of such substances, it is not possible to define their physico-chemical properties with a single value and this has an impact on the ability to model their fate and hazard properties. One way to assess such substances is to group constituents within defined boundaries, which then may be integrated to compile whole substance information. One example of this approach has been applied to petroleum substances. Petroleum substances are complex hydrocarbon UVCBs and potential component numbers can range from 500 (naphtha) to many thousands (heavy fuel oils) (Quann et al., 1992). The approach developed to rationalise such substances is known as the Hydrocarbon Block Method (HBM), developed by Concawe, the European refining technical association (King et al., 1996).

The HBM divides the composition of a complex petroleum substance in blocks, grouped by carbon number in a matrix of hydrocarbon classes (e.g. normal and isoparaffins, napthenics, aromatics). The constituents' physico-chemical, fate and hazard properties are defined using several QSARs (Quantitative Structure-Activity Relationships) developed for this purpose (e.g. Meylan et al., 2005; Howard et al., 2005). Estimates are available for water solubility, Henry's Law Constant, log K_{ow}, log K_{oc} and half-lives in different media. An example of the application of the HBM in ecological risk assessment is given in a case study looking at gasoline hydrocarbons (MacLeod et al., 2004).

To apply relevant blocks to petroleum substances, detailed analytical characterisation is required. The mixture studies presented in Section 3.1.1 of this report relate to substances with compositions covered by the HBM; however, most studies have limited analytical data, making it difficult to interpret the observed effects and use the data for further modelling.

2.1.3 Chemical space analysis: Toxicity

Where available, acute toxicity data were extracted for the sample chemicals presented in Section 2.1.1 above for *Daphnia magna* (48h) and algae (72h). Results are summarised in the plot below (Figure 2). Whilst there are differences in the endpoints (in that *Daphnia* is immobilisation, a surrogate for mortality, and the algal endpoint is based on growth) as expected insecticides exhibit significantly more toxicity towards *D. magna*. The trend was less clear for the herbicides, but in general these chemicals are slightly more toxic towards algae with some exceptions. With few exceptions, the fungicides appear to affect both *Daphnia* and algae to a similar degree, while the PAHs exhibited slightly higher toxicity towards algae.

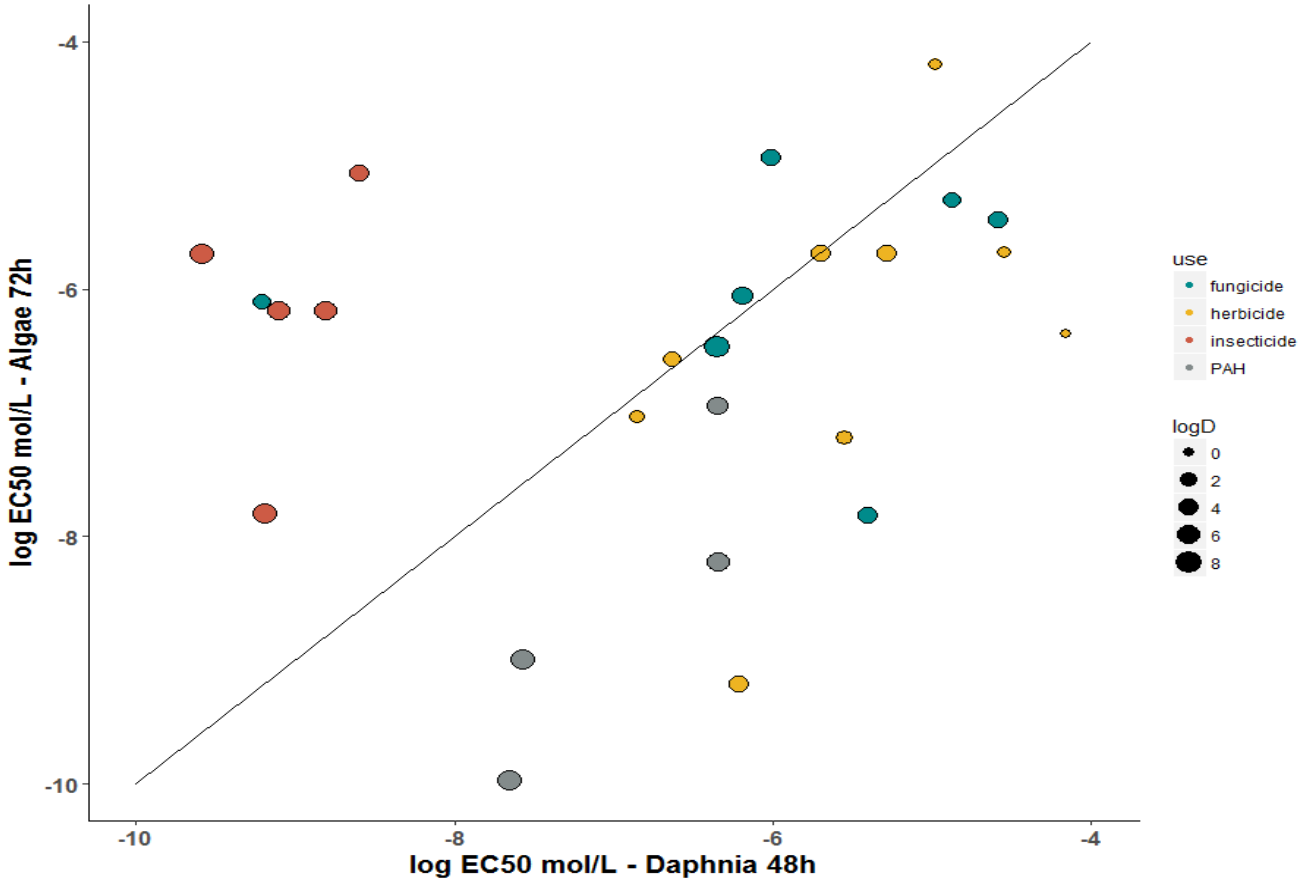

Figure 2: Acute toxicity of a small subset of chemicals to Daphnia and algae

Understanding that different chemical classes affect organisms in different ways is crucial to extrapolating effects from an individual to a community. One approach to classify chemicals and their potential for effects is by mode-of-action.

2.1.4 Short introduction to MoA classes

Some of the first work in classifying organic chemicals by mode-of-action was carried out by Verhaar et al. (1992, 2000). In this early work, four basic chemical classes were identified:

Class I *Inert chemicals.* Chemicals that are not reactive, and that do not interact with specific receptors within an organism.

Class II *Less inert chemicals*. Chemicals that are not reactive but are slightly more toxic than baseline toxicity due to hydrogen bond donor acidity.

Class III *Reactive chemicals*. Chemicals that react unselectively with biomolecules, or substances that are bioactivated via metabolism.

Class IV *Specifically acting chemicals*. Chemicals that interact with receptor biomolecules.

Narcosis spans over class I and class II, where class I is baseline activity (also known as non-polar narcosis). Baseline activity is the minimum level of toxicity that a chemical can exert and is generally thought to be caused by reversible membrane interactions. This mode-of-action is known as baseline toxicity because every

compound will exert this effect if the concentration is high enough. Class II is less inert chemicals (also known as polar narcosis). These compounds are slightly more toxic than class I compounds, but still thought to act through narcosis. Many compounds will also have other more specific modes of action that cause toxicity at much lower concentrations than narcotics. These are class III and class IV chemicals – reactive and specifically acting chemicals. In environmental risk assessment of chemicals, the concern will typically be higher for chemicals of classes III and IV – of course depending on the use and exposure patterns. For this reason, it is a useful tool to classify chemicals by mode-of-action to identify potential chemicals of emerging concern.

The conventional classification schemes proposed by Verhaar et al. (1992) and Russom et al. (1997) provide a low resolution that may not be enough to account for mixture effects in specifically acting chemicals. More recent work by Barron et al. (2015) provides a more detailed database of MoA classification for a selection of 1213 chemicals with respect to acute toxicity to fish and aquatic invertebrates. The chemicals encompassed six broad MoAs (narcosis, neurotoxicity, AChE inhibition, iono/osmoregulatory/circulatory impairment, reactivity, and electron transport inhibition), which were further divided into 31 more specific MoAs based on either chemical structure or known mechanism of action. Non-polar narcosis was the most common MoA (347 of 1213), followed by acetylcholinesterase inhibition (organophosphate and carbamate insecticides 211 and 74 of the 1213, respectively) and neurotoxicity (pyrethroid, neonicotinoid, GABA antagonist, agonist and other insecticidal MoAs, a total of 200 of the 1213), reflecting a bias, common in many ecotoxicology databases towards pesticides, particularly insecticides. One uncertainty highlighted by Barron et al. (2015) as an area for future research, which is particularly relevant for specifically acting compounds, is the applicability of the same MoA across fish and the wide diversity of aquatic invertebrates. Rather than starting with toxicity databases, Busch et al. (2016) looked to assign MoA to 970 chemicals compiled from survey data in EU rivers. They focused on specifically active chemicals and could assign a molecular target to 47% of the compounds which they categorised into 31 mode of action categories. Of these compounds, neuroactive chemicals were again the most common MoA comprising 18% of those with an assigned MoA.

2.2 Environmental emission and exposure aspects

Although the focus of this Task Force work is hazard and risk assessment, there needs to be due consideration of exposure. In risk assessment, whilst the magnitude of exposure is often the only parameter considered, certainly in initial lower tier assessments, spatial and temporal scales are also important. It is not possible to characterise the whole complexity of exposure. However, there have been efforts to bring consideration of these exposure aspects into mixture assessment, examples of which are described below.

The EU 7th framework project 'Solutions for present and future emerging pollutants in land and water resources management (SOLUTIONS)' has developed concepts and tools for the impact and risk assessment of complex mixtures of emerging pollutants (Altenburger et al., 2015). Different research lines of the SOLUTIONS project are leading to innovative approaches. A component-based pathway is based on a European-wide modelling approach ('model train') that includes emission estimation, fate and transport modelling in aquatic systems (Lindim et al., 2016) and consecutive risk modelling. Currently, about 2000 organic pollutants from all areas of use (industrial, pharmaceutical, household, pesticides etc.) can be processed in the model train, where risk is estimated in a classical component-based mixture risk assessment. The challenge is how to consistently deal with the data gaps for emissions and toxicity thresholds. Preliminary results corroborate earlier findings

about the pareto principle, meaning that the risk of a complex mixture can be described by only a few chemicals. However, the identity of the few chemicals changes across locations and for different endpoints (biological groups). Another research line in the SOLUTIONS project investigates whole mixture testing and analysis, providing an alternative to component-based approaches. Effect-based tools and effect directed analysis focus on the detection of toxic equivalents of environmental samples containing mixtures without the *a priori* analysis of the components. Only the most toxic compounds in a mixture are being elucidated by target- and non-target chemical analyses (e.g. König et al., 2017). These methods have been further developed and are currently under discussion for being used in future water quality assessment (Altenburger et al., 2018; Neale et al., 2017; Escher et al., 2018).

In 2015, SETAC (Society of Environmental Toxicology and Chemistry) held a workshop "Simplifying environmental mixtures - An aquatic exposure-based approach via exposure scenarios". The premise is that although mixtures could be complex, specific land uses likely result in exposures to typical sets of chemicals and exposure patterns. A series of papers were published looking at three different scenarios, agriculture (Holmes et al., 2018), domestic wastewater (Diamond et al., 2018), urban run-off (De Zwart et al., 2018) and a scenario combining all three (Posthuma et al., 2018). The focus was on exposure, with the effects-based assessments relying on the default assumptions of concentration addition across all levels of biological organisation, although it mentioned that further effects refinement based on MoA would be a higher tier option.

2.3 Toxicity testing, terminology and data availability

The quantitative basis for any kind of effect modelling is acute and chronic toxicity data. For the registration of chemicals, depending on the type of compound, different legal frameworks and data requirements apply, resulting in differences in the toxicity data available. For industrial chemicals and household and personal care products in the EU, the expected market volume and physico-chemical properties/fate properties, i.e. persistence and bioaccumulation potential, determines initially whether, and subsequently, which, toxicity data are required (REACH Regulation, EC 2006, http://www.prc.cnrs.fr/reach/en/data_requirements.html). Pharmaceuticals undergo a basic Ecological Risk Assessment, initially based on an exposure assessment which can trigger the need for some standard species toxicity data (EMA, 2006). As chemicals which are introduced into the environment with the express purpose of producing a biological effect, pesticides have much more demanding data requirements, with base set data requirements for acute and chronic toxicity to standard organisms, i.e. for the aquatic environment algae and aquatic plants, aquatic invertebrates and fish. Often additional data on both effects and exposure are developed to support higher tier risk assessment refinements (EFSA, 2013).

The lack of effects data is often a limiting factor in the risk assessment and one way to address this is through QSAR (Quantitative Structure Activity Relationship) approaches, to extrapolate to untested chemicals in any mixture. This was first investigated for MoA class 1, narcotics, by Van Leeuwen et al. (1991), who predicted ecosystem no-effect levels (NELs) based on species sensitivity distributions (see Section 3.3.2.2) for narcotic chemicals based on only the octanol-water partition coefficient and molecular weight.

Aquatic toxicity tests done for chemical registration usually follow standard guidelines, typically those of the OECD. Quantitative endpoints such as LC_x or EC_x (e.g. LC_{50}, EC_{50} or EC_{10}) values are statistical descriptors of dose-response relationships. Semi-quantitative endpoints such as NOEC or LOEC values are concentrations that have been tested, but related effect levels are not generally quantitatively defined, i.e. referring to a specific effect level and they depend on experiment-specific factors such as the concentrations tested and variability in control treatments (Crane and Newman, 2000).

These ecotoxicological test datasets from regulatory studies, together with other data from publicly available literature, are the basis for any mixture effects modelling. Databases exist that aim at providing a collection of all such data, e.g., the Ecotox database of the US EPA (United States Environmental Protection Agency; https://cfpub.epa.gov/ecotox/). Despite these values having utility for environmental risk assessment, many of the reported endpoints cannot be used for quantitative modelling of the toxicity of mixtures and effects on communities as they are not quantitative in terms of the effect level. For example, NOEC values are semi-quantitative only, with no descriptor of what % of effect constitutes a NOEC. Most relevant with respect to quantitative modelling are toxicity values which report LC_x for mortality and EC_x for reproduction or growth. The latter group does, however, only contain a small number of toxicity data, most probably because for sublethal effects semi-quantitative NOEC/LOEC values are generally required for regulatory purposes, although, certainly in Europe and for pesticides EC_x values are preferred for these sublethal endpoints (EFSA, 2013).

Because the databases are generally built from regulatory or other laboratory studies following standard test guidelines, the records are dominated by particular test species. Thus, the dimensions of both chemical and species diversity, which can be used to determine mixture effects on communities, can be a seriously limiting element for prospective risk assessment for both single chemicals and mixtures. The limited availability of species-specific chemical toxicity information is further complicated by the lack of availability of ecological information (i.e. traits, see e.g. Baird et al., 2008; Rubach et al., 2012) which is necessary to identify the most vulnerable organisms for risk assessment (Rico et al., 2015) or to parameterise the biological parts of ecological models used for predictions of toxic effects (see Sections 3.3 and 3.4). Some species traits information is available, see for example freshwaterecology.info database (https://www.freshwaterecology.info/index.php), where there are autecological characteristics, ecological preferences and biological traits as well as distribution patterns of more than 20 000 European freshwater organisms belonging to fish, macro-invertebrates, macrophytes, diatoms and phytoplankton.

