IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WHO

IPCS Harmonization Project

Assessment of Combined Exposures to Multiple Chemicals:

Report of a WHO/IPCS International Workshop

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNEP, UNIDO, UNITAR, WHO and OECD

World Health Organization

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization, the International Labour Organization or the United Nations Environment Programme.

Harmonization Project Document 7

ASSESSMENT OF COMBINED EXPOSURES TO MULTIPLE CHEMICALS: REPORT OF A WHO/IPCS INTERNATIONAL WORKSHOP ON AGGREGATE/CUMULATIVE RISK ASSESSMENT

This project was conducted within the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.

Published under the joint sponsorship of the World Health Organization, the International Labour Organization and the United Nations Environment Programme, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

Harmonization Project Documents are a family of publications by the World Health Organization (WHO) under the umbrella of the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP). Harmonization Project Documents complement the Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as authoritative documents on methods for the risk assessment of chemicals.

The main impetus for the current coordinated international, regional and national efforts on the assessment and management of hazardous chemicals arose from the 1992 United Nations Conference on Environment and Development (UNCED). UNCED Agenda 21, Chapter 19, provides the "blueprint" for the environmentally sound management of toxic chemicals. This commitment by governments was reconfirmed at the 2002 World Summit on Sustainable Development and in 2006 in the Strategic Approach to International Chemicals Management (SAICM). The IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter 19, and contributes to the implementation of SAICM. In particular, the project addresses the SAICM objective on Risk Reduction and the SAICM Global Plan of Action activity to "Develop and use new and harmonized methods for risk assessment".

The IPCS Harmonization Project goal is *to improve chemical risk assessment globally, through the pursuit of common principles and approaches, and, hence, strengthen national and international management practices that deliver better protection of human health and the environment within the framework of sustainability.* The Harmonization Project aims to harmonize global approaches to chemical risk assessment, including by developing international guidance documents on specific issues. The guidance is intended for adoption and use in countries and by international bodies in the performance of chemical risk assessments. The guidance is developed by engaging experts worldwide. The project has been implemented using a stepwise approach, first sharing information and increasing understanding of methods and practices used by various countries, identifying areas where convergence of different approaches would be beneficial and then developing guidance that enables implementation of harmonized approaches. The project uses a building block approach, focusing at any one time on the aspects of risk assessment that are particularly important for harmonization.

The project enables risk assessments (or components thereof) to be performed using internationally accepted methods, and these assessments can then be shared to avoid duplication and optimize use of valuable resources for risk management. It also promotes sound science as a basis for risk management decisions, promotes transparency in risk assessment and reduces unnecessary testing of chemicals. Advances in scientific knowledge can be translated into new harmonized methods.

This ongoing project is overseen by a geographically representative Harmonization Project Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
Ah	aryl hydrocarbon
ARfD	acute reference dose
BINWOE	binary weight of evidence
BMD	benchmark dose
BMDL	lower limit on the benchmark dose
BRN	biochemical reaction network
bw	body weight
CEPA 1999	Canadian Environmental Protection Act, 1999
ComDeBDE	commercial decabromodiphenyl ether
ComOcBDE	commercial octabromodiphenyl ether
ComPeBDE	commercial pentabromodiphenyl ether
CSFII	Continuing Survey of Food Intakes by Individuals (USA)
DeBDE	decabromodiphenyl ether
DSL	Domestic Substances List (Canada)
ED_{10}	effective dose for 10% inhibition
EU	European Union
EUSES	European Union System for the Evaluation of Substances
FAO	Food and Agriculture Organization of the United Nations
FQPA	Food Quality Protection Act (USA)
GC-MS	gas chromatography–mass spectrometry
HeBDE	heptabromodiphenyl ether
HI	hazard index
HxBDE	hexabromodiphenyl ether
IPCS	International Programme on Chemical Safety (WHO)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MOA	mode of action
MOE	margin of exposure
MOE_T	combined margin of exposure
NOAEL	no-observed-adverse-effect level
NoBDE	nonabromodiphenyl ether
NOEL	no-observed-effect level
OcBDE	octabromodiphenyl ether

OP	organophosphate
OPP	Office of Pesticide Programs (USEPA)
OSHA	Occupational Safety and Health Administration (USA)
PAH	polycyclic aromatic hydrocarbon
PBB	polybrominated biphenyl
PBDE	polybrominated diphenyl ether
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PeBDE	pentabromodiphenyl ether
POD	point of departure
PODI	point of departure index
POP	persistent organic pollutant
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REP	relative effect potency
RfD	reference dose
RPF	relative potency factor
SAICM	Strategic Approach to International Chemicals Management
TCDD	2,3,7,8-tetrachlorodibenzodioxin
TD_{50}	tumorigenic dose for 50% of test animals
TeBDE	tetrabromodiphenyl ether
TEF	toxic equivalency factor
TEQ	toxic equivalent
TGD	Technical Guidance Document
TLV	threshold limit value
TPH	total petroleum hydrocarbons
TTC	threshold of toxicological concern
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

EXECUTIVE SUMMARY

A World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) International Workshop on Aggregate/Cumulative Risk Assessment (Combined Exposures to Multiple Chemicals) was held in Washington, DC, United States of America (USA), on 19–21 March 2007. The principal objective of the workshop was to initiate development of a framework for the risk assessment of combined exposures to multiple chemicals.

This Harmonization Project Document reports the outcome of this workshop as an introduction to the accompanying extended abstracts to describe the state of the art in this area and delineation of next steps. In addition to recommendations relevant to subsequent development and review of the framework, terminology was considered in order to facilitate communication internationally in this area.

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1. BACKGROUND

To ensure timely consideration of relevant issues in risk assessment, the Steering Committee for the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals periodically considers proposals received from risk assessment agencies and individual experts for new harmonization project activities. As a result of a review undertaken in 2004, it was decided to include work on risk assessment for exposures to multiple chemicals in the Harmonization Project Workplan. The need for further work in this area was also identified as a priority for the health sector in the development of the Strategic Approach to International Chemicals Management (SAICM), which was finalized and adopted by governments in Dubai in February 2006.

As a first step, a workshop was convened, with the output intended to inform future activities by IPCS and others. This report, authored by the members of the Workshop Planning Group, reports the outcome of this workshop, which was held in Washington, DC, USA, on 19–21 March 2007. At this workshop, participants considered relevant questions and considered the relevant contents of a framework for assessment of risks associated with exposures to multiple chemicals, on the basis of a draft prepared in advance of the meeting. The list of participants appears at the front of this document.

The delineated objectives of the workshop were as follows:

- Discuss and review available methods for assessing the combined risk from exposure to multiple chemicals (with or without a common mode of action) via all relevant routes and pathways. This includes risk resulting from concurrent exposures to multiple chemicals or where exposure at different times leads to overlap in the time course of effects as a consequence of their respective toxicokinetics and/or toxicodynamics.
- Review knowledge gained from approaches adopted to date in different sectors (e.g. pesticides, industrial chemicals, therapeutics) and disciplines (e.g. consumer exposure, occupational exposure, environmental exposure).
- Focus on the risks from multiple chemicals and multiple sources, but exclude consideration in combination with other types of agents (e.g. noise, nutrition).
- Develop working definitions for the different types of exposures, effects and risks of multiple chemicals.
- Initiate the development of a framework for assessment of risk to multiple chemicals.
- Define the next steps for work needed to inform the framework, including identification of areas where no (adequate) methodologies exist, where research is needed and where data are lacking, in order to stimulate efforts to fill these gaps.

2. TERMINOLOGY

One of the barriers to the development of harmonized methodology for assessing the effects of exposure to multiple chemicals is variation in terminology adopted in different jurisdictions and associated understanding. To minimize confusion in this area, the workshop recommended that terminology describing various aspects of exposure and effects of multiple chemicals be as precisely descriptive as possible. The workshop did not have time to develop a comprehensive terminology, but agreed on working definitions for key terms and concepts. It should be noted that the abstracts (see Appendix A) are as presented and thus reflect the multiplicity of terms, rather than the terminology subsequently recommended by the workshop.

While not the principal focus of the workshop, exposure to the same substance by multiple pathways and routes is likely best described as "**Single Chemical, All Routes**" (referenced in some jurisdictions as "Aggregate Exposure"). Similarly, it is recommended that exposure to "**Multiple Chemicals by a Single Route**" be distinguished from "**Multiple Chemicals by Multiple Routes**". To this end, the framework being developed addresses "**Combined Exposures to Multiple Chemicals**".

Chemicals that act by the same mode of action and/or at the same target cell or tissue often act in a potency-corrected "**Dose Additive**" manner. Where chemicals act independently, by discrete modes of action or at different target cells or tissues, the effects may be additive ("**Effects Additive**" or "**Response Additive**"). Alternatively, chemicals may interact to produce an effect, such that their combined effect "**Departs from Dose Additivity**". Such departures comprise "**Synergy**", where the effect is greater than that predicted on the basis of additivity, and "**Antagonism**", where the effect is less than that predicted on the basis of additivity.

Relevant also to the development of a framework for risk assessment of combined exposures to multiple chemicals is a common understanding of "**Mode of Action**", which has been defined previously by IPCS, as it figures prominently in approaches to grouping of chemicals for assessment of combined effects. A postulated mode of action is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed effect. Notably, mode of action contrasts with "**Mechanism of Action**", which generally involves a sufficient understanding of the molecular basis for an effect so that causation can be established (Sonich Mullin et al., 2001).

The workshop also recommended that combined exposure to multiple chemicals be defined in the context of whether or not the components act by similar or different modes of action (i.e. "**Single Mode of Action**" or "**Multiple Modes of Action**"). This is a critically important distinction in the context of the framework being developed, in the interest of focusing effort additionally to that implied by the terms "simple" and "complex". These latter terms are often used to distinguish mixtures with limited numbers of substances from those with large numbers of substances; or mixtures of known composition from those of unknown or variable composition.

3. FRAMEWORK APPROACHES

Frameworks for analytical consideration of the weight of evidence for hypothesized modes of action and their human relevance are increasingly being applied within regulatory agencies as a basis to improve transparency for decisions related to risk assessment and associated delineation of critical data gaps. These frameworks are also increasingly being adopted by industrial stakeholders in iteratively considering relevant next steps in data generation, often in collaboration with regulatory authorities (USEPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003; Seed et al., 2005; Boobis et al., 2006, 2008).

4. DEVELOPMENT OF A FRAMEWORK FOR RISK ASSESSMENT OF EXPOSURE TO SINGLE CHEMICALS BY MULTIPLE PATHWAYS

Exposure to single chemicals by multiple pathways was included in the title of the workshop but addressed principally in the context of its relevance to multiple chemical exposures. It was recommended that guidance in this area to address exposure from multiple routes taking into account the relevant populations and timeframes be developed by IPCS in a follow-up initiative.

5. DEVELOPMENT OF A FRAMEWORK FOR RISK ASSESSMENT OF COMBINED EXPOSURES TO MULTIPLE CHEMICALS

The workshop considered a preliminary proposal for a framework for assessment of risks from combined exposure to multiple chemicals, recommended a number of critical aspects for consideration in its further development as described below and adopted it in principle. A revised version of the preliminary framework, which takes into account these recommendations and suggestions, is being developed. It includes introductory criteria as to when the framework is relevant and a proposed tiered approach for assessing effects of exposure to multiple chemicals, which addresses both exposure and hazard.

Application of the proposed framework for consideration of risk from exposure to multiple chemicals is an iterative process, involving stepwise consideration of both exposure and hazard in several tiers of increasingly data-informed analyses. The approach involves decision-based analysis that takes into account relevant information at an early stage as a basis to scope the need or not for additional assessment and recommend any required data generation. Early consideration of potential for exposure (prior to any consideration of hazard) is essential in determining critical next steps, as there is no need for further assessment if there is no or minimal exposure.

The extent of assessment and nature of recommendations for generation of additional data are dependent upon the extent of the knowledge base, the magnitude of public health concern (i.e. taking into account margins between exposure and effect) and the objective of the risk assessment (e.g. implications of potential risk management decisions). It is envisaged, then, that approaches will range from predictive methodologies and conservative assumptions in early tiers to more realistic estimates of risk and rigorous descriptions of uncertainties in later tiers, based on increasingly data-informed and probabilistic approaches.

This tiered approach is considered essential to ensure that the expenditure of resources is proportional to the public health concern and consistent with regulatory objectives. This

includes the purpose of the assessment, such as prioritization for assessment, screening for additional assessment and/or risk management (including bans, controls and other options, such as education).

The framework for consideration of risks from combined exposures to multiple chemicals is also hypothesis driven, involving analysis of available information followed by a conclusion in which a hypothesis is developed and refined (as necessary). This enables transparent and systematic analyses in the context of a "weight of evidence" approach consistent with the IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans (Boobis et al., 2006) and the IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans (Boobis et al., 2008).

A proposed approach to tiered consideration of hazard for exposure to multiple chemicals, developed by one of the workshop breakout groups, is illustrated conceptually in Figure 1. The boxes in Figure 1 represent components of the biological pathway leading to toxicity, including delivery to the target site following exposure, reaction of the ultimate toxicant (parent or metabolite) with the target molecule(s) and a resulting series of biochemical events leading to some manifestation(s) at the cellular and organ level. The proposal as discussed at the workshop is outlined in the next paragraphs. The draft framework prepared after the workshop reflects the further development of the concepts and includes further explanation.

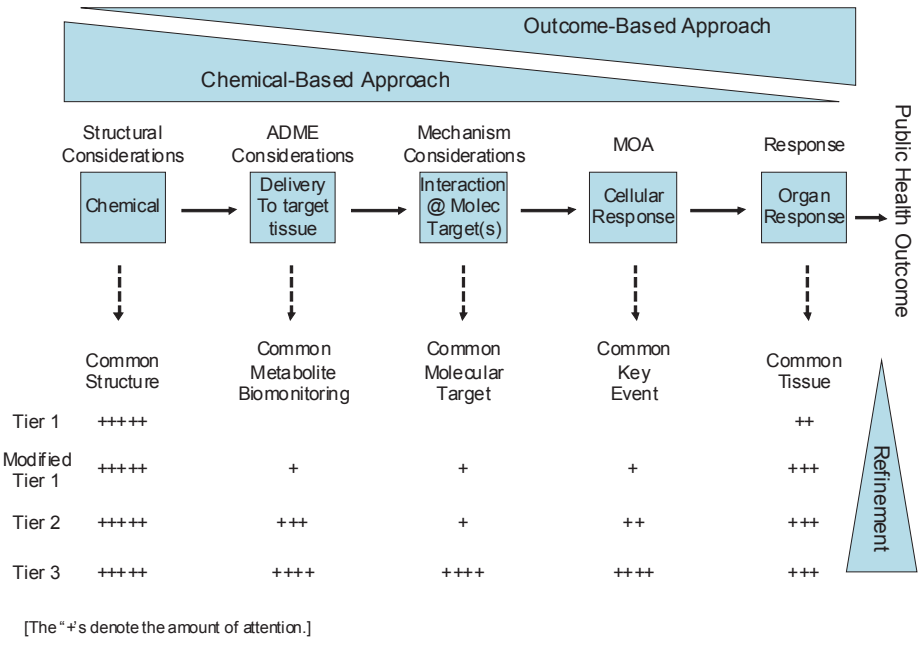

Figure 1: A proposed approach to tiered consideration of hazard for exposure to multiple chemicals (ADME, absorption, distribution, metabolism, excretion; MOA, mode of action).

