Workshop on Combined Exposure to Chemicals 11-12 July 2011, Berlin

Workshop Report No. 22

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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ECETOC WORKSHOP REPORT No. 22

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Workshop on Combined Exposure to Chemicals

CONTENTS

1. EXECUTIVE SUMMARY

In the last 10 years, there has been a significant amount of research into the toxicology of mixtures and co-exposure, which has genuinely increased our understanding. Participants at the workshop generally agreed that the WHO/IPCS framework provides a useful tool for risk assessment of combined exposure to multiple chemicals from multiple sources. A suitable problem formulation at the outset of any risk assessment of combined exposures to multiple chemicals was thought to be a fundamental first step.

There was overall recognition that according to available evidence, in practice, the toxicity of mixtures in the environment is often dominated by a few of their components, and that these can be identified by available approaches. In this regard, where relevant data are available, the Maximum Cumulative Ratio approach is a useful tool for both human health and environmental risk assessments.

The current state of knowledge shows that synergy (exceeding additive effects) is rare and appears to be toxicologically significant only at doses at which there is significant toxicity of one or more of the individual components in the combination. The available data indicate that synergy does not normally occur at environmental concentrations of man-made chemicals. For chemicals that have different modes of action, there is currently very little data to support the occurrence of combination effects below their individual predicted no-effect levels. However, in the absence of information on mode of action, dose / concentration addition can be used as the conservative default.

The discussion at the workshop identified a number of areas that require further research, such as better understanding of mode of action, improved methodologies of exposure assessment including assimilation of better databases and data processing methods. The threshold of toxicological concern approach and non-testing methods were suggested as potentially useful tools that also need further development for use in this context.

In recent years, there has been growing public perception and concern about the possibility of 'cocktail effects' of chemicals at very low doses of the single substances (i.e. below levels deemed to be safe for humans and the environment) which are generally not taken into account in regulatory risk assessment. The current evidence offers little support for this; although some of the workshop participants were of the opinion that the current knowledge on combined exposure and effects was too limited to allow such a conclusion. It is important that combined exposures be considered in risk assessment practice − through the use of science-based, targeted and pragmatic tiered approaches. Such approaches should allow identification of any combinations which may require priority attention.

Whilst recognising the importance of addressing the potential risk of combined exposures to chemicals, this should perhaps be seen in light of the various other scientific areas that are important for protecting and improving human health and the environment. However, some workshop participants felt that this view is a general one, which does not only concern the issue of combined exposure and effects of chemicals. Overall though, the use of a tiered approach is strongly recommended to ensure optimum use of resources.

2. AIM OF THE WORKSHOP

Human and environmental chemical risk assessment is predominantly carried out on individual substances, and this is also reflected in most chemical-related legislation. In reality though, humans, fauna and flora are exposed to a variety of substances concurrently. The toxicology of chemical mixtures has usually been addressed through the concepts of concentration or dose addition and independent action, with synergism being acknowledged as only a rare occurrence. Today there is widespread interest in examining the question of whether current risk assessment procedures are adequate for dealing with combined exposure to multiple chemicals. Development of data and methodology for approaching this issue in a scientific fashion is recognised as very important.

ECETOC held this workshop to review a number of key scientific areas which are relevant in the assessment of the health and environmental impact of combined exposures to chemicals. It focussed on the state of the science and on technical aspects of co-exposure, and discussed reliable and pragmatic approaches to risk assessment of combined exposures to chemicals. Participants were representing academia, industry and regulators. Following presentations on the state of the science, breakout group sessions took place to address specific questions and to discuss where the science may need further developing.

3. PLENARY LECTURES

3.1 Overview on activities on the risk assessment of combined exposures to chemicals

Carlos Rodriguez, Procter & Gamble, Belgium

Humans and the environment are continuously being exposed to multiple combinations of substances both from a natural and synthetic origin. Toxicologists have traditionally mostly focused on studying the toxicology of single substances but have not ignored the need of accounting for combinations of substances (mixtures). The field of toxicology of mixtures is well established but remained mainly of academic and specialised areas' interest until recently. Most regulations on the risk assessment of substances are based on the assessment of individual substances. Broad stakeholder's (regulators, industry, NGOs, media) attention to the need for assessing the risks of combined exposure to multiple substances started to mount in the last decade and has continued to develop with increasing intensity ever since. In the last five or six years a variety of regulatory agencies (including EFSA, US EPA, UK IGHRC, and others) have issued or updated documents proposing guidance for addressing one or more aspects of the assessment of combined exposure to substances. In 2007, the WHO/IPCS held an international workshop on the subject whose main outcome was the establishment of an experts group to develop a framework for risk assessment of combined exposures to multiple chemicals. This WHO/IPCS framework was completed last year and was presented at an OECD/WHO/ILSI-HESI workshop last February (Meek *et al*, 2011). In 2007, the EU DG Environment commissioned a review on the 'State of the Art Report on Mixtures Toxicity' which was completed and published by Kortenkamp *et al* in December 2009. Also in December 2009, the EU Council asked the EU Commission to advise on the adequacy of current legislation for addressing the toxicology of mixtures and to propose appropriate guidance. The three non-food Scientific Committees to the EU Health and Consumer Protection Directorate General took on the scientific task and issued their preliminary opinion in June 2011 (SCHER, 2011). Numerous additional activities in the field have been triggered as a consequence of this keen regulatory interest. ECETOC recently formed two task forces, one to review evidence of toxicological effects as a result of low dose interaction of chemicals and the other to develop guidance for assessing the impact of mixtures of chemicals in the aquatic environment. Both ECETOC task forces are currently completing their work. Last year, Cefic established a 'mixtures team' that has proposed a decision tree for helping address the assessment of mixtures. The Cefic decision tree is based on the WHO/IPCS framework and uses the MCR (Maximum Cumulative Ratio) concept developed by Paul Price (Price and Han, 2011). A few years ago, ILSI-HESI started a 'mixtures group'; they have recently completed a review of the evidence for the magnitude of low-dose synergy and are evaluating the use of TTC as a screening assessment tool (Boobis *et al*, 2011). In February this year, SETAC held a special 'Science Symposium on Prospective and Retrospective Environmental Risk Assessment of Mixtures'. These are some important, recent activities in the field. All of them were discussed in this workshop whose participants included some of their key representatives.

3.2 Effects of low and high doses of combinations of chemicals on human health

Kim Travis, Syngenta, UK

There is no doubt that the public is exposed each day to complex combinations of chemicals, whether natural or synthetic. In the case of chemicals subject to regulatory approval, tests on single chemicals and of products or preparations containing multiple chemicals form the basis by which society seeks to ensure the protection of human health. However, this does not explicitly address concerns about combinations of chemicals in the environment to which the public is exposed. For this reason, the topic of mixture toxicology has been active for many years, with thousands of studies being conducted and hundreds of reviews of the field. This presentation sought to distil some lessons from this large body of work.

Much work on mixtures has been done at doses where individual chemicals in the mixture would already cause an effect if dosed alone. In these circumstances all manner of interactions may be seen. Dose addition and independent action are common, though synergy and antagonism also occur. However, if the public is exposed to any regulated chemical at an effect level when dosed alone, this represents a frank failure of existing regulation or exposure controls. Safety or assessment factors should prevent exposures to single chemicals from approaching effect levels. For this reason, the main focus of a discussion about the protection of human health in the context of chemical mixtures needs to be on lower doses, specifically on the effects of combinations of chemicals where each chemical is below a threshold of toxicity (e.g. NOEL).

An ECETOC Task Force has been reviewing the evidence for the effects of combinations of chemicals when each component is at a dose which would not by itself produce an effect (ECETOC, 2012). A thorough literature review has been conducted, with an emphasis on the identification of primary data sources, and with strict criteria for papers to be accepted as relevant for the review. Three classes of relevant studies were identified:

- Studies where combinations of chemicals were only tested at doses close to (but below) the NOEL of individual components.
- Studies where combinations of chemicals were tested at doses well below the NOEL of every individual component.
- Studies where animals are exposed to real or simulated environmental mixtures.