One issue highlighted in the agricultural scenario from the SETAC workshop “Simplifying environmental mixtures - An aquatic exposure-based approach via exposure scenarios”, but applicable to other situations, is the difference between relatively data rich chemicals, such as plant protection products and those with little ecotoxicology data. Both data rich and poor substances use extrapolation to reach levels at which no community level effects would be expected, but with very different assessments factors to cover the uncertainties. This is always likely to be an issue in mixture risk assessment, with very few chemicals likely to have data beyond some simple acute toxicity data, emphasising the importance of extrapolation and read-across such as that based on MoA.

2.4 Methods for assessing mixture toxicity

There are two main approaches to mixture toxicity, one is the experimental, whole mixture approach, whereby the toxicity is determined in appropriate aquatic toxicity studies. The other is a theoretical, component-based approach, which calculates the toxicity based on the toxicity of the component parts. These approaches have certain advantages and disadvantages

Whole mixture toxicity testing:

- Is suitable for all mixtures of known and unknown composition (described as the "gold standard" by the German Environment Agency (Altenburger et al., 2013))
- Is required for certain substances, pesticides/biocides and other products for risk assessment and classification and labelling
- Considers any interactions (synergism or antagonism) or unknown components
- Doesn't provide information on specific components – but can help identify "drivers"
- Requires experimentation, often accompanied by extensive analytical characterisation to interpret results for use in predictive assessments
- Cannot be used for assessment of mixtures whose composition changes – e.g. due to differential fate and transport

Whereas the component-based approach:

- Is only suitable for mixtures of known composition e.g. products/formulations, effluents
- Requires knowledge of, or assumptions about any interactions (e.g. MoA) and may miss synergism or antagonism
- Requires toxicity information on specific components
- Possibly requires additional testing – although generally not an issue for plant protection products (PPPs)
- Can introduce additional conservatism in effects assessment
- Can be used to evaluate toxicity as composition changes

Both approaches are used in determining mixture toxicity and to some extent the approach applied depends on the type of mixture being assessed. Broadly speaking, environmental mixtures can be categorised into two groups. Firstly, there are "intentional mixtures" which are usually of known composition, for example chemical formulations of two or more substances in specific proportions such as PPPs, biocides, pharmaceuticals, other consumer products, the risks from which are often assessed in the European Union (EU) and elsewhere. Other

examples include mixtures due to the concurrent release in time and/or and space, e.g. effluents, PPP tank mixes, of which there is some regulation. There are also "unintentional mixtures" which may either be of known or unknown composition and which are not generally risk assessed prospectively, although some retrospective assessment may be possible. This can include PPP mixtures resulting from spray programmes at a landscape or watershed level, household and personal care products, pharmaceuticals and biocides released from sewage treatment plants or indeed complex environmental mixtures of different chemicals from both anthropogenic and natural sources.

The component-based approach uses chemical mixture toxicity theory, first outlined by Bliss in 1939 (Bliss, 1939). These concepts were further refined in 1952 by Plackett and Hewlett (Plackett and Hewlett, 1952). The table below from the ECETOC Technical Report No. 111 (ECETOC, 2011) describes the different interactions that can occur between chemicals in mixtures.

Table 1. The four types of joint action for chemical mixtures

	Similar joint action	**Dissimilar joint action**
Non-interactive	A. Simple similar • Concentration addition • Simple addition	B. Independent • Response addition
Interactive	C. Complex Similar • More than additive (synergistic) • Less than additive (antagonistic)	D. Dependent • More than additive (synergistic) • Less than additive (antagonistic)

Where:
Interactive: one substance influences the biological activity of the other substance
Non-interactive: no one substance influences the biological activity of the other substances
Similar joint action: same site of primary toxic action
Dissimilar joint action: different site of primary toxic action
Synergistic: toxic effect more than additive for two or more substances
Antagonistic: less toxicity observed than for the sum of the individual toxicities

The limitations of this classical approach to mixture toxicity include:

- The distinct joint action classes cannot account for several separate overlapping processes
- There is an underlying assumption that a chemical only has one target site
- The assigning of a chemical to a joint action class requires understanding of MoA which is not always available.

The first research into mixture toxicity was mainly concerned with the potential for synergistic action, but very few examples have been demonstrated over the years. Because synergistic interactions are rare, and because antagonistic effects are of little concern with regards to risk, the general recommendation for risk assessment is that the non-interactive models of Concentration Addition (CA) and Independent Action (IA) be used for assessing the risk associated with environmental stressors. In this respect, mode-of-action (MoA) information

is essential. MoA information helps to group chemicals with assumed additive effects and to separate between different MoA groups which are supposed to act independently from each other. MoA information is not suited to identify synergistic effects on the individual level, for that more mechanistic knowledge (e.g. based on AOPs or experimental evidence) is needed. However, having information about the MoA classes for a selection of chemicals allows to account for the potential additive impact of the mixture.

Concentration addition assumes chemicals act in a similar manner, differing only in their potency, and thus all chemicals, regardless of their concentration, will contribute to the overall mixture toxicity. IA on the other hand, assumes that chemicals have different MoAs and those present below the levels where they have an individual effect will not contribute to the overall mixture toxicity.

In many cases MoA is not known for all components of an environmental mixture. For this reason, and because the difference between IA and CA is often negligible in the context of the overall uncertainty, CA is considered to be appropriate as a conservative approach for environmental risk assessment (ECETOC, 2011).

The majority of published studies looking at the aquatic ecotoxicity of mixtures are single species laboratory tests. Because CA is considered to be a reasonable worst case and is used in environmental risk assessment, this is generally the model considered when assessing the results of these studies. For example, Warne (2003) in a review of reviews of mixture toxicity that "*examined the toxicity of 973 predominantly binary, tertiary and quaternary mixtures*" concluded that "*This analysis revealed that the median toxicity of the mixtures was essentially concentration additive, that between 75 and 80% of the mixtures were concentration additive, and 20-25% were antagonistic or synergistic*". However, as stated, the difference between CA and IA would generally be small and the correct conclusion should generally be that the results of the study do not deviate from additivity, rather than an affirmation of concentration addition.

3. ECOLOGICAL COMPLEXITY – EXPERIMENTAL AND MODELLING APPROACHES

The Terms of Reference for this ECETOC Task Force included reviewing the available literature for extrapolation of effects to communities, addressing both indirect and direct effects. This can include both experimental and modelling approaches at and across different levels of biological organisation. Historically, and indeed currently, one of the ways of looking at these direct and indirect effects on communities is experimentally, through community level studies, in model ecosystems known as microcosms or mesocosms. Although these studies have applications across chemical sectors, including petrochemicals and pharmaceuticals, these studies have been used in pesticide registration since the 1980s. There is no strict definition defining microcosms or mesocosms, as might be expected, based on size or volume, and the terms are often used interchangeably. These experimental systems are typically static ranging in volume from < 1 m^3 to > 100 m^3, although flowing systems to simulate streams have sometimes been used. As well as experimental studies, there are also some monitoring studies in the literature which look at the effects of mixtures on communities following hydrocarbon (oil) spills.

Experimental and monitoring approaches are limited in that they only investigate specific sets of mixtures under a certain set of environmental conditions, and as stated previously, the potential permutations of chemicals and conditions are almost limitless. Modelling approaches across different levels of biological organisation, from individual to population, community and ecosystem level can help address this and again perhaps simplify some of the complexity.

3.1 Existing community effect studies for mixtures

3.1.1 MoA class I (narcotics)

Mode-of-action class I refers to baseline toxicity or non-polar narcosis according to the classification scheme introduced by Verhaar et al. (1992) (Section 2.1.4). Effects on organisms exposed to substances with MoA class 1 are generally considered to be related to non-specific disturbance of membranes (Escher & Hermens, 2002). Ecotoxicity tests with mixtures of non-polar narcotics reveal that effects follow a concentration addition paradigm, where total toxicity may be predicted from knowing the concentration and toxicity of individual components (e.g. Hermens et al., 1984). In order to see if the knowledge of how non-polar narcotics act as mixtures can refine the risk assessment of exposed aquatic communities, case studies reporting such exposures sought in the literature have been reviewed and are summarised here.

After reviewing the literature for case studies where communities were exposed to mixtures of substances with MoA class I, it became apparent that studies were based on UVCBs, generally derived from crude oil or petroleum related activity, rather than mixtures of individual, well defined components. This can be considered to be a limitation to our quest to refine risk assessment as effects could not be linked to known concentrations of specific substances. For example, effects on benthic communities following crude oil or fuel oil spills were the most abundant studies of this type (e.g. Widbom et al., 1994; Gomez Gesteira and Dauvin, 2005; Danavaro

et al., 1995; Peterson et al., 2003). Crude oil contains an indefinable number of hydrocarbon components, present in an almost infinite number of combinations of mixtures. Chemical analysis ranged from 'oil in sediment' to generic mixtures of polycyclic aromatic hydrocarbons (PAHs). Also, the very many individual components of crude oil have different intrinsic toxicities and fate properties so that organisms are exposed to variable compositions of toxicants over time. For example, initial exposure to monocyclic aromatic hydrocarbons is important for eliciting acute toxicity effects; however, these volatilise rapidly, and 3-5 ring PAHs become the dominant toxic species in weathered crude oil (Neff et al., 2000). This change in composition over time further complicates the integration of exposure and effects for the refinement of models.

Given the lack of specific exposure information, this summary focuses on the qualitative biological effects concluded from sampling programmes conducted in the field (post crude and fuel oil spills and contamination from oil sand activity), mesocosms (artificial stream and tundra ponds) or laboratory-based studies with natural populations (crude oil, with and without dispersant).

Beginning with post-spill case studies, Widbom et al. (1994) found that ostracod and ampelisca amphipods populations decreased at the site of a spill of no 2-fuel oil in Narragansett Bay. It was not known if the decrease was due to drift or mortality. *Gammarus* sp. did not show signs of sensitivity to the contamination. A reduction in numbers of amphipods was also noted at a site in Spain, contaminated with crude oil spilt from the tanker, the Aegean Sea (Gomez Gesteira and Dauvin, 2005). PAHs were considered to be the components causing most of the observed biological effects. Meiofauna populations were investigated in samples taken from subtidal sediments from an area affected by crude oil spilt from the Agip Abruzzo (Danavaro et al., 1995). There were clear decreases noted in nematode, tubellarian and foraminiferan densities, with the most notable effects seen in non-selective deposit feeding nematodes. Conversely, the number of epibenthic copepods slightly increased as these were able to escape from the hydrocarbon contamination. Long-term effects of crude oil from the Exxon Valdez, on larger scale communities (from algae to shorebirds) were reviewed by Peterson et al. 2003. The review highlighted the need for ecosystem-based toxicology to understand and ultimately predict chronic, delayed and indirect long-term risks and impacts rather than treating each species separately and restricting assessment to acute, short-term impacts.

Examples of controlled exposures of natural planktonic populations to hydrocarbons have also been considered. Federle et al. (1979) exposed natural tundra thaw ponds to Prudoe crude oil and followed the effects on primary production and zooplankton. Primary production and biomass in treated ponds decreased after treatment but recovered; however, the structure of the algal community changed. Oil toxicity to zooplankton resulted in a loss of grazing pressure which caused the elimination of the cryptophyte *Rhodomonas* sp.; chrysophyte species became dominant. Two studies to assess the impact of the oil dispersant, Corexit 9500A were conducted in the laboratory and field, exposing natural populations to dispersed oil (chemically enhanced water accommodated fraction - CEWAF), water accommodated fractions (WAFs) and emulsified oil (Ozhan and Bargu, 2014 and Almeda et al., 2013). The use of dispersant was found to enhance the toxicity of hydrocarbons, presumably through increased hydrocarbon bioavailability. Interestingly, the presence of protozoans in contaminated water reduced the toxic effects of crude oil and bioaccumulation of PAHs in copepods.

An assessment of hydrocarbon toxicity on planktonic and benthic communities was made by dosing artificial streams with refinery effluent (Concawe, 2015). Streams were dosed with raw effluent and with effluent

fortified with kerosene and diesel to ensure that hydrocarbon concentrations were relatively constant and sufficiently high to induce observable effects on the biota. Raw effluent did not impact primary production nor the benthic community; fortified effluents affected benthic communities due to higher hydrocarbon availability. Effects disappeared after 30 days of recovery. The effect of another hydrocarbon mixture (petroleum middle distillate - PMD) on freshwater communities was studies in both stream and pond mesocosms, applied at nominally 0.01, 0.4, 2 and 20 mg/L. Whilst the stream mesocosms were treated with the PMD continuously injected as a Hydrocarbon Emulsion (HE), only the soluble part of PMD, the Hydrocarbon Water Accommodated Fraction (HWAF), was used to treat the ponds to avoid hypoxia due to aerobic biodegradation of the hydrocarbons. Streams were continuously dosed for 3 weeks followed by a 2-month post treatment period and ponds were treated weekly for 4 weeks followed by a 10 month post-treatment period. Effects were studied on macroinvertebrate community structure (Bayona et al., 2015a), the biological and ecological trait responses and leaf litter breakdown (Bayona et al., 2015b), secondary production of zooplankton communities (ponds only, Bayona et al., 2014a) and the structure and biological trait responses of diatom assemblages (Bayona et al., 2104b). There were some responses of the communities at the higher concentrations of 2 and 20 mg/L and evidence for both direct and indirect effects. Although overall responses were often similar, the study showed that there were differences between the pond and stream systems both during treatment and in recovery from any effects. For example, the abundance and biomass of Tanypodinae larvae were significantly reduced in HE-exposed streams whereas they were higher in HWAF-treated ponds as compared to the controls, suggesting some responses were system specific.

Finally, one other case study reviewed, assessed effect of contamination from Canadian oil sand operations on freshwater invertebrate communities (Gerner et al., 2017). The main, bitumen-derived contaminants were PAHs, naphthenic acids and metals and as such included substances with polar and non-polar narcotic mode-of-action as well as specific mode-of-action. However, this reference has been included as it was found that effects on communities were related to levels of PAH contamination, and therefore relevant to the discussion on effects of non-polar narcotic substances on communities. Community effects were evaluated using a trait-based approach to identify bioindicator species (Species at risk approach (SPEAR), Liess and von der Ohe, 2005). Alteration in terms of increased physiological sensitivity and decreased generation time were found at levels of PAH contamination 100 times below the acute sensitivity of *Daphnia magna*.

Summarising, the studies reviewed show complex direct and indirect effects of crude oil hydrocarbon mixtures on aquatic communities. Further analysis of these datasets, however, is hampered by a lack of detailed exposure characterisation. This is a common issue when performing studies for complex substances as constituents have different physico-chemical properties and linking exposure to effects is not evident.

3.1.2 Specific MoA: pesticides and pharmaceuticals

Chemicals with a specific mode-of-action, MoA class 4 (Verhaar et al., 1992), are a diverse group of chemicals targeting specific molecules or receptors. This covers pesticides, biocides and pharmaceuticals (human and veterinary). These are all chemicals selected or designed specifically for biological effects and consequently can have extremely high levels of biological activity. Not surprisingly, this activity along with the potential for environmental exposure mean that these chemicals are amongst the most heavily regulated with respect to their environmental toxicology and potential risk. Whilst they have a MoA which has high biological activity,

it is not necessarily this specific MoA which is relevant for environmental risk assessment. The MoA for the environmental receptors may be different to the specific MoA targeted. This is likely to be true particularly for pharmaceuticals, whereas for pesticides the ecological receptors which drive the risk assessment are more likely to be related to the target.