To develop rudimentary hypotheses for grouping chemicals into a common group in the earliest tiers, considerations could include chemical structure and identification of common potential toxicophores (structural alerts), similarity of target tissue and/or manifestations of toxicity. This proposed tiered approach is being additionally refined.

If, in the earliest tier, there is no evidence of interaction, dose addition is assumed and the combined exposure is an adequate margin below the point of departure of the common

hazard, bearing in mind the purpose of the assessment, the mixture would be considered of low priority for additional consideration of human health effects. However, if the result of this initial consideration is such that the combined risk is not considered acceptable, the initial assessment should be further refined to the extent necessary depending on the overall strength of evidence for co-exposure and dose addition and the purpose of the assessment. Subsequent tiers for hazard could include, for example, additional consideration of the temporal aspect of the common toxic effects (e.g. time to onset/recovery), presence of a common metabolite, key biological events/targets in a hypothesized mode of action for grouped substances and toxicokinetics. In the highest tier, these aspects would be considered in additional detail (e.g. consideration of environmentally relevant exposure mixture ratios and physiologically based pharmacokinetic modelling) as necessary.

More rigorous analysis is based on additional information on the nature of the combined exposure, combined effect, dose addition and greater or less than dose additive effects. Also, the preliminary grouping of chemicals into a common group becomes additionally refined in later tiers, often leading to some substances being dropped or subgrouped.

6. NEXT STEPS

Following peer and public review of the draft framework for consideration of risk from exposure to multiple chemicals, it will be finalized and published. Development of case-studies to illustrate hypothesis generation and refinement, iterative consideration of exposure and hazard and the nature of assessment in tiers of increasing complexity is recommended. Cases are being selected to include groups of substances with additive and greater than additive effects. The workshop identified opportunities to coordinate this work with ongoing national and international efforts.

Dissemination of existing tools relevant to assessment of risk of exposures to multiple chemicals, development and distribution of training materials and compilation of a repository of case-studies were also recommended.

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Appendix A: Workshop Abstracts

WHO/IPCS INTERNATIONAL WORKSHOP ON AGGREGATE/CUMULATIVE RISK ASSESSMENT: OVERVIEW

Kevin M. Crofton

1. BACKGROUND

Humans are constantly exposed to a wide variety of chemicals from food, pharmaceuticals, air and water. A major challenge in risk assessment is to determine the degree of exposure to multiple chemicals, the hazards associated with such combined exposure and the extent to which chemicals interact. Predicting risk from exposure to chemical mixtures is complex, as chemicals in mixtures can interact in terms of both toxicokinetics and toxicodynamics. Such interactions may result in effects that are either antagonistic or synergistic. The temporal nature of the exposures may play a lead role in determining these interactions.

The World Health Organization (WHO) International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals has identified a need to develop a risk framework for assessing the combined risk from exposure to multiple chemicals via all relevant routes and pathways. To begin this process, the WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment was convened to discuss the state of the science for assessing the risk from combined exposure to multiple chemicals.

2. GOALS

The WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment had the following goals:

- Discuss and review available methods for assessing the combined risk from exposure to one or more agents (with or without a common mode of action) via all relevant routes and pathways.
- Review knowledge gained from approaches employed to date in different sectors (e.g. pesticides, industrial chemicals, therapeutics) and disciplines (e.g. consumer exposure, occupational exposure, environmental exposure).
- Focus on the risks from exposure to multiple chemicals and multiple sources, excluding consideration of exposure to and effects of chemicals in combination with other non-chemical factors (e.g. stress, nutrition).
- Develop commonly accepted definitions for the different types of mixtures (e.g. simple, complex) and exposures (e.g. aggregate, cumulative).
- Develop commonly accepted definitions for effect outcomes (e.g. antagonism, synergy).
- Initiate the development of a framework for combined substance risk assessment.
- Define the next steps for work needed to inform the framework, including identifying areas where no (adequate) methodologies exist, where research is needed and where data are lacking, in order to stimulate efforts to fill these gaps.

3. CURRENT METHODS

There are currently a number of methods used to determine the risk of combined exposure to chemicals (ILSI, 1998; USEPA, 2000; Feron & Groten, 2002; DVFA, 2003; Jonker et al.,

2004). These methods can be divided into those that simply add the risk from individual chemicals, those that sum effects based on relative potencies and those that rely only on indirect evidence. Most of these have been developed in response to a regulatory need (e.g. toxic equivalency factor [TEF] and dioxins), and each has advantages and disadvantages.

The *Hazard Index* is the sum of hazard quotients for substances that affect the same target organ or organ system. The hazard quotient is the ratio of the potential exposure to the substance to the level at which no adverse effects are expected (e.g. point of departure, divided by uncertainty factors). A second method, the *Point of Departure Index*, is a simple addition method that adds the no-observed-effect levels (or benchmark doses) of individual chemicals. Neither of these methods includes possible interactions of chemicals that would result in antagonism or synergism.

The *Toxic Equivalent (TEQ)* method was developed for use with compounds that activate the aryl hydrocarbon receptor (Haws et al., 2006; Van den Berg et al., 2006). This is a relative potency method that assumes the additivity of doses of individual components of the mixture after normalization of the response to a reference chemical. The *Relative Potency Factor (RPF)* method (USEPA, 2000) is a generalized form of the TEQ method and has been used for classes of pesticides and other chemicals. This method also uses dose addition as the default assumption for the effects of mixtures.

Two additional methods have been used when data limitations prevent the use of the above-mentioned methods. The *Whole Mixture Approach* (Mumtaz et al., 1993) uses effects data from exposure to the mixture of concern or a sufficiently similar mixture. These data are treated in a risk context similarly to single chemical data. Lastly, the *Threshold of Toxicological Concern (TTC)* has been proposed for use with complex mixtures where no effects data are available (Kroes et al., 2005). This method uses structure–activity relationships to assign exposure thresholds for comparison with the potential exposure level and requires exposure estimates.

The workshop discussed these and other methods in the context of exposure to single or multiple chemicals from all routes. The key objective of the current effort is to harmonize the variety of approaches currently used and to develop a framework in which they can be used.

4. COMMON NOMENCLATURE?

4.1 Interactions

The lack of a common nomenclature to describe the outcome of mixtures testing has been recognized for more than 50 years: “Indeed, the quantitative problems of combined drug-effect still persist unchanged, revolving around the two terms synergism and antagonism—which are in need of clarification as ever before” (Loewe, 1953).

Loewe (1953) listed 10 terms to describe drug interaction, based on whether the outcome was homodynamic or ahomodynamic (de Jong, 1961). Earlier, Bliss (1939) had introduced the concept of independent joint action. Since that time, a plethora of terms, with overlapping definitions, have been used to describe the outcome of testing the effects of mixtures. Wessinger (1986) listed over 20 terms commonly used to describe drug–drug interaction studies. Clearly, a common nomenclature will facilitate the development of a framework for assessing the risk of combined exposures.

4.2 Aggregate and cumulative

For the purposes of discussion at the current workshop, the following definitions of aggregate and cumulative exposure are proposed, each defined within the context of risk:

- *Aggregate exposure*: The demographic, spatial and temporal characteristics of exposure to a single chemical through all relevant pathways (e.g. food, water, residential uses, occupational) and routes (e.g. oral, dermal, inhalation). *Aggregate risk* is the risk associated with multiple pathways/routes of exposure to a single chemical.
- *Cumulative exposure*: Defines the aggregate exposure (see above) to multiple chemicals. *Cumulative risk* is the combined risk from aggregate exposure to multiple chemicals (and may be restricted to chemicals that have a common mechanism of toxicity).

Assessment of aggregate and cumulative exposures must necessarily consider the temporal nature of both the exposures and the effects. While some exposures may be concurrent, simultaneous exposure is not necessary for defining aggregate/cumulative risk. Non-simultaneous exposures to chemicals with disparate toxicokinetics or toxicodynamics may lead to overlap in the time course of the effects or the repair of damage from exposures (Hattis & Shapiro, 1990; Hattis & Crofton, 1995). Temporality of exposure/effects is a key concept in aggregate and cumulative exposure assessments.

5. DEVELOPMENT OF A FRAMEWORK FOR ASSESSING RISK

The major goal of this workshop was to develop a draft decision framework for assessing the combined risk of exposure to multiple substances via multiple routes or pathways. To achieve this goal, the workshop defined the principles and concepts necessary to develop this framework. In addition, the workshop initiated development of a draft framework and identified the next steps needed to inform the framework. As a starting point to help focus discussion, the Workshop Planning Group developed a "straw man" decision framework based on a previous version created by the Interdepartmental Group on Health Risks from Chemicals (IGHRC, 2006). The Workshop Planning Group solicited input (and revisions) to this decision framework.

7. ACKNOWLEDGMENTS

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PRINCIPLES OF AGGREGATE EXPOSURE ASSESSMENT

Marcel T.M. Van Raaij

1. INTRODUCTION

Humans may be exposed to chemicals at different occasions and in a number of different ways. Chemicals may be released into the environment during production or disposal of products and disperse into air, surface water or groundwater, soil, crops and wildlife. Occupational exposure to chemicals may also occur during production or use of a product. Furthermore, chemical exposure may result from the use of a large variety of consumer products. Finally, a large number of chemicals are deliberately used for specific applications (pesticides, biocides, veterinary products, food additives), resulting in exposure through food and other routes. All of these may result in exposure of humans through the inhalatory, dermal and oral routes (Delmaar & Van Engelen, 2006).

2. DEFINITIONS

In the European Union's Technical Guidance Documents (EC, 2003), the term *aggregated exposure*[1] is used solely within the scope of consumer exposure assessment and is defined as exposure to the same chemical from multiple sources. *Combined exposure* is defined as exposure of the same person to the same substance in the same setting via different routes of entry into the body or from different products containing the same substance. In this abstract, "combined exposure" is considered to be synonymous with "aggregate exposure".

3. GENERAL PRINCIPLE

Exposure assessments are conducted for different reasons and with different objectives. For example, the purpose of an assessment may be to get a rough, order-of-magnitude estimate of the maximal level of a chemical to which a population may be exposed. Alternatively, it may be conducted to get a detailed insight into the contribution of specific products to total exposure to a specific chemical. It is clear that these different objectives require different types of methodological approaches. Therefore, the central issue before conducting aggregate exposure assessments is to define the objective of the assessment—that is, which types of questions need to be addressed. It should be stressed that the robustness and level of detail of the exposure assessment should match the objective under study.

4. PERSON-ORIENTED APPROACH

For each aggregate exposure assessment, it is highly important to perform the assessment using a person-oriented approach. This means that an addition of exposures via different pathways and routes should be done for the same individual. In other words, the combination of exposures needs to make sense. The opposite case, where aggregation is started by addition of a per-pathway concept, may lead to erroneous results. In such an approach, it is easy to produce unrealistic situations. For example, the pathway of occupational exposure (generated by model A) could be combined with the hand-to-mouth contact exposure of a

[1] "Aggregate exposure", the term used in this workshop, is defined here as combined exposure to a single chemical through various routes/sources.

toddler (generated by model B). When the assessment is started using a person-oriented approach, first a person is defined, then the likely routes of exposure for that person are defined.

Still, in a person-oriented approach, it is important to define what this person represents. Does this person reflect the average individual, or does he or she reflect a highly exposed (hypothetical) individual simulating a worst-case condition? Another issue that requires attention is the spatial and temporal correlations between exposure events. When combining the exposure from a substance used only on single occasions during summer with occasional exposures from food, the combined occurrences of those two exposures must be realistic, depending on the type of assessment.

5. LEVEL OF DETAIL

Using a highly exposed individual representing the worst-case situation is normally done only for screening-type assessments (e.g. as a first tier). The type of input (data) needed for such an assessment can be very rough or may consist only of a number of assumptions. When such an assessment shows no risk associated with an aggregate exposure, no more detail is needed.

However, when a realistic (mean) scenario needs to be assessed, another level of detail is needed for input into the assessment. Data on various parameters are needed to create a realistic exposure scenario—for example, the mean body weight of an adult in the population at study, the mean amount of product used, etc.

Generally speaking, exposure assessment (including aggregate exposure assessments) can be conducted at various scales. We can use so-called macroassessment, in which only the total exposure is of interest. This is done when using general intake fractions (e.g. the intake assessments for food additives) or actual biomonitoring. In these cases, it is not important to know which routes or which products lead to exposure, but only whether the exposure is at a certain overall level (order of magnitude).

Alternatively, we can use microassessment, in which the exposure assessment tries to reconstruct the exposure by calculating (or measuring) various steps from emission to exposure, resulting in an assessment of the total (systemic) exposure. Some exposure models operate according to this principle (Van Veen et al., 2001), including the ConsExpo model developed by the National Institute for Public Health and the Environment in the Netherlands (Delmaar et al., 2005; see also http://www.rivm.nl/consexpo).

6. DETERMINISTIC VERSUS PROBABILISTIC APPROACHES

Another way of looking at the level of detail is the use of deterministic or probabilistic approaches. Deterministic approaches (point estimates) are usually performed in screening-type assessments. Such calculations normally use quite conservative input values for exposure parameters. The outcome of the assessment will be an exposure that is very likely to be an upper bound of the exposures that occur in reality. When there is a need for a realistic exposure scenario or a need to know the variability in exposure levels, other approaches are required. In a deterministic approach, a mean scenario can be used, providing a point value for estimated mean exposure. A far more sophisticated approach is to use probabilistic exposure modelling. In this way, the “true” variability of the exposure is illustrated.

Using mean deterministic approaches or probabilistic approaches, however, is much more data demanding. It requires representative distributions of exposure factors and detailed information on spatial and temporal correlations of the exposure events. Unfortunately, it is already known that for many exposure factors there is a lack of (adequate) data, or at least the available data are scarce and dispersed. Efforts should be directed towards generating or gathering those exposure factor data as much as possible, with preferences to data that show product use.

7. EXPOSURE MODELS

In order to generate an aggregate exposure assessment, various exposure tools exist, although all of them are developed for a specific type of exposure. The available models can be divided into two groups. The first group of tools models exposure of humans to chemical emissions into the environment (indirect exposure). Models in this group include CalTOX, CSOIL, E-FAST, EUSES, SHEDS and TRIM.

A second group of models has been developed solely for the evaluation of pesticides that are used both in agriculture and in residences. Models in this group include CALENDEX, CARES, LifeLine and Rex2000. In addition, some database models for occupational exposure exist, but these do not take aggregate exposure assessment into account.

All these tools differ in the level of complexity and the number of pathways examined.

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AGGREGATE EXPOSURE TO INDUSTRIAL CHEMICALS AND OTHER CONTAMINANTS: ASSESSMENT OF ENVIRONMENTAL EXPOSURE

Theo Vermeire

1. INTRODUCTION

In the European Union (EU), the risk assessment for new and existing industrial chemicals is performed according to the Technical Guidance Documents (TGDs) (EC, 2003). A software tool, the European Union System for the Evaluation of Substances (EUSES), is available to assist the risk assessor (EC, 2004). This summary explains the EU-TGD approach for the aggregate exposure assessment of industrial chemicals and other contaminants. The EU-TGDs are currently being updated within the framework of the new EU Chemicals Policy, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). In the Netherlands, this approach was also adapted for the exposure assessment of soil contaminants and is used in setting integrated environmental quality standards.