Based on this review, the Task Force is summarising the evidence, and is using it to address the following points in its remit.

- To evaluate whether the evidence on interactions at low doses demonstrates toxicologically relevant effects and determine whether there are any associations with specific modes of action.
- To look at the evidence of chemical interactions and comment on the likelihood of those being important in the context of environmental exposures.

- To evaluate the adequacy of current human safety risk assessment practice in light of the conclusions of the above.

Many searches have been done and from these over 500 papers have been reviewed in detail. Work continues and so only preliminary conclusions were presented. Many papers were rejected as one or more chemicals in the mixtures were already present at an effect level. Of those papers meeting the Task Force's criteria, additivity was common, whilst in a number of studies there were no effects at all. In some studies, the nature of the interaction could not be determined. A few cases of deviation from additivity have been identified, both antagonism and synergy, many of which are from *in vitro* studies. The cases of antagonism identified so far will be subject to additional scrutiny, as these are papers of the greatest interest for risk assessment. There is no clear pattern so far distinguishing endocrine disruption from other kinds of effect.

3.3 Effects of combinations of chemicals in the aquatic environment

Mick Hamer, Syngenta, UK

The potential risk of combinations of chemicals, rather than single chemicals is increasingly being discussed. These combinations of chemicals or mixtures fall into different categories.

- Multi-constituent substances such as petroleum oils, natural dyes and essential oils.
- Chemical formulations and preparations prepared by blending different substances in specific proportions such as plant protection products, biocides, pharmaceuticals and other consumer products.
- Mixtures of chemicals due to co-concurrent release such as effluents, tank mixes of plant protection products.
- Complex mixtures in the environment of unknown composition, consisting of anthropogenic discharges together with natural sources of chemicals mixtures.

An ECETOC Task Force has been looking at ways on how the impact of chemicals on aquatic environments can be assessed (ECETOC, 2011). Numerous laboratory studies have reinforced the position that the long-held concepts of concentration addition and independent action can explain the observed effects. The potential for effects of mixtures of known composition can be determined using these component-based approaches. Whole mixture toxicity testing is another option, indeed it is a requirement for many chemical mixtures such as plant protection products. However, the changing nature of chemical mixtures in the environment due to the differing environmental fate parameters of the component parts, limits the use of these whole mixture approaches in risk assessment. The potential for the impact of chemical mixtures in the environment is even harder to predict or evaluate when not all the components are known and this is where techniques such as whole effluent testing/direct toxicity assessment can be useful,

followed by toxicity identification and evaluation/effects-directed analysis to characterise the chemical(s) driving the effects.

3.4 Human exposure assessment

Jacqueline van Engelen and Marcel van Raaij, RIVM, The Netherlands

The role of exposure assessment in risk assessment has long been underestimated. Fortunately, during the last decades it has been realised that refinement of exposure is a cheap, rapid, animal-friendly and effective tool to improve a risk assessment. It is important that exposure assessors work in co-operation with risk assessors, since the exposure assessment needs to be tailored to the specific goal of the risk assessment. At least four objectives for a risk assessment can be distinguished: 1) safety screening 2) authorisation 3) identification of exposure sources 4) health impact assessment. The focus for the exposure assessment will be different for these different objectives. Rough assumptions will be made for screening purposes, whereas for a health impact assessment a probabilistic assessment will be performed.

The level of detail of the exposure assessment, and consequently the data and/or model, need to be decided on a case-by-case basis. If a low tier, conservative, estimate results in 'no concern', there is then no need for a sophisticated approach. On the other hand, when exposure via various routes and various sources needs to be assessed, this might require high tier models and high quality data. In the ideal case, the high tier exposure assessment results in exposure values that are close to realistic levels. Probabilistic approaches and preferably assessment of internal exposure values, by e.g. application of PBPK models in combination with bio-monitoring, are valuable tools.

The dose metric of the exposure assessment should match with the most relevant human health endpoint identified in the toxicity profile of a substance. When the critical effect is related to the AUC, the exposure needs to be calculated as time-weighted average, whereas peak levels of exposure need to be characterised when the critical effect is related to concentration.

In various domains, like exposure assessment for chemicals in non-food consumer products exposure data, including information on use patterns and time-activity data are scattered and scarce. In recent years, information on specific products and/or use patterns has been made publicly available (e.g. in the US exposure factor handbook, SPIN database, US household product database, RIVM factsheets for the ConsExpo model). However, a comprehensive collection of data, including EU data, is highly welcomed. For a probabilistic exposure assessment information on co-use and use over time needs to be made available or needs to be generated.

3.5 Exposure in the aquatic environment

Chris Holmes, Waterborne Environmental, USA

Gaining a better understanding of the presence of chemical mixtures in the aquatic environment is critical to refined risk assessment. While many methods for estimating the presence and concentration of single chemicals in surface water are available, few approaches attempt to quantify multiple chemicals and/or multiple stressors found in aquatic systems. This can be especially challenging when chemical co-occurrence in space and time is considered. However, it is possible to estimate the presence of multiple chemicals/stressors in spatially- and temporally-explicit assessments.

This talk presented a number of chemical types and associated sources/routes of entry into surface water. Selected exposure models used to estimate presence or concentrations of chemicals were introduced. For point-source emissions, examples of predicting site-specific concentrations of LAS using the GREAT-ER model (Price *et al*, 2009) and triclosan using LowFlows2000-WQX (Price e*t al*, 2010) in the UK, along with comparison to monitored data, were presented. Of particular interest are approaches which incorporate multiple chemicals, sources and/or stressors as part of the aggregate exposure to aquatic environments, specifically highlighting spatial and temporal dimensions. Several examples of modelling for diffuse source pesticide mixtures are referenced in the recently published preliminary opinion of the three non-food Scientific Committees to the EU Health and Consumer Protection Directorate General (SCHER, 2011) on the 'Toxicity and Assessment of Chemical Mixtures', and these were discussed in this presentation. Finizio *et al* (2005) assessed the composition and ecotoxicological relevance of predicted pesticide mixtures emitted from maize in Po River valley for combinations of 16 chemicals. Also referenced in the mentioned Scientific Committees report and presented here, Verro *et al* (2009) collected field survey information for 54 substances and calculated multi-substance environmental concentrations over time for a single point in the River Meolo, Italy. Modelling of diffuse sources of chemicals from the urban environment using UK PSD and US EPA models was presented.

Four case studies were presented as examples of modelling exposure of multiple chemicals in the aquatic environment. These case studies covered a range of examples where multiple chemicals in the environment were characterised, including a variety of scales (catchment to regional), simple to complex temporal variability, similar to diverse chemical types, simple to complex hydrologic modelling, and multiple sources and/or routes of entry. A case study from California/US examined the spatial and temporal co-occurrence of 40 different pesticides over a 10-year timeframe with the presence and relative abundance of nine sensitive species. A second study modelled concentrations for nine plant protection products in three catchments located in East Anglia/UK using complex hydrologic models and compared results to monitoring data. The co-occurrence in time and space of all nine plant protection products were shown. The third case study described an eco-epidemiological approach to investigate the likely causes of poor

biological quality in rivers in England and Wales. Nineteen environmental variables related to waste water treatment plant effluent, water chemistry, pesticides, metals, and land use were combined in space and related to 307 biological monitoring sites for both spring and autumn time periods. Finally, a more comprehensive eco-epidemiological study covering the state of Ohio/US was introduced. Here, over 2,000 biological monitoring sites (fish and aquatic invertebrates) were examined in relation to multiple chemical exposures (including pesticides, pharmaceuticals, hormones, and ingredients from home and personal care products) along with other stressors such as habitat, agriculture, population, soils and water chemistry.

Because of the multiple dimensions in modelling of environmental mixtures (location, time, multiple chemicals, etc.) a simple combination of deterministic endpoints (e.g. a 90^{th} percentile) for each chemical may not be appropriate as these may not occur simultaneously in space and time. More refined analyses, including probabilistic approaches (e.g. Monte Carlo), accounting for multiple chemicals/pathways and utilising improved input data may be desirable, although the relationship between the large number of modelling inputs across multiple models must be well understood.