Pesticides are often introduced directly into the environment through broadcast applications covering large areas, which may result in diffuse entry into aquatic environments through spray-drift, run-off and drainage. Human pharmaceuticals are generally administered as a dose orally, intravenously or perhaps through dermal applications and, as such, generally enter the environment via down-the-drain where the primary pathway to the aquatic environment is via sewage treatment plants (STPs). Veterinary pharmaceuticals, particularly those with farm use may enter the environment more directly though excretion and may even be broadcast, through the use of animal excreta as fertiliser. Biocides can be introduced directly into the environment such as when used as wood treatments or anti-fouling paints, and also have down-the-drain entry, for example when used as household disinfectants.

As mentioned earlier these chemicals are highly regulated with both standard data requirements and risk assessments. These generally follow a tiered procedure using assessment factors to cover such things as differences in sensitivity between and within species, test to test variation and laboratory to field extrapolation, which may potentially include the potential for effects in combination with other chemicals. However, the assessment process focuses mainly on individual active chemical substances, with some consideration of the potential for effects from mixtures if different active substances are combined in a single product.

There is clearly the potential for combined exposure to these chemicals and if there is combined exposure, then the potential for combined effects. Given the specific mode-of-action, this group of chemicals has the potential for the full range of potential mixture toxicity outcomes: antagonism, response addition, concentration addition and synergism. Indeed, synergism from combinations of drugs is exploited in pharmaceutical treatments where the mechanism of action is well understood, and the dose can be controlled with respect to both magnitude and timing (Jia et al., 2009). However, in contrast, the environmental toxicology of pharmaceuticals is poorly understood with respect to the mechanism of toxicity to non-target species and the potential outcome from exposure to mixtures. In a review, Backhaus (2014) concluded that with respect to mixture toxicity of pharmaceuticals, response and concentration addition provided a "*robust scientific footing*", but there were knowledge gaps which required investigation through study in environmentally realistic situations. Similarly, with respect to plant protection products, all potential mixture toxicity outcomes are possible. Synergism can occur but is rarely exploited in the same way as with pharmaceutical treatments, presumably due to the inability to control specific dose levels and timing. However, there are some examples with commercial applications, including insecticide synergists such as PBO (piperonyl butoxide) which inhibits the insect P450 cytochrome system preventing the breakdown of certain insecticides. However, the overall conclusion is that, with the exception of some well documented examples where the underlying biochemical mechanism is understood, additivity is again a reasonable worst-case for environmental risk assessment (Cedergreen, 2014).

The focus with these chemicals has been on the effects of mixture toxicity in laboratory studies and there have been few studies concerned with evaluating community level effects, partly because of the size, complexity

and resources required for such investigations. Richards et al. (2004) studied the effects of a mixture of three frequently prescribed pharmaceuticals at their 95th, 99th and 99.9th percentiles of their monitored concentrations in North American surface waters, using outdoor aquatic mesocosms as experimental systems. Some responses at the community level were observed particularly in the mid and highest-level treatments, but in the absence of studies on the responses from the individual pharmaceuticals, it is not possible to determine if there were any true mixture effects.

More studies have been conducted looking at community level effects from mixtures of pesticides, where, although regulated and assessed as single products, there has been concern for the potential for effects from spray programmes which include applications of herbicides, fungicides and insecticides in combination or in sequence. The potential for mixture effects is used to advocate a precautionary approach to risk assessment of pesticides in the EU, citing it as a reason against recovery arguments in regulatory risk assessment of single products (EFSA, 2013). This is in contradiction to the protection goals which does not allow unacceptable effects (EC, 2009), thus seemingly allowing some effects provided there is recovery.

Many of these studies have previously been summarised by Verbruggen and Van den Brink (2010). The first studies looked at binary mixtures of an herbicide with an insecticide: Hoagland et al. (1993) looked at the individual and combined effects of the herbicide atrazine and the insecticide bifenthrin; Fairchild et al. (1994) looked at the effects of the insecticide esfenvalerate, with and without atrazine. Both studies concluded that whilst the herbicide and insecticide caused effects on the plant and invertebrate communities, respectively, there were no synergisms, neither chemical or through indirect effects, for example effects by insecticidal effects on zooplankton amplified by effects of the herbicide on their algal food supply. Van den Brink et al. (2009) mixed the herbicide atrazine with lindane concluding the "*Effects could well be explained by the effects of the individual chemicals alone, no synergistic effects*".

A number of studies have looked at herbicide mixtures on the community level response. Carder and Hoagland (1998) looked at mixtures of alachlor and atrazine, which have different modes of action, and concluded that the results "*indicate atrazine and alachlor affect stream algal communities in an additive rather than synergistic manner*". Hartgers et al. (1998) mixed atrazine, metolachlor and diuron. Atrazine and diuron both act through blocking electron transport to photosystem II (PS II), whereas metolachlor (like alachlor) is thought to inhibit fatty acid synthesis, thereby inhibiting cell division and growth and increasing cell membrane permeability. Again, the conclusion was that the effects were as expected based on what was known of the individual compounds. Knauert et al. (2008, 2009) mixed atrazine, diuron and isoproturon, all PS II inhibitors at 1/3 of their respective HC30 values (the concentration below which only 30% of the species effect levels are predicted to occur) from a species sensitivity distribution (SSD) for each chemical. The conclusion was that concentration addition described the results, as the effect from the mixture gave very similar effects to the individual compounds at their HC30s. Sura et al. (2012) looked at the effects of six auxin inhibitors (2,4-D, MCPA, clopyralid, dicamba, dichloroprop and mecoprop) on wetland microbial communities using mesocosms. In addition, the six auxin inhibitors were mixed with bromoxynil and glyphosate. The authors concluded the effects of the mixture of the six auxin inhibitors, which showed effects when mixed together at concentrations below water quality guidelines, were explained by concentration addition.

Cuppen et al. (2002) and Van den Brink et al. (2002) reported the effects of a mixture of two insecticides, lindane and chlorpyrifos, and concluded "*The observed effects could be explained from the individual toxicity*

of the insecticides to the invertebrates" and "*Principles for individual compounds ensure protection against chronic exposure of a mixture of insecticides at community level although not always at sp. level"*.

Rather than look at incidental mixtures of pesticides which may or may not occur together, some mesocosm studies have looked at effects at modelled environmental exposures from typical relatively intensive spray programmes on different crops. Arts et al. (2006) looked at the spray program for potatoes in the Netherlands where there was a single application of the herbicide prosulfocarb, followed by a single application of another herbicide metribuzin, then two applications of the insecticide lambda-cyhalothrin and four and eight applications, respectively, of the fungicides chlorothalonil and fluazinam. The authors concluded that "*This suggests that risk assessments based on the individual compounds would in this case have been sufficiently protective for their uses in a crop protection program*" i.e. no mixture effects at the community level. Similar results were found with a spray program for tulips in the Netherlands (van Wijngaarden et al., 2004) and cereals in Northern France (Auber et al., 2011).

Thus, a number of studies with herbicides have shown that if the mode-of-action is the same, effects at a community level are consistent with, or do not deviate from, concentration addition and that effects from mixing together different MoAs can be explained from knowledge of the likely effects of the individual compounds. Synergy is difficult to predict and, without prior knowledge, considered a rare event (Cedergreen 2014). With pesticides, there are some well-documented examples of synergy, the most widely studied being the synergy between azole fungicides which inhibit cytochrome P450s and the pyrethroid insecticides. This synergy has been reported in different organisms in laboratory studies such as bees (Pilling and Jepson 1993) and *Daphnia* (Cedergreen, 2006). By comparing the effects of fenvalerate alone with the effects of fenvalerate mixed with prochloraz at 90 µg/L, Bjergagaer et al. (2011) reported synergism on populations of cladocerans and copepods, increasing toxicity by a factor of three to seven. At the time, it was reported as an "*environmentally realistic concentration*" of prochloraz, which was challenged by Weltje (2013) and in a subsequent publication Bjergager et al. (2017), reported concentrations of azoles up to 0.5 µg/L as being within "*the typically monitored range*". Nevertheless, irrespective of the environmental relevance, it indicates that synergism as found in the lab can be measured at the population and community level in field microcosm studies.

Halstead et al. (2014) investigated the use of community ecology theory as a framework to predict chemical mixture effects on biodiversity and ecosystem properties. They conducted freshwater mesocosm experiments with single chemicals and binary mixtures of a fertiliser, herbicide, insecticide and fungicide on species level and ecosystem level responses. The authors claim responses were predictable based on each functional group sensitivity to the chemical and direct effects, their reproductive (recovery), their interaction with other functional groups (indirect effects) and links to ecosystem properties. The prediction was that fertilisers would increase biomass of primary producers and consequently primary and secondary consumers, herbicides were predicted antagonistically to this, limiting primary production. By decreasing abundance of zooplankton and arthropod consumers, insecticides would be expected to produce positive indirect effects on non-arthropod herbivores and phytoplankton. As general biocides, a fungicide would be expected to produce direct negative effects across all trophic levels. They predicted changes in biodiversity, species richness or abundance would result in measurable changes to ecosystem properties e.g. pH and dissolved oxygen. To validate these predictions, mesocosms were treated at modelled environmental concentrations produced using the US EPA screening model GENEEC v2. Chlorothalonil, atrazine and malathion were treated at concentrations of 164,

102 and 102 µg/L respectively, much higher than typical actual environmental concentrations, but high enough to produce the effects to test the predictions, which the authors claim were validated. This prediction of different community responses to mixtures rather than single chemicals might seem to be contrary to many of the other studies where effects could be predicted on the basis of single chemicals. However, differences are that the chemicals were applied together rather than according to a spray calendar and furthermore concentrations were well above the worst-case modelled concentrations from the EU studies such as those of Van Wijngaarden et al. (2004), Arts et al. (2006) and Auber et al. (2011). Nevertheless, although perhaps not relevant for environmental risk assessment, it does show that these community level experimental systems can detect certain levels of effect from mixtures, should they occur.

Thus overall, whilst examples of synergism have been demonstrated, they are mainly restricted to laboratory studies. Reviewing the available evidence, Cedergreen (2014) concluded that true synergistic interactions between chemicals are rare and when they do occur, it is often at high concentrations i.e. not generally relevant for environmental exposures.

3.2 Relation of experimental community studies of mixture effects to modelling

Community studies of effects from chemical mixtures provide the basis for answering basic risk assessment questions, such as whether risk assessment of single compounds is protective for the mixture. Apart from that basic understanding, community level studies with chemical mixtures can also serve as the basis for testing community model simulations with empirical data. The overview of experimental data in Section 3.1 gives an impression of the number and setup of community studies which were found in the open literature. Despite the use of community level studies in aquatic ecotoxicology being well established for more than 30 years, there are still only a limited number looking at chemical mixtures and these are mostly testing mixtures within chemical sectors e.g. plant protection products or hydrocarbons, rather than mixtures across different sectors. From a regulatory point of view, this is almost certainly because regulation at these higher tiers is concerned with effects from single chemicals. Furthermore, these studies are typically performed in static systems, which although often a worst-case scenario, means results are not easily extrapolated to flowing ecosystems. An additional problem concerning the utility of such community study data for modelling is that population dynamics are rarely measured or reported. This is especially problematic, since most available community- and ecosystem-level models are dynamic (De Laender et al., 2015; Mondy & Schuwirth, 2017) and so validating their capacity to simulate population dynamics correctly will often not be possible. Instead, it is necessary to resort to testing predictions at a coarser level, e.g. time-averages of effect sizes (Spaak et al., 2017, De Laender et al., 2008a). With respect to the monitored taxa, effort is mostly invested in macroinvertebrates, zooplankton and phytoplankton. However, smaller plankton, e.g. ciliates and flagellates, and bacteria are generally not included, and this can pose problems in cases where the systems are fuelled by bacterial production instead of by primary production (De Laender et al., 2010). At the other end of the spectrum, vertebrates are also rarely included, for both ethical and logistical reasons, e.g. they are often not the most sensitive taxa and unlikely to be directly affected, yet can mask direct effects on other taxa. From a modelling perspective, this is less problematic because most available community- and ecosystem models in ecotoxicology focus on systems without longer-lived (vertebrate) organisms.

Whilst some of the listed mesocosm datasets appear relevant and interesting for model testing and validation, overall the number of studies is limited. Because most of the testing has been done within specific sectors of the chemical industry, mixtures of chemicals with specific and non-specific MoAs have not generally been tested experimentally for their effects on communities.

In this section we aim at adding the perspective of ecological modelling to the discussion. The simple idea that provides the basis for our considerations is to assess mixture effects on communities based on MoA by:

- dividing chemicals in a mixture into different MoA groups
- adding concentration-dependent effects of chemicals from one MoA group by using toxic units
- combining different MoA groups by independent action
- linking toxic MoA to effects on single species via dose-response relations or simple TKTD (toxicokinetic-toxicodynamic) models, as parameters are known from toxicity databases
- extrapolating to the community level by the use of multiple population models

In the following section, more details about the extrapolation are discussed.

Key in this approach is to distinguish between extrapolation from single compound to mixture and from single species to community. Single species – single compound (SSSC) toxicity data are available from databases such as the Ecotox database from US EPA (see Section 2.3). The approach to community level mixture toxicity would be to collect building blocks first, i.e. information on SSSC toxicity, and on ecological data, i.e. on the function of species in aquatic ecosystems. To keep also ecosystem function / community level in mind, there are two possible options: first to analyse effects on the taxonomic composition of ecosystems, second to analyse effects on the coarser functional structure of ecosystems. These approaches can probably also be combined with each other, e.g. by including questions for the taxonomic diversity or sensitivity distribution within distinct functional groups into the analyses.

3.3 Modelling approaches from individuals to communities

Unravelling how mixtures affect ecosystems must start with a basic understanding of how individual chemicals affect individual organisms. With this understanding in place we can then begin to look at mixtures of chemicals, and communities of species.

The effect of a chemical on an organism is determined by two key factors, namely the toxicokinetic and toxicodynamic. Toxicokinetic and Toxicodynamic models focus on the quantification of toxic effects at the individual level. In a first step Toxicokinetic (TK) and Physiologically Based Toxicokinetic (PBTK) models describe the time course of chemical uptake, internal distribution, elimination, biotransformation (i.e. what the organism does with the chemical). In contrast to this, Toxicodynamic (TD) models describe the time course of toxic action at the target site, physiological impairment of the organism as a result of the exposure as well as the influence of any compensating mechanisms and toxic effects of the chemical on the organism (i.e. what the chemical does to the organism) (Ashauer and Escher, 2010). Therefore, it follows that TKTD models

consider both kinetic and dynamic aspects of chemical uptake, distribution and effects in a given organism. TKTD models encompass a broad range of models that span the AOP (adverse outcome pathway) framework (see Figure 3 and Ankley et al., 2010), many of which are considered in the General Unified Threshold for Survival (GUTS) framework (Jager et al., 2011). GUTS was designed to unify a wide range of previously unrelated approaches, to clarify their underlying assumptions and to facilitate further improvement in modelling survival under chemical stress.