2. DEFINITIONS

In the EU-TGDs, the term *aggregated exposure* (in this workshop, "aggregate") is used solely within the scope of consumer exposure assessment and is defined as exposure to the same chemical from multiple sources. *Combined exposure* is defined as exposure of the same person to the same substance in the same setting via different routes of entry into the body or from different products containing the same substance. In this summary, "combined exposure" is considered to be synonymous with "aggregate exposure".

3. GENERAL PRINCIPLE

In some cases, it can be relevant to assess aggregate exposure of humans to the same industrial chemical or other contaminant 1) via the environment, 2) through use of consumer products and 3) at the workplace. Attention should be paid to the spatial and temporal scales at which these exposures occur. In general, aggregate exposure can be of particular relevance when long-term exposure to a chemical with widespread use and emissions occurs. Exposures from different scenarios and routes are added, taking into account differences in bioavailability of the chemical via different exposure routes. In the risk characterization, the total daily intake estimated is then compared with a no-effect level for humans at the right spatial and temporal scales.

Below, the approach for exposure of humans to contaminants via the environment is explained.

4. THE APPROACH FOR THE ASSESSMENT OF EXPOSURE VIA THE ENVIRONMENT

In a human environmental exposure assessment, the emissions, pathways and rates of movement of a contaminant or substance are determined in order to estimate the concentration or doses to which human populations are or may be exposed. An exposure is

made up of a source (e.g. factory), a release mechanism (e.g. stack), a transport medium (soil, water, air) and an exposure point (eating, drinking, breathing, touching). An exposure to a substance is direct when it occurs in the transport medium to which the substance is first released. An indirect exposure to a substance occurs when there is at least one intermediate transfer from the medium to which the substance was first released to a second medium before the substance reaches a human. A schematic representation of the indirect exposure routes considered is presented in Figure 1.

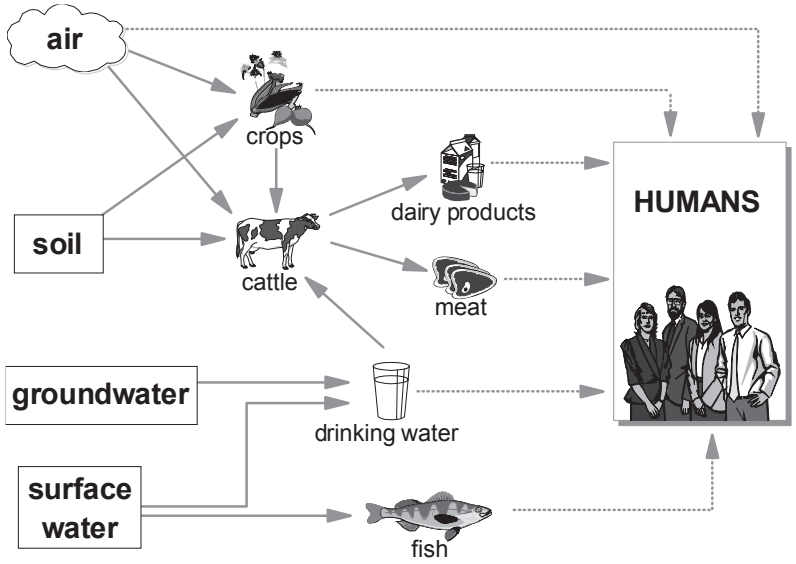

Figure 1: Conceptual model for exposure via the environment in the EU-TGDs. Soil ingestion and dermal contact are normally not included (EC, 2003).

The general formulae used are:

$$DOSE_{air} = \frac{F_{resp} \cdot C_{air} \cdot IH_{air}}{BW} \cdot \frac{BIO_{inh,2}}{BIO_{oral,2}}$$

$$DOSE_{tot} = \sum_{i} DOSE_i$$

$$i \in \{air, drw, fish, leaf, root, meat, milk\}$$

Input

C_i	concentration of chemical [c] in media [wet weight, wwt] for oral intake (drinking-water [drw], fish, leaf and root crops, meat, milk)	$[kg_c \cdot kg_{wwt}^{-1}]$
C_{air}	concentration of chemical [c] in air	$[kg_c \cdot m_{air}^{-3}]$
F_{resp}	respirable fraction of inhaled substance	[–]
IH_i	daily intake of medium *i*	$[kg \cdot d^{-1}$ or $m^3 \cdot d^{-1}]$
$BIO_{oral,2}$	bioavailability for oral intake	[–]
$BIO_{inh,2}$	bioavailability for inhalation	[–]
BW	body weight of (adult) human considered	[kg]

Output

$DOSE_i$	daily dose via intake of medium *i*	$[kg_c \cdot kg_{bw}^{-1} \cdot d^{-1}]$
$DOSE_{tot}$	total daily intake for humans	$[kg_c \cdot kg_{bw}^{-1} \cdot d^{-1}]$

Monitoring data of known quality that are representative for the exposed population are preferred over estimated exposure values calculated using models. However, there is a need for sufficiently accurate models, because there are few field data available on exposure levels and few experimental data on bioconcentration. For a priori hazard assessments (i.e. new chemicals placed on the market), a modelling approach is the only solution. Models can be used to estimate human exposure to environmental concentrations of a chemical that are either measured or estimated with single-medium or multimedia models.

A large number of different models can be used to estimate the concentrations of a chemical in food products. Most often the concentration of a chemical in food is estimated by simple partitioning models that are usually highly dependent on the octanol–water partition coefficient. Although the theoretical basis for these models is sometimes limited, they provide practical tools for risk assessment, especially since they are often applicable to a wide range of substance properties. In these models, bioconcentration, biotransfer and bioaccumulation factors, defined as fixed concentration ratios, are estimated. In a first tier, models can be used, which often are based on generic scenarios and conservative assumptions (Table 1). In a second tier, a more accurate estimate of the indirect exposure can be developed using representative measured data of known quality, when available. Reliable and relevant measured data are always preferred, considering the large uncertainties in the (quantitative) structure–activity relationships. In this way, the uncertainty in the exposure estimate can be decreased for critical exposure routes.

Table 1: Models used for indirect environmental exposure assessment in the EU-TGDs.

Pathways	*Model*
Exposure via air	
a) Biotransfer from air to crops	Trapp & Matthies (1995)
b) Biotransfer via cattle to milk and meat	Travis & Arms (1988)
· Direct inhalation of air	
· Indirect via crops	
Exposure via soil	
a) Biotransfer from soil to crops	Trapp & Matthies (1995)
b) Biotransfer via cattle to milk and meat	Travis & Arms (1988)
· Direct ingestion of soil	
· Indirect via crops	
Exposure via surface water	
a) Purification of drinking-water	Hrubec & Toet (1992)
b) Biotransfer from surface water into fish	Veith et al. (1979); Connell & Hawker (1988)
Exposure via groundwater	
a) Biotransfer via drinking-water of cattle to milk and meat	Travis & Arms (1988)
b) Direct ingestion of drinking-water	–

The estimated concentration in each intake medium and the intake or consumption rates used are dependent on the conservatism of the models used and on monitoring data and assumptions used.

The result can vary from average to worst case. The target for indirect exposure of humans can be set at the exposure level of an average individual in a region. This implies that regional concentrations of the chemical in air, water and soil can be used as input concentrations, and average diets are assessed for the region under consideration. This regional approach accounts for the fact that people do not consume their total food basket from the immediate vicinity of a point source. In a more worst-case approach, the subject receives his total consumption from the contaminated area for each food product and lives near the point source. This exposure scenario is less worst case than it might appear at first glance because, generally, only one or two of all possible routes dominate the total exposure estimation.

5. FINAL REMARKS

Critical evaluations of these EU-TGD models have been performed by Schwartz et al. (1998) and Rikken & Lijzen (2004).

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AGGREGATE/CUMULATIVE RISK OF EXPOSURE TO PESTICIDES

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The 1996 Food Quality Protection Act (FQPA) required the United States Environmental Protection Agency (USEPA) to consider the "available evidence concerning the cumulative effects on infants and children of such residues and other substances that have a common mechanism of toxicity". To implement this regulatory requirement, the Agency needed to interpret the FQPA, define terms, develop guidance/methods/software and compile/analyse/ manage data. Most importantly, because cumulative risk assessment represented a new way of evaluating pesticide risk, the Agency needed to ensure rigorous peer review of the methods and transparency of the process.

The USEPA's Office of Pesticide Programs (OPP) defines *aggregate risk* as the risk associated with all pathways and routes of exposure to a single pesticide and *cumulative risk* as the risk of a common toxic effect associated with concurrent exposure by all relevant pathways and routes of exposure to a group of chemicals that share a common mechanism of toxicity. This abstract focuses on the pesticide cumulative risk process and is structured to address the questions posed by the Steering Committee for the World Health Organization International Programme on Chemical Safety (IPCS) Workshop on Aggregate/Cumulative Risk Assessment.

1. ELEMENTS OF THE APPROACH

Information on how the USEPA's OPP conducts cumulative risk assessment for pesticides can be found at http://www.epa.gov/pesticides/cumulative/. The basic steps in the OPP's cumulative risk assessment process are briefly described below.

1.1 Prioritizing and identifying common mechanism of toxicity groups

A cumulative risk assessment under the FQPA begins with the identification of a group of chemicals that induce a common toxic effect by a common mechanism of toxicity. Common mechanism of toxicity determinations should follow a weight-of-evidence approach, as described in the OPP's *Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity* (USEPA, 1999a). Since that guidance, other useful weight-of-evidence approaches for conducting mode of action analyses have been published, including the human relevance frameworks of the International Life Sciences Institute Risk Sciences Institute (Meek et al., 2003; Seed et al., 2006) and IPCS (Boobis et al., 2006, 2008). Examples of the OPP's common mechanism of toxicity determinations include the organophosphate (Mileson et al., 1998; USEPA, 1999b), *N*-methyl carbamate (ILSI, 1999; USEPA, 2001a), chloroacetanilide (USEPA, 2001a,b) and triazine pesticides (USEPA, 2002).

To focus cumulative risk to a manageable number of assessments, it was important to develop criteria for addressing priority assessments. Legislative language (Federal Food, Drug and Cosmetic Act section 408(q)(2)) directed the USEPA to give priority in the tolerance reassessment process to "tolerances or exemptions that appear to pose the greatest

[1] This abstract contains the views of the listed author and does not necessarily represent the decisions or the stated policy of the United States Environmental Protection Agency.

risk to public health" (i.e. worst first). In 1997, the OPP published the list of active ingredients that would be given priority in tolerance reassessment—principally, organophosphate, carbamate, organochlorine and carcinogenic pesticides (see http://www.epa.gov/fedrgstr/EPA-PEST/1997/August/Day-04/p20560.htm). Potential health impact on and exposure of infants, children and other sensitive subpopulations were taken into account.

1.2 Problem formulation and scoping phase

The OPP developed guidance for evaluating and estimating the potential human risks associated with multichemical and multipathway exposures to pesticides operating by a common mechanism of action (i.e. cumulative risk) (USEPA, 2001c). This 2001 guidance was viewed as providing an important starting point for the OPP's cumulative risk assessment process. However, it was realized that the cumulative risk assessment process would continue to evolve with experience and increasing knowledge.

Before the conduct of a pesticide cumulative risk assessment, risk assessors and risk managers engage in a planning dialogue that includes discussion of management goals and the scope and complexity of the cumulative risk assessment. During this planning phase, available hazard and exposure information for each pesticide and information gaps are identified. Relevant exposure scenarios and pathways (routes) that need to be addressed in the cumulative risk assessment are determined. Initial projections are made regarding which pesticides could potentially be major contributors to the problem based on a preliminary evaluation of their hazard potency and exposure (i.e. consideration of per cent reference dose and aggregate risks of each pesticide, detection in exposure monitoring programmes, registered uses, tolerances, use patterns, percentage of crops treated, usage information).

A plan for analysing data and characterizing risk is developed that identifies the appropriate approach/methods/models and data sources. If critical missing data are identified, default options are discussed and plans are proposed as to how best to obtain that information (required testing, in-house research). The peer review process and the timeline for completing the assessment are also proposed.

1.3 Analysis phase

Data are selected and gathered as input parameters used in modelling that will characterize exposure to the common mechanism chemicals by all relevant pathways/durations/routes, which may allow for prediction of a combined risk (due to the overlapping of exposures and/or effects). Dose addition is assumed. In other words, for each route of interest, relative potency factors are used to normalize the potency of each chemical to an "index chemical", and points of departure for the index chemical are established. Probabilistic (Monte Carlo) procedures are used to generate exposure distributions from consumption data from dietary consumption surveys and pesticide residue data from either supervised field trials or residue monitoring programmes. For the drinking-water assessment, vulnerable regions are identified. For the residential pathway, use patterns, frequencies and their associated probabilities are evaluated.

Margins of exposure (MOEs) are used to integrate the different pathways/routes of exposure. A regional approach is used to evaluate the residential and drinking-water exposure assessments, and the food exposure assessment is assumed to be nationally representative. A

hypothetical population of approximately 20 000 individuals is constructed (based on the Continuing Survey of Food Intakes by Individuals, or CSFII) to reflect real-world exposures across the United States throughout the year that capture geographic, temporal and demographic variability and actual co-occurrence of pesticides. Calendar-based models (DEEM/Calendex™, LifeLine™, CARES™, SHEDS) are used to preserve and maintain geographic, temporal and demographic specificity. The effects data are appropriately matched and integrated with the exposure data by consideration of the timeframe of effects and exposure. The outcome of cumulative risk assessment is not a single number but a series of daily MOE distributions arrayed as distributions (common percentiles generated are 95th, 99th and 99.9th) across time (1 year) for specific age groups. MOEs are developed such that the exposures from pesticides in foods, in drinking-water and from residential uses are all calculated simultaneously for each individual in the hypothetical population.

It should be emphasized that a cumulative risk assessment is conducted using an iterative and tiered process to balance resources against the need to refine the assessment and reduce uncertainty. Sensitivity analyses are done throughout the development of the cumulative risk assessment to identify major contributors of risk and to refine the inputs where appropriate.

1.4 Interpretation and risk characterization phase

The ultimate goal of pesticide cumulative risk assessments is to guide further risk mitigation activities. Thus, a critical aspect of the approach is the ability to track back and identify major contributors at the high-end exposure tails by pesticide, crop and pesticide/crop combinations and to perform sensitivity analyses by subtracting out specific pesticides, specific crops and specific pesticide/crop combinations.

Because multiple chemical/pathway assessments are associated with substantial uncertainties and data limitations, it is critical in the risk characterization phase to clearly distinguish conclusions based on actual data versus policy choices/assumptions and to provide qualitative and quantitative descriptions of the uncertainties and variability.

2. INCLUSION CRITERIA AND KEY ISSUES

2.1 Sources, pathways and routes

Aggregate exposure to each pesticide within the common mechanism group is considered in the cumulative risk assessment (i.e. multiple pathways—food, drinking-water, residential; and multiple routes—oral, dermal, inhalation). To ensure accuracy of risk estimates and to guide appropriate risk mitigation decisions for remaining uses, only registered uses of pesticides are considered, not those uses that have been cancelled or phased out. Occupational exposures and ecological effects are not included.

