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A decision tree for assessing effects from exposures to multiple substances

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Abstract

Background: The Cefic Mixtures Industry Ad-hoc Team (MIAT) has investigated how risks from combined exposures can be effectively identified and managed using concepts proposed in recent regulatory guidance, new advances in risk assessment, and lessons learned from a Cefic-sponsored case study of mixture exposures.

Results: A series of tools were created that include: a decision tree, a system for grouping exposures, and a graphical tool (the MCR-HI plot). The decision tree allows the division of combined exposures into different groups, exposures where one or more individual components are a concern, exposures that are of low concern, and exposures that are a concern for combined effects but not for the effects of individual chemicals. These tools efficiently use available data, identify critical data gaps for combined assessments, and prioritize which chemicals require detailed toxicity information. The tools can be used to address multiple human health endpoints and ecological effects.

Conclusion: The tools provide a useful approach for assessing risks associated with combined exposures to multiple chemicals.

Keywords: Combined exposures, Cumulative exposures, Mixtures, Maximum cumulative ratio, Human health, Ecotoxicity

Background

Responding to the challenge of exposures to multiple chemicals from multiple sources

Human and ecological receptors are continuously exposed to multiple chemicals; however, chemicals have traditionally been regulated on a chemical-by-chemical basis. As a result, there is a possibility that are instances where chemicals independently do not cause adverse effects, but in combination they could pose a risk to human health and the environment. A number of organizations have investigated the effect of combined exposures to multiple chemicals and provided guidance on how risks from these exposures could be assessed [1-5].

The Mixtures Industry Ad-hoc Team (MIAT) was created by the European Chemical Industry Council (*Conseil Européen des Fédérations de l'Industrie Chimique,* Cefic) to help address the concerns associated with combined exposures to multiple chemicals. In 2010 the MIAT began development of a decision tree to guide the assessment of risks from combined exposures. This paper presents the results of this effort. The tree draws on concepts developed by the World Health Organization (WHO) [1,2], the European Commission Non Food Scientific Committees (SCs) [6], and industry-sponsored research on the use of the Maximum Cumulative Ratio (MCR) [7,8]. An earlier version of the tree was applied to an assessment of human health and ecological risks from the combined exposure to chemicals measured in surface waters or municipal effluents discharged to surface waters. The feedback from this project led to the current version of the decision tree. The results from the application project are the subject of a companion paper to this publication [9].

Terminology and methodology

The combined effects of exposures to multiple chemicals have been the subject of discussion, research, and regulation by a number of organizations for more than

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50 years. One unfortunate result of this history is the proliferation of confusing and sometimes conflicting terminology [1,2]. In this paper the term "combined exposures" is defined as a receptor's exposure (where a receptor could be a person or another organism) to multiple chemicals that are received from either one or more sources by one or more routes. When doses of multiple chemicals are received from one source (multiple chemicals co-occurring in a medium or a commercial product) they can be referred to as a mixture exposure. Thus mixture exposures are a subset of combined exposures.

A number of quantitative methods are available for assessing the risks from combined exposures. In this paper we have used the Hazard Index (HI) and Hazard Quotient (HQ) approach [3,4]. Finally, dose additive models are used to assess risks to humans and concentration additive models are used to assess risks to ecological receptors. In this paper both models are referred to as additive models.

WHO/IPCS tiered approach to assessing combined exposures

One of the sources for the decision tree is the framework for risk assessment of combined exposures to multiple chemicals that was developed by the World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) [1,2]. The framework is based on a series of four tiers that begins with simple and conservative screening assumptions and moves to higher tiers as necessary (Figure 1). Each of the higher tiers involves a more refined assessment (i.e., less conservative and more accurate) than the previous tier but requires more resources, including additional exposure and toxicity data. The aim of the tiered approach is to screen out combined exposures of low concern as efficiently as possible so that resources can be focused on assessing exposures of greater concern. However, the approach does not outline a specific process for assessing a combined exposure.

Maximum Cumulative Ratio (MCR)

A second component of the decision tree is the use of the MCR to identify the optimum approaches for assessing and managing risks from combined exposures. The MCR is the ratio of the total toxicity a receptor receives from its combined exposures to multiple chemicals divided by the largest toxicity it receives from any single chemical [7,8]. MCR values range from one to n (where n is the number of chemicals that reach a receptor). The chemical with the largest contribution is referred to as the primary chemical. Values close to one indicate that the primary chemical dominates the toxicity of the exposures. Values less than two indicate that the primary chemical provides more than 50 percent of the toxicity for the receptor. Values of n indicate that the receptor is exposed to equitoxic doses of all chemicals.

European Commission (EC) Scientific Committees' Approach for assessing mixtures

In June 2011, an approach was developed for the assessment of mixtures in the form of a decision tree by the Scientific Committee (SCs) of EC and published in final form (Figure 2) at the end of 2011 [6]. The decision tree is intended to address combined exposures to multiple chemicals from one or more sources; however, many of the steps in the tree are only appropriate for the evaluation of mixtures exposures. For human health, the decision tree proposes to first use the threshold of toxicological concern (TTC) to screen out mixture

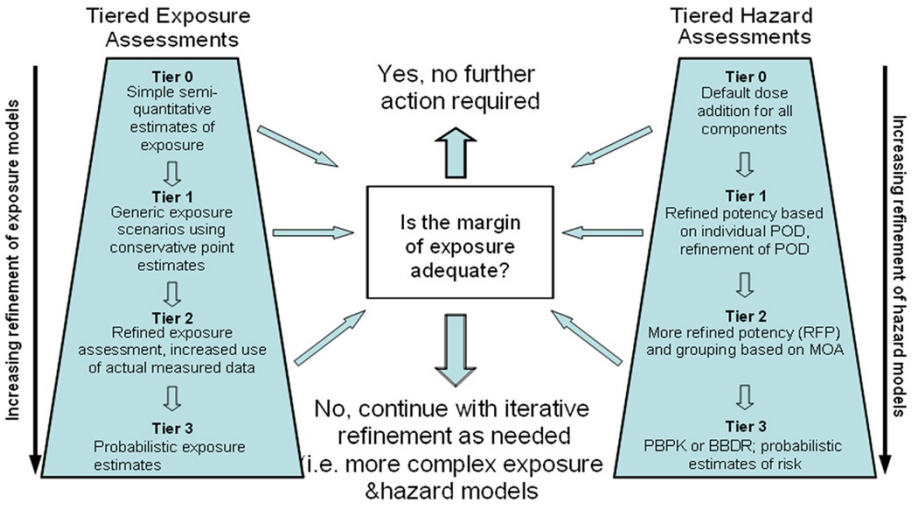

Figure 1 WHO/IPCS framework for assessing combined exposures to multiple chemicals from multiple sources [1].