3.6 Conventional approaches to risk assessment of mixtures

John C. Lipscomb, U.S. Environmental Protection Agency, USA

Humans are never exposed to a single agent; human exposure patterns are dynamic and complex and they modulate the ensuing response(s) among the population. Cumulative risk assessment (CRA) approaches are employed to assess the risks to a given population from multiple agents emanating from multiple sources. Two key concepts in CRA are the suite of agents and the population of interest. In some instances, exposures can be reasonably well characterised, even if the nascent toxic properties of the individual agents cannot. The extent to which the agents have been toxicologically characterised is a major determinant of the choices that are made in a CRA. The initial phase of a CRA is based on the principles (and practice) of mixtures toxicology, where the major issue is characterising the degree to which the mode(s) of action of mixture components (agents) are similar or dissimilar. The concept of using similarity versus independence of MOA has traditionally been used as a means of judging the extent to which one agent is likely to add to the toxicity (response) elicited by a second agent. When both agents act through the same, or similar MOA, then the anticipated response is estimated as a function of the combined individual doses of the agents, adjusted for potency. When two agents act through dissimilar or independent MOAs, then the combined response is predicted as the sum of the separate responses produced by the individuals. While some CRA endeavours have limited the inclusion of chemicals to those acting through the same MOA (e.g. cholinesterase inhibition), CRA applications do not require similarity of MOA. There may be merit in also assessing the commonality among major effects, even when these effects are not key components of the MOA (e.g. enzyme induction). The second phase is to identify the population of interest and characterise the exposures. The

anticipated response in individuals may vary within a population, and it is necessary to define the population of interest. CRA may be applied to small, well-defined populations, such as those living near a contaminated site, or to geographically-scattered and diverse populations such as those subjected to national-level regulations. In either case, it is important to define the toxicologically-relevant characteristics of the population and account for their variability in CRA. The presentation provided an overview of CRA and its application, as well as laid out some issues for additional consideration.

Disclaimer: The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the US EPA.

3.7 WHO/IPCS Framework on risk assessment of combined exposures to multiple chemicals

Bette Meek, University of Ottawa, Canada

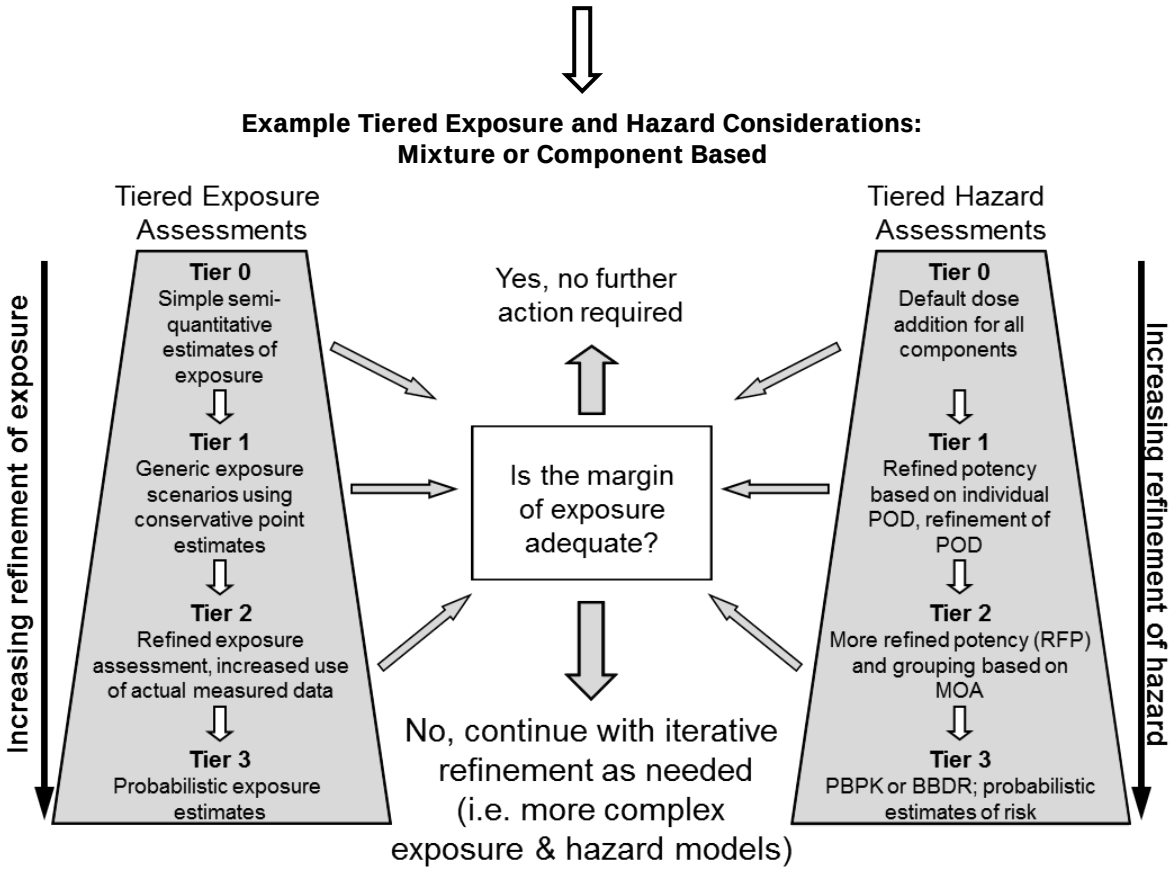
More efficient methodology for assessing the impact of combined exposures to multiple chemicals has been considered in a project of the World Health Organization (WHO) International Programme on Chemical Safety (IPCS).

Following an initial international workshop to review advances in this area, a draft framework for assessment of combined exposures was developed which includes problem formulation, followed by stepwise consideration of both exposure and hazard in several tiers of increasingly data-informed analyses (Meek *et al*, 2011). These analyses build on recent developments in assessment in a range of international programmes, incorporating predictive approaches in early tiers and increasingly refined, more data-informed and probabilistic analyses in later tiers.

The framework, authored by M.E. (Bette) Meek, Alan R. Boobis, Kevin M. Crofton, Gerhard Heinemeyer, Marcel Van Raaij and Carolyn Vickers, is illustrated by a number of case studies which provide examples of assessments in different tiers. The framework and case studies were recently considered in a second international workshop for which proposed follow-up includes international co-ordination in the development of a broader range of more encompassing case studies.

Recommendations regarding terminology and the status of development of the framework, its content, review and application have been described. Evolving experience in its application has been illustrated by examples with special emphasis on the critical content of problem formulation, the role of predictive tools in grouping of chemicals for consideration and the importance of explicit delineation of relative uncertainty and sensitivity for tiered exposure assessment.

Figure 1: A conceptual representation of the framework (Meek **et al***, 2011)*



3.8 Review of the evidence for the magnitude of low-dose synergy by ILSI/HESI

Alan R. Boobis, Imperial College London, UK

Exposure to chemicals almost never occurs in isolation; rather there is co-exposure to several chemicals. As it is not feasible to test all potential combinations, default assumptions are used in the risk assessment of relevant assessment groups. The most common such assumptions are dose or response addition, as appropriate, depending on available information on the mode of action of the mixture components. The ILSI Health and Environmental Sciences Institute convened a tripartite working group comprising scientists from academia, government and industry, to explore the development of a screening level risk assessment as part of a tiered approach in the risk assessment of co-exposures to chemicals. Specifically, the threshold of toxicological

concern (TTC) was investigated for this purpose. In such a screening approach, chemicals that could conceivably share a mode of action would be assumed to exhibit dose addition, as this would be more conservative than assuming response addition. However, before applying such an approach, it was necessary to take into account any synergistic interactions that might occur at low, environmentally relevant exposure levels.