2.2 Level of complexity

Not all cumulative assessments need to be of the same depth and scope; thus, early in the process, it is necessary to determine whether a full-scale and refined assessment is needed by considering the number of chemicals involved and the exposure scenarios in conjunction with the magnitude/duration of exposure. In the case of the triazine draft cumulative risk assessment (USEPA, 2006a), it was reasonable to assume that the members of the common

mechanism of toxicity group (simazine, propazine and the metabolites desethyl-s-atrazine, desisopropyl-s-atrazine and diaminochlorotriazine) were of lesser potency than or equal potency to (on a molar basis) atrazine. This conservative approach was viewed as health protective and minimized the possibility of underestimating risk. Thus, dose–response modelling and the establishment of relative potency factors for the members of the common mechanism group were not done, and the point of departure based on atrazine's no-observed-adverse-effect level (NOAEL) was used in the assessment. In other cases, NOAELs were also used rather than benchmark dose (BMD) modelling for potency estimates, as was the case for the chloroacetanilide cumulative risk assessment (USEPA, 2006b).

2.3 Key issues

BMD modelling is preferred over NOAELs/lowest-observed-adverse-effect levels (LOAELs) for the determination of relative potency factors and points of departure. Although there are extensive required data for the registration of food use pesticides, these standard studies are not designed for purposes of dose–response modelling. The OPP was able to combine cholinesterase data sets for the organophosphate pesticides to increase the reliability of the BMD modelling. For some of the *N*-methyl carbamates, cholinesterase data were also combined from multiple studies (submitted by registrants) for dose–response modelling; for other carbamates, however, the OPP relied heavily on data generated in-house for the establishment of BMDs. Beyond these chemical classes, future pesticide cumulative risk assessments will likely be hampered by lack of appropriate hazard and dose–response data, as well as insufficient knowledge of mechanisms and limited resources.

The food exposure pathway is considered to be a refined assessment given the availability of extensive, high-quality pesticide residue monitoring (e.g. the United States Department of Agriculture's pesticide data programme; the United States Food and Drug Administration's residue monitoring programme and total diet study) and consumption data (CSFII) in the United States. There are significant conservatisms and assumptions used in the residential and drinking-water assessments. The drinking-water assessment poses a challenge given the great diversity of geographic-, climate- and time-dependent factors that affect residue levels; thus, there is a large reliance on modelling, with some limited monitoring data to evaluate the model results. Although there are some reliable data on how pesticides are used and how people may come into contact with them, there is still limited knowledge of the actual homeowner activities for our residential assessment. Also, there is a current lack of longitudinal data on relevant food residues and residential pesticide uses.

3. ORGANIZATIONS USING APPROACHES AND STATUS OF IMPLEMENTATION

The USEPA has long considered chemical mixtures (USEPA, 1986, 1989, 2000). For example, the hazard index approach has been used to evaluate the combined risks from exposure to hazardous air pollutants or hazardous waste, toxic equivalency factors (TEFs) have been developed to assess dioxins and ecological assessments have been conducted on multiple stressors. However, pesticide cumulative risk assessments represent full-scale quantitative analyses that evaluate multiple chemicals and multiple pathways/routes of exposure and incorporate BMD modelling, probabilistic exposure distributions and sensitivity analyses and have been considered in regulatory decision-making. The OPP has finalized its organophosphate cumulative risk assessment (USEPA, 2006c) and is in the process of

finalizing the cumulative risk assessments on *N*-methyl carbamates (USEPA, 2007), triazines (USEPA, 2006a) and chloroacetanilides (USEPA, 2006b).

4. FUTURE NEEDS

Cumulative risk assessment is an evolving process, and there is still a great deal of research needed to produce data and methods. Given the complexity and number of chemicals that need to be considered and the scarcity of resources, the development of more efficient and reliable screening methods is needed.

The support of biomonitoring systems, exposure databases and disease registries is also critical in order to provide better data, to help guide prioritization of assessments and to evaluate the benefits of cumulative assessments.

Well designed real-world mixture studies are needed to evaluate combined toxicity and chemical interactions. There is hope that technologies such as "omics" will advance our ability to efficiently discern relevant pathways of toxicity and contribute to our understanding of mechanisms of toxicity and that the development of physiologically based toxicokinetic modelling approaches will better address the dynamic nature of effects and characterize dose across species and routes.

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CUMULATIVE RISK ASSESSMENT: PRINCIPLES AND CONCEPTS

Alan R. Boobis

Within the field of mixture toxicology, assessment of chemicals that exhibit simple similar action—i.e. dose or concentration additivity—has probably received the most attention. As a consequence, approaches to such assessments are relatively mature, at least compared with other mixture assessments. To date, the focus has been on groups of compounds with a well defined mechanism of action. There is some concern that this might mean that exposures to other groups of compounds, where such information is currently lacking or is more difficult to obtain, may be such that the risk is greater than that assumed on the basis of their individual assessments.

The basis for simple similar action is rooted in receptor occupancy/ligand binding site theory. This states that a receptor (or some other allosteric binding site) does not distinguish between occupancy by different agonists. Occupancy is determined by affinity; for any agonist, 50% occupancy occurs at K_D, the dissociation constant, which is the reciprocal of the affinity constant, K_a. Fractional occupancy is determined by the ratio $[D]/([D]+K_D)$, where $[D]$ is the concentration of the ligand. Hence, agonists of different potencies will occupy the receptor to an extent determined only by their concentration and respective K_D. In general, the magnitude of the biological response is proportional to receptor occupancy. Hence, the fractional response $E/E_{max} = [D]/([D]+K_D)$, where E = response at $[D]$ and E_{max} = maximal response. On this basis, the fractional response of the mixture is determined from the potency-normalized concentrations of each agonist. In effect, a given fractional receptor occupancy, and hence fractional response, will be determined only by $[D]$ and K_D. Hence, when K_D changes, the same fractional occupancy will be obtained at a corresponding different concentration. While a simplification, this theory has been adequate for the cumulative risk assessments performed to date. It is possible to modify the assumptions for more complex receptor interactions and maintain the same overall approach.

Compounds acting as agonists at the same binding site—that is, with the same mechanism of action—are said to belong to the same common mechanism group. However, the information required to establish membership of a common mechanism group is substantial, and only a relatively few common mechanism groups have been determined. Nevertheless, failure to consider together compounds that could exhibit simple similar action may not be adequately protective of public health. One strategy to address this would be to use a tiered approach, in which the lowest tiers are based on relatively broad assumptions and the higher tiers are based on chemical-specific data, either on hazard or on exposure. Progress to successive tiers would be based on estimated combined exposure exceeding some metric reflecting the combined effect of the putative members of the chemical group. Hazard in the lower tiers could be based on action at the same target organ, same cell type, same mode of action or same biological process. It is important in any tiered approach not to assume that exceedance of some exposure limit in lower assessments necessarily means that there is a risk to public health. Rather, it means that negligible risk cannot be assumed without further refinement of the assessment.

As indicated above, whereas pragmatically it can be useful to assume simple similar action even for compounds not yet shown to share a common mechanism of action, this does mean that as the hazard assessment is refined, it will become apparent that this is not the case. As the mechanisms of action are determined, it may become obvious that compounds belong to

different common mechanism groups, as there is more than one molecular target. Alternatively, it may be that compounds show simple dissimilar action (i.e. effect addition) or interaction (i.e. potentiation or antagonism). This would then require a different type of assessment, covered in the abstracts by Teuschler and by De Rosa and Mumtaz.

A number of methods have been proposed that can be used to assess the joint hazard of compounds that share a common mechanism of action (USEPA, 2000; Feron & Groten, 2002; DVFA, 2003; Jonker et al., 2004). These can be divided into those that simply add the risk from the individual chemicals and those that sum effects based on potency-corrected exposures. None of these methods is ideal, and each has advantages and disadvantages. The Hazard Index (HI) is the sum of the exposure to each chemical divided by its respective reference dose (RfD) (i.e. $HI = Exposure_1/RfD_1 + Exposure_2/RfD_2 + \ldots + Exposure_n/RfD_n$). It includes the use of uncertainty factors in the derivation of the individual RfDs. This method is likely to overestimate risk, but is relatively simple to apply. It could therefore be useful in a lower-tier assessment.

The Point of Departure Index (PODI) and the Combined Margin of Exposure (MOE_T) also add risks of individual chemicals. They are very similar to each other, one being the reciprocal of the other. The $PODI = Exposure_1/POD_1 + Exposure_2/POD_2 + \ldots + Exposure_n/POD_n$. The point of departure (POD) is normally the no-observed-adverse-effect level (NOAEL), benchmark dose (BMD) or lower limit on the BMD (BMDL). Here, a single overall uncertainty factor is used to compare the PODI or MOE_T with actual or predicted exposure. Again, these methods are relatively simple to apply and may be more appropriate to lower tiers of assessment.

Potency normalization approaches have been developed—the Toxic Equivalent (TEQ) method (Haws et al., 2006; Van den Berg et al., 2006) for dioxins and other aryl hydrocarbon receptor agonists and the Relative Potency Factor (RPF) method (USEPA, 2000) for pesticides. The RPF method is more generalized than the TEQ method and has been used for classes of chemicals in addition to pesticides, such as polycyclic aromatic hydrocarbons (Pufulete et al., 2004). Both methods depend on selection of an index chemical against which the potencies of all other members of the group are normalized. For example, if compound X is 10% as potent as the index compound, concentrations will be corrected by a factor of 0.1. The activity of the mixture is determined from the sum of the potency-normalized concentrations relative to the acceptable exposure to the index compound, which could be the RfD for that compound.

Higher-tier approaches are possible should these be necessary. These include physiologically based toxicokinetics (PBTK) and toxicodynamics and probabilistic approaches. PBTK enables the internal concentration of each component of the mixture, and indeed the concentration at the target site, to be determined. Hence, differences in absorption and disposition can be taken into account (Jonker et al., 2004). This can be linked to a toxicodynamic model of the toxicological response, where known (El-Masri, 2007). Few such applications in cumulative risk assessment have yet been reported. Probabilistic approaches have been applied more to exposure assessment, but there is increasing interest in their use in hazard characterization (Bosgra et al., 2005). In the area of cumulative risk assessment, such an approach could provide a refined higher-tier method, where necessary, but it is likely that considerable development work would be necessary before this could be possible.

A number of the methods above use RfDs in determining the combined hazard of chemicals in a common mechanism group. However, it should be recognized that even if the end-point that serves as the basis for the common mechanism group does not drive the RfD, it will be necessary to include such a compound from the combined assessment in the common mechanism group. This is because when exposure to this compound is combined with exposure to other members of the common mechanism group, the total exposure may exceed that which is considered acceptable. In such circumstances, it would be necessary to calculate a putative RfD on the assumption that the common mechanism group end-point is the critical one in the risk assessment. For the other approaches—for example, those relying on the POD—no such recalculations would be necessary.

One of the assumptions of cumulative risk assessment at low exposures is that the PODs identified in experimental studies may not be thresholds, but responses below the limit of detection of the study. In particular, approaches such as the HI add small incremental effects so that inclusion of a large number of compounds in the group will increase the likelihood of exceeding acceptable exposure. As an example, 100 compounds with individual exposures at 2% of their respective RfDs would result in a combined exposure that exceeded the acceptable limit by 2-fold. However, at such low doses, it is highly likely that the compounds are below their true biological thresholds. Hence, exposure would have to increase appreciably before a biologically significant response would be produced. Such considerations should be taken into account in problem formulation—that is, which compounds to prioritize for consideration in the assessment.

In undertaking a cumulative risk assessment, it is the total occupancy of the molecular target that is of concern. While the foregoing has considered the possibility of multiple agonists combining to increase receptor occupancy, it is important to consider multiple sources of exposure to the same chemical. Estimation of the total exposure to a chemical by all pathways has been described as aggregate exposure. Hence, a complete cumulative risk assessment would include aggregate exposure assessments for all of the chemicals in the group. Although a deterministic approach could be used for this purpose, this would be extremely conservative, and a probabilistic approach would be much more preferable (Price et al., 2001).

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CUMULATIVE RISK ASSESSMENT OF ANTICHOLINESTERASE PESTICIDES (ORGANOPHOSPHATES AND CARBAMATES) IN THE UNITED KINGDOM

Ian Dewhurst

1. BACKGROUND

In 1998, the United Kingdom initiated a review of national authorizations of all anticholinesterase pesticides. One of the ministerial requirements was that a cumulative risk assessment should be performed at the end of the process. By the time the United Kingdom review was coming to an end, the United States Environmental Protection Agency (USEPA) was well on its way to completing its cumulative/aggregate risk assessment of organophosphates (OPs), and a USEPA officer was invited to the United Kingdom to discuss the USEPA approach and the lessons learnt. (Details of the United States approach can be found in the abstract by Dellarco.)

2. METHODOLOGY

The United Kingdom cumulative risk assessment included both OPs and carbamates that inhibited acetylcholinesterase. Although the kinetics are very different, there was the potential for co-exposures from the same meal or to a carbamate after an OP, and the potential for combined toxicity could not be discounted. The common mechanism group included some low-potency inhibitors (e.g. the fungicide tolcofos-methyl) as well as the more potent insecticides. Initially, the common mechanism group contained only pesticides authorized in the United Kingdom, but it was later expanded to include those found during residue surveillance exercises.

Various options for assessing relative potency were discussed. These included no-observed-adverse-effect levels (NOAELs) for erythrocyte or brain cholinesterase inhibition in studies of various durations and species; benchmark dose estimations of effective doses for 10% inhibition of acetylcholinesterase activity (ED_{10}s); and using the acceptable daily intake (ADI) or acute reference dose (ARfD), corrected if necessary to exclude non-cholinergic end-points.

The exposure model was run using two different approaches. The first one was a toxic equivalency factor (TEF)–type approach using the NOAEL for erythrocyte cholinesterase inhibition in 90-day rat studies (or, for some carbamates, a modern acute study that minimized reactivation), with chlorpyrifos as the reference compound. Chlorpyrifos had the best database of the compounds authorized in the United Kingdom. For the second approach, the plan was to determine exposures as percentages of the reference doses and then sum these, but this would not work with the modelling software, so a TEF approach using ADIs and ARfDs was adopted.

After some initial in-house work using "@Risk", the exposure assessment was performed using probabilistic software (MCRA) from Jacob Van Klaveren's group based at the Institute of Food Safety (RIKILT) in the Netherlands. This used Dutch consumption data (data from recent United Kingdom surveys were not in a suitable format and would have taken a lot of resources to load into the model) and some relatively old United Kingdom residue data. The

old residue data were used, as this was an extensive data set looking at individual units and thus avoided issues associated with composite sample data.

3. RESULTS

On investigating the early results, some problems were identified—for example, high residue levels on individual fruits before processing being matched with high consumption data for fruit juices (other data show that residues in fruit juice are routinely very low). A sensitivity analysis showed that the method of determining relative potency had minimal impact on the overall outcome; the main contributors were the absolute potency, residue levels and consumption values. The results from the modelling were reassuring and in line with findings in other countries, showing that acute exposures on about 0.1% of consumer days might exceed the ARfD.

4. FUTURE WORK

Now that the European Union reviews of anticholinesterase compounds have been completed, the plan is to rerun the modelling using updated toxic potency values, new United Kingdom consumption data and contemporary residue data from United Kingdom surveillance schemes and to include some assessment of uncertainty.