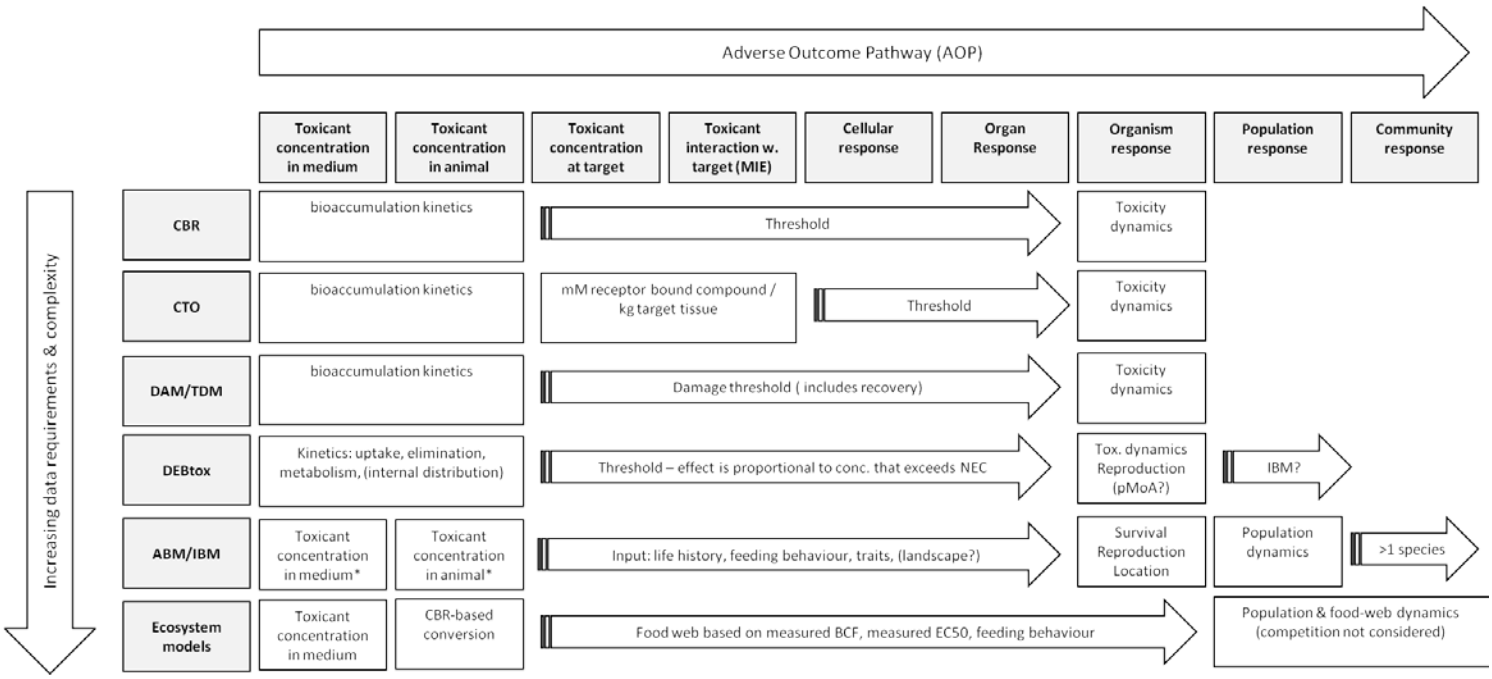

Figure 3: Overview of ecological models relevant to environmental risk assessment in the AOP framework [CBR = Critical Body Residue, CTO = Critical Target Occupation, DAM = Damage Assessment Model, TDM = Threshold Damage Model, DEB = Dynamic Energy Budget, ABM = Agent Based Model, IBM=Individual based model]

***ABM and IBM take very different approaches depending on the organism in question, therefore toxicant concentration in medium/organism is only considered in some applications.**

3.3.1 Modelling individual level effects

3.3.1.1 Critical Body Residue (CBR) and Critical Target Occupation (CTO)

The Critical Body Residue (CBR) approach provides a simple mechanistic link between exposure time and ecotoxicological effect by assuming that an organism dies when an internal threshold concentration has been exceeded (McCarty and Mackay, 1993). The concept is applicable to reversibly acting chemicals and has been particularly popular for predicting the toxicity of narcotically acting compounds (for which the internal threshold has been narrowed down to between 2-8 mmol/kg) (van Wezel et al., 1995). A recent review of the CBR approach by McCarty et al. (2013) found that a lack of information about metabolite toxicity, lipid content/composition, other modes of toxic action, and lack of steady-state status, can cause variability in CBR estimates of up to three orders of magnitude. As a result, the authors suggest that it may be necessary to design experiments specifically for CBR estimations. Using chemical activity to predict baseline narcosis was suggested by Mackay et al. (2009), where it was found that narcosis will occur at an activity exceeding

approximately 0.01. The activity approach is consistent with the CBR concept, but avoids the variability caused by differing lipid contents in organisms.

A limitation of the CBR approach is that it cannot be used when irreversibly acting chemicals react with specific receptors. The Critical Target Occupation (CTO) model was therefore developed as an extension of the CBR concept to address such compounds (Legierse et al., 1999). CTO assumes that mortality occurs when a critical number of targets are irreversibly occupied.

Using the CBR and CTO approach can reduce the variability in toxicity metrics by orders of magnitude (Ashauer and Escher, 2010).

3.3.1.2 Damage Assessment Model (DAM) and Threshold Damage Model (TDM)

The Damage Assessment Model (DAM) is an extension of the CBR concept that has been developed predominantly for agrochemicals. DAM takes recovery into account and assumes that death occurs when the cumulative damage reaches a critical point. Damage is assumed to accumulate in proportion to the accumulated residue and damage recovery in proportion to the cumulative damage when damage is reversible (Lee et al., 2002).

The Threshold Damage Model (TDM) is a similar model that unlike DAM (which is based on an individual tolerance distribution) is based on the concept of stochastic death to simulate survival following fluctuating or pulsed exposures (Ashauer et al., 2007).

The advantage of the DAM and TDM models over the simpler CBR and CBO models is that compounds that act reversibly can be modelled more realistically with intermediate recoveries rather than the extremes of CBR where instantaneous recovery is assumed, or CTO where interactions are irreversible.

3.3.1.3 Toxicokinetic (TK) models and Physiologically-Based Toxicokinetic (PBTK) models

It is important to consider toxicokinetics (uptake, distribution, biotransformation and elimination) in an organism as a chemical must enter an organism and reach a target site in order to elicit a toxic effect. Two groups of TK models generally exist: models based on a one-compartment assumption and those which follow a multi-compartment approach (e.g. PBTK). Whilst one-compartment models assume that the chemical concentration is the same throughout the organism, PBTK models allow for the determination of organ specific accumulation. This might be important to understand toxic pathways specific to target sites located in certain organs and for food chain bioaccumulation if predators preferentially consume particular organs.

A comparison of a PBTK model which predicts chemical concentrations in the whole fish as well as in various tissues, outperformed the one-compartment models with respect to simulating chemical concentrations in the whole body (Stadnicka et al., 2012). Whilst a PBTK model requires physiological data and is slightly more complicated to estimate whole body concentrations than a one-compartment model, comparative studies indicate that it is more accurate and, therefore, where data are available PBTK may be used in preference to TK models.

3.3.1.4 GUTS framework

The GUTS (General Unified Threshold model for Survival) model is an integration of existing TKTD (toxicokinetic-toxicodynamic) models e.g., critical body residue, critical target occupation, damage assessment, DEBtox survival, threshold distribution. The driver for building the model was to increase the application of TKTD models in ecotoxicological research as well as environmental risk assessment of chemicals. One of the main features of the GUTS framework is that it accounts for time-variable exposure to the stressors (Jager and Ashauer, 2018).

3.3.1.5 Excess toxicity approaches

Two of the most common approaches for identifying compounds that have excess toxicity relative to baseline toxicity are the toxic ratio approach and the chemical activity approach. The toxic ratio approach is based on QSAR predictions for baseline toxicants relative to the measured toxicity, while the activity approach is a thermodynamic approach based on chemical potentials.

Toxic Ratio

The toxic ratio (TR) approach is a simple ranking technique based on how much toxicity a chemical exerts relative to baseline toxicity (as predicted by Quantitative Structure-Activity Relationship models (QSARs)).

$$TR = \frac{LC50\ baseline\ QSAR}{LC50\ measured}$$

Verhaar et al. (2000) found that inert chemicals have TR values close to 1.0 (class 1: non polar narcosis) while less inert substances have TR ratios ranging from 5-10 (class 2: polar narcosis). Reactive (class 3) and specifically acting (class 4) chemicals have much higher TR ratios, ranging from 10 to 10000, but cannot be clearly distinguished from one another by the TR approach. Russom and co-workers found similar ranges in TR values for nonspecific and specific modes of action (Russom et al., 1997).

Activity based approaches

The concept of using chemical activity rather than concentrations in relation to toxicity was first suggested by Ferguson in his seminal paper from 1939 (Ferguson, 1939). Since then, several studies have discussed and tested the relationship between toxicity and chemical activity – particularly for baseline toxicants. Although the lethal activity may also depend on compound structure and properties, the use of activity reduces the window of uncertainty from several orders of magnitude (for concentration-based approaches) to a single order of magnitude (Mackay et al., 2011).

Similar to the TR approach the activity approach can be used to rank chemicals. This can be done either by plotting measured EC_{50} values against the solubility, or by calculating the activity directly:

$$activity = \frac{EC50\ (\frac{mol}{L})}{Solubility\ (\frac{mol}{L})}$$

3.3.1.6 Target Lipid Model (TLM) and its application for hazard assessment of UVCB petroleum substances

Though the exact mechanism of toxicity is not clear, substances that display narcosis as a mode-of-action are believed to act on the lipid of an organism. This assumption is based on the observation that critical body burdens increase linearly with the lipid concentration of the organism (van Wezel et al., 1995). The target lipid model is based on the relationship between the effect concentration (LC_{50}) and the octanol-water partition coefficient (K_{ow}) that is well established for substances that act via narcosis:

$$\log(\mathrm{LC50}) = \mathrm{m}\log(\mathrm{K_{OW}}) + \mathrm{b}$$

where m and b are the slope and intercept, respectively, of the regression line relating log LC_{50} and log K_{ow}.

Di Toro et al. (2000) found that a single slope describes the relationship for all species, while the y-intercept varies for each species. They rationalised this finding by suggesting that the slope describes a linear free energy relationship between target lipid (e.g., site of action) and octanol, which appears to be the same for all species in the database. The slope was denoted as the universal narcosis slope. The intercept of the regression was equal to the critical body burden. At the y-intercept, where log K_{ow} = 0, the concentration of chemical in octanol is equal to the concentration of chemical in exposure water. Since octanol is used as a surrogate for organism lipid, at the y-intercept the concentration of chemical in water is equal to the concentration of chemical in organism lipid. Di Toro et al. (2000) defined the y-intercept as the Critical Target Lipid Body Burden (CTLBB), with units of µmol/g octanol. The CTLBB is endpoint and test species-specific. If the toxicity data are expressed in terms of LC_{50} values, then the y-intercept is equivalent to the concentration in the organism lipid that will cause 50% mortality of test species (i.e., the LC_{50} expressed as an internal lipid normalised concentration or body burden). To convert the acute concentration to a chronic concentration an acute to chronic ratio (ACR) of 5.09, a geometric mean value derived from data for 6 species comprising a total of 20 chemicals, is applied. In the development of the TLM, the log LC_{50}-log K_{ow} relationship was evaluated for 33 aquatic species and more than 140 narcotic compounds (Di Toro and McGrath, 2000).

For petroleum hydrocarbons, the TLM is applied in the hazard assessment tool, PETROTOX. PETROTOX uses the concept of HBM (see section 2.1.2) and mimics the partitioning behaviour of complex petroleum substances in simulated Water Accommodated Fraction (WAF) experiments (Redman et al., 2012). The TLM is then used to estimate LC/EC_{50}s for all constituents, calculated to be present in the aquatic phase. The principle of concentration addition is assumed to combine constituent values to derive a measure of hazard for the whole petroleum substance, i.e. it uses the Toxic Unit concept to compute the loading at which a petroleum substance will exert toxicity for 50% of the population.

3.3.1.7 Summary: Modelling individual effects

As the above model descriptions indicate, modelling individual level effects and internal concentrations can be achieved using a suite of approaches. Most of these approaches rely heavily on the understanding of chemical fate in the organism and are rooted in toxicokinetics. The table below is a brief summary of the approaches, their advantages, disadvantages, and examples of implementation from the literature.

Table 2. Overview of individual effect models advantages, disadvantages and example:

	Advantage	Disadvantage	Applied examples
CBR and CTO	Potential for exploring the exposure-based toxicity metrics for solutions of multiple chemicals. Potential use as a screening tool for separating non-target, baseline toxicants from the specifically acting chemicals.	Does not consider lipid dynamics, intrinsic potency processes that alter toxicant potency, metabolites, phototoxicity, individual fitness, and species and life stage specific sensitivities.	CBR: Neutral Organics (McCarty et al,. 1992) (McCarty et al,. 2013) Pentochlorophenol (Hickie et al,. 1995) CTO: Organophosphorus Pesticides (Legierse et al., 1999)
DAM and TDM	Considers cumulative damage as a result of pulsed exposure (though this may not be relevant for steady-state emissions).	Does not consider lipid dynamics, intrinsic potency, processes that alter toxicant potency, metabolites, phototoxicity, organism health, and species and life-stage specific sensitivities. For accurate predictions the mode-of-action must be known.	DAM: PAHs in *Hyalella azteca* (Lee et al., 2002) TDM: Two pesticides in aquatic invertebrates (Ashauer et al., 2007)
TK / PBTK	TK and PBTK models both offer a way to simulate internal concentrations when predicting toxic effects in organisms. Determining where a chemical accumulates in the body enables a link to be made to specific MoA.	Most PBTK models are developed for fish species, additional models need to be parameterised for a range different species Biotransformation and metabolism can be difficult to estimate for different species.	Stadnicka et al., 2012 – Comparison of one-compartment and multi-compartment TK models in fish Nichols et al., 1990 – PBTK model for the uptake and deposition of waterborne organic chemicals in fish Gergs et al., 2015. Body size-dependent toxicokinetics and toxicodynamics could explain intra- and interspecies variability in sensitivity in aquatic invertebrates Ducrot et al., 2016. Used a TK-TD model to predict the time-course of fish survival for relevant FOCUS SW exposure scenarios.
GUTS	Takes time course data into account. Integrates different peer reviewed models. Has been ring tested.	Cannot take sub-lethal effects into account – limiting is use for modes of action that do not directly affect survival.	Dohmen et al., 2016 - The general unified threshold model for survival (GUTS) model was linked to 3 individual-based models (IBM) for aquatic invertebrates, translating individual survival of sensitive organisms into population-level effects

3.3.2 Modelling community level effects

The extrapolation of impacts of single chemicals to higher ecological complexity levels can be based on different models (Figure 4). On the one hand, detailed population models such as IBMs (individual based models) use information on traits that influence population dynamics and on chemical toxicity (e.g. Van den Brink et al., 2007; Baveco et al., 2014; Martin et al., 2014). Such models provide the advantage that they can account for lethal and sublethal effects at the same time, and are usually built on first principles, implying that they can predict population growth at various temperatures and resource availabilities. These population models can be combined into multi-species models that provide a high level of ecological complexity, accounting for the ecological interactions such as competition and predation (Viaene, 2016). In combination with the species level toxicity modelling, such multi-species approaches provide a very high level of ecological complexity and can be challenging in terms of parameter needs, computation times and analyses of simulation runs. However, if used in the right way they could give very realistic pictures of effects of single chemicals and their mixtures at levels of communities and food-webs.

On the other hand, functional food-web models focus on relationships between functional groups but largely ignore species diversity within groups (e.g. De Laender et al., 2015). This limitation challenges the linking of such models to toxicity databases, which contain data for specific species. In consequence, it is challenging for such functional models to directly guide the regulatory risk assessment of chemicals. An advantage of such functional food-web models, however, is that they are computationally undemanding, account for indirect effects, and link to ecosystem functions and related services. These two model types, along with others, are discussed below.