A systematic literature review on the occurrence and magnitude of synergy in mammalian test systems was therefore performed (Boobis *et al*, 2011). The emphasis was on studies undertaken at doses close to the points of departure (PODs) for the mixture components. The search identified 90 studies on mixture additivity. However, few of the papers included a quantitative estimate of the magnitude of any synergy at low doses. Only 11 papers included such information. The methodology used varied amongst the studies, including the null hypothesis tested, the POD used to investigate synergy and whether or not the slope of the dose-response curve was considered. The method used for calculating the magnitude of the synergistic interaction also varied amongst the studies. Based on the findings of this review, it was concluded that consistent approaches should be applied for quantification of synergy, including: defining synergy in terms of departure from dose addition; development of harmonised procedures for assessing synergy at low exposure levels; and standardisation of the POD used when calculating synergy.

Only six studies provided useful, quantitative estimates of synergy. The magnitude of synergy at low doses, but exceeding the PODs of the mixture components, observed in these studies ranged from 1.5 to 3.5-fold, i.e. the effects observed were not more than 3.5-fold those predicted by dose addition. Such information suggests that it should be possible to use the TTC approach for screening and prioritisation of co-exposures to chemicals, as part of a broader, tiered approach for mixture toxicology.

Acknowledgement: The work described reflects the efforts of the ILSI HESI Risk Assessment Methodology working group on Mixtures.

3.9 Cumulative risk assessment and MCR approach

Paul Price, Dow Chemical, USA

Human and environmental receptors are exposed to multiple chemicals from multiple sources. Despite widespread discussion of the need to consider cumulative exposures when determining chemical safety, there has been little investigation into how much greater the cumulative toxicity would be than the toxicity from individual chemicals. Because of the considerable level of resources required by cumulative assessments, it is useful to determine when this difference is large and a cumulative assessment is needed and when it is sufficiently small that a chemical-by-chemical approach is adequate.

In this talk the use of a simple tool was described, the Maximum Cumulative Ratio (MCR), that provides a quantitative measure of the magnitude of the toxicity that is 'missed' by not performing a cumulative risk assessment (Price and Han, 2011). The MCR can be applied whenever there are sufficient data to use either a TEQ (toxicity equivalents)-based approach or Hazard Index/Hazard Quotient approach for the evaluation of cumulative toxicity of individuals in a population. By calculating the MCR for individuals with known cumulative exposures, the tool can determine if there is value in performing cumulative risk assessments for similar populations or if certain groups of chemicals consistently have larger or smaller need for a cumulative risk assessment. This information can be used to guide future collections of data and risk management decisions.

A case study was presented of the application of MCR to human exposures to mixtures of pesticides in surface waters. In this study it was shown how the MCR can be used within the WHO/IPCS tiered approach for assessing the toxicities of mixtures (Meek *et al*, 2011). Specifically, the example examines how MCR values from exposures to mixtures of pesticides change as the mixtures are assessed under simple assumptions of additivity (Tier 1), when mechanism of action is considered (Tier 2), and when probabilistic models of non-cancer toxicity are used (Tier 3). In the examples presented MCR values found in lower tiers declined in the higher tier assessments. This suggests that findings of low MCR values in Tier 0 and 1 assessments are valid indications of a limited need for a cumulative assessment that do not change with refinements in the estimates of exposure or toxicity.

3.10 Evaluating impacts of chemicals in the environment

Scott D. Dyer, Procter & Gamble, USA

Logical frameworks by which measured biological impacts can be attributed to chemical mixtures have been developed (ECETOC, 2011). The foundation from which these frameworks derive is the definition of impact. For environmental risk assessment, impact is typically defined as a measured deviance from reference conditions. Reference conditions typically refer to sites in which biological communities are minimally affected by human influences. Large deviances from reference are indicative of large impacts. The Water Framework Directive (WFD) defines reference as sites that are of 'good ecological status'. Several methods have been developed for determining ecological status (e.g. multi-metric indices such as the index of biotic integrity as well as ecological-modelling based approaches such as the River Invertebrate Prediction and Classification System). These methods take into account landscape and local characteristics such as latitude/longitude, altitude, river size and in-stream habitat quality. An important caveat in understanding ecological status is land use. For instance, Barbour *et al* (2006) showed that biological community potential is decreased with increased urbanisation. Hence, chemical mixtures investigations using ecological status information must take urbanisation into account. Using principles found within the WFD, there are two pathway sources that can contribute to

potential chemical mixture effects: discrete point sources and dispersive sources. Point sources include poor municipal wastewater treatment, industrial discharge and pour points corresponding to agricultural runoff. Dispersive sources include down the drain consumer products, as they are discharged via multiple wastewater treatment plants (WWTPs), as well as less discrete non-point source runoff from agriculture and other land covers.

The ECETOC framework incorporated both discrete and dispersive sources. For discrete sources, biologically directed toxicity tests (e.g. whole effluent toxicity tests) may be used to determine the potential causes. A case study was presented from >14,000 acute and chronic whole effluent toxicity tests (using the invertebrate [*Ceriodaphnia dubia*] and the fish [*Pimephales promelas*]) collected from hundreds of municipal wastewater treatment plants in four states in the USA. It indicated that <5% of these tests detected toxicity. Wastewater plants that failed were highly related to the proportion of industrial flow as well as having consistent permit violations, insinuating that plants that fail were poorly run plants. Hence, there appeared to be a lack of evidence that well-run municipal plants were delivering toxic effluents (e.g. includes down the drain consumer product chemicals) to receiving waters. An upstream / downstream study conducted in the state of Ohio verified this finding (Dyer and Wang, 2002). Nearly 200 WWTPs were split into two groups: urban and rural with both upstream and downstream data including fish and invertebrate community status, in-stream habitat, dilution and measured conventional water quality parameters. Via t-tests it was shown that urban upstream and downstream sites were significantly adversely impacted compared to rural sites. Downstream urban sites were significantly more impacted than upstream urban sites. Interestingly, there were no significant decreases in any biological metric downstream from rural WWTPs as compared to upstream sites. Importantly, there was no significant difference in the in-stream dilution of urban vs. rural sites. That is, large WWTPs (urban) discharge to large rivers and rural WWTPs typically discharge to small streams. The implications of this study are considerable as one considers the potential mixture effects of down the drain consumer product chemicals. Assuming the per capita use of down the drain product chemicals is the same in urban vs. rural areas, one would have expected both areas to show adverse effects to the ecological status downstream of their respective WWTPs. However, this was not found to be the case. Instead, it appeared that urban discharges contain other chemicals/factors that may be responsible for poorer downstream ecological status.

Since rivers typically receive chemical mixtures from multiple discharges and non-point sources, eco-epidemiological studies are utilised to assess relative causality. A case study from Ohio, USA, was presented where a ‘golden dataset’ of ~2000 sites containing diverse data such as – hydrology, land use/land cover, soil characteristics, measured conventional pollutants, modelled pesticide, pharmaceutical, hormone and consumer product riverine chemical concentrations (>300 total chemicals), toxic pressure as well as biological community status and local habitat quality – were compiled into a geographically referenced database. Since some variables were

highly correlated, they were eliminated or grouped within several statistical methods – including classification and regression trees and the effect and probable-cause method (de Zwart *et al*, 2006). Preliminary analyses from data collected for the 2000-2008 time period suggest that a key factor in appropriate ecological diagnostics is to make sure that reference sites are indeed at reference condition. Within the time period studied, the state had experienced both hurricanes and severe droughts. Preliminary analyses clearly showed that overall the ecological condition of the state's rivers and streams has improved compared to that analysed by de Zwart *et al* (2006). The potential effect of mixtures to reduced ecological status did not appear to be additive across all >300 chemicals, but due to a small number. The 2000-2008 case studies are currently in draft and will be submitted for publication in 2012.