5. PROBLEMS IDENTIFIED AND LESSONS LEARNT

Data limitations included consumption and residue issues mentioned above, plus difficulties in determining relative potency factors for pesticides not authorized in the United Kingdom but present on imported produce. The latter was crucial to the outcome, as one of the main contributors to exposure was an active substance that had never been authorized in the United Kingdom, and it was necessary to rely on third-party summaries of the toxicity data.

Lessons learnt included the following:

- Discussions with others are very important; the United Kingdom evaluators learnt much from the USEPA work.
- Benchmark dose approaches are not suited to standard toxicity study designs; 95% confidence intervals were often so large as to make the derived values meaningless.
- The modelling outputs should not be taken at face value; it must be confirmed that major and minor contributors make sense.
- It is resource intensive to set up the model.
- It is important to find a way to handle inter-unit variability within composite samples.
- Some assessment of uncertainty must be included.

CUMULATIVE CONCEPTS: THE DIOXIN TOXIC EQUIVALENCY FACTOR (TEF) CONCEPT

Angelika Tritscher

1. INTRODUCTION

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), commonly called "dioxins", are by-products of combustion and of various industrial processes. Polychlorinated biphenyls (PCBs) were manufactured in the past for a variety of industrial uses, notably as electrical insulators, dielectric fluids and specialized hydraulic fluids. Most countries banned the manufacture and use of PCBs in the 1970s; however, past improper handling of PCBs constitutes a continuing source of these compounds in the environment, and disposal of equipment containing these compounds poses some risk of further contamination (WHO, 2002). PCDDs, PCDFs and PCBs are persistent organic pollutants (POPs) under the Stockholm Convention on Persistent Organic Pollutants (http://www.pops.int/) and are omnipresent in the global environment.

Exposure to PCDDs, PCDFs and coplanar PCBs can occur occupationally, accidentally or through the environment (background). Exposure to background contamination can occur by inhalation, ingestion or contact with contaminated soil. Exposure assessments in Europe and North America demonstrated that over 90% of the exposure of a typical person came from food, predominantly from animal fat (WHO, 2002).

2,3,7,8-Tetrachlorodibenzodioxin (TCDD), the most potent congener of this group of related compounds, is considered one of the most potent toxicants and carcinogens known to date. PCDDs, PCDFs and PCBs occur as complex mixtures in food and feed; hence, efforts have been undertaken to develop an approach that allows the cumulative assessment (exposure and risk) of this group of related compounds.

2. PRINCIPLES OF DIOXIN TOXIC EQUIVALENCY FACTORS

During the past few decades, data from many experimental studies with mixtures of these compounds indicated that the effects were additive, although deviations up to a factor of 2, and sometimes more, were described. As a result of this generally accepted concept of "additivity of effects", the toxic equivalency concept was developed during the mid-1980s. It uses the relative effect potency (REP) determined for individual PCDD, PCDF and PCB compounds for producing toxic or biological effects relative to a reference compound, usually 2,3,7,8-TCDD. REP values are derived from experimental in vitro and in vivo studies and sometimes epidemiological studies. From the range of REP values for each individual compound, the toxic equivalency factor (TEF) is derived. TEF values are estimates and are expressed on a defined scale as steps of, for example, half log increments.

The total toxic equivalent (TEQ) of a mixture is operationally defined as the sum of the products of the concentration of each compound multiplied by its TEF value and is an estimate of the total 2,3,7,8-TCDD-like activity of the mixture.

Use of the TEF approach is based on the assumption that PCDDs, PCDFs and coplanar PCBs have a common mode of action, which involves binding to the aryl hydrocarbon (Ah)

receptor, an intracellular receptor protein. This binding is considered to be the necessary, but not sufficient, first step in the expression of the toxicity of these compounds.

3. HISTORY OF WHO DIOXIN TEFS

Since the early 1990s, the World Health Organization (WHO) has organized expert meetings with the objective of international harmonization of the TEFs for dioxins and dioxin-like compounds, thereby giving recommendations to national and regional regulatory authorities. In 1993, the first evaluation resulted in human and mammalian WHO TEFs for all 17 PCDDs and PCDFs for which this approach can be applied, but also a recommended TEF value for several PCBs (Ahlborg et al., 1994). A WHO TEF (re)evaluation was done in 1997, which led to the revision of several mammalian TEF values of important congeners and withdrawal of the di-*ortho*-PCBs from the TEF concept for dioxin-like compounds. In addition, the first WHO TEF values for birds and fish were proposed (Van den Berg et al., 1998), resulting in the "WHO 1998 TEF values". In support of this meeting, the Karolinska Institute in Stockholm, Sweden, prepared a database with all studies from which REP values were derived as a basis for the TEF derivation. This REP database was recently updated and expanded to become a much more extensive database of REP values (Haws et al., 2006). It was used as the starting point for the third WHO expert meeting held in 2005 to reevaluate current mammalian TEF values (Van den Berg et al., 2006) (see Table 1).

4. RECENT REEVALUATION OF MAMMALIAN DIOXIN TEFS

Besides the reevaluation of the WHO 1998 TEF values, the validity, criteria and correct use of the TEF/TEQ concept, methods for proper identification of TEF values and possible compounds for future inclusion were discussed.

Additivity is an important prerequisite of the TEF concept, and this aspect was revisited in detail by the 2005 expert panel. Several new in vivo mixture studies were reviewed, and it was concluded that the results are consistent with additivity and support the TEF approach. In particular, new results from extensive studies by the United States National Toxicology Program generally supported effect additivity and parallel dose–response curves for complex and long-term neoplastic and non-neoplastic end-points (Van den Berg et al., 2006).

The criteria for inclusion of a compound in the dioxin TEF concept were reconfirmed. Compounds have to:

- show a structural relationship to the PCDDs and PCDFs;
- bind to the Ah receptor;
- elicit Ah receptor–mediated biochemical and toxic responses;
- be persistent and accumulate in the food-chain.

A systematic scheme to derive dioxin TEF values from the REP database was developed and applied to each congener. This approach was developed to provide more transparency and consistency to the TEF derivation process. Within this scheme, significant expert judgement is required, since the REP database is highly variable from congener to congener. This limitation also did not allow the statistical derivation of a TEF value from the range of REPs for each congener in a consistent and comparable way.

Table 1: Summary of WHO 1998 and WHO 2005 TEF values (Van den Berg et al., 1998, 2006).

Compound	*WHO 1998 TEF value*	*WHO 2005 TEF value*[a]
Chlorinated dibenzo-*p*-dioxins		
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.000 1	**0.000 3**
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.000 1	**0.000 3**
Non-ortho-substituted PCBs		
PCB 77	0.000 1	0.000 1
PCB 81	0.000 1	**0.000 3**
PCB 126	0.1	0.1
PCB 169	0.01	**0.03**
Mono-ortho-substituted PCBs		
PCB 105	0.000 1	**0.000 03**
PCB 114	0.000 5	**0.000 03**
PCB 118	0.000 1	**0.000 03**
PCB 123	0.000 1	**0.000 03**
PCB 156	0.000 5	**0.000 03**
PCB 157	0.000 5	**0.000 03**
PCB 167	0.000 01	**0.000 03**
PCB 189	0.000 1	**0.000 03**

CDD, chlorodibenzodioxin; CDF, chlorodibenzofuran; Hp, hepta; Hx, hexa; O, octa; Pe, penta

[a] Bold values indicate a change in TEF value.

Another important aspect considered was the uncertainty associated with each TEF value. The previous WHO expert consultation estimated the uncertainty to be within 1 order of magnitude. The 2005 consultation investigated the feasibility of describing the specific uncertainty associated with the TEF value for each congener. However, for the reasons explained above (i.e. highly variable REP database from congener to congener), this was not possible. The decision was taken to assign TEFs as half order of magnitude estimates (on a logarithmic scale), since this may be useful in describing, with statistical methods, the

uncertainty of TEFs in the future. Thus, as a default, all TEF values are assumed to vary in uncertainty by at least 1 order of magnitude, depending on the congener and its REP distribution. Thus, the TEF is a central value with a degree of uncertainty assumed to be at least ±0.5 log. For example, a TEF of 0.1 infers a degree of uncertainty bounded by 0.03 and 0.3.

5. APPLICATION AND USE OF THE DIOXIN TEF/TEQ APPROACH

The current dioxin TEF scheme has been derived mainly based on REP studies using oral exposure. As indicated above, food is the main source of human exposure to dioxins; hence, application of the TEF scheme and TEQ methodology to human exposure from food is most appropriate for human risk assessment. Moreover, the current scheme is frequently applied to various other matrices, often complex environmental matrices such as soil, sediment, industrial wastes, soot and fly ash. Applying the current TEF/TEQ scheme to these complex matrices allows the contamination levels of environmental samples by dioxin-like chemicals to be characterized and compared. However, the total TEQs of such environmental matrices cannot be used directly in human risk assessment, without taking factors such as fate, transport and bioavailability from each matrix into account. Nevertheless, application of this scheme to environmental matrices is an important tool to characterize and compare samples and to prioritize intervention actions.

The dioxin TEF/TEQ scheme is used in many national and international recommendations, legislations and conventions. Two examples are given below.

The Stockholm Convention (http://www.pops.int/) is a global treaty to protect human health and the environment from POPs. In implementing the Convention, governments take measures to eliminate or reduce the release of POPs into the environment. The Convention specifically mentions the WHO TEF scheme to be applied in measuring total TEQ. In applying the dioxin TEF scheme, current environmental and human contamination levels can be determined, and the effectiveness of intervention action can be monitored.

The European Commission implemented legislation to reduce dioxin contamination in food and feed. Two directives were established that set limits in food and feed and establish analytical requirements. Both directives use the WHO TEF scheme as a basis. This allows for international comparison of analytical results and monitoring for compliance (EC, 2002, 2003).

6. CONCLUSIONS

The dioxin TEF concept has a long history and has been developed to assess total TEQ of a complex mixture of structurally related compounds with a common mode of action. The TEF scheme has been reviewed and updated several times to take account of new scientific information. The current 2005 WHO TEFs are based on a relatively large database; however, the database is highly variable from congener to congener. The uncertainty of each individual TEF value cannot be expressed specifically, but is assumed to be within 1 order of magnitude.

The underlying principle of effect additivity has been confirmed by recent in vivo studies.

An extensive database is necessary to derive TEF values for groups of compounds in a reliable manner. Despite the elaboration of a detailed scheme for the dioxin TEF derivation in a consistent and transparent manner, significant expert judgement is necessary in the final decision-making process.

Many uncertainties exist in applying the dioxin TEF approach to the assessment of human health risk, but it is considered the most feasible approach currently available for this class of compounds. Moreover, this approach allows (international) comparison of relative toxic potency in a variety of matrices and the prioritization of intervention actions and control of their effectiveness.

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SCREENING-LEVEL RISK ASSESSMENT OF MIXTURES—AN EXAMPLE: POLYBROMINATED DIPHENYL ETHERS (PBDES)

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1. INTRODUCTION

Canada was the first country to introduce a legislative requirement for systematic priority setting of all chemicals in commerce. The Canadian Environmental Protection Act, 1999 (CEPA 1999) required "categorization" (priority setting) from among all of the approximately 23 000 "existing substances" on the Domestic Substances List (DSL) by September 2006, to identify those that should be considered further in screening assessments. This requirement was additional to the continuing provision to conduct full assessments on designated "priority substances" under the legislation.

CEPA 1999 delineates, then, three different levels of priority setting and assessments of increasing complexity for existing substances: categorization, screening and full (priority substances) assessments. This presentation addresses the intermediate stage—namely, a screening-level risk assessment for polybrominated diphenyl ethers (PBDEs) conducted as part of a pilot project for the categorization and screening of the substances on the DSL.

The principal objectives of screening assessments are to efficiently identify those substances that can be set aside as non-priorities for further work, those for which risk should be more fully characterized in assessments of priority substances and those to recommend for risk management.

Consistent with the principal objective of screening to increase efficiency in priority setting for full assessment and/or risk management, the degree of inherent conservatism is considerable. As a result, associated specified uncertainties are explicitly taken into account in drawing conclusions concerning the need and/or priority for further action on the substance under CEPA 1999 (Health Canada, 2004).

This assessment is presented as an example of a potential approach to maximizing use of available data in the screening of groups or mixtures of substances as mandates worldwide expand to require more efficient and inclusive consideration of larger numbers of existing substances. The approach involved comparison of upper-bounding estimates of exposure through environmental media and consumer products to a range of PBDE congeners present in commercial mixtures used in Canada with a conservative effect level for the most toxic congener, based on information available as of July 2003. The magnitude of the resulting margin of exposure is considered along with the confidence and uncertainties in the database upon which the assessment is based. As such, this screening assessment is illustrative of those that could be adopted in early tiers of consideration.

2. IDENTITY, USE AND SOURCES OF EXPOSURE

PBDEs are a class of substances that contain an identical base structure but differ in the number of attached bromine atoms (n = 1–10). Selection of the seven PBDE congener groups considered in this assessment was based on their potential use in Canada (i.e. their designation as existing substances included on the DSL) (Table 1). The three main

commercial mixtures (indicated using the prefix Com) containing these seven isomers are commercial pentabromodiphenyl ether (ComPeBDE, usually containing a mixture of PBDEs with 4–6 bromines), commercial octabromodiphenyl ether (ComOcBDE, usually containing a mixture of PBDEs with 6–9 bromines) and commercial decabromodiphenyl ether (ComDeBDE, usually containing a mixture of PBDEs with 9–10 bromines).

Table 1: List of PBDEs considered in the assessment (Health Canada, 2006).

Congener group	*Acronym*	*Chemical Abstracts Service No.*	*No. of individual congeners*
Tetrabromodiphenyl ether	TeBDE	40088-47-9	42
Pentabromodiphenyl ether	PeBDE	32534-81-9	46
Hexabromodiphenyl ether	HxBDE	36483-60-0	42
Heptabromodiphenyl ether	HeBDE	68928-80-3	24
Octabromodiphenyl ether	OcBDE	32536-52-0	12
Nonabromodiphenyl ether	NoBDE	63936-56-1	3
Decabromodiphenyl ether	DeBDE	1163-19-5	1

The commercial mixtures are used as flame retardants in many consumer products, including the foam stuffing used in furniture, the plastic for computer and television casings and carpet backings.

These seven isomers were assessed as a group in light of their identical base structure, the overlap in congeners within the commercial mixtures, similarities in uses and common target organs and effects. Limitations of available data also precluded their being considered separately; to the extent that available data permitted comparison, trends in physical/chemical properties and toxicity varied consistently with increasing degree of bromination.

3. EXPOSURE ASSESSMENT

Available data upon which to base estimates of population exposure to PBDEs are quite disparate, ranging from concentrations in specific media of individual congeners or congener groups to concentrations of total PBDEs, without further identification of specific congeners. In view of the limitations of the data with which to meaningfully estimate exposure to individual congeners or congener groups and the limited objectives of a screening assessment, conservative upper-bounding estimates of total intake of PBDEs were derived based on maximum levels in air, water, dust, food and human breast milk and standard intake values for six age groups within the Canadian population.