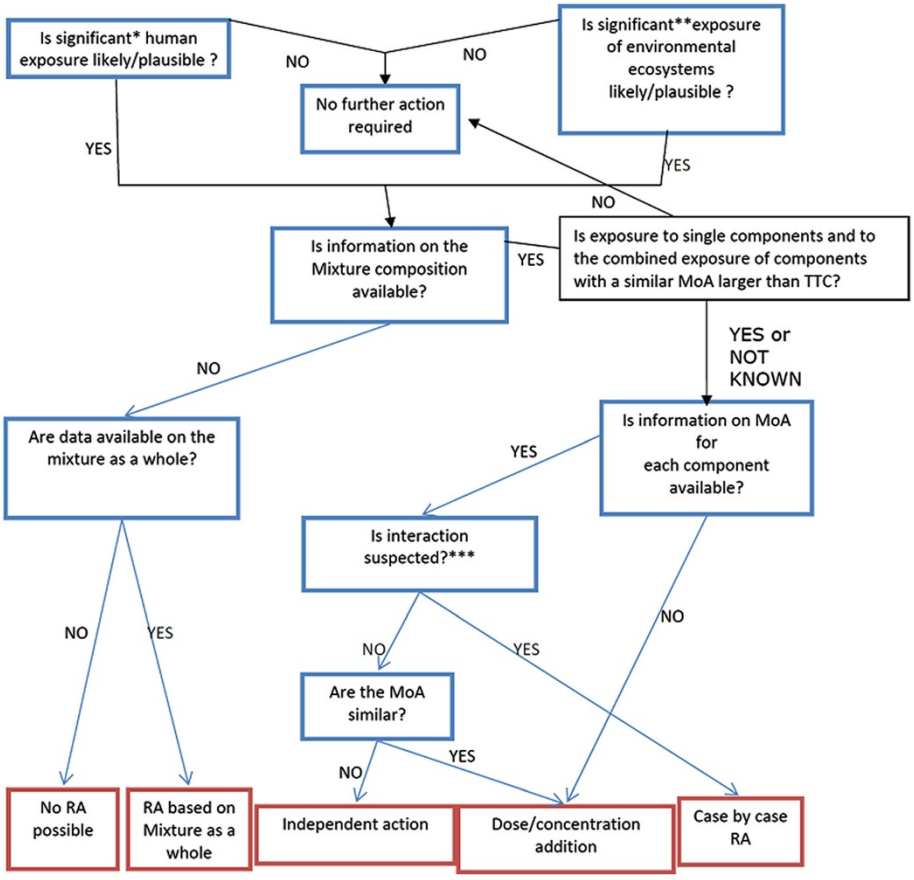

Figure 2 Scientific committees decision tree for the risk assessment of mixtures [6].

exposures of low concern. Existing toxicity data are then used to recommend specific approaches for characterizing mixture toxicity (addition models, independence models, case-by-case assessments, or use of whole mixture toxicity data). The tree does not address the challenges of choosing between addition or independence models when only minimal data are available nor does it provide options for further refining assessments when addition models are preferred.

Cefic LRI project

In early 2011, Cefic through its Long Range Initiative (LRI) program funded a project to apply an earlier version of the decision tree to a real world example of combined exposures. This project was performed by a team of experts from industry, consulting, and academia [9]. In this project, the decision tree was applied to mixture exposures for humans and environmental receptors. The mixtures were measured in surface waters and municipal effluents discharged to surface waters. During this project, a number of issues with the early version of the tree were identified by project members. This led to a revision of the tree over the course of the project. The revised version was used in the publication of that project [9] and this paper.

One of the results of the LRI project was the recognition that data on MCR and HI values obtained from a WHO/IPCS Tier 1 (or Tier 0) assessment could be used to group combined exposures into categories that facilitated risk assessment and risk management decisions. Four groups of combined exposures were identified.

- *Group I* are combined exposures that are a concern because one or more individual chemicals are a concern (dose or concentration of one or more chemicals exceed the corresponding reference value (RV)).
- *Group II* are combined exposures where there is a low concern for both individual chemicals and for their combined effects (HI is less than one); and
- *Group III* are combined exposures where there is a low concern for individual chemicals but there is a concern for the combined effects (all HQs are less than one but the HI is greater than 1). This is the critical group for further assessments since the concern for these exposures cannot be identified

using a chemical-by-chemical approach. This group is further divided into *Group IIIA* where one chemical provides the majority of toxicity of the combined exposures (MCR is less than two) and *Group IIIB* where no one chemical dominates the toxicity of the exposures (MCR is greater than two).

The value of this exercise that each of the four groups requires different strategies for managing combined effects. ***Group I*** exposures would have been identified as a concern on a chemical-by-chemical basis. Efforts to refine the assessment of combined exposures or to reduce the receptors' exposure need to focus on the chemicals that are a concern before addressing risks from the combined exposures. **Group II** exposures can be set aside as a low concern. ***Group IIIA*** exposures have one chemical that is responsible for the majority of the toxicity received by a receptor and that chemical should be the focus of either refining the risk assessment or reducing exposure. In ***Group IIIB*** exposures no one chemical dominates the concern for risks from combined exposures. These exposures are most affected by the default assumption that all chemicals follow addition models. Therefore, refining the assessment for these exposures should focus on determining the modes of action (MoAs) for the chemicals that drive the toxicity of exposures and using these data to refine the assessment.

Results

Based upon the information discussed above, the Cefic MIAT and the LRI project team have developed tools for assessing risks from combined exposures. These include a decision tree, a conceptual framework for addressing combined exposures, and a method of graphically presenting the results of the assessments of the risks from combined exposures.

The toxicological effects of combined exposures can be evaluated using a number of methodologies including the Hazard Index/Hazard Quotient (HI/HQ), Margin of Exposure, Toxicity Equivalents, and Toxic units [5]. This paper will use the HI/HQ approach.

Description of the decision tree

Figure 3 presents the proposed decision tree. The components of the tree are colour coded to indicate the different portions of the tree are derived from prior efforts to assess mixtures and combined exposures. This identification does not imply that a given portion of the tree is an exact copy of the earlier work but that the portion was based on the earlier work and is intended to perform a similar function. The red portion is based on the SCs approach [6], blue is based on the WHO Framework [1,2], and black is based on concepts related to the MCR [7,8].