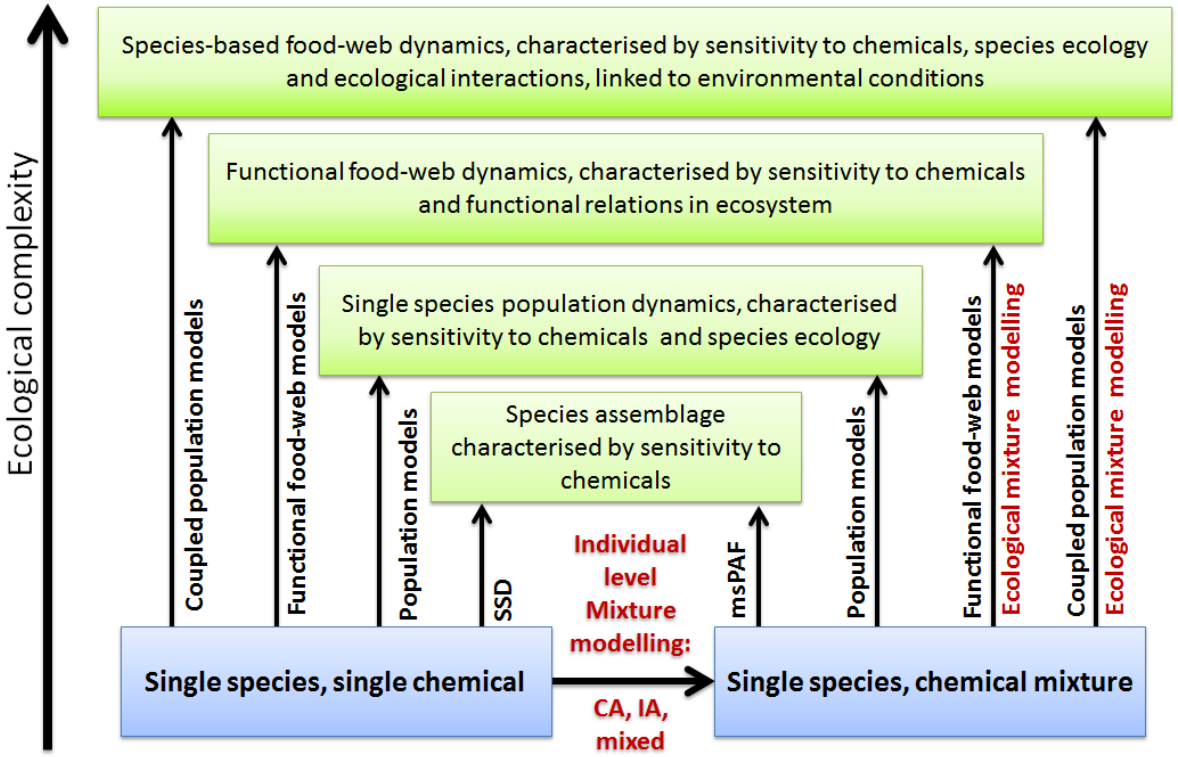

Figure 4: Overview of different ways to extrapolate single species, single chemical toxicity to mixture toxicity and higher ecological complexity levels [SSD = species sensitivity distribution, msPAF = multi-substance potentially affected fraction, CA = concentration addition, IA = independent action]

As well as extrapolation from single species to communities for single chemicals, a modelling toolbox needs to account for mixture toxicity. Using mixture effect modelling, in combination with mode-of-action information, mixture effects at single species levels can be calculated using single compound toxicity data. The application of mixture toxicity modelling can provide estimates of the expected effects of a mixture on survival, but, given that toxicity data are available, also for sublethal effects. These effects can then enter the ecological models to translate these individual-level effects to higher levels of organisation.

3.3.2.1 Definition of single species and ecological mixture toxicity

Single species mixture toxicity: Multiple chemicals co-occur in organisms of one species. Some of the chemicals have targets that are related to the same mode-of-action (e.g. multiple acetylcholine-esterase inhibitors, or multiple compounds showing narcotic effects). Also, groups of chemicals can co-occur that have independent targets. Toxic effects of combinations of multiple chemicals at levels of single species can be described using mixed toxicity models. Mixture toxicity modelling is in a broad sense reduced to providing the necessary toxicity and species ecology data, linked to appropriate single species ecological models. Most relevant mixtures could be defined by the compounds that add to the most critical MoA groups, in terms of their toxicity.

Ecological mixture toxicity: When considering multiple species within a community or an ecosystem, mixture effects can occur which can be referred to as ecological mixture toxicity. Toxic effects of chemicals can affect

different targets within an ecosystem, and the interactions of these ecosystem elements can potentiate, mitigate or not affect single species level mixture effects. Toxic effects of multiple chemicals at the food-web or ecosystem levels can be described using mixed toxicity models in combination with appropriate functional or species-based food-web models. Most relevant ecological mixtures can be defined as combinations of compounds that result in potentiated effects at the ecosystem level.

An illustrative example for ecological mixture toxicity might be the mixture of a broad-spectrum insecticide and a broad-spectrum herbicide. When such compounds are considered in isolation, typically indirect effects at the ecosystem level are expected. The herbicide could e.g. result in decreased algae and periphyton biomass, so that macroinvertebrate grazers would be affected in their densities, too, because they do not find enough food. In turn, when insecticides would reduce the densities of grazers in an ecosystem, algae and periphyton would be consumed less and thus increase in abundance. When considering mixture effects of such compounds, mixture effects at single species levels will not likely be induced. This can be different when considering ecosystems as a whole. The insecticide can affect the densities of macroinvertebrate grazers within an aquatic ecosystem, and the herbicide can suppress growth of all algae. In summary, indirect effects of the insecticide are dampened out by the herbicide when considering mixture effects at ecosystem levels. Vice versa in this example, it might be that effects of the insecticide on the abundances of macroinvertebrate grazers are more pronounced when food sources, e.g. periphyton, are decreased. While dampening of indirect effects can be seen as antagonistic at ecosystem levels, it is also possible that effects of multiple chemicals show synergistic effects at ecosystem levels, i.e. effects of mixtures are significantly stronger than the sum of single compounds. Such a situation is further exemplified in a theoretical example in section 3.4. A number of the mesocosm studies reported in Section 3.1 have looked at mixtures of broad-spectrum insecticides and herbicides, but they mainly report a lack of synergism. Van den Brink et al. (2009) looking at a mixture of lindane and atrazine, did report that atrazine produced fewer effects than expected, probably due to decreased grazer stress on the algae as a result of the lindane application, somewhat supporting the concept of ecological mixture toxicity.

3.3.2.2 Species sensitivity distribution modelling

A species sensitivity distribution (SSD) can be used to predict the potential effect of a stressor or stressors, such as chemicals, on a community. The SSD concept is based on the observation that species have different sensitivities to stressors and that the distribution of sensitivities can be described with a statistical distribution (Stephan et al., 1985). SSDs have been used since the 1980s for the derivation of environmental quality standards and thresholds (Stephan et al., 1985; Van Straalen and Denneman, 1989) and have become an important tool in ecotoxicology and environmental risk assessment (Posthuma et al., 2002; De Zwart et al., 2002; EFSA, 2013).

Using the available toxicity data, a log-normal or log-logistic equation is typically used to derive the SSD, which is a theoretical distribution of the sensitivities of species tested for a given chemical. From this curve, it is possible to derive a point estimate which can be used as the protection level or to derive the protection level, for the community. Generally, this point estimate is the HC5, the concentration which will exceed the effect levels for no more than 5% of the species. The HC5 can be used directly as a quality standard, or it may have an additional assessment factor added to account for uncertainties in the assessment. Some of this uncertainty when it comes to potential for effects on communities is based on the fact that the SSD only considers direct

effects based on the sensitivity to the stressor and does not account for any of the ecological interactions between species within communities. In addition, for a known concentration, the corresponding potentially affected fraction (PAF) for the exposure level can be calculated based on the SSD, giving an indication of what percentile of a community is potentially impacted.

The SSD concept has been extended for mixtures, calculating multi-substance potentially affected fractions (ms-PAF) considering the combined concentration addition and independent action models (De Zwart and Posthuma, 2005). Both the SSD and the ms-PAF approaches have been used frequently to characterise the effects of chemicals and chemical mixtures on communities, e.g. Maltby et al., 2009, Kon Kam King et al., 2015, Ramo et al., 2016.

SSDs are undoubtedly useful because of their simple parameterisation and dual use. In some regulatory schemes, assessment factors are used together with SSDs to extrapolate no effect levels i.e. no community level effects. However, a limitation of the SSD and ms-PAF approaches is that they do not consider any potential ecological interactions. They do not explicitly account for anything other than the sensitivity and ignore other factors which could have an impact on intensity and duration of possible community effects, i.e. species ecology and their interactions. However, for PPPs Maltby et al. (2009) compared HC5 values from acute SSDs for aquatic invertebrates to community NOECs and demonstrated that, together with an appropriate AF, SSDs could be used in risk assessment to be protective of community level effects.

3.3.2.3 Population models

Single species population models have been used for many years in ecotoxicology and other related fields such as pest pressure in agriculture and fisheries management. The main target of the use of ecological population models for risk assessment of chemicals is the combination of information on sensitivity to chemicals and species ecology and population dynamics, respectively. One of these models is MASTEP (Metapopulation model for assessing spatial and temporal effects of pesticides) (Van den Brink et al., 2007). In the MASTEP approach, dose-response toxicity modelling was combined with individual growth and reproduction in a spatially explicit setting by the means of individual-based models (IBM). The MASTEP model was used for a number of studies in the context of chemical risk assessment, for example for the characterisation of landscape aspects of effects of chemicals (Galic et al., 2012, Galic et al., 2013), for the combination with exposure patterns at landscape scales (Focks et al., 2014a) or for the assessment of exposure to multiple chemicals (Focks et al., 2014b). Growth and reproduction in the MASTEP model were, however, imposed as time-dependent functions, and not based on first principles.

A more fundamental way of describing population dynamics based on external driving forces such as food availability was provided by the fusion of the individual-based modelling (IBM) paradigm and the dynamic energy budget (DEB) theory, which allowed the simulation of population dynamics based on energy intake at individual levels (Martin et al., 2012, Martin et al., 2013). Other IBM approaches in the context of environmental risk assessment of chemicals were developed for *Daphnia* (Preuss et al., 2009), but also for mammals (Wang and Grimm, 2007, Liu et al., 2013). In general, examples of single species population models accounting for effects of mixtures are scarce (e.g. Focks et al., 2014b). Their application for environmental risk assessment of chemicals leads to the consideration of more ecology, but the risk assessment remains limited to a single species, hence leaving out ecological interactions.

3.3.2.4 Community models

Community ecology studies how multiple abiotic variables and species interactions determine species coexistence, community composition and structure, and various aspects of biodiversity (Chesson 2000). Community level effect models in the context of chemical risk assessment are rare and focus on chemicals as abiotic variables. Simple two-species individual-based models have been developed to examine the role of species interactions on pesticide effects and subsequent recovery (Liess et al., 2013; Kattwinkel and Liess, 2014). Such models are useful to assess risk in species-poor communities and their main advantage is their simplicity. Most communities, however, consist of many species, especially communities that occupy lower trophic levels, such as plankton. Despite being traditionally considered as ecologically equivalent and of similar sensitivity, the species occupying such trophic levels can have very different contributions to community dynamics and ecosystem functions and span a wide range of sensitivities (De Laender et al., 2014a; Baert et al., 2016a; Mensens et al., 2017). In addition, the diversity within such trophic levels determines recovery (Baert et al., 2016b). Thus, their separate inclusion in models is warranted. At present, few models are available to do so: one model correctly predicts chemical effects on algal richness and evenness (De Laender et al., 2014a), one is able to correctly predict effects on algal community composition (Baert et al., 2016b), and one has been developed for stream food-webs (Kattwinkel et al., 2016).

An important challenge to the development and uses of community models is the number of processes driving species dynamics. This is because the objective of such a model would be to simulate effects on up to several dozens of species, each potentially having a distinct biology (e.g. presence of distinct life stages with potentially different environmental responses). Unlike models that consider fewer (or only single) species but represent species' biology in a more detailed way (Grimm and Martin, 2013), not all this complexity can be accounted for. This is because model implementation would no longer be technically feasible, results difficult to interpret, and parameters poorly identifiable. In practice, model developers will have to decide what mechanisms to include in community models and where to simplify. Methods such as approximate Bayesian computation are useful tools to identify what mechanisms contribute most to observed patterns and thus to optimise model complexity (Hartig et al., 2011). Another challenge to community level effect model development and use is calibration (identifying parameter values) and validation (comparing predictions with observations not used during calibration). Because of the level of biological organisation considered, model calibration and validation is cumbersome in practice. Indeed, micro- and mesocosm studies will not always be available for a given chemical, let alone 'cosm studies that investigate ecological responses for different environmental scenarios. An alternative option is to carry out detailed studies that examine how processes that are key to community composition or ecosystem functioning (e.g. competition, predation) combine with chemicals in affecting simplified study systems consisting of few species (Viaene et al., 2015; Liess and Foit, 2010; De Hoop et al., 2013). Because the scale of such experiments is smaller than that of community- and ecosystem-level studies they can more realistically be repeated for a selection of environmental scenarios.

3.3.2.5 Food-web and ecosystem models

Ecosystem ecology is concerned with fluxes of matter and energy between functional groups and the abiotic environment. When functional groups cover multiple trophic levels, food-webs are used to represent predator-prey relationships. Food-web and ecosystem level models are thus used to simulate effects on such fluxes

(ecosystem functioning) and on the size of functional groups (ecosystem structure). These models therefore do often not consider specific species and cannot be used to study effects on biodiversity or community composition. These models have been used to study indirect chemical effects (Fleeger et al., 2003) and the influence of environmental variables on toxicant effects. In their simplest form, food-web and ecosystem models are a set of ordinary differential equations, extended with concentration-response relationships (e.g. De Laender et al., 2008b; De Laender et al., 2015; Park et al., 2008; Lombardo et al., 2015). The highest level of ecosystem model complexity has been developed during the Cefic ECO19 project (ChimERA). This modelling framework consists of building networks of IBMs to simulate ecosystem dynamics, starting from individual level processes (De Laender et al., 2014b).

Because of the level of biological organisation considered, calibration and validation of community and ecosystem models is cumbersome in practice. As for the models discussed in 3.3.2.4, detailed studies can be designed to examine how key processes combine with chemicals in affecting simplified study systems.

3.4 An illustration with a minimal model – Additivity at single species level does not imply additivity at community level

The ecological interactions following exposure to a chemical stressor are difficult to interpret and are not always what might be expected from single species data. Consider two substances A and B that act through independent action in single species tests with a prey (endpoint: growth rate) and predator (endpoint: feeding rate):

$$\text{growth} = \text{growth}_{\max} \cdot \frac{1}{1 + \left({C_A}/{EC_{50\text{prey,A}}}\right)^{s_A}} \cdot \frac{1}{1 + \left({C_B}/{EC_{50\text{prey,B}}}\right)^{s_B}}$$

$$\text{feeding} = \text{feeding}_{\max} \cdot \frac{1}{1 + \left({C_A}/{EC_{50\text{pred,A}}}\right)^{s_A}} \cdot \frac{1}{1 + \left({C_B}/{EC_{50\text{pred,B}}}\right)^{s_B}}$$

Using a simplified version of an existing predator-prey model, it can be tested if the mixture of A and B also acts through independent action on prey biomass density at the community level:

$$\frac{\text{dPrey}}{\text{dt}} = \text{Prey} \cdot (\text{growth} - \text{Prey} - \text{feeding} \cdot \text{Pred})$$

$$\frac{\text{dPred}}{\text{dt}} = \text{Pred} \cdot (-\text{mortality} - 0.1 \cdot \text{Pred} + \text{feeding} \cdot \text{efficiency} \cdot \text{Prey})$$

The test was done by exposing the predator-prey community to substances A and B alone, and to their mixtures, always in concentrations from 0 to 10. Since A and B are hypothetical substances, units are irrelevant here. Effects caused by A and B on equilibrium prey biomass density were calculated when administered alone. The effects expected of the mixture of A and B under independent action is simply the product of these two effects. We compared this expected effect with the effect observed in the mixture simulations.