Based on reported concentrations of PBDEs in ambient and indoor air, water, various foodstuffs, human breast milk and dust, along with standard reference values, an upper-bounding estimate of daily intake of total PBDEs (i.e. the tetra to deca congeners considered here) ranged from 0.2 to 2.6 μg/kg body weight (bw) per day for six different age groups of the general population, including breastfed infants, in Canada (Health Canada, 2006). Food (including breast milk) represents the principal source of exposure for the majority of the age groups (although dust was the principal source of exposure for the 0- to 6-month-old non-breastfed age group). The age group with potentially the greatest exposure was 0- to 6-month-old breastfed infants, with breast milk accounting for 92% of the exposure.

These upper-bounding estimates of exposure were considered conservative, in that they were based on summed estimates for all congeners for which data were available and highest measured concentrations for many media. Quantitative implications of this degree of conservatism were taken into account in determining the adequacy of the margin of exposure (see section 5 below).

Upper-bounding estimates of intake in food for subpopulations consuming more traditional or country foods were not substantially greater (i.e. less than 2-fold). Similarly, estimates of intake from dermal contact with dust or oral contact with household products treated with flame retardants containing the penta and octa congeners were also negligible in comparison with intake from food (Health Canada, 2006).

4. HAZARD CHARACTERIZATION

The majority of identified data on the toxicity of PBDEs relate to the commercial mixtures, with much less information being available for individual congeners. Although a full range of toxicity studies was not available for all congeners or commercial mixtures, target systems and organs for PBDEs are similar, including the liver, the thyroid and early behavioural development. Based on preliminary assessment of the available toxicological data, the critical effects and effect levels for the ComPeBDE, ComOcBDE and ComDeBDE commercial mixtures, as well as each of the congener groups considered in this assessment (where possible), are presented in Table 2. Critical effects of PBDEs were those that occur on the liver and on neurobehavioural development. Owing to the limited nature of the database for some substances, confidence in the assessment for each PBDE congener group and commercial mixture varies.

Table 2: Overview of critical health effects and effect levels for PBDE congener groups and commercial products (Health Canada, 2006).

PBDE	*LOEL (mg/kg bw per day)*	*End-point*	*Reference*
TeBDE	11	Neurobehavioural development (mouse)	Eriksson et al. (2001)
PeBDE	0.8	Neurobehavioural development (mouse)	Eriksson et al. (1998, 2001)
HxBDE	0.9	Neurobehavioural development (mouse)	Viberg et al. (2002)
HeBDE	–	–	
OcBDE	–	–	
NoBDE	–	–	
ComPeBDE	2	Liver histopathology: subchronic dietary study (rat)	Great Lakes Chemical Corporation (undated)
ComOcBDE	5	Liver weight: subchronic dietary study (rat)	Great Lakes Chemical Corporation (1987)
ComDeBDE/ DeBDE	2.2	Neurobehavioural development (mouse)	Viberg et al. (2001a, 2001b, 2003); Viberg (2002)

LOEL, lowest-observed-effect level

The selected critical effect level (Health Canada, 2006) was the conservative value of 0.8 mg/kg bw per day (for PeBDE), based on neurobehavioural effects consisting of changes

in locomotion, rearing and total activity in a dose- and time-related manner observed in neonatal mice administered a single oral dose by gavage on postnatal day 10 and observed for a subsequent 5-month period (Eriksson et al., 1998, 2001). Selection of this critical effect level was supported by additional information on similar effects being observed in mice exposed to the penta congener via maternal administration and in neonatal mice administered single, relatively low doses of the tetra, hexa and deca congeners by the same investigators (Eriksson et al., 1998, 2001). A somewhat lower lowest-observed-effect level (LOEL) of 0.44 mg/kg bw per day for ComPeBDE, based on alterations in hepatic enzyme activities, was not considered critical based on the lack of observation of histopathological changes in the liver at this or higher doses (Health Canada, 2006).

5. RISK CHARACTERIZATION

As a basis for development of conservative margins for the purposes of screening and in light of the similarity of health effects associated with the various PBDEs considered here, the selected critical effect level was compared with an upper-bounding estimate of exposure to total PBDEs (i.e. the tetra to deca congeners considered here) for the potentially most highly exposed subgroup.

Comparison of the critical effect level (i.e. 0.8 mg/kg bw per day for neurobehavioural effects in mice following neonatal exposure) with the upper-bounding deterministic estimate of exposure for the intake of total PBDEs (2.6 µg/kg bw per day in breastfed infants) resulted in a margin of exposure of approximately 300.

Margins based on available biomonitoring data were approximately 10-fold less. These were estimated through back-calculation of intakes by first-order kinetic modelling of limited data on levels in blood of the general population and comparison of estimated body burden for the critical study in animals with that for breastfed infants. However, confidence in these estimates was considered to be less, owing to the considerable limitations of the relevant data on biological half-lives of PBDEs in humans and their seeming inconsistency with what would be expected based on relevant physical/chemical properties.

The degree of conservatism in this margin is relevant to its interpretation. One critical aspect is the large interindividual variability in levels of PBDEs in breast milk within the general population. It should be noted that mean and median values for levels in breast milk were as much as 400- and 200-fold less, respectively, than the maximum values on which the estimates of exposure were based. In addition, the critical effect level with which the estimate of exposure was compared was that for the most sensitive effect for the most toxic congener. In comparison, effect levels in chronic studies for the same congener were approximately 100 times greater than that used in the margin of exposure.

The margin of exposure does not, however, take into account the potential continuing increase in body burden of PBDEs (based on data for breast milk), should similar use patterns continue. Based on limited data, levels of PBDEs in human breast milk in Canada appear to be increasing with time (e.g. there was a 9-fold increase in mean concentration between 1992 and 2001). Prediction of trends in body burdens is precluded by the limited information on the toxicokinetics of PBDEs in humans and animals and transfer from human breast milk to infants, as well as the uncertainty in half-lives for removal processes for PBDEs in environmental media.

Determination of the adequacy of the derived margin to address elements of uncertainty associated with limitations of the database for health effects and population exposure (in which confidence overall is considered to be moderate), intraspecies and interspecies variations in sensitivity, as well as the biological adversity or severity of the effects deemed critical was found to require additional in-depth evaluation of the relevant data. Development of additional, more meaningful information on population exposure to PBDEs was also considered desirable.

However, in view of the smaller margin between the most conservative estimated critical values for exposure and effects on the environment in comparison with that for human health and the resulting recommended action to protect the environment, in-depth evaluation of PBDEs from a human health perspective was considered a low priority, unless information becomes available to indicate that measures recommended to control exposure of environmental organisms to PBDEs will not be protective for human health. This conclusion is consistent with experience in other countries where risk management actions to protect the environment have resulted in a reduction of exposure of humans. It also contributes to increasing efficiency in the assessment and management of prioritized chemical substances.

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USE OF BIOMARKERS OF EFFECT IN THE EVALUATION OF CUMULATIVE EXPOSURE: NEPHROTOXICITY OF SOLVENTS AT THE WORKPLACE

Inge Mangelsdorf

1. EXPOSURE

It has been estimated that in the United States, about 4 million workers are exposed to chemicals that, at least from data from animal experiments, are suspected to show nephrotoxic effects. Among these chemicals, solvents form a structurally heterogeneous group of chemicals with a widespread use in a variety of products and at different workplaces. Depending on the vapour pressure of the individual solvents, workers may be exposed by inhalation. Exposure concentrations may be very high, at least for some procedures (e.g. cleaning). In addition, dermal exposure may contribute considerably to the overall exposure. Exposure may occur despite the use of protective clothing, because solvents may penetrate gloves or cloth. Solvent mixtures are frequently used. Therefore, effects may also be caused by combined exposures.

Threshold limits in the air, established by several national and international institutions, enable control of a large number of solvents in air at the workplace. For some compounds, threshold values for excretion of metabolites in urine (biomarkers of exposure) are also available for evaluating dermal exposure. Combined exposures are, however, usually not assessed.

2. EFFECTS

In animal studies, most solvents lead to liver and kidney damage at high concentrations. Human studies also give indications of nephrotoxic effects. Renal damage (primarily Goodpasture syndrome or other glomerulonephritides) after acute exposure to solvents has been described in several case-reports, which have prompted numerous case–control studies. According to an overview of all case–control studies up to 2000, a positive relationship between solvent exposure and non-systemic glomerulonephritides was found in 19 out of 24 studies, and a meta-analysis revealed a significantly, albeit weakly, increased odds ratio of 1.6 (95% confidence interval: 1.2–2.0) for all studies combined (Ravnskov, 2000). A study published after this review again showed a positive association (Huber et al., 2000). No specific solvents could be identified as risk factors in these studies.

Unlike case–control studies, cohort mortality studies, including a meta-analysis of 55 mortality studies, failed to show a relationship between solvent exposure at the workplace and kidney disease (Chen & Seaton, 1996). It is likely that the study power was not sufficient for analysis, as glomerulonephritis is a rare disease. Thus, the results of the cohort studies do not contradict the results of the case–control studies.

Cross-sectional studies provide additional evidence that kidney disease may be associated with solvent exposure (Voss et al., 2003, 2005). Studies have been carried out in groups of workers exposed to various hydrocarbon mixtures, toluene or toluene/xylene mixtures, styrene, methyl ethyl ketone, butoxyethanol, ethylene glycol, tetrachoroethene, trichloroethene, 1,3-dichloropropene and various mixtures, including organochlorine compounds. In

these studies, various biomarkers were measured in the urine to detect early functional alteration of the kidney. No parameter was consistently increased after exposure to a certain chemical, and no dose–response was found. Furthermore, as for the case–control studies, no specific solvent or group of solvents could be identified as a risk factor. However, it was observed that, although the differences in mean or median levels of the biomarkers between exposed and unexposed workers were relatively small, maximum levels of the biomarkers in individuals were in some studies considerably higher in exposed workers compared with non-exposed workers. Therefore, the frequency of occurrence of high levels of various biomarkers was explored in a meta-analysis. As Table 1 shows, a statistically significant difference was found for the frequency of high albumin levels in urine in the exposed group compared with the unexposed group. For other biomarkers, the database was not sufficient, or no increase was found.

Table 1: Exploratory statistical analysis of the frequency of "high" values for urinary excretion of albumin in cross-sectional studies with solvent exposure.

Variable	*Cut-off limit*	*Frequency of groups containing individuals with "high" values*[a]		*p-value*[b] *(Fisher's exact test, one-tailed)*
		Exposed	*Non-exposed*	
Albumin	37 mg/g creatinine[c] or 19 mg/l urine[c]	14/14 (set to 100%)	7/12 (58%)	**0.01**
	100 mg/g creatinine or 100 mg/l urine	9/14 (64%)	2/12 (17%)	**0.02**
	20 U/g creatinine	3/9 (33%)	0/7 (0%)	0.2

[a] Number of studies (groups within a study) in which the upper limit of the range of individual values is greater than the cut-off limit, related to the number of studies for which the range of individual values is given in the paper.

[b] Comparison exposed versus non-exposed. Boldface type indicates statistical significance at $p < 0.05$.

[c] Upper limit of the normal range.

As albumin is a marker of glomerular damage, the cross-sectional studies also support an association of glomerulonephritis and solvent exposure, although the overall database is weak.

3. DISCUSSION AND CONCLUSIONS

Kidney effects are found frequently in animals exposed to high concentrations of solvents. However, while predominantly tubular effects are observed in animal studies, presumably the result of direct toxic effects of reactive metabolites from the solvents, glomerulonephritis is observed in humans. As glomerulonephritis is also described in case-reports, where single high exposure concentrations were encountered, one may conclude that predominantly peak exposures may lead to glomerular disease. As the disease is very rare, despite the exposure of a large population, it is possible that only a few susceptible individuals are affected.

It seems that glomerulonephritis from solvent exposure is an unspecific effect, which may be caused by any solvent. The hypothesis for the mode of action is that glomerulonephritis is mainly immunologically mediated, either through antibodies reacting with autoantigens of the kidney or by the deposition of immune complexes within renal structures. The solvent-induced reaction with autoantigens is supported by the findings of antiglomerular basement

membrane antibodies in workers exposed to hydrocarbons (Stevenson et al., 1995). However, further research is needed to investigate how exactly the damage may be induced.

Under conditions of chronic solvent exposure, renal damage may remain clinically silent for many years owing to the large functional reserve capacity of the kidney. During this time, alterations may gradually progress through a cascade of events, from early biological effects that may be of no clinical significance through initially reversible functional and/or structural alterations to focal damage, and finally to manifest, clinically detectable disease. At a later stage, the disease can be irreversible and end up in end-stage renal failure. It is therefore important to detect kidney defects as early as possible to prevent progression of the disease. Therefore, the use of albumin as a "biomarker of effect" would be a very useful tool for detecting effects at an early stage. This is supported by studies in which it was shown that effects were indeed reversible if the exposure was stopped/reduced. In these studies, the degree of reversibility depends on the severity of the disease. Albumin as a biomarker of effect would allow the identification of sensitive individuals or individuals with high exposures in the past. A proposal has been made for systematic workplace surveillance (Brinkmann et al., 2004), which also includes measurement of albumin in the urine (microalbuminuria) in a stepwise procedure. This marker would include integrated effects from exposure by inhalation and dermal exposure, as well as effects from all types of solvents, potentially leading to kidney damage.

As liver effects are also common effects from high exposures to solvents, measurements of liver enzymes in serum should be included in such systematic surveillance as well.

In conclusion, biomarkers of effect present a valuable tool for assessing and controlling combined exposures from different routes as well as from different compounds and should be further explored. Biomarkers may also reveal the etiology of diseases; for example, urinary excretion of isoprostanes may serve as a marker for lipid peroxidation (Uchida, 2007). A further advantage of the use of biomarkers is that they can be measured in humans; therefore, uncertainties referring to species differences do not apply in this case.

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COMBINATION TOXICITY: EVALUATING CHEMICAL MIXTURES WHEN COMMON MODE OF ACTION ASSUMPTIONS DO NOT APPLY

Linda K. Teuschler

1. INTRODUCTION

Environmental mixtures may be assessed using evaluations of 1) simple defined mixtures, using additivity concepts and data on toxicological interactions; 2) whole mixtures, using data on the chemical mixture of concern or on a sufficiently similar mixture; and 3) partial mixtures, ranging from using one chemical component to estimate the toxicity of the entire mixture to evaluating fractions of the mixture. Within these general categories, many types of information may be used, including toxicological data, epidemiological information, physiologically based pharmacokinetic (PBPK) model results, statistical models and inference, and in silico toxicology.

Additivity concepts are often used to estimate risk or hazard from exposure to a simple defined mixture of perhaps a dozen or fewer components. Simple similar action refers to chemicals that cause toxicity through a common toxic mode of action (MOA) and are thus evaluated using dose addition approaches (Feron & Groten, 2002). However, a common MOA assumption may require an extensive database and will often not be biologically supportable. Thus, the subject of this abstract is to present concepts and methods for evaluating chemical mixtures when the assumption of common MOA does not apply and dose addition–based procedures are not appropriate.