The following is a detailed discussion of the individual steps for the tree.

Step 1. Is human/ecological exposure likely/plausible?

A combined exposure assessment begins with the definition of the exposed population that is the subject of the assessment. Beginning with exposure allows the avoidance of assessments of groups of chemicals that have common endpoints but do not co-occur in the real world. To achieve this, an assessor should follow the WHO/IPCS problem formulation step [1,2] and define the scope of the assessment including the timeframe, sources, and routes of exposures, together with the chemicals involved. This Step does not require a determination that the co-exposures are significant on a risk basis, since this would require consideration of toxicological information. Such issues are considered in Steps 4, 11, 13, and 16 of the decision tree. At the end of this Step 1 the assessor should have determined that a group of receptors are likely to be co-exposed to specific chemicals over an appropriate time frame.

Step 2. Using whole mixture toxicity data

This Step determines if the assessment of the cumulative exposures can be evaluated using toxicity data for a specific mixture of chemicals. The step reflects the relative importance of whole mixture toxicity data in assessments of combined exposures. When performing an assessment of combined exposures to a discrete mixture, such as a formulated consumer product, whole mixture toxicity data has historically been given a higher priority than component-based approaches for human health [4,10]. The reason for preferring whole mixture data is that such data capture any effects due to interactions that may occur between chemicals and contributions from compounds that have not been measured or for which toxicity information is lacking (e.g. formulation additives in plant protection products) [11].

Whole mixture toxicity data play an important role in assessing human exposures to mixtures with fixed compositions (e.g., a specific combination of chemicals in air, water, a food item, or consumer product). Whole mixture data are relatively common for intentional mixtures such as formulations (e.g., household or plant protection products) or extracts from natural sources such as plants. Data are also available for commercial mixtures where compositions of the mixtures are relatively constant over time (e.g., petroleum products).

Whole mixture toxicity data on specific formulations are less relevant in the assessment of effects from combined exposures to environmental receptors. Generally formulations do not reach environmental receptors in the way that a consumer product reaches humans. Waste water discharges or air emissions emitted to the

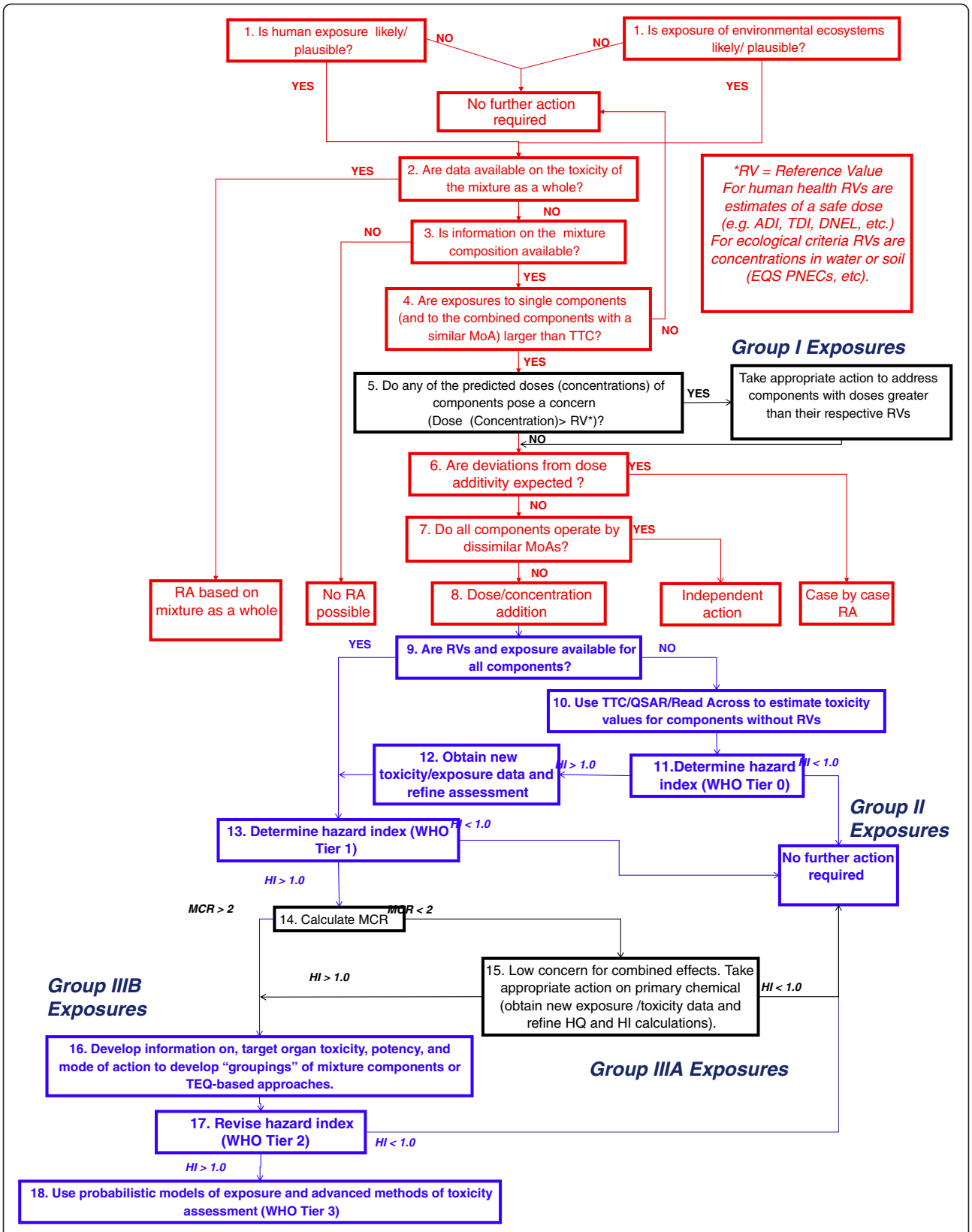

Figure 3 The MIAT decision tree. Colours indicate the origin of steps. Red are steps based on SCs decision tree [6]. Blue are based on the IPCS/WHO framework [1,2] and black on MCR [7,8].

environment tend to be complex time-varying combinations of chemicals from many sources. Once released, the different environmental fate properties of the various components of a mixture result in changes in composition as the mixture moves through the environment.

