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Harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals

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BRIEFING NOTES FOR DISCUSSION GROUPS

These briefing notes are prepared to provide participants with the relevant background information so as to be prepared for an interactive exchange of views and expertise during the Colloquium.

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Background

Human and ecological risk assessment of combined exposure to multiple chemicals ("chemical mixtures") poses several challenges to scientists, risk assessors and risk managers, particularly from the large number of chemicals involved and their associated exposure patterns and toxicological profiles in humans and other species present in the environment. In principle, methodological frameworks for human and ecological risk assessment use tiered approaches for exposure assessment, hazard assessment and risk characterisation. Overall the tiers range from qualitative/semi-quantitative tiers to fully probabilistic tiers – the choice of the tier often depending on data availability – and the purpose of the risk assessment.

In the human health area, EFSA's Panel on Plant Protection Products and their Residues (PPR) and the scientific Panel on Contaminants in the Food Chain (CONTAM) have developed risk assessment methodologies for pesticides with a similar and a dissimilar mode of action (MoA) and methodologies for the human risk assessment of combined exposure to multiple contaminants respectively. Recently, a scientific report of EFSA on "Modern methodologies and tools for human hazard assessment of chemicals" illustrated the potential applications of physiologically-based models, OMICs and in silico tools for the hazard assessment of combined exposure to multiple chemicals.

In the environmental risk assessment area, a number of activities at EFSA have proposed methodologies to deal with combined toxicity of pesticides in bees, including the scientific opinion on "The science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees)" by the PPR panel and the recent EFSA report "Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations".

The development of harmonised methodologies for combined exposure to multiple chemicals is an important element in EFSA's Science Strategy 2012-2016 and a number of activities have been undertaken over the years at EFSA to support such harmonisation. In this context, a recent EFSA report reviewed the available "international frameworks dealing with human risk assessment of combined exposure to multiple chemicals" and made a number of recommendations for future work in the area to move towards harmonisation of methodologies. These recommendations were identified in consultation with EFSA's panels, units and Scientific Committee (SC) and included data collection in the area of human, animal and environmental toxicology of mixtures for substances of relevance to EFSA.

These recommendations have already been taken on board and two procurements are ongoing in this area. Furthermore, the SC of EFSA has identified the topic of risk assessment of combined exposure to multiple chemicals as a priority topic for guidance development. It is therefore proposed to further support the SC in this area through an EFSA scientific colloquium on the harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals.

Objective

International experts will gather for an open scientific debate on the harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals (chemical mixtures). Discussions will focus on the following topics of relevance to both human and ecological risk assessment of combined exposure to multiple chemicals: i) mechanistic models for hazard assessment; ii) harmonisation of methods for combined exposure assessment; iii) the use of OMICs and in silico methods for risk assessment; and iv) application of science-based uncertainty factors and approaches for risk characterisation using mechanistic approaches.

Organising Committee

Marco Binaglia, Diane Benford (overall chair), Jean-Lou Dorne, Andrea Germini, Georges Kass, Tobin Robinson, Joseph Schlatter, David Spurgeon (overall rapporteur), Jose Tarazona, Andrea Terron

DISCUSSION GROUP 1 - Tiered approaches in hazard assessment of combined exposure to multiple chemicals in human and ecological risk assessment: from default assumptions to mechanistic models

INTRODUCTION

In the context of combined exposure to multiple chemicals, tiered approaches have been proposed to deal with combined toxicity for hazard assessment. These tiered approaches range from qualitative/semi-quantitative estimates to full probabilistic models as described in the WHO framework (Meek et al., 2011). Within these tiers, combined toxicity is addressed using either a whole mixture approach or component-based approaches. The whole mixture approach is applied when toxicological data are available either for the mixture itself or for a sufficiently similar mixture which can then be used as a surrogate for the mixture under evaluation. Component-based approaches are the most common approaches to deal with combined toxicity, for example, and by evaluating toxicity data for a limited number of individual chemicals to set Cumulative Assessment Groups (CAGs)/Assessment Groups (AGs). Ideally, MoA information can be used as the scientific basis to group compounds into CAGs/AGs, however, such MOA data are still rarely available (EFSA, 2013, 2014).