Both substances A and B, when administered alone, affect prey biomass in a community context (Figure 5A and B). Note that low to intermediate levels of B actually stimulate prey biomass density because predators are more sensitive for B than for A. Even though the mixture acted through independent action at the single species level, the mixture effects observed in the simulations did not obey independent action (Figure 5C). This illustrates that additivity at the level of single species tests does not necessarily inform about the mixture effects expected in a community context.

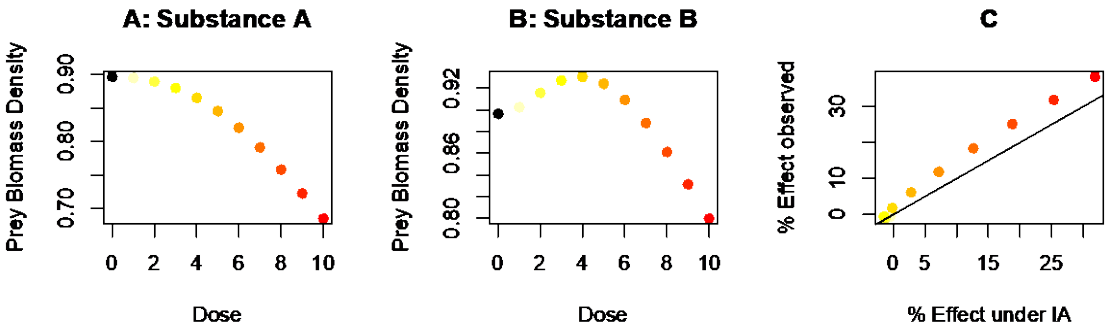

Figure 5: Panels A and B: Substance A and B, when administered alone but in the predator-prey community, both affect prey biomass density. Panel C: Independent action (IA) at the single species level does not imply independent action at the community level. Colour codes: control (black), increasing C_A and C_B (white to red). Simulations were done with $EC_{50prey,A} = 15$; $EC_{50prey,B} = 20$; $EC_{50pred,A} = 10$; $EC_{50pred,B} = 5$; $s_A = s_B = 2$.

4. DISCUSSION, RECOMMENDATIONS & KNOWLEDGE GAPS

The risk assessment for mixtures is continually raised as a major concern and continues to be the focus of many projects and workshops. However, it is not dealt with explicitly within prospective risk assessment frameworks. For example, under the REACH Regulation the protection goal is based on no effects, whereas for Plant Protection Products (EC, 2009), it is no unacceptable effects, implicit within this is that some effects are acceptable. There is a clear need to clarify specific protection goals with definition of the level of acceptability of effect, with specificity as to the size and type of effect as well as the temporal and spatial scale (EFSA, 2010). However, the setting of such specific protection goals is a risk management concern and it should not affect the risk assessment itself, the extrapolation from individual through population to community and ecosystem level using experimental and modelling techniques.

Studying mixture effects at higher levels of biological organisation (i.e. populations, communities, ecosystems) is a challenging task. It requires information about the chemical mixture of concern, the species likely to be exposed and other factors, such as the abiotic environment, as this will have an impact on both the chemical and biological components of the system. One could consider a seemingly unlimited number of chemical mixtures, various species combinations, and broad ranges of - for example - temperature or nutrient loading. However, considering all combinations of all these factors is not feasible nor is it necessary. Instead, we propose a strategy that is based on (1) classification; (2) the ecological scenarios paradigm; and (3) model-aided synthesis and design of informative experimenting.

Classification allows collapsing the high dimensionality that characterises the mixture toxicity challenge. Grouping chemicals according to their mode-of-action is a well-known example. Are similar classification efforts possible for the exposed species? Could traits help to reduce the number of ecologically distinct species combinations? These are open questions that have received some attention for single chemicals but remain to be explored for mixtures.

Not all combinations of the factors listed above are to be found in nature. For example, certain species (or trait) combinations will only be found in certain environmental conditions. Then, how informative and efficient is it to consider these combinations in mixture toxicity testing? This point connects to the paradigm of ‘ecological scenarios’; the idea that specific environmental conditions will harbour specific species and exposure conditions, and that these ‘scenarios’ are not equally distributed across space and time. A sensible first step is therefore to focus on those scenarios that are most frequently encountered, of course with the caveat that rare scenarios could contribute in unique ways to landscape-level ecosystem services, which needs to be carefully considered. Rico et al. (2015) propose the development of ecological scenarios for prospective risk assessment for pesticides in Europe, where exposure scenarios to estimate worst-case chemical exposure for risk assessment have long been part of the regulatory paradigm (FOCUS 2001). The development of ‘environmental scenarios’ incorporating combined exposure and effects to estimate worst-case risk would seem to be a logical step to take from here, an approach advocated for down-the-drain chemicals by Franco et al. (2017). This approach is thus starting to be considered within different sectors i.e. pesticides and down-the-drain chemicals, at the appropriate scale to satisfy their own needs, regulatory and otherwise. Exposure scenarios incorporating combined pesticide, down-the-drain and urban run-off exposure was considered by

Posthuma et al. (2018). It should therefore be possible to combine this approach to effects linking through ecological scenarios.

To get to this position of a holistic approach to exposure and effects requires linking the different components together using exposure and effects models, an approach started in the ChimERA project (De Laender et al., 2014b). Models can inform risk assessment of mixtures by contributing to intelligent testing design. They offer insight into which ecological scenarios exposed to which chemical mixture are thought to, according to current knowledge, represent the highest risk. Such analyses could inform experimental work, e.g. by triggering tests targeted to explore toxicity at those combinations of community, mixture composition, and scenario that are deemed 'most hazardous' by a model (i.e. 'worst case' identification). Such 'intelligent' or 'model-based' testing would thus enable identifying critical mixture effects at the community level and helps limiting experimental effort. In addition, they can complement experimental efforts by theoretically testing general hypotheses on which risk assessment is based. For example, the simplified example model in Section 3.4 illustrates that mixture effects at the level of individuals could misjudge mixture effects at the community level. Obviously, more work is required to properly interpret and explain this result, test how it changes when changing model parameters (i.e. sensitivity analysis), test how these results depend on the type of model considered (from simple to complex), and – most importantly – whether these results are supported by independent data from community level studies with mixtures.

Concrete ways forward to put the proposed strategy into practice would consist of different steps. A first step would be coupling the toxicity information and mode-of-action data to trait data and ecological scenarios. This is followed by the identification of the trait combinations which would be affected by a given mixture of MoAs and by defining whether or not responses are additive. Finally, community and ecosystem models are employed to scale up effects to true community- and ecosystem-level.

Recommendation 1: ***Couple the toxicity information and mode-of-action data to trait data and ecological scenarios***

For coupling toxicity information and mode-of-action data to trait data and ecological scenarios available toxicity databases (e.g. US EPA Ecotox database) and collated MoA information needs to be considered. Busch et al. (2016) partly adopted this approach to develop effect-based tools for water monitoring of EU rivers under the Water Framework Directive. Although similar to Barron et al. (2015), it was highlighted that the primary MoA, for example the target site for an herbicide might be known in plants, but the MoA in different taxonomic groups might be different and is often unknown. Furthermore, for many compounds no MoA information, primary or otherwise, exists at all. Thus, a key knowledge gap is on the MoA of different compounds across different taxonomic groups, beyond the primary MoA described in schemes such as those of Verhaar et al. (1992) and Barron et al. (2015).

Recommendation 2: ***Identification of trait combinations affected by given mixtures of MoAs, and identify whether additivity of effects would be expected***

Although there are currently limitations due to the availability of ecological traits data across taxa, available work on trait-sensitivity correlations (e.g., Rico and Van den Brink, 2015) can be used to identify which mixtures of MoAs would affect which trait combinations. The MoA information would further be used to identify whether effects are likely to be additive through concentration or response addition and thus whether direct effects are likely. Mechanistic information on MoAs could also identify any combinations where more than additive responses are a possibility.

Recommendation 3: ***Use of models to scale up effects to community and ecosystem level***

The community and ecosystem models listed and discussed in Section 3.3 can be used and are being further developed to scale up such effects to true community- and ecosystem-level variables such as biodiversity, community composition, and ecosystem function, for a range of species interactions, incorporating both direct and indirect effects, any potential synergisms or antagonisms through ecological interactions.

In summary, we propose a strategy that works in principle. However, putting it into practice requires that the limitations and data gaps outlined above are addressed and that the workability of the strategy is underpinned with more examples. To that end we consider it advisable to explore available data to reduce the complexity surrounding the assessment of mixture effects on communities and ecosystems, by focusing on key descriptors of chemical toxicity (MoA) and species sensitivity (traits). Collating this information into models of multiple interacting species is a way forward towards understanding how ecological interactions may cause interactive effects of chemical compounds on variables at higher levels of biological organisation. Including the ecological scenario paradigm could allow examining theoretically in which environmental conditions certain chemicals are expected to interact. Such a research agenda can then feed into an intelligent experimental design of mixture studies that focuses on testing key assumptions and predictions of the models. Doing so will produce knowledge on the mechanisms driving the effects of multiple chemicals on endpoints of global concern such as biodiversity and ecosystem functions.

ABBREVIATIONS

ABM	Agent based model
ACR	Acute to chronic ratio
AOP	Adverse outcome pathway
CA	Concentration addition
CBR	Critical body residue
Cefic	European Chemical Industry Council
CEWAF	Chemically enhanced water accommodated fraction
CTLBB	Critical target lipid body burden
DAM	Damage assessment model
DEB	Dynamic energy budget
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
FOCUS	Forum for Coordination of Pesticide Fate Models and their Use
GUTS	General unified threshold for survival
HBM	Hydrocarbon block method
HC5	5% hazardous concentration (the concentration which will exceed the effect levels for no more than 5% of the species)
HC30	30% hazardous concentration
IA	Independent action
IBM	Individual based model
MASTEP	Metapopulation model for assessing spatial and temporal effects of pesticides

MoA	Mode-of-action
ms-PAF	Multi-substance potentially affected fraction
PAF	Potentially affected fraction
PAH	Polycyclic aromatic hydrocarbon
PBTK	Physiologically based toxicokinetic
PPP	Plant protection products
PS II	Photosystem II
QSAR	Quantitative structure-activity relationship
SETAC	Society of Environmental Toxicology and Chemistry
SSD	Species sensitivity distribution
SSSC	Single species – single compound
STP	Sewage treatment plant
TD	Toxicodynamic
TDM	Threshold damage model
TK	Toxicokinetic
TKTD	Toxicokinetic-toxicodynamic
TLM	Target lipid model
TR	Toxic ratio
US EPA	United States Environmental Protection Agency
UVCB	Unknown, variable composition, complex reaction products and biological materials
WAF	Water accommodated fraction

BIBLIOGRAPHY

Almeda R, Wambaugh Z, Wang Z, Hyatt C, Liu Z. 2013. Interactions between Zooplankton and Crude Oil: Toxic Effects and Bioaccumulation of Hydrocarbons. *PLoS ONE* 8(6):e67212.

Ashauer R, Boxall ABA, Brown CD (2007). A new ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environmental Science and Technology*, 41 (4), 1480-1486

Altenburger R, Arrhenius A, Backhaus T, Coors A, Faust M, Zitzkat. 2013. Ecotoxicological combined effects from chemical mixtures. Part 1: Relevance and adequate consideration in environmental risk assessment of plant protection products and biocides. Texte 92/2013

Altenburger R, Ait-Aissa S, Antczak P, Backhaus T, Barceló D, Seiler T. B, de Aragão Umbuzeiro G. 2015. Future water quality monitoring—adapting tools to deal with mixtures of pollutants in water resource management. *Science of the Total Environment* 512-513:540-551.

Altenburger R, Scholze M, Busch W, Escher BI, Jakobs G, Krauss M, Seiler TB. 2018. Mixture effects in samples of multiple contaminants–An inter-laboratory study with manifold bioassays. *Environment International* 114: 95-106.

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry.* 29(3): 730–741.

Arts GHP, Buijse-Bogdan LL, Belgers DM, van Rhenen-Kersten CH, van Wijngaarden RPA, Roessink I, Maund SJ, Van Den Brink PJ, Brock TCM. 2006. Ecological impact in ditch mesocosms of simulated spray drift from a crop protection program for potatoes. *Integrated Environmental Assessment and Management* 2: 105-125.

Ashauer R, Escher BI. 2010. Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment. *Journal of Environmental Monitoring* 12(11): 2056 – 2061.

Auber A, Roucaute M, Togola A, Caquet T. 2011. Structural and functional effects of conventional and low pesticide input crop-protection programs on benthic macroinvertebrate communities in outdoor pond mesocosms. *Ecotoxicology* 20: 2042–2055.

Backhaus T. 2014. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. *Philosophical Transactions of the Royal Society B: Biological Sciences* 19: 369 (1656)

Baert JM. 2016a. Per capita interactions and stress tolerance drive stress-induced changes in biodiversity effects on eco- system functions. *Nature Communications* 18(7): 12486.

Baert JM, De Laender F, Sabbe K, Janssen CR. 2016b. Biodiversity increases functional and compositional resistance but decreases resilience in phytoplankton communities. *Ecology* 97(12): 3433-3440.

Baird DJ, Rubach MN, Van den Brink PJ. 2008. Trait-based ecological risk assessment (TERA): The new frontier? *Integrated Environmental Assessment and Management* 4(1): 2-3.

Barron MG, Lilavois CR, Martin TM. 2015. MOAtox: a comprehensive mode-of-action and acute aquatic toxicity database for predictive model development. *Aquatic Toxicology* 161: 102–107.

Baveco JM, Norman S, Roessink I, Galic N, Van den Brink PF. 2014. Comparing population recovery after insecticide exposure for four aquatic invertebrate species using models of different complexity. *Environmental Toxicology and Chemistry* 33: 1517-1528.

Bayona Y, Roucaute M, Cailleaud K, Lagadic L, Bassères A, Caquet T, 2015a. Effect of thiram and petroleum distillate on freshwater macroinvertebrate communities in outdoor stream and pond mesocosms: I. Study design, chemicals fate and structural responses. *Ecotoxicology* 24: 1976-1905.

Bayona Y, Roucaute M, Cailleaud K, Lagadic L, Bassères A, Caquet T, 2015b. Effect of thiram and petroleum distillate on freshwater macroinvertebrate communities in outdoor stream and pond mesocosms: II. Biological and ecological trait responses and leaf litter breakdown. *Ecotoxicology* 24: 1933-1946.

Bayona Y, Roucaute A, Roucaute M, Gorzerino C, Cailleaud K, Bassères A, Lagadic L, Caquet T 2014a Secondary production of freshwater zooplankton communities exposed to a fungicide and to a petroleum distillate in outdoor pond mesocosms. *Environmental Toxicology and Chemisrty* 33: 836–846

Bayona Y, Roucaute A, Roucaute M, Gorzerino C, Cailleaud K, Bassères A, Lagadic L, Caquet T 2014b Structural and biological trait responses of diatom assemblages to organic chemicals in outdoor flow-through mesocosms. *Environmental Pollution* 192: 186–195

Bjergager MA, Hanson, ML, Lissemore L, Henriquez N, Solomon KR, Cedergreen N. 2011. Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate. *Aquatic Toxicology* 101(2): 412–422.