Whole mixture toxicity data are valuable when used in combination with component based approaches for retrospective risk assessments. For example whole mixture data may give a better estimate of the environmental risks from estrogenic mediated effects than a component based approach. Because the effect concentrations of human or synthetic hormones are often below the limits of quantitation (LOQs) of analytical methods, these substances are analytically difficult to monitor but their effects can be measured with specifically designed assays [12].

Two issues need to be kept in mind when using whole mixture data in either human health or ecological assessments. First, such data are only relevant when a receptor's exposures come from a single source. When combined exposures come from multiple sources the relative doses of the chemicals will vary across exposed receptors. As a result, no single mixture can represent the receptors' exposures. Second, whole mixture toxicity data can only be used for endpoints that are measured in the study of the mixture. As a result, when a component-based approach identifies a critical endpoint of concern that was not addressed in the whole mixture toxicity study, a component-based approach should be used.

In summary, when whole mixture toxicity data are available on relevant endpoints, they should be considered since they provide information on the toxicity of all chemicals present in a given medium. If they are not available, or are not sufficient, the assessment of combined exposures should move to Step 3.

Step 3. Mixture composition

In this Step of the decision tree the assessor determines if data on the identity and quantity of the chemicals reaching the population of concern are adequate to support a risk assessment. The absence of data could be due to the lack of information on the composition of chemicals released from a specific source, the number and nature of the sources of exposure, or the doses that the receptor receives from a source. If such data on identity and quantity are not available, and if whole mixture toxicity data are not available (or not applicable), an assessment of the combined exposures is not possible. In these situations, the assessor should collect additional data on the sources, exposed populations, and exposure pathways, and perform additional monitoring or modelling to determine the doses of chemicals reaching a receptor.

Step 4. Are exposures larger than the TTC?

This Step is based on the recognition that if the doses reaching a receptor are sufficiently small, it may be possible to reach a conclusion that no further action is required despite the absence of specific data on the toxicity of the compounds. Such a Step is based on the assumption that there are doses of little or no concern for all chemicals. In order to perform Step 4 it is necessary to perform a combined exposure assessment to determine the doses that reach a receptor. This exposure assessment will typically use a conservative screening approach that requires minimal information on the exposure. These doses are then compared to levels that are believed to be of minimal concern. The value of Step 4 is that assessors can set aside combined exposures to chemicals that occur at very low doses. If a level of minimal concern cannot be defined or it is exceeded by the exposures, the assessor will skip this Step and go to Step 5.

The SCs proposed to exclude combined exposures to chemicals if all chemicals, when grouped by common MoAs, do not result in doses that exceed the relevant Threshold of Toxicological Concern [13,14]. This approach is a reasonable example of using an existing tool to identify exposures of low concern based only on the chemicals' structure and has been shown to be protective for mixtures [15]. It must be noted that, while several researchers have proposed to establish a TTC for ecological effects [12,16], there is currently no widely accepted equivalent of the TTC in ecotoxicology and methods for setting levels of minimal concern for ecological effects need further development. In the human health area, the applicability range of the TTC will benefit from modifications and extensions of the tool that address inhalation and dermal routes of exposure, see for example the work of Escher et al., [17].

Step 5. Do any chemicals pose unacceptable risks?

Step 5 is based on the concept that assessments of exposures to multiple chemicals should include the determination of whether the receptor is exposed to one or more chemicals at levels that raise concern, i.e., levels that exceed chemicals' reference values (RVs). Such combined exposures are called ***Group I*** exposures. There is no need to perform an assessment of the combined effects of the Group 1 to determine if they pose a concern, since if any one chemical poses a concern, the effects of the combined chemical exposures will always pose a concern when either an additive or independent model is used. This finding is made using the screening exposure assessment performed in Step 4.

Instead of proceeding further with the Group 1 exposures the assessor should determine if the concerns from the individual chemicals in the screening exposures

assessment are warranted. This will be done by performing a higher tier exposure or toxicity assessment on those individual chemicals that exceed their RVs. If the chemical-specific concerns for a receptor are removed by the refined assessments, the assessor would not assume that the combined exposure was without concern. This is necessary, because refining the risk assessments for chemicals that had individual concerns will not necessarily result in acceptable risks from combined exposures. As result the refined exposures would move on to step 6. Finally, exposures to chemicals without RVs are not evaluated in this step. These exposures are evaluated in Steps 11-13 of the decision tree.

Step 6. Considering data on toxicological interactions that deviate from dose additivity

In Step 6, the decision tree considers if there are data on the components that suggest an interaction other than dose additivity (i.e. antagonism, potentiation, or synergy). If interactions are known to occur, the risk assessment should be done on a case-by-case basis that incorporates these data. The SCs suggested, for example, that if whole mixture toxicity data are available it could be used as a test for a supra-additive response [9].

As discussed by ECETOC [18,19] and Kortenkamp [20], supra-additive responses are expected to be rare. Boobis et al. [21] provided additional support for this finding in a detailed review of the literature on the toxicity of mixtures. Therefore, this Step does not require a finding that such interactions do not occur; it merely requires that if interaction data are available they will be considered in the assessment. In the absence of interaction data, the exposures move to Step 7.

Step 7. Consideration of independent action models of toxicity

In Step 7, the assessor determines if independent action (also called response addition) should be assumed for the combined exposures. Such a decision requires information on the mode of action (MoA) of each chemical in the assessment and an evaluation that all the chemicals' effects are independent of one another. Such data could be available for certain formulations (e.g. consumer products, pesticides, etc.) where all of the chemicals have their respective data sets. In the absence of such data the assessment would move to Step 8.

The decision to use an independent action model will result in different modelling approaches depending on the endpoint. Following an independent action model, joint exposure to chemicals where each chemical's dose is below a receptor's threshold for the chemical's effects will result in no adverse effect to the receptor. For human health risks, where RVs are generally well below the thresholds for sensitive humans [22], such a finding would indicate that no further action is required. This occurs because all exposures that reach this point in the decision tree have been shown to produce doses of chemicals that are lower than the chemicals' RVs (see Step 5).

This may not be the case, however, for certain ecological effects. The goals for protecting the environment are often based on population- or community-level effects. Consequently, the RV may be at a level at which some effects on individual organisms are tolerated. As a result, the effects from chemicals that operate independently may still accumulate because of response addition. Calculation of the impact of response addition is difficult for many chemicals due to the extensive dose-response dataset being required. As a consequence, concentration addition models have been used as a conservative default approach for characterizing the impact of response addition [9,19].