In the human health area, EFSA has proposed to set CAGs based on common phenomenological effects. (EFSA PPR, 2013a). In this context, EFSA has recommended to use dose addition as a default assumption for the assessment of combined toxicity for pesticides, provided they produce a common adverse outcome (EFSA, PPR 2013b). In ecological risk assessment, the three non-food Committees of the European Commission have proposed the default assumption of dose addition for hazard characterisation using existing acute or chronic toxicity endpoints (SCCS, SCENHIR, SCHER, 2012).

Even though evidence of interactions in combined toxicity is rare, methodologies and mechanistic models have also been proposed to deal with either synergistic effects (increasing combined toxicity) or antagonism (decreasing combined toxicity) (US-EPA, 2007). In the human health area, these include mechanistic models such as physiologically-based toxicokinetic (PB-TK) models and physiologically-based toxicokinetic-toxicodynamic (PB-TK-TD) models, which have been proposed and developed for tier 3 assessments (EFSA, 2013, 2014). In the ecological risk assessment, recent mechanistic models include dynamic energy budget toxicity (DEB) models use TK and TD principles to improve prediction of combined toxicity of multiple chemicals (Baas et al., 2010).

The aim of this discussion group is to critically discuss available tiered approaches (from default assumptions to mechanistic models and probabilistic approaches) for the harmonisation of hazard assessment of combined exposure to multiple chemicals in human and ecological risk assessment. In addition the discussion group will identify datagaps and research needs.

DISCUSSION POINTS

1. Discuss tiered approaches, default assumptions, mechanistic models and probabilistic approaches that are available/used in human and ecological hazard assessment of combined exposure to multiple chemicals to provide an overview.
2. Discuss strengths and limitations of the tiers, default assumptions, mechanistic models and probabilistic approaches.
3. Do these hazard assessment methodologies give the opportunity to risk assessors to harmonise human and ecological hazard assessment when dealing with exposure to multiple chemicals? Strengths and limitations should be included.

4. What are the current data gaps and future research needs to further improve the hazard assessment methodologies to deal with combined exposure to multiple chemicals so that they can be applied in a harmonised way for both human and ecological hazard assessment?

BACKGROUND DOCUMENTS

- Baas J, Jager T, Kooijman B. A review of DEB theory in assessing toxic effects of mixtures. Sci Total Environ. 2010 Aug 15;408(18):3740-45.

- EFSA Panel on Plant Protection Products and their Residues (PPR), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 2013, 11(7),1-131. Available online: http://www.efsa.europa.eu/en/search/doc/3293.pdf

- EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013b. Scientific Opinion on relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12): 3472, 40 pp. doi:10.2903/j.efsa.2013.3472

- EFSA (European Food Safety Authority), 2013. International Framework Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals. EFSA Journal 2013;11(7):3313, 69 pp. doi:10.2903/j.efsa.2013.3313

- EFSA (European Food Safety Authority), 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 2014;12(4):3638, 13 pp. doi:10.2903/j.efsa.2014.3638

- Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV and Vickers C, 2011. Risk assessment of combined exposure to multiple chemicals, A WHO/IPCS framework. Regulatory Toxicology and Pharmacology, 60, Supp 2, S1-S14.

- SCCS, SCENHIR, SCHER, 2012. Toxicity and Assessment of Chemical Mixtures. 50 pp. Available online: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf.

- US-EPA (U.S. Environmental Protection Agency), 2007. Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects, A Resource Document. EPA/600/R-06/013F.