3.11 Direct measurements of human exposure to priority air pollutants – the AIRMEX project

Dimitrios Kotzias, Diana Rembges and Josefa M. Barrero-Moreno, Joint Research Centre Ispra, Italy

The European Indoor Air Monitoring and Exposure Assessment Project (AIRMEX) (2003-2008) was designed with the aim to identify and quantify the principal air contaminants present in public buildings. These include indoor environments frequented by children, such as schools and kindergartens. The project also evaluates to what extent people's exposure to these pollutants is affected whilst working and/or remaining in these areas (Kotzias *et al*, 2009). Within this frame, measuring campaigns in eleven European cities located in Southern, Central and Northern Europe were carried out to monitor indoor/outdoor and personal exposure concentrations of selected volatile hydrocarbons (VOCs) including low molecular weight carbonyls (CARB). In total, about 1000 samples from 182 working environments (offices, class rooms, waiting halls) in public buildings, schools and kindergartens, from 103 private (home) places and from adult volunteers (148 samples) were analysed for VOCs and CARBs (database AIRMEX: http://web.jrc.ec.europa.eu/airmex/). The campaigns were carried out twice at each site in different seasons to evaluate possible climate-related variations in indoor, outdoor and exposure concentrations. The results indicate that indoor air pollution values are higher than the respective outdoor ones for the chemical families this study focused on. Personal exposure concentrations were higher or similar to indoor concentrations and significantly higher than outdoor concentrations. In some cases, home indoor concentrations by far exceeded public building and school/kindergarten levels and dominated personal exposures indicating the presence of strong indoor sources at home. For some compounds (e.g. benzene, formaldehyde) median or mean average and 95th percentile personal exposures and indoor concentrations are well above health benchmarks, so that outdoor concentration measurements alone would underestimate long-term health risks from human exposure to these pollutants. The non-cancer effect of the combined exposure to the main VOCs at the levels measured in the campaigns was investigated using whole genome gene expression micro-arrays (toxicogenomics). The results show that the presence of

toluene in indoor air mixtures comprising benzene and other single aromatic compounds enhances non-carcinogenic responses like inflammation.

3.12 Toxicity and the assessment of mixtures of chemicals (Opinion of DG SANCO SCs)

Helmut Greim, SCHER and Technical University Munich, Germany

The three non-food Scientific Committees of DG SANCO, i.e. the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) have been asked by the EU Commission to advise on six issues related to chemical mixture. The Commission's concern is to ensure that EU's chemical legislation takes proper account of the latest scientific information on mixture toxicity. The preliminary opinion, having also received input from expert of other relevant EU agencies, was published this July for public commenting (SCHER, 2011).

The presentation introduced the structure and overall content of the report, such as principles of mixture toxicology and methodological aspects of effects and exposure assessment as well as specific aspects relating to ecological effects. Detailed answers to the six issues raised can be summarised as follows (taken from the SCHER, 2001, report):

"

1. Under certain conditions, chemicals may act jointly in a way that the overall level of toxicity is being affected.
2. Chemicals with common modes of action may act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. These effects can be described by dose/concentration addition.
3. For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health concern if the individual chemicals are present at or below their zero-effect levels.
4. Interactions (including antagonism, potentiation, synergies) usually occur at medium or high dose levels (relative to the lowest effect levels). At low exposure levels, they are either not occurring or toxicologically insignificant.
5. In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of initial filter to allow a focus on mixtures of potential concern is necessary. Several criteria for such screening are offered.
6. With regard to the assessment of chemical mixtures, a major knowledge gap at the present time is the rather limited number of chemicals for which there is sufficient information on their mode of action. Currently, there is neither an agreed inventory of mode of actions, nor a defined set of criteria how to characterise a mode of action for data-poor chemicals.

7. If no mode of action information is available, the dose / concentration addition method should be preferred over the independent action approach. Prediction of possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis.

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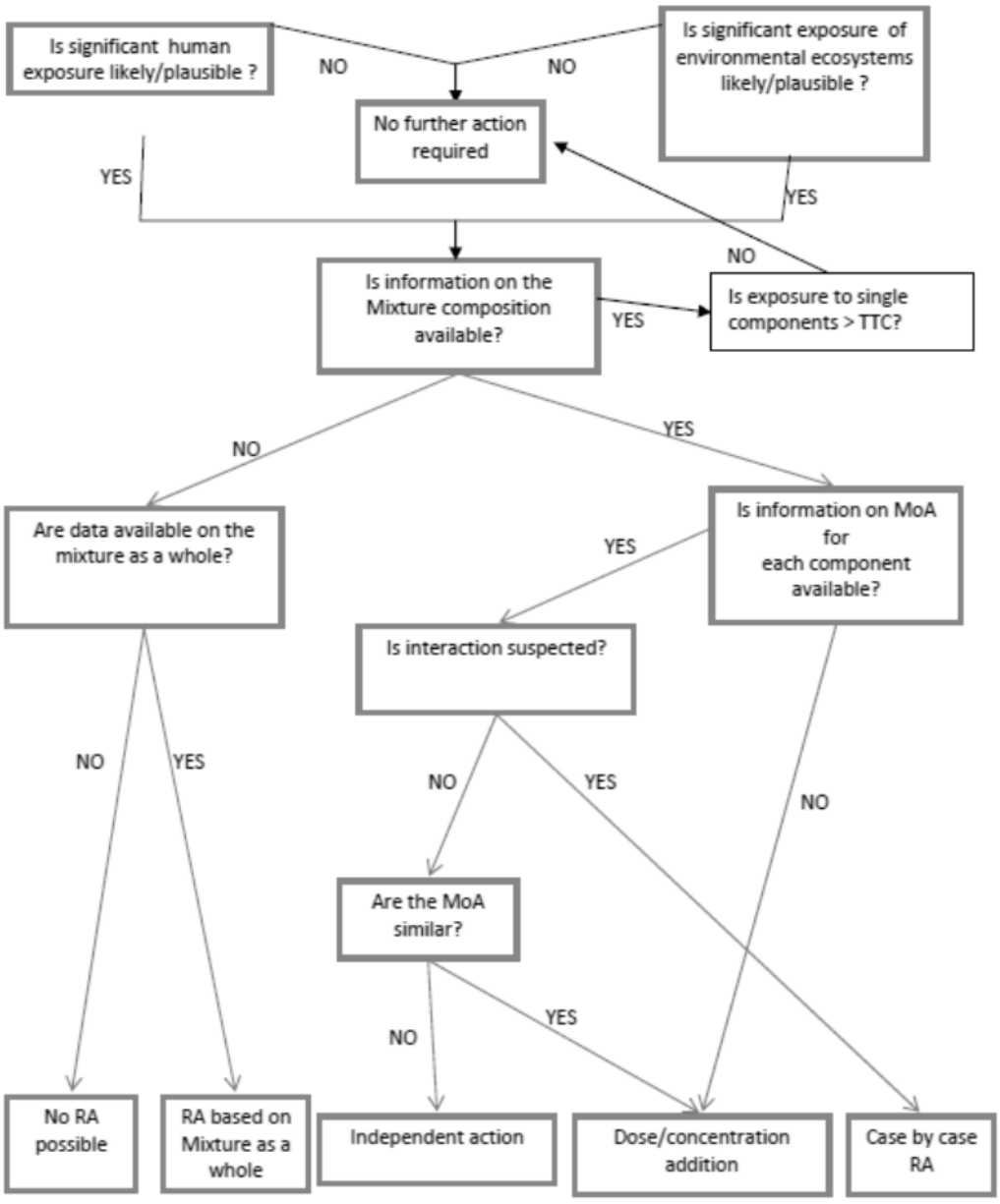
Based upon these conclusions, a decision tree for evaluating the risk of chemical mixtures is proposed.

Figure 2: Decision tree for the Risk Assessment of Mixtures (SCHER, 2011)



4. BREAKOUT GROUP SESSIONS

The Workshop participants discussed specific questions under three broad themes in breakout groups. The following feedback was given from the discussions.

4.1 Breakout Group I: Assessment of combined toxicity

Moderator: Thomas Backhaus
Rapporteur: Alan Boobis

Other members of the breakout group included:
Herman Autrup, Paola Cassanelli, Catherine Clapp, Eric Debruyne, Jean-Lou Dorne, Katherine Flynn, Frédéric Frère, Helmut Greim, Christa Hennes, Per Johansson, Marion Junghans, Markus Junker, Reinhard Kreiling, Marie-Louise Meisters, Karina Petersen, Paul Price, Chantal Smulders, Volker Soballa, Henrik Tyle, Erik Van Miert, Carolyn Vickers.

The discussion within the breakout group was organised along the following major issues:

When do we need to consider combined effects?