Steps 8 and 9. Evaluation of available toxicity and exposure data

In Steps 8 and 9 the decision is made to perform a WHO/IPCS Tier 0 screening assessment (Step 10) or, if there are sufficient toxicity and exposure data, to support a WHO/IPCS Tier 1 assessment (Step 13) [1,2]. Both steps use a dose addition model for all chemicals in the assessment. Tier 0 assessments are the preferred option for combined assessments when specific toxicity or exposure data are not available for some, or all, of the chemicals of interest. (A Tier 1 assessment (Step 13) requires RV for each chemical and information on exposure to estimate the dose for each chemical). In addition, a Tier 0 assessment can help minimize the effort of performing a Tier 1 assessment by screening out exposures that produce low concern based on a finding of very low doses for all chemicals reaching a receptor. However, because Tier 1 assessments are more effective than Tier 0 assessments in screening out exposures of low concern, assessors typically choose to skip a Tier 0 assessment and perform a Tier 1 assessment when necessary data are available (Step 13).

Steps 10-12. Performing a Tier 0 assessment

An example of a useful tool for conducting a Tier 0 assessment for human health effects is the use of the TTC to estimate the RVs for chemicals with missing toxicity information [2,15]. Other approaches could be used including QSARs or read across arguments [2]. Combined exposures that are screened out (HI values less than one) are placed into ***Group II*** (combined exposures of low concern). Combined exposures not screened out by a Tier 0 assessment (HI values greater than) should be evaluated to determine if additional information on exposure and/or toxicity can be identified or developed that would support a Tier 1 assessment.

Step 13. Performing a Tier 1 assessment

In this step, a screening assessment is performed using the RV for each chemical and using conservative estimates of exposure that would be reasonably protective of the most exposed individual receptors in an exposed population [1,2]. If a Tier 0 assessment cannot be performed for the whole combination of chemicals and RVs are missing for some of the chemicals, a Tier 1 assessment can be performed on the remaining chemicals. However, the results of such an assessment will be uncertain due to the absence of the contributions of the chemicals with no RVs. If the fraction of chemicals whose RVs are missing is small, this uncertainty may be acceptable. If the fraction is large, a combined assessment may not be possible. Combined exposures that are screened out (HI values less than 1) are placed into ***Group II*** (combined exposures are of minimal concern) and remaining exposures move to Step 14.

Step 14. Determination of MCR values

Combined exposures reaching this Step have been shown to have passed a chemical-by-chemical assessment (Step 5), but failed a screening assessment of toxicity of combined exposures (Step 13). These exposures are placed into ***Group III***. This group should be the focus of assessments of combined exposures since they are the exposures of potential concern that are missed by chemical-by-chemical approaches. In Step 14, the Maximum Cumulative Ratio (MCR) is determined for these chemicals. Exposures with MCR values of less than two moves to Step 15 and those with values greater than two move to Step 16.

Step 15. Evaluation of primary chemicals for Group IIIA

Combined exposures with MCR values less than two are placed into ***Group IIIA***. These exposures have one chemical (the primary chemical) that contributes the majority of the toxicity of the mixture. Efforts to refine the estimates of toxicity and/or exposure (WHO Tier 2 or 3 assessment), or to reduce exposures should focus on this primary chemical. Once this is done, the HI value is revised (Step 16). If the revised value is less than 1, the exposure is moved to ***Group II***; if not, the combined exposure is moved to Step 17.

Steps 16 and 17. Performing Tier 2 combined exposure assessment on Group IIIB

Combined exposures that have MCR values greater than two are placed in ***Group IIIB***. The estimates of combined exposure are driven by the contributions of multiple chemicals. Refining the risk estimates for these exposures will require additional toxicity and exposure data on multiple compounds. Risk estimates for these exposures are very dependent on whether the effects of individual chemicals follow additive models or follow independent action models. Therefore, the chemicals in these combined exposures that reach this step should be the focus for efforts to use MoA data. In Step 17, the assessor groups chemical exposures based on data on the MoA of the chemicals (WHO/IPCS Tier 2 assessment). Grouping chemicals based on MoA allows the refinement of the assumption of additivity [2,5]. Following this grouping, the exposures are reassessed (Step 18).

In Step 17, MoA information is used for the second time in the decision tree. Step 7 generally assessed whether all components of a co-exposure can be assumed to act dissimilarly. In this application, the MoA is used on the chemicals where MoA information has been shown to make a difference in the assessment. This is because in many instances the vast majority of chemicals contribute little to an estimate of the toxicity for the receptor. Whether these chemicals are assumed to follow additive or independent models will not significantly affect the estimate of the toxicity associated with the combined exposure. As a result, MoA information on these chemicals is of little value to risk managers. In Step 17, the MoA is only applied on the chemicals where MoA information has been shown to make a difference in the assessment. As shown in the example in the companion paper [9], this subset may only involve a small fraction of the chemicals that reach an individual.

Ecotoxicity assessments performed in this Step differ from the human health assessments in two ways. First, as discussed above, when setting up groups based on MoA, assessors need to address the issue of response addition for chemicals that have different MoAs. Second, if the ecological risk assessment is at an ecosystem level (i.e., using RVs such as PNEC or EQS values) consideration should be given to refining the assessment by performing assessments on specific receptors (e.g. algae, macrophytes, invertebrates, and fish). As discussed above, the RVs for ecological effects are usually based on the most sensitive receptor which varies across different chemicals. As Backhaus and Faust indicated, it is a crude simplification to add the impact of a chemical where the most sensitive receptor is fish to one where the most sensitive receptor is an invertebrate (25). However, the decision to refinement should also consider the potential for indirect effects on ecological systems.

Once the MoA data have been used to make the groupings, the assessor determines the HI values for each group. If the largest HI of any of the groups is less than one, the exposures are moved to ***Group II***. If one or more groups are greater than one, the combined exposures move on to a WHO Tier 3 assessment (Step 18).

Step 18. Use of advanced dose response modelling and probabilistic models to account for variations in exposure and toxicity

In this step, the assessor focuses on the chemicals and groups of chemicals that drive toxicity. Advanced techniques such as biologically-based dose response models are used to refine the RVs and probabilistic models to account for uncertainties and variabilities in exposure and dose-response are used to refine the risk estimates for the chemicals that drive the value of HI for the combined exposures.