DISCUSSION GROUP 2 - Harmonisation of combined exposure assessment to multiple chemicals in humans and the environment: from environmental fate to internal dose

INTRODUCTION

Humans and ecosystems are exposed to a complex and variable combination of chemicals from different sources and overall exposure of a particular individual depends on multiple factors including dietary, behavioural and environmental factors. Furthermore, the exact chemical composition of a mixture or a group of multiple chemicals is often unknown and the levels of particular components will vary with time and environmental conditions. Combined exposure assessment generally uses relevant available data, such as emissions data, measurement of the components (or a lead component) in environmental media, and ideally biomarker information. In the human health area, combined exposure assessment requires a clear definition of objectives and priorities in the problem formulation and covers three main elements; dietary exposure, environmental exposure (i.e. inhalation, dermal), and other exposures, such as occupational exposure (SCCS, SCENHIR, SCHER, 2012). The US-EPA, the WHO, EFSA and other institutions have developed a number of methodologies to assess such combined exposure assessment for multiple chemicals (US-EPA, 2007; Meek et al., 2011; EFSA PPR panel, 2012; EFSA, 2013). In principle, these methods combine chemical occurrence data with food consumption or environmental concentrations using tiered approaches, as is the case for hazard assessment, i.e ranging from qualitative/semi-quantitative tier 0 to fully probabilistic tier 3 models. The choice of the tier depends on data availability, the purpose of the risk assessment and resources available (Meek et al., 2011; EFSA, 2014; SCCS, SCENHIR, SCHER, 2012).

A simple example is the assessment of combined acute exposure to multiple chemicals in a single combined exposure, e.g. combination of chemicals measured in the same food item). The complexity of the assessment of combined exposure can increase, e.g. multiple chemicals in the same food items or all chemical residues consumed in a single meal. In more complex scenarios such as repeated, long-term exposure assessments taking into account multiple routes of exposure (aggregate exposure assessment for multiple chemicals), each source may lead to a variable combination of multiple chemicals, including simultaneous and/or successive exposure. In order to move from exposure (external dose) to internal dose metrics, toxicokinetic information is a key element for assessing different exposure routes. Multi route physiologically-based TK models have been developed to address this issue (Andersen et al., 2007).

For environmental receptors, combined exposure assessment requires the identification of exposure sources, environmental fate and exposure pathways. The properties responsible for the environmental fate may vary between chemicals emitted from the same source. In addition the relevant exposure pathways for different organisms may also differ significantly according to their biological and ecological characteristics. As a consequence, parallel estimations for each species are required and introduce several levels of complexity. Estimation of the exposure of an organism present in the environment from the environmental fate of the compound to the internal dose, is also complex partly because of the diversity of species and their associated species-specific traits regarding TK processes (absorption, distribution, metabolism and excretion pathways). However, TK models are also increasingly used to determine internal dose in ecological risk assessment particularly in vertebrates such as fish and birds and invertebrate such as crustaceans (Galic et al., 2013). Recently, approaches and recommendations on exposure metric selection have been recently published for aquatic organisms (Schafer et al., 2013).

The aim of this discussion group is to critically discuss methods for combined exposure assessment of multiple chemicals in the human health and ecological areas. In addition, methods to measure, estimate or model internal dose in both areas will also be discussed. Challenges and opportunities for

harmonisation of these methodologies between the two fields will be discussed, and datagaps/research needs will be identified.

DISCUSSION POINTS

1. Which methods are currently available for assessment of exposure to multiple chemicals in the human health and the environmental field measure, estimate or model occurrence, food consumption or environmental exposure of combined exposure? Discuss the methods under different tiers (default values to probabilistic methods). Strengths and limitations should be included.
2. What methods are currently available to measure, estimate or model internal dose to single and multiple chemicals in the human health and the environmental fields?
3. Do risk assessors currently have opportunities between the human health and the ecology field to harmonise exposure assessment methods and methods to integrate internal dose /toxicokinetic processes?
4. What are the current data gaps and future research needs to be filled in order to harmonise methodologies for assessment of exposure and internal dose to multiple chemicals in the human health and environmental fields?