The participants agreed that the fundamental premise for combination effects to warrant attention is the co-occurrence of several chemicals in sufficient proximity in time and space. Whether this is the case has to be decided on the basis of appropriate exposure scenarios, e.g. from an occupational health perspective, based on monitoring data from a particular environmental compartment, exposure modelling efforts or prospective thresholds set for e.g. food and feed. It was stressed that new legislative efforts, e.g. the new regulation on plant protection products or the upcoming biocide regulation, might pose specific legal requirements to consider combined effects.

The threshold of toxicological concern (TTC) concept was discussed as a cut-off criterion for the consideration of mixture effects. It was argued that the TTC describes an appropriate 'no-effect' concentration, and hence a mixture of compounds with dissimilar modes/mechanisms of action can be considered inherently safe if all compounds are present at or below their individual TTC. This, however, was debated. It was also argued that it is principally impossible to prove the absolute absence of an individual effect at the TTC ("absence of proof is not proof of absence") – and hence a mixture might still show biological activity, even if the compounds are present only at their individual TTCs, depending on the number of involved compounds. It was agreed, however, that if the compounds share the same mode/mechanism of action, appropriate fractions of TTC concentrations could be used to ensure that the mixture does not reach a critical effect threshold.

Although an ecoTTC concept which follows the philosophy of the TTC concept has been suggested for ecotoxicologial purposes (de Wolf *et al*, 2005), this work needs further refinement and validation. In particular, its potential use in the context of environmental mixture toxicity assessment needs further analysis.

It was a general consensus that there is a lack of appropriate case studies, both in human toxicology and ecotoxicology.

When is it appropriate to apply the available concepts?

It was unanimously agreed that component-based concepts (mainly concentration / dose addition and independent action) play important roles in the risk assessment of chemical mixtures, given the continuous change in exposure profiles (even with respect to well-defined products). Component-based concepts, by virtue of their very nature, always assume that the chemical composition of the mixture of interest is known or can be estimated with reasonable certainty. Both concepts are based on the idea of a non-interaction of the mixture components, an assumption that needs critical reflection for each assessed mixture. Both are also only applicable to chemicals that are toxic as individual compounds, although perhaps only at a concentration higher than the one present in the mixture.

The quantity and quality of available data and the consideration of uncertainties in the various models were also put forward as critical issues for the application of both concepts. Finally, it was pointed out that it is often a policy decision whether the data available for a particular exposure scenario are regarded as being sufficient for risk assessment and management.

Dose addition was identified as a suitable default in a tier 0 risk assessment for the environmental as well as the human health risk assessment of chemical mixtures. In higher tiers, more sophisticated approaches based on advanced modelling approaches (e.g. PBPK modelling) and the detailed study of physiological and/or ecological interactions are appropriate.

For the environmental hazard and risk assessment it was suggested to use the sum of PEC/PNEC values as a starting point, while keeping in mind that this is potentially violating the basic assumption of dose addition, i.e. that all considered toxicity values refer to the same species and endpoint. However, due to its conservatism and ease of use it was suggested to employ the sum of PEC/PNECs ratios as a first filter, in order to analyse whether there is actually a case to answer. If this is the case, a scientifically more robust Toxic Unit summation (species-by-species or trophic level by trophic level) can be employed as a follow up. In subsequent tiers, more data demanding methods based on species sensitivity distributions could then be used for the environmental assessment of mixtures.

It was finally stressed that a complete and adequate documentation of results from studies on individual compounds is critical for the subsequent use of component-based approaches in mixture toxicity assessments. This includes in particular the appropriate description of complete dose-response relationships (instead of providing only NOEC/NOELs or EC_{50} values). The absence of adequate toxicological or ecotoxicological information was identified as a typically limiting factor for the application of dose addition (DA) and independent action (IA).

Are there adequate criteria for grouping substances?

Dose addition and independent action are based on competing, mutually exclusive assumptions on the pharmacological similarity and dissimilarity of the mixture components, respectively. DA is based on the idea that the compounds share a similar mode/mechanism of action, while IA assumes that the compounds in a mixture still contribute to the same endpoint, but do that via different modes and mechanisms of action. However, it has also been argued that due to the mathematical relationship between the two concepts, the quantitative differences between the DA- and IA-predicted mixture toxicities are often too small to be of any regulatory concern.

It was hence discussed whether, under which circumstances and to what extent, mode or mechanism of action information is needed for applying the concepts, respectively for selecting the most suitable concept for a given mixture. Although no final consensus was reached, the general feeling was that mode/mechanism of action information plays a more prominent role in human health oriented assessments. This might be because such assessments are usually organ/receptor based, whereas environmental assessments usually focus more on integral effects on the whole organism and how this influences the population. There was agreement that there is value in applying multiple models to a given set of data, given the on-going debate and the often considerable knowledge gaps with respect to the available information on modes/mechanisms of action of the mixture components. It is recognised that this might be difficult if limited data are available since for IA calculations the actual effects of the single mixture components at their given concentration in the mixture need to be known – while usually only the lethal effect concentrations are known (e.g. LC/EC_{50}-values). For such limited data it is possible to calculate the maximum possible factor by which the application of CA as a default approach might overestimate the toxicity if not all substances act through the same mechanism of action. This is done by dividing the highest single toxic unit by the sum of all toxic units in the mixture.

The task of adequately grouping compounds might be simplified if only a comparatively small number of compounds drive the expected toxicity of a mixture. The Maximum Cumulative Ratio (MCR) that has recently been put forward by Price and Han (2011) was discussed as a tool for deciding how much could be gained by an assessment of the mixture components together, as opposed to that of any single dominant compound. A general need was identified for more case studies in order to determine the impact of different grouping criteria on the outcome of the risk

assessment within the different regulatory contexts. Further work is particularly needed on developing criteria on how broad assessment groups should be.

How can the developing methodologies be used in the future e.g. systems biology, adverse outcome pathways?

There is currently considerable effort in developing a 'bottom-up' approach to toxicity testing for potential effects on human health, rather than the conventional 'top-down' approach, based on studies in laboratory species. In the evolving paradigm, chemicals would be tested for their effects on toxicity pathways, using *in silico* (e.g. QSAR) and *in vitro* approaches, with only limited, if any, testing *in vivo*. Hence, adverse health effects would be predicted on the basis of effects on intermediate processes. These developing methodologies could be used in the assessment of combined exposures to chemicals in several ways. As they will be based on toxicity pathways, or key events, similarities and dissimilarities in mode of action will be more readily identified, aiding the grouping of compounds and the selection of the appropriate prediction concept. The development of relatively inexpensive and rapid assays would enable more combinations of chemicals to be tested experimentally. As part of the evolving paradigm involves the development of effective methods for extrapolation from effects *in vitro* to *in vivo,* for example by using physiologically-based toxicokinetics, higher tier assessments, which rely on estimates of combined target tissue concentration, will be more readily performed. It should be stressed however, that whilst substantial resources are being committed to the development of such methodologies, it is by no means certain which will be available in a sufficiently reliable form in the near future.

For the environmental assessment of mixtures, new tools need to be developed and validated that can describe and predict the impact of chemical mixtures on population processes and recovery phenomena. QSARs and chemometric approaches were discussed as surrogates for bridging gaps in single substance data and a general lack of information on modes and mechanisms of action.

General comments

- Methods should:
  - Generate information relevant to adverse health effects and have the ability to test a wide variety of chemical combinations.
  - Provide quantitative data on dose-response (qualitative hazard identification is not informative for combined risk assessments) for use in defining common assessment groups and effects of concern that occur at relevant exposure levels.

- Apply new methods to problem formulation.

- Toxicity testing in the 21st century: QSARs etc. [Implications for the types of models that will be used].

- Prediction/estimation of parameters for PBPK models:
  - HTS technique.
  - QSAR and other chemometric approaches.

- Value of knowledge of key events, accessible by *in vitro* and other 'Tox 21' approaches in combined risk assessments.

- Possible applications in ecotoxicology e.g. by enabling wider range of species sensitivities to be determined, using *in vitro* assays?

- Systems biology and 'omics in ecotoxicology and toxicology.

- Improved approaches to assess population dynamics and recovery in ecotoxicology.