Framework for applying the decision tree to assessing combined exposures

As discussed above, combined exposures present a challenge to regulators because of their inherent complexity. Combined exposures depend not only on the characteristics of the sources, but also on those of the populations of receptors. Each individual in these populations will have a unique relationship to their sources of exposure [23,24]. Thus, a decision tree needs to be designed for evaluation of populations of exposed receptors and not around a specific source (or sources). Such populations could be exposed to multiple combinations of discrete mixtures within a relevant time frame. The implications of this finding have not always been recognized by the earlier approaches. Earlier approaches have tended to treat all combined exposures as if they were due to a mixture exposure and were a property of a specific source.

Assessments of combined exposures should begin by defining the population that is the subject of the assessment. The population can be defined as groups of humans that are in contact with a specific source in a specific way or can be defined in terms of demographics [23]. The populations of ecological receptors can be based on the trophic levels relevant for a source or sources of exposure. This implies that multiple combined exposure assessments will be required for human health and ecological receptors and multiple assessments maybe required for both human health and ecological effects. Human health assessments may need to address the combined exposures that occur by different routes and over different durations. For example, separate assessments may be required for local acute exposure (e.g., dermal and eye effects) and for chronic systemic effects because the RVs and exposures received by the exposed population will be different for the different endpoints. Separate human health assessments may be required if human exposures cannot be represented by a single population.

Ecological assessments may also need to be performed separately for each of the taxonomic groups that are used as the receptors. This will be a challenge for ecological assessments since Environmental Quality Standards (EQS) can be based on the protection of the most sensitive taxonomic group and that group will vary across chemicals. Using EQS values based on multiple receptors, however, could be used as a screening assessment for ecotoxicity endpoints (Steps 11 and 13) and refined later in the decision tree (Step 18).

Because multiple applications of the decision tree will be required for assessing exposures from a source or group of sources, the decision tree may lead to different approaches for assessing combined risks for human and ecological assessments. For example whole mixture toxicity data may be available for dermal or eye effect in humans or endocrine activity in ecological receptors, while component based approaches may be used for the evaluation of systemic human health or ecological effects. An example of the use of different approaches for human health and ecological effects can be seen in the companion paper [9].

Maximum cumulative ratio-hazard index plots

As discussed above, the decision tree divides combined exposures into four groups (I, II, IIIA, and IIIB) These four groups can be graphically depicted by plotting the combined exposures' values of MCR against the corresponding HI values (an MCR-HI plot). In these plots, MCR values are plotted in a linear scale with a minimum value of 1 on the vertical axis. Since HI values typically vary over several orders of magnitude, HI values are plotted in log scale and are plotted on the horizontal axis.

A combined exposure that falls into ***Group I***, requires that Maximum Hazard Quotient (MHQ) must exceed one. Since MCR is defined as HI/MHQ, MHQ will only exceed one when MCR is less than HI. Therefore, ***Group I*** exposures fall into the region to the right and below the function MCR = HI (since this is a log-linear plot, this linear function appears as a curve instead of a line). ***Group II*** exposures fall to the left of the vertical line, HI =1. Group III exposures fall into the region between the two lines. ***Group IIIA*** exposures are in the portion of this region below the horizontal line, MCR = 2, and ***Group IIIB*** falls in the portion of this region that is above the line.

Figure 4 presents the MCR-HI plot for the assessment of the ecological effects from chemicals in surface waters and in effluents reported in the companion paper [9]. As the figure indicates, the 68% of the values of the reported mixtures fell into **Group I**, 19% fell into **Group II**, 6% fell into ***Group IIIA*** and 6% into ***Group IIIB***.

Discussion

The decision tree builds on, and is consistent with, existing approaches for mixture and combined exposures

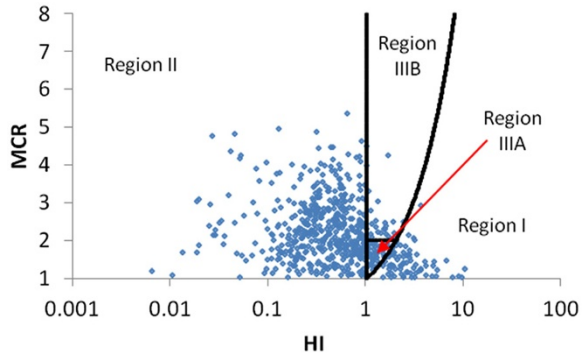

Figure 4 MCR/HI plot of MCR versus HI for ecological effects of mixtures of chemicals measured in surface water or WwTP effluents [9].

proposed by the WHO/IPCS, the European Commission, and other organizations. The decision tree is intended to address a wide range of combined exposures that include exposures to multiple chemicals from single or multiple sources that occur over time. The decision tree adopts precautionary assumptions to account for known sources of uncertainty. For example, the model follows the WHO/IPCS and European Commission SCs in adopting additive models as a default screening assumption when MoA information is not available on the relevant chemicals.

The decision tree adopts the tiered approach in the WHO/IPCS framework [1,2] to efficiently assess combined exposures. The tree allows the assessor to screen out combined exposures that are too low to pose a risk to humans or the environment when the appropriate tools are available (Step 4) and then use conservative assumptions to screen out combined exposures that can be shown to pose minimal risks (Steps 12, 13, 16, and 18). As a result, the exposures that are brought forward to the most detailed and resource-intensive assessments (Tiers 2 and 3) are minimized.

The combined exposures that are subject to higher tier assessments also focus on the chemicals that drive the toxicity of the combined exposures. This is a novel concept [7,8] not discussed in prior approaches for mixtures and combined exposures. This ability to focus is performed on a quantitative basis using the MCR. MCR values for exposures in ***Group IIIB*** give an indication of how many chemicals will drive the toxicity associated with the exposures. Values less than two indicate that only a few chemicals make important contributions to toxicity. When MCR values approach n (the number of chemicals that reach a receptor) all n chemicals will need to be investigated. In the example provided in Figure 4, the average value of MCR and n for ***Group IIIB*** exposures is 1.7 and 22 [9] indicating that toxicity is dominated by a few chemicals. In addition, it was shown that seven of the 89 chemicals analysed made significant contributions to ecotoxicity of the combined exposures of the mixtures. This finding can be confirmed by summing the HQ values of the components in order to cover a specific fraction of the total toxicity [25]. MoA data are only necessary for chemicals that drive toxicity, as the remaining chemicals can be conservatively assumed to follow additive models and not change the predicted HI values [26].