Bjergager MA, Dalhoff K, Kretschmann A, Nørgaard KB, Mayer P, Cedergreen N. 2017. Determining lower threshold concentrations for synergistic effects. *Aquatic Toxicology* 182: 79–90.

Bliss CI. 1939. The toxicity of poisons applied jointly. *Annals of Applied Biology* 26:585–615.

Brown AR, Whale G, Jackson M, Marshall S, Hamer M, Solga A, Kabouw P, Galay-Burgos M, Woods R, Nadzialek S, Maltby L. 2017. Toward the definition of specific protection goals for the environmental risk assessment of chemicals: A perspective on environmental regulation in Europe. *Integrated Environmental Assessment and Management* 13(1):17-37.

Busch W, Schmidt S, Kühne R, Schulze T, Krauss M, Altenburger R. 2016. Micropollutants in European rivers: A mode-of-action survey to support the development of effect-based tools for water monitoring. *Environmental Toxicology and Chemistry* 35(8): 1887-1899.

Carder JP, Hoagland KD. 1998. Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. *Environmental Toxicology and Chemistry* 17:1415-1420.

Cedergreen N, Kamper A, Streibig JC. 2006. Is prochloraz a potent synergist across aquatic species? A study on bacteria, daphnia, algae and higher plants. *Aquatic Toxicology* 78(3):243-252.

Cedergreen N. 2014. Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies within Environmental Toxicology. *PLoS ONE* 9(5): e96580. doi: 10.1371/journal.pone.0096580

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31: 343–366

Concawe. 2012. REACH – Analytical characterisation of petroleum UVCB substances. Concawe Report No.7/12. https://www.concawe.eu/wp-content/uploads/2017/01/rpt_12-7-2012-05443-01-e.pdf

Concawe. 2015. Evaluation of Whole Effluent Bioassays Assessment of Hydrocarbon Ecotoxicity: Phase III Stream Study Report. Concawe Report No.7/15. https://www.concawe.eu/wp-content/uploads/2017/01/rpt_7-15.pdf

Crane M, Newman M. 2000. What level of effect is a no observed effect? *Environmental Toxicology and Chemistry* 19(2): 516–519.

Cuppen JGM, Crum SJH, Van Den Heuvel HH, Smidt RA, Van Den Brink PJ. 2002. Effects of a mixture of two insecticides in freshwater microcosms: I. Fate of chlorpyrifos and lindane and responses of macroinvertebrates. *Ecotoxicology* 11: 165-180.

Danavaro R, Fabiano M, Vincx M. 1995. Meiofauna Response to the Agip Abruzzo Oil Spill in Subtidal Sediments of the Ligurian Sea. *Marine Pollution Bulletin* 30(2): 133–145

De Hoop L, De Troch M, Hendriks AJ, De Laender F. 2013. Modelling toxic stress by atrazine in a marine consumer-resource system. *Environmental Toxicology and Chemistry* 32(5): 1088-1095.

De Laender F, De Schamphelaere KAC, Vanrolleghem PA, Janssen CR. 2008a. Validation of an ecosystem modelling approach as a tool for ecological effect assessments. *Chemosphere* 71(3): 529-545.

De Laender F, De Schamphelaere KAC, Vanrolleghem PA, Janssen CR. 2008b. Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems? An examination of a theoretical assumption underlying species sensitivity distribution models. *Environment International* 34(3): 390-396.

De Laender F, Van Oevelen D, Soetaert K, Middelburg JJ. 2010. Carbon transfer in herbivore-and microbial loop-dominated pelagic food-webs in the southern Barents Sea during spring and summer. *Marine Ecology Progress Series* 398: 93-107.

De Laender F. 2014a. The contribution of intra- and interspecific tolerance variability to biodiversity changes along toxicity gradients. *Ecology Letters* 17(1): 72-81.

De Laender F, Van den Brink PJ, Janssen CR, Di Guardo A. 2014b. The ChimERA project: coupling mechanistic exposure and effect models into an integrated platform for ecological risk assessment. *Environmental Science and Pollution Research* 21:6263–6267. DOI 10.1007/s11356-014-2605-5

De Laender F, Morselli M, Baveco H, Van den Brink PJ, Di Guardo A. 2015. Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling. *Environment International* 74: 181-190.

De Zwart D, Posthuma L, Suter II GW, Traas TP. 2002. Observed regularities in SSDs for aquatic species. Species sensitivity distributions in ecotoxicology. Lewis Publishers, Boca Raton, FL, USA. Pages 133-154.

De Zwart D, Posthuma L. 2005. Complex mixture toxicity for single and multiple species: Proposed methodologies. *Environmental Toxicology and Chemistry* 24(10):2665-2676.

De Zwart D, Adams W, Galay Burgos M, Hollender J, Junghans M, Merrington G, Muir D, Parkerton T, de Schamphelaere KAC, Whale G, Williams R. 2018. Aquatic exposures of chemical mixtures in urban environments: Approaches to impact assessment. *Environmental Toxicology and Chemistry* 37(3):703–714.

Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and PAH criteria. I. Water and tissue. *Environmental Toxicology and Chemistry* 19:1951-1970.

Di Toro DM, McGrath JA. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. 2. Mixtures and sediments. *Environmental Toxicology and Chemistry* 19:1971-1982.

Diamond J, Altenburger R, Coors A, Dyer SD, Focazio M, Kidd K, Koelmans AA, Leung KMY, Servos MR, Snape J, Tolls J, Zhang X. 2018. Use of prospective and retrospective risk assessment methods that simplify chemical mixtures associated with treated domestic wastewater discharges. *Environmental Toxicology and Chemistry* 37(3): 690–702.

Ducrot V, Ashauer R, Bednarska AJ, Hinarejos S, Thorbek P and Weymany G. 2016. Using Toxicokinetic-Toxicodynamic Modeling as an Acute Risk Assessment Refinement Approach in Vertebrate Ecological Risk Assessment. *Integrated Environmental Assessment and Management* 12(1)32–45.

Dulio V, Bavel B, Brorström-Lundén E, Harmsen J, Hollender J, Schlabach, M, Koschorreck, J. 2018. Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. *Environmental Sciences Europe* 30(1): 5.

EC. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency

EC. 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309/1, 24.11.2009, pp. 1–50.

EC. 2013. Scientific opinion SCCS 131. SCHER (Scientific Committee on Health and Environmental Risks), SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCCS (Scientific Committee on Consumer Safety), Addressing the New Challenges for Risk Assessment. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_131.pdf

ECETOC. 2011. Development of guidance for assessing the impact of mixtures of chemicals in the aquatic environment. Technical report No.111. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium. http://www.ecetoc.org/taskforce/development-of-guidance-for-assessing-the-impact-of-mixtures-of-chemicals-in-the-aquatic-environment

EFSA. 2010. Scientific opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). *EFSA Journal* 8(10):1821.

EFSA. 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal* 11(7):3290

Escher BI, Hermens JLM. 2002. Modes of action in ecotoxicology: Their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environmental Science and Technology* 36(20): 4201–4217.

Escher BI, Aït-Aïssa S, Behnisch PA, Brack W, Brion F, Brouwer A, Hettwer K. 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Science of the Total Environment* 628-629: 748-765.

European Medicines Agency. 2006. Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA/CHMP/SWP/4447/00). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessment-medicinal-products-human-use-first-version_en.pdf

Fairchild JF, La Point TW, Schwartz TR. 1994. Effects of an herbicide and insecticide mixture in aquatic mesocosms. *Archives of Environmental Contamination and Toxicology* 27(4): 527-533.

Federle TW, Vestal JR, Hater GR, Miller MC. 1979. Effects of Prudhoe Bay crude oil on primary production and zooplankton in arctic tundra thaw ponds *Marine Environmental Research* 2: 3–18.

Ferguson J. 1939. The use of chemical potentials as indices of toxicity. *Proceedings of the Royal Society of London. Series B* 127:387–404.

Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment* 317(1-3): 207–233.

Focks A, Horst MT, Van den Berg E, Baveco H, Van den Brink PJ. 2014a. Integrating chemical fate and population-level effect models for pesticides at landscape scale: New options for risk assessment. *Ecological Modelling* 280: 102-16.

Focks A, Luttik R, Zorn M, Brock T, Roex E, Van der Linden T, Van Den Brink PJ. 2014b. A simulation study on effects of exposure to a combination of pesticides used in an orchard and tuber crop on the recovery time of a vulnerable aquatic invertebrate. *Environmental Toxicology and Chemistry* 33(7): 1489-98.

FOCUS. 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2, 245 pp.

Franco A, Price OR, Marshall S, Jolliet O, Van den Brink PJ, Rico A, Focks A, De Laender F, Ashauer R. 2017. Toward refined environmental scenarios for ecological risk assessment of down-the-drain chemicals in freshwater environments. *Integrated Environmental Assessment and Management* 13(2): 233–248.

Galic, N, Baveco H, Hengeveld GM, Thorbek P, Bruns E, Van den Brink PJ. 2012. Simulating population recovery of an aquatic isopod: Effects of timing of stress and landscape structure. *Environmental Pollution* 163: 91-99.

Galic, N, Hengeveld GM, Van den Brink PJ, Schmolke A, Thorbek P, Bruns E, Baveco HM. 2013. Persistence of Aquatic Insects across Managed Landscapes: Effects of Landscape Permeability on Re-Colonization and Population Recovery. *PLoS ONE* 8(1): e54584. https://doi.org/10.1371/journal.pone.0054584

Gergs A, Kulkarni D, Preuss T. 2015. Body size-dependent toxicokinetics and toxicodynamics could explain intra- and interspecies variability in sensitivity. *Environmental Pollution* 206: 449-455

Gerner NV, Koné M, Ross MS, Pereira A, Ulrich AC, Martin JW, Liess M. 2017. Stream invertebrate community structure at Canadian oil sands development is linked to bitumen-derived contaminants. *Science of the Total Environment* 575: 1005-1013.

Gesteira JL, Dauvin JC. 2005. Impact of the Aegean Sea oil spill on the subtidal fine sand macrobenthic community of the Ares-Betanzos Ria (Northwest Spain). *Marine Environmental Research* 60(3): 289–316.

Grimm V, Martin BT. 2013. Mechanistic effect modelling for ecological risk assessment: where to go from here? *Integrated Environmental Assessment and Management* 9(3): 58-63.

Halstead NT, McMahon TA, Johnson SA, Raffel TR, Romansic JM, Crumrine PW, Rohr JR. 2014. Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity an ecosystem property. *Ecology Letters* 17(8): 932 – 941.

Hartgers EM, Aalderink GH, Van den Brink PJ, Gylstra R, Wiegman JWF, Brock TCM. 1998. Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms. *Aquatic Ecology* 32: 135-152.

Hartig F, Calabrese JM, Reineking B, Wiegand T, Huth A. 2011. Statistical inference for stochastic simulation models--theory and application. *Ecology Letters* 14: 816–827.

Hermens JLM, Canton H, Janssen P, de Jong R. 1984. Joint effects of a mixture of 14 chemicals on mortality and inhibition of reproduction of Daphnia magna. *Aquatic Toxicology* 5: 315-322.

Hickie BE, McCarty LS, Dixon DG. 1995. A residue-based toxicokinetic model for pulse-exposure toxicity in aquatic systems. *Environmental Toxicology and Chemistry* 14: 2187–2197.

Hoagland KD, Drenner RW, Smith DJ, Cross DR. 1993. Freshwater community responses to mixtures of agricultural pesticides: Effects of atrazine and bifenthrin. *Environmental Toxicology and Chemistry* 12: 627-637.

Holmes CM, Brown CD, Hamer M, Jones R, Maltby L, Posthuma L, Silberhorn E, Teeter JS, Warne MSJ, Weltje L. 2018. Prospective aquatic risk assessment for chemical mixtures in agricultural landscapes. *Environmental Toxicology and Chemistry* 37:674–689

Howard P, Meylan W, Aronson D, Stiteler W, Tunkel J, Comber M, Parkerton T. 2005. A new biodegradation prediction model specific to petroleum hydrocarbons. *Environmental Toxicology and Chemistry 24 (8): 1847-1860.*

Jager T, Ashauer R. 2018. Modelling survival under chemical stress. A comprehensive guide to the GUTS framework. Version 1.0. Toxicodynamics Ltd., York, UK. Available from Leanpub: *https://leanpub.com/guts_book*

Jia J, Zhu F, Ma X, Cao Z, Cao ZW, Li Y, Li YX, Chen YZ. 2009. Mechanisms of drug combinations: interaction and network perspectives. *Nature Reviews Drug Discovery* 8:111–128 doi:10.1038/nrd2683

Kattwinkel M, Liess M. 2014. Competition matters: Species interactions prolong the long-term effects of pulsed toxicant stress on populations. *Environmental Toxicology and Chemistry* 33(7): 1458–1465.

Kattwinkel M, Reichert P, Rüegg J, Liess M, Schuwirth N. 2016. Modeling Macroinvertebrate Community Dynamics in Stream Mesocosms Contaminated with a Pesticide. *Environmental Science and Technology* 50: 3165-3173.

King DJ, Lyne RL, Girling A, Peterson DR, Stephenson R, Short D. 1996. Environmental risk assessment of petroleum substances: the hydrocarbon block method. Concawe report No. 96/52. https://www.concawe.eu/wp-content/uploads/2017/01/rpt_96-52-2004-01719-01-e.pdf

Knauert S, Escher B, Singer H, Hollender J, Knauer K. 2008. Mixture toxicity of three photosystem II inhibitors (atrazine, isoproturon, and diuron) toward photosynthesis of freshwater phytoplankton studied in outdoor mesocosms. *Environmental Science and Technology* 42(17): 6424-6430.

Knauert, S, Dawo U, Hollender J, Hommen U, Knauer K. 2009. Effects of Photosystem II inhibitors and their mixture on freshwater phytoplankton succession in outdoor mesocosms. *Environmental Toxicology and Chemistry* 28(4): 836-845

Kon Kam King G, Larras F, Charles S, Delignettemuller ML. 2015. Hierarchical modelling of species sensitivity distribution: development and application to the case of diatoms exposed to several herbicides. *Ecotoxicology and Environmental Safety* 114:212–221.

König M, Escher BI, Neale PA, Krauss M, Hilscherová K, Novák J, Ahlheim J. 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. *Environmental Pollution* 220: 1220-1230.

Lee JH, Landrum PF, Koh CH. 2002. Toxicokinetics and time-dependent PAH toxicity in the amphipod Hyalella azteca. *Environmental Science and Technology* 15;36(14): 3124-3130.

Legierse KCHM, Verhaar HJM, Vaes WHJ, De Bruijn JHM, Hermens JLM. 1999. Analysis of the time-dependent acute aquatic toxicity of organophosphorus pesticides: The critical target occupation model. *Environmental Science and Technology* 33:917–925.

Liess M, Von der Ohe PC. 2005. Analysing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry* 24(4): 954-965.

Liess, M, Foit K. 2010. Intraspecific competition delays recovery of population structure. *Aquatic Toxicology* 97: 15-22.

Liess M, Foit K, Becker A, Hassold E, Dolciotti I, Kattwinke M, Duquesne S. 2013. Culmination of low-dose pesticide effects. *Environmental Science and Technology* 47(15): 8862–8868.

Lindim C, Van Gils J, Cousins IT. 2016. A large-scale model for simulating the fate & transport of organic contaminants in river basins. *Chemosphere* 144: 803-810.