2. ADDITIVITY: BEYOND DOSE ADDITION

There are several other ways to evaluate simple defined mixtures using additivity. Simple dissimilar action is assumed when chemicals cause a common health effect, but by a different toxic MOA. In this case, the toxic responses are thought of as biologically and statistically independent events. Methods include either summing the probabilistic risks of an adverse effect for the mixture components (response addition) or summing the actual biological measurements of the adverse effect for the mixture components (effects addition). When a group of mixture components causes a common health effect, but can be classified into different MOA subgroups, then dose addition and response addition methods can be integrated to assess risk (Teuschler et al., 2004). Finally, the joint toxic action of a defined mixture can be evaluated statistically without making MOA assumptions (Gennings et al., 2005). In this case, if the slope of the dose–response curve of a chemical is not altered in the presence of another chemical, then these chemicals can be said to combine additively regardless of their MOA; conversely, if the slope is altered, then, depending on the direction of the change, the result is a greater- or less-than-additive response (i.e. an interaction effect).

3. TOXICOLOGICAL INTERACTIONS

A common concern for evaluating chemical mixtures is the potential for toxicological interactions to occur from co-exposures. Types of toxicological interactions include chemical–chemical reactions and pharmacokinetic and pharmacodynamic interactions. In the United States, the United States Environmental Protection Agency (USEPA, 2000) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2004) define toxicological

interactions as responses that deviate from those expected under a specified definition of additivity. Interactions may generally be referenced as effects that are greater than additive or *synergistic* (e.g. increased carcinogenicity for co-exposures to asbestos and tobacco smoke) or less than additive or *antagonistic* (e.g. decreased cadmium toxicity through co-exposure to dietary zinc, which reduces cadmium absorption). More specific terms include *inhibition* and *potentiation*, which are defined as when a component that does not have a toxic effect on a certain organ system decreases or increases, respectively, the apparent effect of a second chemical on that organ system (ATSDR, 2004). Finally, the term *masking* is used when the components produce opposite or functionally competing effects on the same organ system and diminish the effects of each other, or one overrides the effects of the other (ATSDR, 2004).

Dose dependence of interactions requires consideration of changes in the type of adverse effect that is manifested, as well as changes in the type of joint toxic action. This typically ranges from additivity at low doses to synergism in the mid-range of the dose–response curve to antagonism at high doses close to a maximum biological response. Interactions data can be used in risk assessment based on the strength of evidence for these requirements: 1) adequate toxicity data are available on dose–response and MOA, 2) data on the same route of exposure should be used across components or similar mixtures, 3) data on components should be from comparable studies (e.g. same species, end-point, study duration) and 4) observed interaction effects should be toxicologically significant (USEPA, 2000).

Most of the interactions data in the toxicological literature are from studies of binary mixtures. Consequently, risk assessment approaches developed to date use binary data to estimate changes in the additive Hazard Index (HI) attributable to interactions. ATSDR (2007) has published 11 interaction profiles for simple defined chemical mixtures that evaluate health effects, dose–response and toxicological interactions data. Within these interaction profiles, a qualitative binary weight of evidence (BINWOE) is developed that evaluates mechanistic evidence, strength of interactions data, influence of exposure duration and route, and sequence of exposure for each pair of chemicals. The BINWOE is used to qualitatively modify the HI. A method also exists to quantitatively modify the HI, using factors that account for interaction weight of evidence, interaction magnitude, fraction of toxic hazard of each interacting chemical pair and relative proportions of the chemicals (USEPA, 2000).

Newer methods for evaluating toxicological interactions include the use of PBPK models and biochemical reaction network (BRN) modelling. Haddad et al. (2001) used PBPK models to compare an interaction-based HI for central nervous system effects with an additive HI (both computed for internal doses) over a range of exposure concentrations for different mixtures of dichloromethane, benzene, toluene, ethylbenzene and *m*-xylene, showing greater-than-additive effects at the higher total dose levels of the mixture. Also, Krishnan et al. (2002) used PBPK modelling to predict the kinetics of chemicals in complex mixtures by accounting for binary interactions alone within a binary interaction–based PBPK modelling structure. PBPK models developed for the mixture components were interconnected at the level of the tissue where metabolic interaction occurred (e.g. competitive inhibition for hepatic metabolism). Once interconnected at the binary level, the PBPK framework simulated the kinetics of all mixture components, accounting for interactions at various levels in more complex mixtures. This method was then validated using laboratory data.

BRN approaches are computer algorithms that use a systems biology approach to model chemical interactions (Mayeno & Yang, 2005). These computer models simulate interactions for mixtures composed of a large number of components and link computer simulation techniques with PBPK models. They predict the formation of metabolites from mixture exposures, including chemical and metabolic interactions, and interconnect metabolic pathways by common metabolites. As such models continue to be developed and are validated, they will aid in predicting metabolism and toxicity and in understanding MOA.

4. WHOLE MIXTURES

Estimating dose–response and characterizing risk for complex mixtures (e.g. diesel emissions, dioxins) is difficult because of variability in chemical composition due to different environmental sources or weathering of mixtures in the environment. Risk assessment of a complex mixture may include using information on the environmental mixture or its concentrate, a sufficiently similar mixture, fractions of the mixture or component data. Toxicity values can be determined for the complex mixture based on epidemiological or toxicological data on the whole mixture or a sufficiently similar mixture. For example, the USEPA's Integrated Risk Information System (http://epa.gov/iriswebp/iris/index.html) contains reference doses for the whole mixtures Aroclor 1016 and Aroclor 1254, based on a study in rhesus monkeys and an inhalation cancer slope factor for coke oven emissions based on human occupational exposures.

Chemical and toxicological similarities are needed to support the use of toxicity data from a known complex mixture as surrogate data to evaluate potential toxicity of an environmental mixture. Two mixtures are sufficiently similar if there are small differences in their components and in the proportions of their components (USEPA, 2000). An example method by Eide et al. (2002) uses pattern recognition techniques and multivariate regression modelling to characterize and analyse the chemical composition and toxicity of extracts of soot particles. This approach identifies variables on gas chromatography–mass spectrometry (GC-MS) chromatograms of the complex mixtures and uses multivariate analysis to identify the peaks that co-vary with mutagenicity. Thus, the mutagenicity of a new mixture of exhaust particles can be predicted from its GC-MS chromatogram.

Several methods have been used to evaluate highly variable complex mixtures. These include 1) assuming that a single component's toxicity can represent the toxicity of the entire mixture, which is a simple approach, but likely inaccurate, as it poorly characterizes exposure; 2) identifying and quantifying a subset of known components and using an additivity approach, which characterizes potential exposures to known components well, but is resource intensive and may be analytically difficult; and 3) using analytical chemistry to fractionate the mixture and assign toxicity values to these fractions for use in additivity calculations, which is a flexible method for characterizing chemical composition and toxicity of a complex mixture. An example of this third approach is the evaluation of total petroleum hydrocarbons (TPH) at contaminated sites by dividing the TPH into several analytically defined aromatic and aliphatic hydrocarbon fractions and then assigning each fraction an oral and inhalation toxicity value (MADEP, 2002, 2003).

5. CONCLUSIONS

The most commonly used chemical mixture methods are based on additivity assumptions, usually dose addition or response addition, are easy to populate with data and require simple

calculations. These methods are widely applied to various environmental media, in both human and ecological risk assessment, and in many countries around the world. For complex exposures and toxicity data, including interactions data, more sophisticated models have been developed, but are usually applied only when the expenditure of resources is justified by the risk assessment need. Validation of models and discussion of uncertainties are important aspects of chemical mixture risk assessments and need to be incorporated into the risk characterization of a chemical mixture.

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MIXTURES RISK ASSESSMENT IN PUBLIC HEALTH PRACTICE

C.T. De Rosa and M.M. Mumtaz

1. INTRODUCTION

All populations are exposed to ubiquitous hazardous chemicals present in their environment. However, the communities in the vicinity of hazardous waste sites are especially vulnerable and increasingly aware that they are at increased risk in the absence of benefit. For decades, the development of health criteria and chemical regulation has focused on single chemicals. Often a government agency, international organization or advisory body identifies a chemical or group of chemicals of concern. This concern can be generated by legislative mandate, evidence of existing human risk or some other process of prioritization. For each chemical of concern, the existing toxicological database pertinent to human and environmental health, including epidemiological, occupational, animal and in vitro data, is thoroughly reviewed, and an "acceptable" exposure level is derived and documented.

However, exposures in the real world are seldom, if ever, to a single chemical, but are usually to mixtures of chemicals. The United States National Toxicology Program's Annual Report on Carcinogens (NTP, 2005) lists 11 mixtures, including environmental tobacco smoke, coal tar, diesel exhaust particulates, mineral oils, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and polycyclic aromatic hydrocarbons (PAHs). Thus, in some instances, complex exposures occur to mixtures whose exact composition often is not even characterized (e.g. coke oven emissions, diesel exhaust, asphalt fumes and welding fumes). In such cases, the available toxicological information on the mixture is reviewed, the complex mixture is for the most part treated as a single chemical and direct risk assessments are carried out for the purposes of deriving a criteria or regulatory standard.

Thus, it is recognized that human exposure to chemicals in the environment will involve exposures to multiple chemicals. Exposure to complex mixtures can be sequential and/or simultaneous with a variety of compounds in food, medications and recreational or abused substances (e.g. ethanol and tobacco). These chemicals have the potential to enhance, inhibit or exacerbate the health risks posed by the mixtures.

2. MIXTURES OF CONCERN AT HAZARDOUS WASTE SITES

Exposure to chemicals in the environment could be accidental, episodic or chronic—the latter often due to their presence in environmental media, such as air, water and soil at hazardous waste sites. Some of the hazardous waste sites can contribute to significant exposures and hence are put on a national priorities list, indicating their potential hazard. Such sites are further characterized for the hazard they pose by identifying completed exposure pathway(s) (ATSDR, 2007a). By definition, a completed exposure pathway exists when there is direct evidence or a strong likelihood that people have in the past come or are currently coming in contact with site-related contaminants. For a route to be categorized as a completed exposure pathway, the source of contamination, environmental fate and transport, exposure point and exposed population all have to be identified. If a completed exposure pathway is identified, a plan to mitigate exposures that could compromise human or environmental health is often developed and implemented. Of the 1706 hazardous waste sites that the Agency for Toxic Substances and Disease Registry (ATSDR) has analysed, 743 have been found to have

completed exposure pathways, of which 588 are for mixtures of chemicals. Seventy-nine per cent of these latter sites are for at least two chemicals, and 64% are for at least three chemicals. These data underscore the concerns of exposure to mixtures of environmental chemicals.

3. CONSIDERATIONS OF INTERACTIONS IN RISK ASSESSMENTS

Several of the approaches available for the risk assessment of chemical mixtures have been summarized in previous reviews (USEPA, 2000; ATSDR, 2001; De Rosa et al., 2004). The most direct and accurate is the "mixture of concern" approach. This approach is used if toxicological data on the mixture of interest are available. This approach has been used to derive United States Occupational Safety and Health Administration (OSHA) exposure limits, as well as American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs) (ACGIH, 2006). A modification of the mixture of concern approach is the "similar mixture" approach, which is used if data are unavailable for the mixture of concern but are available for a mixture that is considered to be similar in terms of its characteristics, particularly its toxicity. There is no set criterion for determining the similarity, but due considerations are given to the qualitative and quantitative aspects of the composition of the mixture. This approach is used on a case-by-case basis. In both the above approaches, the mixture is treated as a single chemical because, to some extent, data are available on the whole mixture.

The third and most often used approach attempts to estimate the toxicity of a mixture based on the information available on the individual components of the mixture. This, the "hazard index" approach, is used if data are not available on the whole mixture. This approach relies heavily on some form of additivity—potency-weighted dose or response additivity. The dose or response additivity models employ assumptions concerning modes of action or mechanisms of action that may not be thoroughly understood. Thus, the number of mixtures to which such approaches can be applied is also limited. This approach has been applied to some very important classes of environmental contaminants, such as PAHs, PCBs and dioxins, using the toxic equivalency factors (TEF) method (Safe, 1998).

Most toxicologists agree that there exists ample information, both empirical and mechanistic, suggesting that chemicals do interact, and this information should not be disregarded. However, there is no clear agreement and hence limited guidance on the use of this information in risk assessment. The potential significance of interactions in joint toxicity assessments can be regarded as one of many sources of uncertainty in the risk assessment of exposure to mixtures. Most of the information available on compound interactions has not been and is not amenable to existing statistical analysis and cannot be used to quantify interactions. A weight-of-evidence scheme proposed by Mumtaz & Durkin (1992) provides a framework for systematically assessing the weight of evidence for the qualitative determination of interactions (i.e. whether the mixture is likely to be more or less toxic than anticipated based on the assumption of additivity). The scheme also suggests ways to consider the magnitude of the interaction and quantitatively adjust risk assessments using dose–response or dose–severity (Table 1). Nonetheless, in the field, it is rarely used for quantitative risk assessment. In summary, the weight-of-evidence evaluation is a qualitative judgement based on empirical observations and mechanistic data. The scheme characterizes the plausibility of joint toxicity of pairs of toxicants—that is, how a chemical's toxicity can be influenced by the presence of a second toxicant. The weight-of-evidence scheme yields an alphanumeric identifier that takes into consideration several factors, including the quality of

the data, its mechanistic understanding, its toxicological significance and factors, such as route and duration of exposure, that could play a critical role in the expression of the overall integrated joint toxicity of the mixture.

Table 1: Weight-of-evidence scheme for the qualitative assessment of chemical interactions (Mumtaz & Durkin, 1992).

Determine if the interaction of the mixture is additive (=), greater than additive (>) or less than additive (<).

Classification of Mechanistic Understanding

I. Direct and Unambiguous Mechanistic Data:
The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.

II. Mechanistic Data on Related Compounds:
The mechanism(s) by which the interactions could occur is not well characterized for the compounds of concern, but structure–activity relationships, either quantitative or informal, can be used to infer the likely mechanisms and the direction of the interaction.

III. Inadequate or Ambiguous Mechanistic Data:
The mechanism(s) by which the interactions could occur have not been well characterized, or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.

Classification of Toxicological Significance

A. The toxicological significance of the interaction has been directly demonstrated.
B. The toxicological significance of the interaction can be inferred or has been demonstrated in related compounds.
C. The toxicological significance of the interaction is unclear.

Modifiers

1. Anticipated exposure duration and sequence
2. A different exposure duration or sequence
    a. In vivo data
    b. In vitro data
        i. The anticipated route of exposure
        ii. A different route of exposure

Applying this weight-of-evidence methodology to available data on a mixture of four chemicals—atrazine, simazine and diazinon, all pesticides, as well as nitrate, a common contaminant resulting from fertilizers and human and animal waste—yielded the summary matrix shown in Table 2. This mixture was chosen based on an analysis of frequently occurring mixtures in groundwater, its ubiquity in the environment and its potential to illustrate interactions of mixtures of similar and dissimilar modes of action. Other mixtures of environmental relevance have also been analysed using this methodology and are available on the ATSDR web site (ATSDR, 2007b). Based on the information (Table 2), it could be concluded that reproductive effects will be additive—that is, interactions might not play a significant role in the joint toxicity assessment of some binary mixtures. This conclusion is based on strong evidence. In contrast, interactions might influence genotoxicity, and neurological effects assessment (i.e. the hazard index), based on additivity, might underestimate risk. The evidence for these effects is somewhat weak and is based on inference of the data. Several data gaps are identified in the matrix (question marks) for certain binary combinations of this mixture, pointing to data gaps and future research needs (ATSDR, 2006).

Table 2: BINWOE determinations for a mixture of chemicals found in drinking-water.