A second novel concept in the decision tree is the inclusion of Step 5. In this Step, the decision tree identifies combined exposures that are a concern because one or more chemicals exceed their RVs. This Step is a recognition that no further refinement of the combined exposures will result in a finding of acceptable risk unless the toxicity and exposure information on the identified chemicals are found to be individually acceptable. Thus, these combined exposures should first focus on the chemicals that exceed their RVs. Separating out these exposures allows the assessor to focus on combined exposures that would have been missed using a chemical-by chemical based approach.

Finally the tree introduces the four groups of combined exposures (I, II, IIIA, and IIIB) and the MCR/HI plot as a tool for organizing and presenting combined exposures data and how exposures vary across individuals in an exposed population. Or, as shown in Figure 4, how exposures vary across reported mixtures of chemicals in an environmental medium [9].

The decision tree also highlights areas where future work on assessing combined assessments is needed. Firstly, better tools are needed for screening out combined risks from low doses of multiple chemicals (Step 4) and for performing Tier 0 assessments. Currently, there is no widely accepted tool for predicting thresholds of ecological concern that would support a Tier 0 assessment. Secondly, performing a Tier 1 assessment of the risk from combined exposures requires RVs for all of the chemicals in the assessment. In many instances, the assessments will include one or more chemicals without RVs. As a result, combined assessments increase the demand for RVs for chemicals. Development of RVs remains a resource intensive process requiring extensive animal testing. More efficient and lower cost methods need to be developed to set RVs that can be used in Tier 1 assessments.

Conclusions

The decision tree described in this paper can be used as a helpful tool for conducting assessments of the risks from combined exposures to multiple chemicals. The decision tree can provide information on current combined exposures and identify when combined exposures are of potential concern and when using chemical-by-chemical assessments are sufficiently protective of human

health and the environment. The decision tree can be helpful for prioritization in assessing and managing risks associated with combined exposures to multiple chemicals.

Methods

The decision tree presented here is largely based on the sources discussed above [1,2,7-9]. However, other trees developed by U.S. EPA [4], European Food Safety Agency [27] and The Intergovernmental Group on Health Risks from Chemicals [3] were also evaluated. In general, these trees are consistent with the WHO/IPCS framework and the European Commission's SCs tree. As discussed above, the decision tree was developed over 2010-2012 based on wide ranging discussions between MIAT members. These experts had extensive experience with evaluation of combined exposures and risks. In addition, comments on the tree were provided by the research team for the application project [9].

Finally, we note that many of the concepts in the decision tree are included in a recent publication of Backhaus and Faust [28] on environmental risk assessment for chemical mixtures. These include the use an additive model as an initial tier, and refining the assessment using receptor specific assessments.

Definitions

Addition models Chemicals have the same effect on the target organism and differ only in potency; hence the combined effect of two agents can be estimated from the potency weighted total dose of both agents. For ecological effects additive models are referred to as concentration addition models.

Combined exposures The measurements of the doses of multiple chemicals that reach an individual or an ecological receptor from one or more sources by one or more routes at the same time or within a period of time sufficiently short that the substances simultaneously evoke effects on the organism [24].

Effluents Discharges to surface water from waste water treatment plants (WWTP) following treatment.

Independent joint action If two or more substances elicit the same endpoint via different modes of action the combined effect can be estimated by the Bliss independence model [29]. Also called response-additivity.

Mixture A combination of chemicals present in a medium (e.g., a consumer product or an environmental medium).

Mixture exposure The combination of doses received by a receptor from exposure to a mixture.

Primary chemical The chemical that contributes most to the toxicity of a mixture (e.g., the chemical with the largest HQ value for a receptor).

Receptor For human health a population of individuals receiving the cumulative exposure. For ecological assessments-an organism, taxonomic group or environmental compartment.

Tier 0 Assessment Defined in WHO approach to mixtures as an initial approach to assessing toxicity (or exposure) where data gaps are filled using conservative assumptions [2].

Tier 1 Assessment Defined in WHO approach to mixtures as an initial approach to assessing toxicity (or exposure) where data on toxicity and exposure data are available for each component of a mixture [2].

Tier 2 Assessment Defined in WHO approach to mixtures as a refined assessment that only assumes additivity within groups of chemicals where a common mode of action occurs [2].

Tier 3 Assessment Defined in WHO approach to mixtures as an advanced assessment of cumulative risks that uses techniques such as biologically based dose response and probabilistic models of variation in dose and susceptibility [2].

Abbreviations

Cefic: Conseil Européen des Fédérations de l'Industrie Chimique (European Chemical Industry Council); EC: European Commission; HI: Hazard Index: The sum of the hazard quotients when dose addition is assumed; HQ: Hazard Quotient: The ratio of the dose of a chemical reaching an individual human or a population of organisms to the reference value of the chemical.; IPCS: International Programme on Chemical Safety; LOQ: Level of Quantification; MCR: Maximum Cumulative Ratio: The toxicity from the combined exposures to multiple chemicals divided the maximum toxicity from any one chemical; MHQ: Maximum Hazard Quotient: The largest HQ for a receptor; MIAT: Mixtures Industry Ad Hoc Team; MoA: Mode of Action: a common set of physiological and behavioural signs that characterize a type of adverse biological response; RV: Reference Value: In human health risk assessment, RVs are doses that are not anticipated to cause adverse effects in humans and are developed based on a point of departure divided by "uncertainty" or "adjustment" factors. Examples of human RVs are an ADI, TDI, or DNEL. In ecological risk assessment, RVs are concentrations in environmental compartments (soil, sediments, or water) that are not anticipated to cause adverse effects for receptors in the environmental compartments. Examples of RVs are Environmental Quality standards (EQS) or predicted no effect concentrations (PNECs). RV values may also be conservatively estimated using approaches such at the Threshold for Toxicological Concern or QSARs; SCs: EU Commission Scientific Committee on Consumer Safety, Scientific Committee on Health and Environmental Risks, and Scientific Committee on Emerging and Newly Identified Health Risks; WHO: World Health Organization; WwTP: Wastewater Treatment Plant.

Competing interests

Paul Price and Csilla Magyar, Ellen Dhein, Mick Hamer, Marjoke Heneweer, and Holger Penning, are employees of various companies that manufacture chemicals. Carlos Rodriguez is an employee of Procter & Gamble. Procter & Gamble uses chemicals in manufacturing many consumer products. The products of these companies are chemicals or contain chemicals that could be addressed in cumulative exposure assessments. The decision tree, however, is not about any specific chemical. The authors therefore declare that they have no competing interests. The remaining authors declare that they have no competing interests.