Liu, C, Sibly RM, Grimm V, Thorbek P. 2013. Linking pesticide exposure and spatial dynamics: An individual-based model of wood mouse (*Apodemus sylvaticus*) populations in agricultural landscapes. *Ecological Modelling* 248: 92-102.

Lombardo A, Franco A, Pivato A, Barausse A. 2015. Food-web modelling of a river ecosystem for risk assessment of down-the-drain chemicals. *Science of the Total Environment* 508: 214-227.

Mackay D. 2004. Finding fugacity feasible, fruitful and fun. *Environmental Toxicology and Chemistry* 23(10):2282–2289

Mackay D, Arnot J, Petkova E, Wallace K, Call D, Brooke L, Veith G. 2009. The physicochemical basis of QSARs for baseline toxicity. *SAR and QSAR in Environmental Research* 20:393–394.

Mackay D, Arnot JA, Wania F, Bailey RE. 2011. Chemical activity as an integrating concept in environmental assessment and management of contaminants. *Integrated Environmental Assessment and Management* 7:248–255

MacLeod M, McKone TE, Foster KL, Maddalena RL, Parkerton TF, Mackay D. 2004. Applications of contaminant fate and bioaccumulation models in assessing ecological risks of chemicals: A case study for gasoline hydrocarbons. *Environmental Science and Technology* 38: 6225-6233.

Maltby L, Brock TCM, Van den Brink PJ. 2009. Fungicide risk assessment for aquatic ecosystems: Importance of interspecific variation, toxic mode-of-action and exposure regime. *Environmental Science and Technology* 43: 7556–7563

Martin BT, Zimmer EI, Grimm V, Jager T. 2012. Dynamic Energy Budget theory meets individual-based modelling: A generic and accessible implementation. *Methods in Ecology and Evolution* 3(2): 445-449.

Martin BT, Jager T, Nisbet RM Preuss TG, Grimm V. 2013. Predicting population dynamics from the properties of individuals: A cross-level test of dynamic energy budget theory. *American Naturalist* 181: 506-519.

Martin BT, Jager T, Nisbet NM, Preuss TG, Grimm V. 2014. Limitations of extrapolating toxic effects on reproduction to the population level. *Ecological Applications* 24: 1972-1983.

McCarty LS, Mackay D, Smith AD, Ozburn GW, Dixon DG. 1992. Residue based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Neutral narcotic organics. *Environmental Toxicology and Chemistry* 11: 917–930.

McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modelling and assessment: Body residues and modes of toxic action. *Environmental Science and Technology* 27:1719–1728.

McCarty LS, Arnot JA, Mackay D. 2013. Evaluation of critical body residue for acute narcosis in aquatic organisms. *Environmental Toxicology and Chemistry* 32(10): 2301-2314.

Mensens C, De Laender F, Janssen C, Sabbe K, De Troch M. 2017. Different response-effect trait relationships underlie contrasting responses to two chemical stressors. *Journal of Ecology* 105(6): 1598-1609.

Meylan WM, Howard PH. 2005. Estimating octanol–air partition coefficients with octanol–water partition coefficients and Henry's law constants. *Chemosphere* 61: 640 – 644.

Mondy CP, Schuwirth N. 2017. Integrating ecological theories and traits in process-based modelling of macroinvertebrate community dynamics in streams. *Ecological Applications* 27(4):1365-1377. doi:10.1002/eap.1530. Epub 2017 Apr 21. https://www.researchgate.net/publication/314270789_

Moschet C, Wittmer I, Simovic J, Junghans M, Piazzoli A, Singer H, Hollender J. 2014. How a complete pesticide screening changes the assessment of surface water quality. *Environmental Science and Technology* 48(10): 5423-5432.

Neale PA, Munz NA, Aït-Aïssa S, Altenburger R, Brion F, Busch W, Seiler TB. 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. *Science of the Total Environment* 576: 785-795.

Neff JM, Ostazeski S, Gardiner W, Stejskal I 2000. Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. *Environmental Toxicology and Chemistry* 19: 1809–1821.

Nichols JW, McKim JM, Andersen ME, Gargas ML, Clewell HJ III, Erickson RJ. 1990. A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. *Toxicology and Applied Pharmacology* 106: 433–447.

Ozhan K, Bargu S. 2014. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant Corexit Ec9500A under different nutrient regimes. *Ecotoxicology* 23:370–384

Park RA, Clough JS, Coombs Wellman M. 2008. AQUATOX: Modelling environmental fate and ecological effects in aquatic ecosystems. *Ecolical Modelling* 213:1-15.

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB. 2003. Long term ecosystem response to the Exxon Valdez oil spill. *Science* 302: 2082-2086

Pilling ED, Jepson PC. 1993. Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis-mellifera*). *Pest Management Science* 39: 293–297

Plackett RL, Hewlett PS. 1952. Quantal responses to mixtures of poisons. *Journal of the Royal Statistical Society* B14:141–163.

Posthuma L, Suter GW II, Traas TP, eds. 2002. Species-Sensitivity Distributions in Ecotoxicology. Lewis, Boca Raton, FL, USA

Posthuma L, Brown CD, De Zwart D, Diamond J, Dyer SD, Holmes CM, Marshall S, Burton Jr GA. 2018. Prospective mixture risk assessment and management prioritizations for river catchments with diverse land uses. *Environmental Toxicology and Chemistry* 37: 715–728

Preuss, TG, Hammers-Wirtz M, Hommen U, Rubach MN, Ratte HT. 2009. Development and validation of an individual based Daphnia magna population model: The influence of crowding on population dynamics. *Ecological Modelling* 220: 310-329.

Quann RJ, Jaffe SB. 1992. Structure-oriented lumping: describing the chemistry of complex hydrocarbon mixtures. *Industrial & Engineering Chemistry Research* 31 (11) 2483–2497.

Ramo RA, Van den Brink PJ, Rupert C, Castillo LE, Gunnarsson S. 2016. Environmental risk assessment of pesticides in the River Madre de Dios, Costa Rica using PERPEST, SSD, and msPAF models. *Environmental Science and Pollution Research* 25(14):13254-13269. doi: 10.1007/s11356-016-7375-9.

Redman AD, Parkerton TF, McGrath JA, Di Toro DM. 2012. PETROTOX: An aquatic toxicity model for petroleum substances. *Environmental Toxicology and Chemistry* 31:2498–2506

Rico A, Van den Brink PJ, Gylstra R, Focks A, Brock TCM. 2015. Developing Ecological Scenarios for the Prospective Aquatic Risk Assessment of Pesticides. *Integrated Environmental Assessment and Management* 12(3): 510–521.

Rico A, Van den Brink PJ. 2015. Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode-of-action. *Environmental Toxicology and Chemistry* 34(8): 1907-1917.

Richards SM, Wilson CJ, Johnson DJ, Castle DM, Lam M, Mabury SA, Sibley PK, Solomon KR (2004) Effects of pharmaceutical mixtures in aquatic microcosms. *Environmental Toxicology and Chemistry* 23:1035–1042

Rubach MN, Baird DJ, Boerwinkel MC, Maund SJ, Roessink I, Van den Brink PJ. 2012. Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos. *Ecotoxicology* 21(7): 2088-2101.

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond DA. 1997. Predicting modes of action from chemical structure: Acute toxicity in the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* 16(5): 948-967.

SETAC. 2008. Extrapolation Practice for Ecotoxicological Effect Characterisation of Chemicals. Solomon KR, Brock TCM, De Zwart D, Dyer SD, Posthuma L, Richards S, Sanderson H, Sibley P, Van den Brink PJ. SETAC Press and CRC Press, Boca Raton, FL. ISBN 978-1-4200-7390-4 380 pp.

Spaak JW, Baert JM, Baird DJ, Eisenhauer N, Maltby L, Pomati F, Radchuk V, Rohr JR, Van den Brink PJ, De Laender F. 2017. Shifts of community composition and population density substantially affect ecosystem function despite invariant richness. *Ecology Letters* 20(10): 1315-1324.

Stadnicka J, Schirmer K, Ashauer R. 2012. Predicting Concentrations of Organic Chemicals in Fish by Using Toxicokinetic Models. *Environmental Science and Technology* 46(6): 3273-3280.

Stephan CE. 1985. Are the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Life and Its Uses" based on sound judgments? In Cardwell, R.D., R. Purdy, and R.C. Bahner, Eds., Aquatic Toxicology and Hazard Assessment, Seventh Symposium, American Society for Testing and Materials, Philadelphia, PA, USA: 515–526.

Sura S, Waiser M, Tumber V, Farenhorst A. 2012. Effects of herbicide mixture on microbial communities in prairie wetland ecosystems: a whole wetland approach. *Science of the Total Environment* 1;435-436:34-43.

Van den Brink PJ, Hartgers EM, Gylstra R, Bransen F, Brock TCM. 2002. Effects of a mixture of two insecticides in freshwater microcosms: II. Responses of plankton and ecological risk assessment. *Ecotoxicology* 11: 181-197.

Van den Brink PJ, Baveco JM, Verboom J, Heimbach F. 2007. An individual-based approach to model spatial population dynamics of invertebrates in aquatic ecosystems after pesticide contamination. *Environmental Toxicology and Chemistry* 26: 2226-36.

Van den Brink PJ, Crum SJH, Gylstra R, Bransen F, Cuppen JGM, Brock TCM. 2009. Effects of an herbicide–insecticide mixture in freshwater microcosms: Risk assessment and ecological effect chain. *Environmental Pollution* 157: 237-249

Van Leeuwen CJ, Van Der Zandt PTJ, Aldenberg T, Verhaar HJM, Hermens JLM. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. *Environmental Toxicology and Chemistry* 11(2): 267-282.

Van Straalen NM, Denneman CAJ. 1989. Ecotoxicological evaluation of soil quality criteria. *Ecotoxicology and Environmental Safety* 18:241-251

Van Wezel AP, De Vries DAM, Kostense S, Sijm DTHM, Opperhuizen A. 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquatic Toxicology* 33:325-342.

Van Wezel AP, Opperhuizen A. 1995. Narcosis due to environmental pollutants in aquatic organisms: Residue-based toxicity, mechanisms, and membrane burdens. *Critical Reviews in Toxicology* 25:255–279

Van Wijngaarden RPA, Cuppen JGM, Arts GHP, Crum SJH, Van den Hoorn MW, Van Den Brink PJ, Brock TCM. 2004. Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. *Environmental Toxicology and Chemistry* 23: 1479-1498.

Verbruggen EMJ, Van den Brink PJ. 2010. Review of recent literature concerning mixture toxicity to aquatic organisms. RIVM report 601400001/2010.

Verhaar HJM, van Leeuwen CJ, Hermens JLM. 1992. Classifying environmental pollutants. 1: Structure- activity relationships for prediction of aquatic toxicity. *Chemosphere* 25(4):471-491.

Verhaar HJ, Solbé J, Speksnijder J, van Leeuwen CJ, Hermens JL. 2000. Classifying environmental pollutants: part 3. external validation of the classification system. *Chemosphere* 40: 875–883.

Viaene, KPJ, De Laender F, Rico A, Van den Brink PJ, Di Guardo A, Morselli M, Janssen CR. 2015. Species interactions and chemical stress: Combined effects of intraspecific and interspecific interactions and pyrene on Daphnia magna population dynamics. *Environmental Toxicology and Chemistry* 34: 1751-59.

Viaene K. 2016. Improving ecological realism in the risk assessment of chemicals: development of an integrated model. PhD Thesis, Ghent University, Belgium.

Wang M, Grimm V. 2007. Home range dynamics and population regulation: An individual-based model of the common shrew *Sorex araneus*. *Ecological Modelling* 205: 397-409.

Warne MSJ. 2003. A review of the ecotoxicity of mixtures, approaches to, and recommendations for, their management. Proceedings, Fifth National Workshop on the Assessment of Site Contamination, Adelaide, Australia, May, 2002, pp 253–276.

Weltje L. 2013. Letter to the Editor - No proof of synergy at environmentally realistic concentrations of prochloraz and esfenvalerate - A reaction on “Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate” by Bjergager et al. (Aquatic Toxicology 101 (2011), 412–422). *Aquatic Toxicology* 140–141: 466–468.

Widbom B, Oviatt CA. 1994. The 'World Prodigy' oil spill in Narragansett Bay, Rhode Island: acute effects on macrobenthic crustacean populations. *Hydrobiologia* 291: 115–124.

MEMBERS OF THE TASK FORCE

Louise Camenzuli (from September 2017)	ExxonMobil Petroleum & Chemical BE – Machelen
Andreas Focks (until May 2018)	Wageningen University & Research NL – Wageningen
Mick Hamer (**Task Force Chair**)	Syngenta UK – Bracknell
Frederick De Laender	University of Namur BE – Namur
Laurent Lagadic	Bayer AG DE - Monheim
Miriam Leon Paumen (until September 2017)	ExxonMobil Petroleum & Chemical BE – Machelen
Aaron Redman (from June 2018)	ExxonMobil Petroleum & Chemical BE – Machelen
Joy Worden (until January 2019)	Shell International UK – Manchester

MEMBERS OF THE SCIENTIFIC COMMITTEE

(Peer Review Committee)

B. van Ravenzwaay (Chair) Senior Vice President - Experimental Toxicology	BASF DE – Ludwigshafen
R. Bars Scientific Director - Toxicology	Bayer CropScience FR – Sophia Antipolis
P. Botham Principal Science Advisor	Syngenta UK – Bracknell
T. Gant Visiting Professor, Environmental Toxicology	Kings College London UK - London
H. Greim Institute of Toxicology and Environmental Hygiene	Technical University München DE – München
A. Häner Environmental Risk Assessor	F. Hoffmann-La Roche Ltd CH – Basel
J. Hermens Associate Professor, Institute for Risk Assessment Sciences	University of Utrecht NL – Utrecht
H. Hollnagel Regulatory Toxicologist	Dow CH – Horgen
P. Lemaire Head of Product Stewardship and Sustainable Development	Total Fluides FR – Paris La Défense Cedex
L. Maltby Professor of Environmental Biology	University of Sheffield UK – Sheffield
M.L. Meisters Global Chemical Legislation Technical Leader, Corteva, Agricultural Division	Corteva BE – Brussels
M. Pemberton Director	Systox UK – Wilmslow

MEMBERS OF THE SCIENTIFIC COMMITTEE (cont'd)

C. Rodriguez Principal Toxicologist, Corporate Central Product Safety	Procter and Gamble BE – Strombeek-Bever
A. Redman Senior Environmental Scientist	ExxonMobil Petroleum & Chemical BE - Machelen
G. Sanders Principal Scientist	Givaudan CH – Vernier
G. Swaen Associate Professor	Maastricht University NL – Maastricht
J. Tolls[#] Director Ecology	Henkel AG & Co. KGaA DE -Düsseldorf
J. Urbanus Manager Exposure & Health Analysis Sciences	Shell BE – Brussels
K. van Leeuwen[#] Chief Science Officer/Professor	KWR Water Research Institute NL – Nieuwegein
E. Van Miert Toxicological and Environmental Risk Assessment Manager	Solvay BE – Brussels

_____

[#] Responsible for primary peer-review

ECETOC PUBLISHED REPORTS

The full catalogue of ECETOC publications can be found on the ECETOC website:

http://www.ecetoc.org/publications

Responsible Editor:
Mr Olivier de Matos
ECETOC AISBL
Rue Belliard 40
B-1040 Brussels, Belgium
VAT: BE 0418344469
www.ecetoc.org
D-2020-3001-259

Since 1978 ECETOC, is a collaborative space for leading scientists from industry, academia and governments to develop and promote practical, trusted and sustainable solutions to scientific challenges which are valuable to industry, as well as to the regulatory community and society in general.