BACKGROUND DOCUMENTS

- EFSA (European Food Safety Authority), 2012. Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. EFSA Journal 2012;10(10):2839, 95 pp. doi:10.2903/j.efsa.2012.2839

- EFSA (European Food Safety Authority), 2013. International Framework Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals. EFSA Journal 2013;11(7):3313, 69 pp. doi:10.2903/j.efsa.2013.3313

- Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV and Vickers C, 2011. Risk assessment of combined exposure to multiple chemicals, A WHO/IPCS framework. Regulatory Toxicology and Pharmacology, 60, Supp 2, S1-S14.

- SCCS, SCENHIR, SCHER, 2012. Toxicity and Assessment of Chemical Mixtures. 50 pp. Available online: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf.

- Andersen ME, Dorman DC, Clewell HJ 3rd, Taylor MD, Nong A (2010) Multi-dose-route, multi-species pharmacokinetic models for manganese and their use in risk assessment. J Toxicol Environ Health A. 73(2):217-234.

- Schäfer RB, Gerner N, Kefford BJ, Rasmussen JJ, Beketov MA, de Zwart D, Liess M, von der Ohe PC .2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities? Environ Sci Technol. 47, 7996-8004.

- Galic N, Ashauer R, Baveco H, Nyman AM, Barsi A, Thorbek P, Bruns E, Van den Brink PJ. Environ Toxicol Chem. 2013. Modeling the contribution of toxicokinetic and toxicodynamic processes to the recovery of Gammarus pulex populations after exposure to pesticides.

DISCUSSION GROUP 3 – Applying biologically-based models, *in silico* tools and OMICs to human and ecological risk assessment of combined exposure to multiple chemicals

INTRODUCTION

In principle, biologically-based models, *in silico* tools and OMICs that have been applied to human and ecological risk assessment in the case of single compounds can provide useful methods and tools to assess the combined exposure to multiple chemicals.

Biologically-based models such as physiologically based toxicokinetic (PB-TK) models are mathematical models simulating the relationship between exposure (external dose) and chemical concentration in biological matrices (internal dose) over time. They can be applied to both human and ecological risk assessment. PB-TK models ideally take into account Absorption, Distribution, Metabolism and Excretion (ADME) of chemicals and their metabolite(s) and integrate different physiological parameters. PB-TK models can also be combined with dose response data to produce a PB-TK-Toxicodynamic (PB-TKTD) model (WHO, 2010; EFSA, 2014). In the human health area, examples of such models include PB-TK models addressing metabolic interactions (Cheng and Bois, 2011) and PB-TK-TD models addressing combined toxicity of multiple pesticides such as organophosphates (Lee et al., 2011) and carbamates (Pelekis and Emond, 2009). In ecological risk assessment, Dynamic Energy Budget (DEB) models have been applied to chemical mixtures to integrate effects on growth, reproduction and survival. These models incorporate exposure time and biology of the organisms (e.g feeding, maintenance, growth, development and reproduction) and provide opportunities to identify potential interactions and investigate their mechanism(s) .(Bass et al., 2010).

(Quantitative) Structure Activity Relationships (QSARs) are *in silico* mathematical models that relate the structure of chemicals to their biological/toxicological activities. The molecular descriptors of a chemical are generally its inherent physicochemical properties such as atomic composition, structure, sub-structures, hydrophobicity, surface area charge, and molecular volume. QSARs are typically used as *in silico* tools in combination with other non-testing (e.g. read-across) and testing (e.g. *in vitro*) methods (OECD, 2009a; EFSA, 2014). Examples of QSAR include the investigation of toxicity of complex mixtures of petroleum products in combination with PB-TK-TD modelling for human risk assessment and the modelling of combined ecotoxicity (Verhaart et al; 1997; Altenburger et al., 2003).