4.2 Breakout Group II: Exposure and risk assessment – human health

Moderator: Bette Meek
Rapporteur: Martin Wilks

Other members of the breakout group included:
Karine Angeli, Jim Bridges, Neil Carmichael, Jacques de Gerlache, Claudia Drucker, Alexius Freyberger, Hans Ketelslegers, Dimitris Kotzias, Barry Maycock, Britta Michalski, Pierre Nord, Mark Pemberton, Carlos Rodriguez, Erik Tielemans, Jacqueline van Engelen, Corinna Weinz, Frederike Wiebel.

Are the available methods/tools for assessing combined exposure to chemicals adequate and, if not, what is needed?

- Approaches to assessments of combined exposures to multiple chemicals may vary from those for exposure to individual chemicals from a variety of sources.

- Current methods are available for single chemical assessment. The issue is whether these are applicable/need to be changed to assess combined exposures.

- Towards improving predictability, better use should be made of the tools and data that are already available by verifying their applicability to combined exposures. For example, through targeted monitoring data and by using the principles applied in 'data-rich' situations (e.g. pesticides) and related fields (e.g. ecotoxicology) for 'data-poor' situations (e.g. low volume chemicals).

- A stepwise approach is essential.

How can exposure considerations inform on the need for conducting risk assessment of combined exposures?

- It is important to establish that co-exposure does in fact occur.

- In relation to establishing the potential for co-exposure, use profiles (such as source, frequency, duration of use) are more relevant than, for example, production volume.

- Essential information for the prioritisation of combined exposure assessments also includes routes of exposure, physico-chemical properties (such as stability and volatility) and the population likely to be exposed (e.g. consumer versus non-consumer use).

What type of data (quantity and quality) are required for each level of the assessment tiers for combined exposure, and how can uncertainty be accounted for?

- Moving through the tiers makes predictions more realistic and reduces the need for conservatism. Worst case assumptions are made in the lower tiers. However the level of protection does not change and must be sufficient at all levels of the tiered assessment to protect human health.

- Uncertainty is related to the degree of precision of the available information.

- There is a need for better communication. Terminology is crucial in establishing a common understanding of the terms used, for example 'conservative' or 'sensitivity analysis'.

- Generic approaches are adequate in the early tiers with more specific information being required in the later tiers.

- A consideration of sensitivity is critical, particularly in relation to the drivers at the lower tiers.

- Additional data allows the refinement of models. Data are more important in the higher tiers.

What technologies would be helpful in the future?

- Currently, developing technologies show more promise in relation to hazard assessment (e.g. identification of mode of action) than to exposure assessment.

- With regard to hazard assessment, better identification of 'mode of action' is needed to refine groupings.

- With regard to exposure assessment:

    - additional development and testing of methodology in relation to tiered approaches for combined exposures are desirable;
    - 'exposome' is currently a theoretical concept rather than a methodology;
    - communication is important (e.g. in ensuring that the difference between exposure and risk is fully understood).

- Coordinated monitoring strategies are required for verifying early tier models and for improving higher tier assessments. In a tiered approach it is important to have a series of increasingly comprehensive, data-rich exposure models available.

4.3 Breakout Group III: Exposure and risk assessment – environment

Moderator: Theo Brock
Rapporteur: Tobias Frische

Other members of the breakout group included:
Erwin Annys, Abigail Bartram, Peter Day, Klaas den Haan, Axel Dinter, Scott Dyer, Malyka Galay Burgos, Vladimir Garkov, Anne Gourmelon, Mick Hamer, Chris Holmes, Reinhard Länge, John Lipscomb, Maximillian Mühe, Louise Pope, Johannes Tolls, Kim Travis, Marco Vighi, Paul Whitehouse.

What tools are available for the assessment of environmental exposure to mixtures?

A general observation was that the situation is complex and different with regard to prospective or retrospective risk assessment. With regard to prospective risk assessment the definition of environmentally relevant mixtures (i.e. mixture composition: number, identity and concentrations of individual substances) was generally considered easier than for retrospective risk assessment but not without problems. Prospective environmental risk assessment of technical mixtures is (at

least partly, i.e. especially for acute exposure) covered by current legislation on plant protection products, biocides, pharmaceuticals and REACH, while a focus on retrospective assessment came with the Water Framework Directive. In considering the tools available and/or required for mixture risk assessment, each was considered separately and as indicated below. The discussion was restricted to the aquatic compartment.

A. Prospective assessment

- Reasonable prioritisation criteria are needed to establish which chemicals should be included in a prospective mixture risk assessment for a given exposure scenario.
- There are adequate models for point and non-point sources for a mixture exposure assessment.
- There is a lack of access to basic input data to run the exposure models.
- Existing exposure models have limitations for prospective exposure assessment for complex (i.e. less well defined mixtures, see above) technical mixtures (apart from petroleum products). Retrospective assessment using monitoring was considered the only way forward for such complex mixtures.
- No specific guidance is yet available in relation to mixture exposure assessment (i.e. for the derivation of a PECmix). Overly conservative assumptions should be avoided, given that typically they have already been made for single substances exposure assessment.
- Time-variation in exposure towards mixtures should be considered, such as peak versus time-weighted average.
- Responsibility in the case of formulated products/technical mixtures rests with the producer/notifier.

B. Retrospective assessment

- Emission data are largely missing.
- Data in relation to point sources and chemicals emitted are available for certain catchment areas.
- There is a need for improved co-operation between the agencies involved in assessment and management, particularly with regard to data sharing.

What tools are available for the assessment of environmental effects of mixtures?

A. Prospective assessment

- In relation to the predictive toxicity assessment of mixtures, concentration addition (CA) is a pragmatic but a relatively worst-case to realistic worst-case tier 1 screening approach.
- Questions arise in relation to how to proceed at higher tier levels (e.g. mesocosms, field studies) and of the applicability of CA for long-term effects/chronic exposure.

- PEC/PNEC summation (as an even more pragmatic approach for mixture risk assessment): It is crucial to know how the PNEC is derived to ensure scientific integrity of the PEC/PNEC summation. More debate around this issue is needed.
- Data-poor situations need to be considered (e.g. input of QSAR data).

B. Retrospective assessment

- As with prospective assessment, CA is suited for a screening assessment of the effect of mixtures in the environment.
- ms-PAF [multi-substance potentially affected fraction of species, as calculated from a species sensitivity distribution for a defined mixture] is also a suitable tool, but the ecological significance of this approach has to be further explored.
- A definition of representative environmental reference conditions is crucial.

Discussion on 'problem formulation'

- "As environmental quality is constantly improving, is there really a problem with mixtures?" ["Maybe, maybe not!"]

- With regard to the prospective risk assessment of technical mixtures/products (especially those with active ingredients), a consideration of mixture toxicity is generally needed, but there is a lack of explicit guidance on how to conduct the mixture risk assessment (i.e. through different tiers and for different legislations).

- With regard to using retrospective risk assessment data from several in-put sources, there is a question of whether focus is on the relevant mixtures and on the substances that contribute. Other environmental stressors should be considered if risk management is required.

- It is recommended that exposure modelling is verified by chemical monitoring.

4.4 Question posed to each of the groups

Can these approaches be applied in a regulatory context?

- Approaches such as those of WHO/IPCS and DG SANCO can be applied in a regulatory context, but application will depend on the problem formulation.
- Problem formulation is essential in human health risk assessment towards identifying priority groupings.
- Regulatory drivers and regulatory context are important.
- There is a need to be pragmatic.

- Lower tier assessments are useful to identify a need for critical data.
- Concentration addition can be used as a default in a regulatory context as worst case.
- There should be some conclusion/communication in a tiered approach when a lower tier is not passed.

4.5 Plenary discussion

Following the reports from the breakout sessions, a general discussion took place led by Mark Pemberton. The points raised were:

- Problem formulation is essential in considering the combined exposure to chemicals and towards establishing under which conditions there is a real/theoretical problem, i.e. there is a need to identify populations of interest and to characterise exposure.

- Prioritisation is essential and a step-wise approach required.