Authors' contributions

The following were members of the MIAT subgroup charged with developing the decision tree HP, PP, MH, HM, and CR. The tree is largely a product of their joint efforts. MH, CR, and HM performed research on existing regulatory policies on mixtures. PP was the primary writer for the

manuscript. MJ and PK provided extensive comments and recommendations on an earlier version of the tree that resulted in major changes in the tree with a main focus on environmental risk assessment. All of the authors made significant contributions to the drafting and editing of the manuscript. All authors read and approved the final manuscript.

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References

1. World Health Organization: *Harmonization Project Document 7; Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment*. Geneva: WHO; 2009.
2. Meek M, Boobis A, Crofton K, Heinemeyer G, Van Raaij M, Vickers C: **Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework.** *Regul Toxicol Pharmacol* 2011, **60**(S1-S14).
3. IGHRC: *Chemical Mixtures: A Framework for Assessing Risk to Human Health (CR14). The Intergovernmental Group on Health Risks from Chemicals*. Cranfield: Institute of Environment and Health, Cranfield University; 2009. Available at: http://ieh.cranfield.ac.uk/ighrc/Chemical%20Mixture%20Final%20May%202009.pdf.
4. U.S. EPA: *Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Office of Research and Development*. Washington, DC: EPA; 2000. 630/R-00-002.
5. U.S. EPA: *Concepts, methods and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: A resource document*. Cincinnati: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment; 2007 [EPA/600/R-06/013F].
6. Scientific Committees Scientific Committee on Consumer Safety (SCCS) Scientific Committee on Health and Environmental Risks (SCHER) Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR): *Toxicity and Assessment of Chemical Mixtures, European Commission*. Brussels: DG Health & Consumers; 2011.
7. Price P, Han X: **Maximum Cumulative Ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment.** *Int J Environ Res Public Health* 2011, **8:**2212–2225. doi:10.3390/ijerph8062212. http://www.mdpi.com/1660-4601/8/6/2212/.
8. Han X, Price P: **Determining the maximum cumulative ratios for mixtures observed in ground water wells used as drinking water supplies in the United State.** *Int J Environ Res Public Health* 2011, **8**(12):4729–4745. doi:10.3390/ijerph8124729. http://www.mdpi.com/1660-4601/8/12/4729/.
9. Price P, Han X, Junghans M, Kunz P, Watts C, Leverett D: *An application of a decision tree to the assessment of human and ecological effects from exposures to chemical mixtures observed in surface waters and waste water effluents*. Europe: Submitted Environmental Sciences; 2012.
10. UNECE: *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*. Fourthth edition. Geneva, Switzerland: United Nations Economic Commission for Europe; 2011.
11. Coors A, Frische T: **Predicting the aquatic toxicity of commercial pesticide mixtures.** *Environmental Sciences Europe* 2011, **23:**22. doi:10.1186/2190-4715-23-22.
12. Gross M, Daginnus K, Deviller G, De Wolf W, Dungey S, Galli C, Gourmelon A, Jacobs M, Matthiessen P, Micheletti C, Nestmann E, Pavan M, Paya-Perez A, Ratte H-T, Safford B, Sokull-Kluttgen B, Stock F, Stolzenberg H-C, Wheeler J, Willuhn M, Worth A, Comenges J, Crane M: **Thresholds of toxicological concern for endocrine active substances in the aquatic environment.** *Integrated Environmental Assessment and Management* 2010, **6**(1):2–11.
13. Kroes R, Renwick A, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A: **Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet.** *Food Chem Toxicol* 2004, **42**(1):65–83.
14. Munro I, Ford R, Kennepohl E, Sprenger J: **Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern.** *Food Chem Toxicol* 1996, **34**(9):829–867.
15. Price P, Hollnagel H, Zabik J: **Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity.** *Risk Anal* 2009, **29**(11):1534–1548.
16. De Wolf W, Siebel-Sauer A, Lecloux A, Koch V, Holt M, Feijtel T, Comber M, Boeije G: **Mode of action and aquatic exposure thresholds of no concern.** *Environ Toxicol Chem* 2005, **24**(2):479–485.
17. Escher SE, Tluczkiewicz I, Batke M, Bitsch A, Melber C, Kroese ED, Buist HE, Mangelsdorf I: **Evaluation of inhalation TTC values with the database RepDose.** *Regul Toxicol Pharmacol* 2010, **58**(2):259–274.
18. ECETOC: *Effects of Chemical Co-exposures at Doses Relevant for Human Safety Assessments. Technical Report No. 115*. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 2012. ISSN-0773-8072-115.
19. ECETOC: *WR 22: Workshop on Combined Exposure to Chemicals*. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 2011. ISSN-2078-7219-22.
20. Kortenkamp A, Backhaus T, Faust M: *State of the Art Report on Mixture Toxicity Final Report. U.K.*. European Commission, DG Environment Contract Number 070307/2007/485103/ETU/D.1, 22: 2009.
21. Boobis A, Budinsky R, Collie S, Crofton K, Embry M, Felter S, Hertzberg R, Kopp D, Mihlan G, Mumtaz M, Price P, Solomon K, Teuschler L, Yang R, Zaleski R: **Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment.** *Crit Rev Toxicol* 2011.
22. Gaylor D, Kodell R: **Percentiles of the product of assessment factors for establishing probabilistic reference doses.** *Risk Analysis* 2000, **20**(2):245–250.
23. Price P, Chaisson C: **A conceptual framework for modeling aggregate and cumulative exposures to chemicals.** *J Expo Anal Environ Epidemiol* 2005, **15:**473–481. doi:10.1038/sj.jea.7500425.
24. Rice G, MacDonell M: **Chemical Mixtures in the Environment: Exposure Assessment Chapter 2**. In *The Principles and Practice of Mixtures Toxicology*. Edited by Moiz Mumtaz. UK: Wiley-VCH; 2010.
25. Kortenkamp A, Faust M: **Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment.** *Internat J Androl* 2010, **33:**463.
26. Junghans M, Backhaus T, Faust M, Scholze T, Grimme L: **Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures.** *Aquat Toxicol* 2006, **76:**93–110.
27. EFSA: **Guidance document on risk assessment for birds and mammals.** *EFSA journal* 2009, **7**(12):1438. European Food Safety Authority, Parma, Italy.
28. Backhaus T, Faust M: **Predictive environmental risk assessment of chemical mixtures: a Conceptual framework.** *Enviro Sci Technol* 2012, **46:**2564–2573.
29. Bliss C: **The toxicity of poisons applied jointly.** *Ann Appl Biol* 1939, **26:**585–615.

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