The term ‘OMICs’ refers to a broad field of studies in biology, ending in the suffix ‘-omics’, such as transcriptomics, proteomics, metabolomics, and associated ‘bioinformatics’ (US-EPA, 2002). Transcriptomics addresses the expression level of mRNAs in a given tissue, organ or other cell population, using DNA microarray and other high-throughput technologies that can estimate the quantities of mRNAs. Proteomics deals with cell and tissue-wide expression of proteins encoded by a genome. After transcriptomics, proteomics is the next step in OMICs studies. The OECD refers to metabolomics as the discipline that deals with endogenous metabolite profiles of tissues or organs derived from mass spectrometry or nuclear magnetic resonance spectrometry analyses of plasma, urine or homogenates. Metabolic profiling can give an immediate picture of the physiological state of the tissue (OECD, 2009b). OMICs technologies have been applied to chemical mixtures in both the human health and the ecological risk assessment area (EFSA, 2014). Examples include investigation of the combined toxicity of the ‘Northern contaminant mixture’ (NCM) which includes methylmercury (MeHg), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in rats (Padhi et al., 2008) using transcriptomics or metabolomics to test independent action and concentration addition of multiple chemicals in earthworms (Baylay et al., 2012).

The aim of this discussion group is to discuss how biologically-based models, *in silico* tools and OMICs can be applied in the future for human and ecological risk assessment and the opportunities they provide for harmonisation in both fields. Current data gaps and future research needs will also be discussed.

DISCUSSION POINTS

1. How can biologically-based models (e.g TK and DEB models) and *in silico* tools (QSAR) contribute to human health and ecological risk assessment of combined exposure to multiple chemicals. Strengths and limitations should be included.
2. How can OMICs contribute to the human and ecological risk assessment of combined exposure to multiple chemicals? Strengths and limitations should be included.
3. What are the opportunities to harmonise the use of these methods in both the human and ecology fields when dealing with combined exposure to multiple chemicals? How might they contribute to reduce animal testing?
4. What are the current data gaps and future research needs to move towards the use of integrated testing strategies when dealing with combined exposure to multiple chemicals.

BACKGROUND DOCUMENTS

- Altenburger R, Nendza M, Schüürmann G. 2003. Mixture toxicity and its modeling by quantitative structure-activity relationships. Environ Toxicol Chem. 22(8):1900-1915.
- Baas J, Jager T, Kooijman B. A review of DEB theory in assessing toxic effects of mixtures. Sci Total Environ. 2010 Aug 15;408(18):3740-45.
- Baylay AJ, Spurgeon DJ, Svendsen C, Griffin JL, Swain SC, Sturzenbaum SR, Jones OA. (2012) A metabolomics based test of independent action and concentration addition using the earthworm Lumbricus rubellus. Ecotoxicology. 21(5):1436-1447.
- Cheng S and Bois FY, 2011. A Mechanistic Modeling Framework for Predicting Metabolic Interactions in Complex Mixtures. Environmental Health Perspectives, 119, 1712-1718.
- EFSA (European Food Safety Authority), 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 2014;12(4):3638, 13 pp. doi:10.2903/j.efsa.2014.3638
- Lee S, Poet TS, Smith JN, Hjerpe AL, Gunawan R and Timchalk C, 2011. Impact of repeated nicotine and alcohol coexposure on *in vitro* and *in vivo* chlorpyrifos dosimetry and cholinesterase inhibition. Journal of Toxicology and Environmental Health A, 74, 1334-1350.
- OECD (Organisation for Economic Co-Operation and Development), 2009a. Guidance document for using the OECD (Q)SAR Application Toolbox to develop chemical categories according to the OECD Guidance on grouping of chemicals. ENV/JM/MONO(2009)5, Series on Testing and Assessment No. 102.
- OECD, 2009b. Report of the second survey on available omics tools. ENV/JM/MONO(2008)35. Series on testing and assessment Number 100. Available online: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282008%2935&doclanguage=en