		ON TOXICITY OF			
		Atrazine	Simazine	Diazinon	Nitrate
EFFECT OF	Atrazine		=IA repro	>IIB neuro	>IIC geno
	Simazine	=IA repro		>IIB neuro	>IIC geno
	Diazinon	?	?		?
	Nitrate	>IIC geno	>IIC geno	?	

Geno, genotoxic; neuro, neurological; repro, reproductive
See Table 1 for definition of classifications and modifiers.

4. CONCLUSION AND FUTURE DIRECTIONS

Because of resource and time limitations, experimental toxicology probably will never be able to provide direct information on all the possible mixtures to which humans or other target species are exposed. Hence, implicit in every single chemical toxicity/risk assessment is the assumption that exposures to other compounds are insignificant. Even when a great deal of information is available on certain chemicals, several questions often still remain. How can the dose–response and dose–severity relationships be evaluated? Do all the toxic effects have thresholds? If so, can the errors associated with these thresholds be estimated and the consequences of exceeding these thresholds be measured? All of these questions have a very direct and significant impact on the risk assessment of mixtures.

Pragmatic and realistic risk assessments can be done only by considering issues beyond single chemicals within the risk assessment paradigm of exposure assessment, hazard identification, dose–response assessment and risk characterization. Data needs are realized at every step of this paradigm. Environmental monitoring, biomonitoring, surveillance and population surveys are essential to an accurate exposure assessment, the fundamental basis of every risk assessment. The entirety of exposures to chemicals and environmental factors must be assessed for a determination of total integrated exposure through multiple routes and for multiple chemicals. The next phase of hazard identification and evaluation takes one of the several options available and is driven by the quality and quantity of the toxicity data. Understanding the mechanisms and modes of action is essential for any advances in the joint toxicity assessment of mixtures. Only then can predictive models be developed for relevant complex exposures that occur in real life. The “omic” techniques seem to be an exciting and promising venue to explore their utility in advancement of methods development. Such methods will illustrate up- and downregulations of genomic sequences that might have implications for chemical exposure and human health.

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FLAVOURS IN THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

John Christian Larsen

1. INTRODUCTION

At its 37th meeting in 1990, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered three flavouring agents that were allyl esters of fatty acids and concluded that a group acceptable daily intake (ADI) should be allocated to the three esters on the basis of the allyl alcohol moiety, because they are rapidly hydrolysed and the observed toxic effects are due to the allyl moiety. At the same time, it was recognized that a large number of other allyl esters of fatty acids, also used as flavouring agents, are hydrolysed in a similar manner, so the same considerations should apply to them. JECFA therefore concluded that the safety evaluation of a specific flavouring agent would be facilitated by consideration of a structurally related group as a whole (WHO, 1991).

At its 39th meeting in 1992, JECFA again drew attention to the desirability of structurally related compounds being evaluated at the same time, since data derived from one member of a group of such compounds may assist in the evaluation of another (WHO, 1992). Therefore, in order to speed up the safety evaluation of the approximately 3000 flavouring agents potentially used in foods, JECFA adopted a new "Procedure for the Safety Evaluation of Flavouring Agents" at its 46th meeting and used it for the evaluation of a number of flavouring substances that were simple esters (WHO, 1997). The Procedure was first discussed at the 44th meeting of JECFA (WHO, 1995), based on a paper prepared by Dr I.C. Munro (Munro et al., 1996; WHO, 1996), and was further modified at the 49th meeting of JECFA to include the acceptance of a general threshold of toxicological concern (TTC) of 1.5 μg/person per day (WHO, 1998, 1999).

2. THE PROCEDURE

The Procedure is intended to be used on groups (or subgroups) of chemically closely related flavouring agents predicted to share similar efficient metabolic pathways and takes account of available information on intake from current uses, structure–activity relationships, metabolism and toxicity data. Some flavouring agents have simple chemical structures and are efficiently metabolized to innocuous products, occur as normal constituents of mammalian tissues or are metabolized to form such constituents. Such compounds are considered safe if human intake is low, but should be evaluated on the basis of toxicity data if human intake is high. The safety evaluation may also rely, at least in part, on toxicity data on substances of closely related structure. However, the evaluation of compounds not known to be metabolized to innocuous end products must be based on toxicity data, even if intake is low. There should be an adequate margin of safety between human intake and the no-observed-effect level (NOEL) for the substance or a closely related substance. Finally, for those flavouring agents currently in use for which no toxicity data or metabolic data exist, but where the intake is extremely low, it might be possible to specify a threshold below which intake is considered safe.

2.1 Thresholds of toxicological concern (TTC)

The flavouring substances are divided into three structural classes based on increasing structural complexity and structural alerts (classes I, II and III), according to Cramer et al. (1978). Class I substances have simple chemical structures and are efficiently metabolized by high-capacity pathways. Class II substances are "intermediate" substances with less innocuous structures but without structural features suggestive of toxicity. Class III substances have chemical structures that do not permit presumption of safety or even suggest toxicity or reactivity.

Munro and co-workers (Munro, 1990; Munro et al., 1996; WHO, 1998) established a comprehensive database containing conservative NOELs (2941) for a number of toxicological end-points for a large number of different chemicals (613). This database was used to establish the 5th-percentile NOELs for each structural class. By applying the conventional default safety factor of 100 on the 5th-percentile NOELs, the following human intake TTCs were obtained: for structural class I, 1.8 mg/person per day; for class II, 0.54 mg/person per day; and for class III, 0.090 mg/person per day. Later work increased the number of chemicals in the database without altering the cumulative distributions of NOELs, adding further reassurance about the validity of using this database to derive the TTC values (Barlow, 2005).

The Procedure finally includes the acceptance of a general TTC of 1.5 μg/person per day. Flavouring agents for which insufficient data are available for evaluation by earlier steps in the Procedure, but for which the intake would not exceed 1.5 μg/person per day, would, in the judgement of JECFA, not be expected to present a safety concern (WHO, 1999). This general TTC was based on an evaluation of the carcinogenic potency (tumorigenic dose for 50% of test animals, or TD_{50}) of a large number of substances tested in long-term toxicity/carcinogenicity studies in rats and mice included in the Gold et al. (1989) cancer potency database. The lowest TD_{50} was selected from the most sensitive site, species and sex for each substance, and the distributions of these lowest TD_{50}s were transformed into the corresponding distribution of the 10^{-6} risks for the carcinogens in the database using linear extrapolation from the TD_{50} to the origin (Munro, 1990; WHO, 1998). However, JECFA recognized that the Procedure should not be used for flavouring agents with unresolved toxicity problems. Therefore, flavouring agents such as furfural, menthol and *trans*-anethole were not evaluated through the Procedure, and compounds with structural alerts/evidence for genotoxicity and carcinogenicity would not be considered under the Procedure.

Expanded databases and cumulative distributions of the NOELs have also been developed for the specific end-points neurotoxicity (82 substances), immunotoxicity (37 substances), developmental neurotoxicity (52 substances) and developmental toxicity (81 substances) in order to see whether these end-points were more sensitive than those for structural class III compounds in the original database of Munro et al. (1996), and to see whether the general TTC of 1.5 μg/person per day derived from the carcinogenic potency database adequately covered such end-points. It was concluded that only the neurotoxicity of organophosphate pesticides (threshold of 18 μg/person per day) would not be covered by the class III threshold of 90 μg/person per day, but it would be covered by the general threshold of 1.5 μg/person per day (Munro et al., 1999; Kroes et al., 2000, 2004).

The TTC concept has been further refined by the suggestion to also include specific TTCs for groups of particular chemical carcinogens (Kroes et al., 2004), taking advantage of the work

done by Cheeseman et al. (1999). However, this has not yet been included in the Procedure by JECFA.

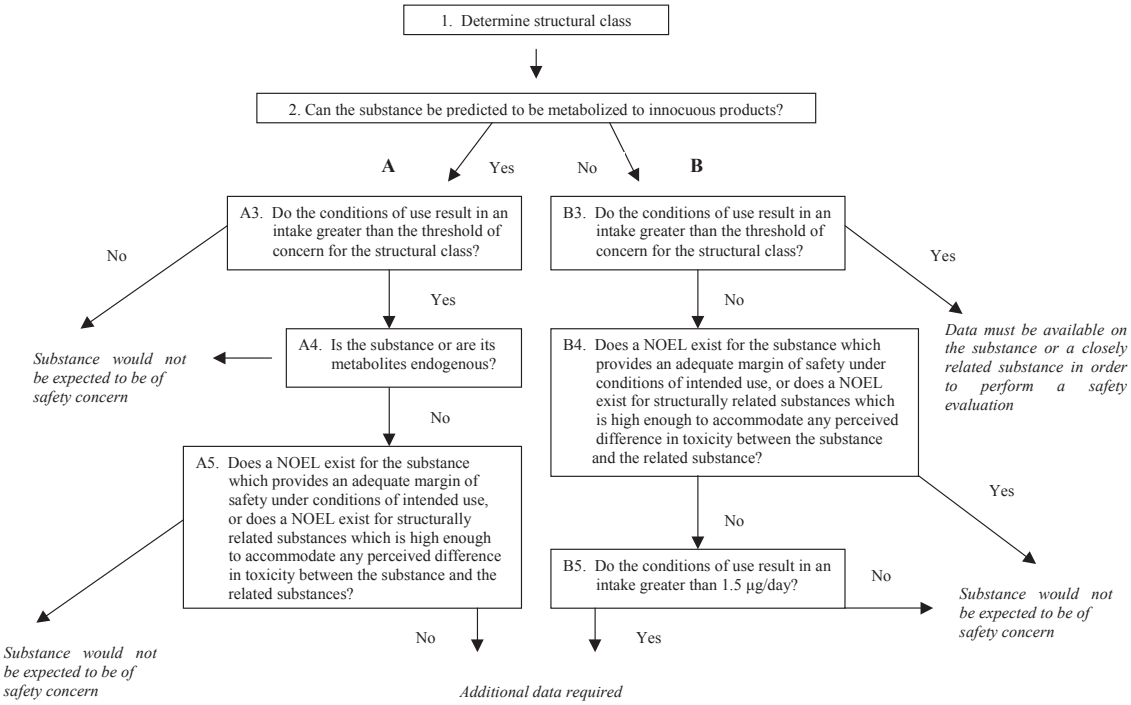
2.2 Intake estimates

In the absence of other suitable and better methods, JECFA uses the per capita × 10 method for its intake estimates based on comprehensive surveys on annual poundage data from the United States, Europe and Japan, respectively. The estimates are based on the assumption that the surveys accounted for only 80% of the production and that the entire amount produced is consumed by only 10% of the population ("consumers only"). JECFA has recommended that information on intake be periodically updated to ensure the validity of the evaluations.

2.3 Applying the Procedure

The Procedure for the Safety Evaluation of Flavouring Agents proceeds through a number of steps in which several questions have to be answered (Figure 1).

Figure 1: Procedure for the Safety Evaluation of Flavouring Agents.



So far, JECFA has evaluated 55 groups of flavouring agents containing a total of more than 1600 substances using the Procedure. The groups of flavouring agents evaluated are listed in Annex I at the end of this abstract.

2.4 Combined exposures

For each group of flavouring agents, JECFA performs an evaluation of the combined exposure in the (unlikely) event that all of the substances in the group (or a subgroup) were simultaneously consumed on a daily basis. In the case that the estimated combined daily per capita human intake does not exceed the thresholds of concern for the structural classes in

question, JECFA concludes that there would be no safety concerns associated with the combined intake.

However, in the case that the estimated combined daily per capita human intake exceeds the thresholds of concern for the structural classes in question, JECFA would base its evaluation on the known or predicted metabolism of the compounds and in some cases also compare the estimated combined intake with NOELs from toxicity studies:

- If all of the substances in the group (or subgroup) and their metabolites are predicted to be endogenous, JECFA considers whether the estimated combined intake would give rise to perturbations outside the physiological range and thus be of concern.
- If all of the substances in the group (or subgroup) are predicted to be efficiently metabolized via commonly known metabolic pathways, such as high-capacity, conjugation pathways, to innocuous metabolites, JECFA considers whether the estimated combined intake would saturate the available metabolic pathways and thus be of concern.
- In the case that the metabolic routes cannot easily be predicted for a group (or subgroup) of chemically related compounds, JECFA would require that the estimated combined intake be compared with a NOEL of the substance(s) or a closely related substance, even if its intake does not exceed the relevant threshold of concern.

At its 63rd meeting, JECFA recognized that the current Procedure to estimate the combined intake for all congeners of one congeneric group of flavouring substances reflects an unlikely situation in which the same individuals are consumers of all the substances. Nevertheless, this results in conservative estimates that allow evaluations to be completed. JECFA has therefore recommended the establishment of a working group to develop a more adequate approach (WHO, 2005).

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Annex I. Groups of flavouring agents evaluated by JECFA under the Procedure

- Ethyl esters (15)
- Isoamyl alcohol and related esters (11)
- Allyl esters (21)
- Saturated aliphatic linear primary alcohols, aldehydes and acids (38)
- Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids (25)
- Aliphatic lactones (35)
- Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids (32)
- Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids (67)
- Esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids (26)
- Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters (39)
- Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters (42)
- Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances (23)
- Carvone and structurally related substances (9)
- Ionones and structurally related substances (21)
- Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones (22)
- Substances related to menthol (13)
- Simple aliphatic and aromatic sulfides and thiols (137)
- Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups (47)
- Cinnamyl alcohol and related flavouring agents (55)
- Furfuryl alcohol and related flavouring agents (15)
- Phenol and phenol derivatives (48)
- Pulegone and related flavours (6)
- Pyrazine derivatives (41)
- Aromatic substitutes secondary alcohols, ketones and related esters (74)
- Benzyl derivatives (37)
- Hydroxy- and alkoxy-substituted benzyl derivatives (46)
- Aliphatic acyclic diols, triols and related substances (31)
- Aliphatic acetals (10)
- Alicyclic primary alcohols, aldehydes, acids and related esters (26)
- Phenylethyl alcohol, aldehyde, acid and related acetals and esters and related substances (43)
- Sulfur-containing heterocyclic compounds (30)
- Sulfur-substituted furan derivatives (33)
- Alicyclic ketones, secondary alcohols and related esters (25)
- Aliphatic secondary alcohols, ketones and related esters (39)
- Alicyclic, alicyclic-fused and aromatic-fused ring lactones (16)
- Aliphatic alicyclic linear α,β-unsaturated di- and trienals and related alcohols, acids and esters (26)

- Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters (32)
- Aliphatic and aromatic ethers (29)
- Hydroxypropenylbenzenes (9)
- Simple aliphatic and aromatic sulfides and thiols (12)
- Pyridine, pyrrole and quinoline derivatives (22)
- Aliphatic and alicyclic hydrocarbons (20)
- Aromatic hydrocarbons (5)
- Aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters (37)
- Monocyclic and bicyclic secondary alcohols, ketones and related esters (32)
- Amino acids and related substances (20)
- Tetrahydrofuran and furanone derivatives (18)
- Phenyl-substituted aliphatic alcohols and related aldehydes and esters (22)
- Maltol and related substances (7)
- Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (40)
- Eugenol and related hydroxyallylbenzene derivatives (7)
- Anthranilate derivatives (19)
- Miscellaneous nitrogen-containing substances (16)
- Epoxides (9)
- Aliphatic and aromatic amines and amides (37)

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