- There is a strong need to develop relevant exposure data in all areas. Improved exposure data banks are needed with better coordination between them.

- A tiered approach is necessary in the risk assessment of co-exposures to chemicals. The ECETOC TRA tool is an example of a first level tool. What is also needed is the next level tool for all three, i.e. environment, worker and consumer exposure.

- The TRA approach involves a high degree of 'conservatism'. An agreement on the levels of conservatisms is needed, especially for consumer exposure.

- The TTC concept should be developed further, particularly in relation to environmental assessments.

- There is a problem of communication, e.g. a clear understanding of the terminology used is needed.

- There is a substantial amount of data available on case studies. These data could inform under which conditions there is a problem and which end points to study. More use should be made of existing knowledge and more case studies studied to identify the gaps in knowledge. Combined exposure assessments should be performed only when there is justification that they are needed, and with a tiered and targeted approach.

5. CONCLUSIONS AND RECOMMENDATIONS

Concluding remarks from Jim Bridges

Professor Jim Bridges presented his views as a participant on the outcome of the workshop.

Scope of the topic
It is important to establish the scope of the issue, i.e. whether one should be looking at everything or focusing on one aspect – and if so how the focus should be decided. For example is this in relation to chemicals alone or chemicals in combination with biological and physical stressors? In addition are these mixtures from specific sources or all situations involving combined exposure, and are these all combinations and mixtures, or only selected ones? Finally, is exposure only simultaneous or also sequential?

Prerequisites for an assessment
Prerequisites for an assessment are transparent and robust criteria for identifying combinations to be given priority for assessment, and suitable measured and/or modelled exposure data. This raises an important question of what is acceptable imprecision in exposure data, beyond which no assessment is worthwhile.

General conclusions
Some general conclusions can be drawn at this stage. General categorisation of interactions has been agreed, but not the terminology. Synergy or antagonism are uncommon, but might occur at a relatively low magnitude. In an investigation of a combined exposure, a few chemicals may have a predominant impact, thus it is important to identify such chemicals at an early stage. Critical issues are framing the problem, and communication of the findings.

Procedures
With regard to procedures, a tiered approach beginning with limited data and a high degree of conservatism is required, moving if necessary to a requirement of more realistic data, with a reduction in conservatism and increased expert input. This raises the question, however, of what is 'realistic' conservatism. TTC or equivalent is a potentially important element of lower tier assessment.

Promising developments
There are currently a number of promising developments. These include an increasing understanding of MOA in risk assessment, and improving data bases/data processing methods and their use, for example, in QSAR.

Particular challenges

Particular challenges are how to group chemicals for both exposure and hazard characterisation, establishing what would constitute a realistic tiering of exposure methods to fit the WHO/IPCS framework[1], how to address major data gaps that are not readily filled, and how the increasing dependence on *in vitro* methods for data generation might influence the future strategy.

What next?

The next steps are suggested as the development of transparent, scientifically-valid criteria for prioritising combinations that need to be assessed, and considering interactions where exposure to biological or physical stressors is currently near the threshold for effects. A much greater priority should be given to exposure assessment, which is also vital for TTC. A need has also been identified for a range of case studies to validate/improve the assessment of mixtures. Finally guidance should be formulated on how to address combinations of chemicals across the various domains.

Departing discussion point

Finally, as a departing discussion point, perhaps some consideration should be given to establishing the position of the risk assessment of mixtures among the various priority areas for improving the contribution of the sciences to protecting human health and the environment.

[1] During the Workshop, it was suggested that more case studies should be useful to illustrate the WHO/IPCS framework including methods for tiered exposure assessments.

ABBREVIATIONS

AIRMEX	European Indoor Air Monitoring and Exposure Assessment Project
CA	Concentration addition
CARB	Low molecular weight carbonyls
Cefic	European Chemical Industry Council
CRA	Cumulative risk assessment
DA	Dose addition
DG	Directorate General
DG SANCO	EU Health and Consumer Protection Directorate General
EFSA	European Food Safety Authority
EU	European Union
GREAT-ER	Geographically-referenced Regional Exposure Assessment Tool for European Rivers
HESI	Health and Environmental Sciences Institute
HTS	High throughput screening
IA	Independent action
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
LAS	Linear alkylbenzene sulfonate
MCR	Maximum Cumulative Ratio (approach)
MOA	Mode of action
ms-PAF	Multi-substance potentially affected fraction
NGO	Non-governmental organisation
NOEL	No observed effect level
OECD	Organisation for Economic Co-Operation and Development
PBPK	Physiologically based pharmacokinetic (modelling)
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
POD	Point of departure
PSD	Pesticides Safety Directorate
QSAR	Quantitative structure activity relationship
REACH	Registration, evaluation, authorisation and restriction of chemicals
RIVM	Dutch National Institute for Public Health and the Environment

SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SETAC	Society of Environmental Toxicology and Chemistry
SPIN	Substances in Preparations in Nordic Countries (database)
TEQ	Toxicity equivalents
TRA	Targeted risk assessment
TTC	Threshold of toxicological concern
UK IGHRC	United Kingdom Interdepartmental Group on Health Risks from Chemicals
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organisation
WWTPs	Waste Water Treatment Plants

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APPENDIX 1: WORKSHOP PROGRAMME

Monday 11th July 2011 – morning

08.30 – 09.00	*Registration*	
09.00 – 09.10	Welcome and introduction	Neil Carmichael ECETOC
	Chair: Christa Hennes, ECETOC	
09.10 – 09.30	Overview on activities on the risk assessment of combined exposures to chemicals	Carlos Rodriguez P&G

HAZARD EVALUATION

09.30 – 10.00	Effects of low and high doses of combinations of chemicals on human health	Kim Travis Syngenta
10.00 – 10.30	Effects of combinations of chemicals in the aquatic environment	Mick Hamer Syngenta
10.30 – 10.45	Questions	
10.45 – 11.25	*Coffee break*	

EXPOSURE EVALUATION

11.15 – 11.45	Human exposure assessment	Jacqueline van Engelen RIVM
11.45 – 12.15	Exposure in the aquatic environment	Chris Holmes Waterborne Environmental
12.15 – 12.30	Questions	
12.30 – 13.30	*Lunch*	

Monday 11th July 2011 – afternoon

IMPACT AND RISK ASSESSMENT

13.30 – 14.00	Conventional approaches to risk assessment of mixtures	John Lipscomb US EPA
14.00 – 14.30	WHO/IPCS Framework on risk assessment of combined exposures to multiple chemicals	Bette Meek University of Ottawa
14.30 – 15.00	Review of the evidence for the magnitude of low-dose synergy by ILSI/HESI	Alan Boobis Imperial College London
15.30 – 15.15	Questions	
15.15 – 15.45	*Coffee break*	
15.45 – 16.15	Cumulative risk assessment and MCR approach	Paul Price Dow Chemicals
16.15 – 16.45	Evaluating impacts of chemicals in the environment	Scott Dyer P&G
16.45 – 17.15	Toxicity and the assessment of mixtures of chemicals (Opinion of DG SANCO SCs)	Helmut Greim SCHER
19.00 – 22.00	*Workshop Dinner*	

Tuesday 12th July 2011

09.00 – 09.15	Introduction to breakout groups	Christa Hennes ECETOC
09.15 – 09.30	Project briefing	Dimitris Kotzias JRC

Direct measurements of human exposure to priority air pollutants - the AIRMEX project

09.30 – 12.30 **BREAKOUT GROUP DISCUSSIONS**

(10.30 – 11.00 *Coffee break*)

Breakout Group I: Assessment of combined toxicity
Moderator: Thomas Backhaus *Rapporteur: Alan Boobis*

Breakout Group II: Exposure and risk assessment – human health
Moderator: Bette Meek *Rapporteur: Martin Wilks*

Breakout Group III: Exposure and risk assessment – environment
Moderator: Theo Brock *Rapporteur: Tobias Frische*

12.30 – 13.30	*Lunch*	
13.30 – 14.45	Report of the breakout groups and panel discussion	Moderator: Mark Pemberton Lucite
14.45 – 16.00	Conclusions	Jim Bridges University of Surrey

Close of Workshop

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