- Pelekis M and Emond C, 2009. Physiological modeling and derivation of the rat to human toxicokinetic uncertainty factor for the carbamate pesticide aldicarb. Environmental Toxicology and Pharmacology, 28, 179-191
- Padhi BK, Pelletier G, Williams A, Berndt-Weis L, Yauk C, Bowers WJ and Chu I, 2008. Gene expression profiling in rat cerebellum following in utero and lactational exposure to mixtures of methylmercury, polychlorinated biphenyls and organochlorine pesticides. Toxicology Letters, 176, 93-103.
- US-EPA (U.S. Environmental Protection Agency), 2002. Science Policy Council. Interim Policy on Genomics. Available online: http://www.epa.gov/OSP/spc/genomics.pdf
- Verhaar HJ, Morroni JR, Reardon KF, Hays SM, Gaver DP Jr, Carpenter RL, Yang RS. (1997) A proposed approach to study the toxicology of complex mixtures of petroleum products: the integrated use of QSAR, lumping analysis and PBPK/PD modeling. Environ Health Perspect. 105 Suppl 1:179-195.
- WHO (World Health Organization), 2010. Characterization and application of physiologically based pharmacokinetic models in risk assessment. Available online: http://www.who.int/ipcs/methods/harmonization/areas/pbpk_models.pdf?ua=1

DISCUSSION GROUP 4 – Harmonisation of uncertainty factors and risk characterisation for human and ecological risk assessment using mechanistic descriptors

INTRODUCTION

Uncertainty factors (UFs) are applied to data on toxicity of single substances to derive health-based guidance values (HBGV) for human health or environmental standards for ecotoxicological risk assessment. Over the last 50 years, a 100-fold default UF has been applied in human risk assessment to allow for interspecies differences and human variability in toxicokinetics (TK) and toxicodynamics (TD). In ecological risk assessment, UFs are applied to deal with inter- and intraspecies, acute-to-chronic, lowest- to no-observed-effect concentration (NOEC), and laboratory-to-field extrapolations (e.g., extrapolation of laboratory results to the field) (Chapman et al., 1998). The nature of the UFs that are applied within a risk assessment will depend on the amount of hazard data available. Hence, these can range from a factor of 1000 or more, when only QSAR predictions of toxicity or limited acute data are available; to factors as small as 3 when extensive chronic toxicity data are available for a range of species from different trophic groups under a range of environmental conditions. In a combined exposure context, these HBGVs for human health or environmental standards for ecotoxicological risk assessment are ideally grouped into assessment groups (AGs) and combined with exposure estimates for risk characterisation. Over the last twenty years, many efforts have been made to refine UFs in the human health area including chemical-specific adjustment factors (CSAFs). These CASFs can be derived from mechanistic models such as physiologically-based (PB) models describing interspecies differences and/or human variability in toxicokinetics (TK) and toxicodynamics (TD) (WHO, 2005). In the ecological risk assessment area, efforts have also been made to refine the use of default UFs using mechanistic descriptors including interspecies correlation analysis (Golstejin et al., 2012). Investigation of the scientific and mechanistic basis of these UFs gives a number of opportunities and options to refine and harmonise their use in both human and ecological risk assessment of multiple chemicals. Examples of options to refine UFs range from a) integration of basic knowledge of TK and/or TD for specific groups of compounds for human risk assessment to derive process-related UFs and/or to set AGs b) integrate taxa-specific traits related to TK and/or TD processes for ecological risk assessment, c) derive CSAFs for high tier chemical-specific assessment (EFSA, 2013, EFSA PPR, 2012).

For risk characterisation, the probability of observing a toxic response for each chemical component in the mixture is first estimated and components are then summed to estimate total risk from the combined exposure. In the human health area, the most common risk characterisation method is the hazard index approach (HI), which combines the HBGVs for the individual chemicals in an AG with exposure estimates using dose addition as the default assumption. Variants of the HI method include use of a target-organ toxicity dose, a reference point index/point of departure index, the relative potency factor and the toxicity equivalency factor (US-EPA, 2007; EFSA, 2013). In ecological risk assessment, the Toxic Unit (TU) has been proposed as a conservative default approach for risk characterisation. Individual TUs for each compound in the mixture can be added to calculate a TUm (toxic unit of the mixture) using dose addition as the default assumption (SCCS, SCENHIR, SCHER, 2012; EFSA PPR Panel, 2012; EFSA, 2013).

In the case of TK or TD interactions, combined toxicity of multiple chemicals is categorised as either less than additive (antagonism, inhibition, masking) or greater than additive (synergism, potentiation). In the human health area, methods for deriving risk estimates for interactions include interaction-based HI and HI modified for binary interactions. For high tier risk assessment (tier 3), PB-TK can also be applied to the derivation of interaction-based Hazard Index using tissue doses accounting for multiple TK interactions between the chemicals (ATSDR, 2004; US-EPA, 2007; EFSA, 2013). In the ecological risk assessment area, the PPR panel of EFSA proposed an approach to take into account

synergistic interactions in the risk characterisation of multiple pesticides in bees. The proposal involves first testing combined pesticides at relevant levels of environmental exposure to generate full dose–response studies in adult bees and larvae in order to measure the magnitude of interaction. The magnitude of interaction can be taken into account in the risk characterisation using a modified interaction-based toxic unit approach (EFSA PPR Panel, 2012).

Once risk characterisation has been performed, uncertainty analysis is a key component to identify the sources and magnitude of uncertainty in a tiered manner (qualitative, semi-quantitative or probabilistic) associated with exposure and hazard estimates and the risk characterisation itself. Uncertainty analysis also provides a means to identify strengths and limitations of the assessment, data gaps and needs for further refinements, and underpins recommendations for future research (EFSA, 2013).

The aim of this discussion group is to critically discuss challenges and opportunities to refine and harmonise uncertainty factors, risk characterisation methods for human and ecological risk assessment of combined exposure to multiple chemicals using mechanistic information. In addition the discussion group will discuss uncertainty analysis and identify data gaps and research needs.

DISCUSSION POINTS

1. What are the challenges and opportunities to refine and harmonise uncertainty factors for human and ecological risk assessment of combined exposure to multiple chemicals using mechanistic information? Discuss potential options
2. How can we refine and harmonise risk characterisation of human and ecological risk assessment of multiple chemicals using current knowledge of mode of action? Discuss potential options
3. How can we report uncertainties in the context of human and ecological risk assessment using current knowledge of mode of action? Discuss potential options
4. What are the data gaps and research needs to improve such refinements, and lead to harmonisation of uncertainty factors and risk characterisation methods where appropriate?

BACKGROUND DOCUMENTS

- ATSDR (Agency for Toxic Substances and Disease Registry), 2004. Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. US Agency for Toxic Substances and Disease Registry. Division of Toxicology. May 2004. Available online: http ://www.atsdr.cdc.gov/interactionprofiles/ipga.html.

- Chapman, P. M.; Fairbrother, A.; Brown, D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ. Toxicol. Chem. 17, 99−108.

- .EFSA Panel on Plant Protection Products and their Residues (PPR), 2012a. Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal (2012);10(5):2668, 275 pp. doi:10.2903/j.efsa.2012.2668

- EFSA (European Food Safety Authority), 2013.International Framework Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals. EFSA Journal 2013;11(7):3313, 69 pp. doi:10.2903/j.efsa.2013.3313

- Golsteijn L, Hendriks HWM, van Zelm R, Ragas AMJ, Huijbregts MAJ. 2012. Do interspecies correlation estimations increase the reliability of toxicity estimates for wildlife? Ecotoxicology and Environmental Safety 80, 238-243.

- SCCS, SCENHIR, SCHER, 2012. Toxicity and Assessment of Chemical Mixtures. 50 pp. Availableo online: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf.

- US-EPA (U.S. Environmental Protection Agency), 2007a. Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects, A Resource Document. EPA/600/R-06/013F.

- WHO, 2005. International Programme on Chemical Safety , Chemical-specific Adjustment Factors for Interspecies Differences and Human Variability , Guidance Document for Use of Data in Dose/concentration Response Assessment. World Health Organization, Geneva. Available online: http://www.who.int/ipcs/methods/harmonization/areas/uncertainty/en/index.html