CHEMICAL MIXTURES

IN SOURCE WATER AND DRINKING-WATER

World Health Organization

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GLOSSARY OF TERMINOLOGY, ACRONYMS AND ABBREVIATIONS

A lack of common terminology across governmental and industrial sectors is one of the recognised barriers currently impeding the harmonization of methods to assess and manage the risk associated with combined exposure to multiple chemicals. The World Health Organization – International Programme on Chemical Safety (WHO/IPCS) framework, described in detail in this document, adopts an explicit descriptive terminology as a means to increase understanding of relevant terms and to facilitate harmonization of multiple chemical risk assessment methodologies (WHO, 2009; Meek et al., 2011; Meek, 2013).

The following table summarises the descriptive terminology and associated definitions used throughout this document, and are those described or adapted from key documents from USEPA, 2000; ECETOC, 2002; EFSA, 2008; IGHRC, 2009; WHO, 2009; Meek et al., 2011; Meek, 2013; EFSA, 2013. These definitions may differ from definitions used in different jurisdictions (e.g. cumulative exposure). Of importance to note is that the framework described in this document addresses **combined exposure to multiple chemicals** (abbreviated to **'chemical mixtures'** throughout).

GLOSSARY

Acceptable daily intake (ADI)	Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed, by all routes, daily over their lifetimes without appreciable health risk. The ADI is expressed in milligrams of the chemical per kilogram of body weight (the default assumption of bodyweight by WHO is 60 kg).
Additivity	**Dose additivity** is applied in the case of chemicals in a mixture that act by the same mode of action and/or at the same target cell, tissue or organ and differ only in their potencies. Commonly used dose-additive methods include the hazard index (HI) approach (sum of the ratios of exposure of each component to a health endpoint – see HI method) and two index chemical approaches (sum of the doses of each mixture component that can induce the same response/effect, if appropriate scaled by their toxic potency relative to the index chemical; see relative potency factor - RPF and toxic equivalency factor - TEF methods). **Response additivity** is applied in the case of chemicals that act via independent modes of action to elicit the same response. The toxic response (rate, incidence, risk or probability of effects) from the combination is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities.
Adverse effect	Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.
Aggregate exposure	The combined exposures to a single chemical across multiple routes (oral, dermal, inhalation) and across multiple pathways (e.g. food, drinking-water, residential). Also known as multi-media or route exposure.
Antagonism	Chemicals that interact to produce an effect less than that predicted on the basis of additivity. Also referred to as inhibitive, sub-additive, infra-additive.
Assessment group	Substances grouped together for evaluation of combined exposure.

Benchmark dose (BMD); Benchmark concentration (BMC)	A dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response - BMR) compared to background. Generally a dose that is generally derived from the modelled dose-response relationship for a substance and is associated with a specified low incidence of risk (e.g. 1–10%) of a health effect.
Combined action	The joint effects of two or more chemicals.
Combined exposure to multiple chemicals	Exposure to multiple chemicals by a single route and exposure to multiple chemicals by multiple routes. It covers both temporal and non-temporal co-exposure. This document is focused on exposure to multiple chemicals through drinking-water (temporal co-exposure) and has abbreviated the term to 'chemical mixtures' throughout.
Constituent	A substance (e.g. calcium) which is frequently naturally present in drinking-water and is considered to be a normal part of the chemical make-up of natural water, which is not purely H_2O.
Contaminant	A substance that is unintentionally present in drinking-water or in the environment.
Dose	Total amount of a substance administered to, taken up by or absorbed by, an organism, system or (sub)population.
Dose additivity	See Additivity.
Dose-response	Relationship between the amount of an agent administered to, taken up by or absorbed by, an organism, system or (sub)population and the change developed in that organism, system or (sub)population in reaction to the agent.
Exposure	Concentration or amount of a particular agent that reaches a target organism, system or (sub)population in a specific frequency for a defined duration.
Exposure assessment	Evaluation of the exposure of an organism, system or (sub)population to an agent (and its derivatives). Exposure assessment is one of the steps in the process of risk assessment.
Hazard	Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent.
Hazard assessment	A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub)population could be exposed. The process includes hazard identification and hazard characterization. The process focuses on the hazard, in contrast to risk assessment, where exposure assessment is a distinct additional step.
Hazard characterization	The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second step in risk assessment.
Hazard identification	The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)population. Hazard identification is the first stage in hazard assessment and the first step in the process of risk assessment.

Hazard index (HI)	The sum of the exposures to each of the component compounds of an assessment group divided by their respective reference values. As such, it represents risk-based summation of exposures to individual components, adjusted by their relative hazard. If HI > 1, the total concentration (or dose) of mixture components exceeds the level considered to be acceptable.
Hazard quotient (HQ)	The ratio of the potential exposure to the substance to the level at which no adverse effects are expected (e.g. acceptable daily intake - ADI or tolerable daily intake - TDI). Used in calculation of HI.
Independent action	Occurs if chemicals act independently from each other, usually through discrete modes of action that do not influence each other, or at different target cells, tissues or organs.
Interaction	The situation in which individual chemicals in a mixture influence the way the body responds to other chemicals present. Interactions can lead to under or over-estimation of risk.
Lowest observed adverse effect level (LOAEL)	The lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.
Margin of exposure (MOE)	Ratio of the no observed adverse effect level (NOAEL) or benchmark dose (BMD) or its lower confidence limit for the critical effect to the theoretical, predicted or estimated exposure dose or concentration.
Maximum cumulative ratio (MCR)	Ratio of the toxicity of the most potent chemical component to the total toxicity of all chemical components of mixture.
Mixture	Temporal co-exposure to any combination of two or more chemicals that may jointly contribute to actual or potential effects in a receptor population.
Mode of action	Describes the sequence of key cytological and biochemical events leading to an observed effect. Each key event must be both measurable and necessary to the observed effect.
Maximum Residue Level (MRL) (for pesticides)	The maximum concentration of a pesticide residue (expressed as milligrams per kilogram) recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feed. These levels are in place to ensure the lowest possible consumer exposure.
No observed adverse effect level (NOAEL)	Greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.
No observed effect level (NOEL)	Greatest concentration or amount of a substance, found by experiment or observation, that causes no significant alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Physiologically-based pharmacodynamic modelling (PBPD)	PBPD modelling is a model that simulates the toxicological effects of chemicals in the cell or tissue in response to a chemical that is delivered to and interacts with the target site or molecular initiating event site.
Physiologically-based pharmacokinetic modelling (PBPK)	A model that estimates the dose to target tissue by taking into account the rate of absorption into the body, distribution and storage in tissues, metabolism and excretion on the basis of interplay among critical physiological, physicochemical and biochemical determinants.
Potency equivalency factor (PEF)	See relative potency factor.
Point of departure (POD)	Dose or concentration selected as the point for comparison with exposure estimates as a basis for consideration of risk. Examples include no observed adverse effect level, lowest observed adverse effect level and benchmark dose level.
Quantitative structure-activity relationships (QSAR)	Computational tools that enable the toxic effects of chemicals to be predicted based on an analysis of the chemical structure.
Relative potency factor (RPF)	A numerical indicator of the toxicological potency of a chemical in relation to that of an index chemical (often the most toxic from the same chemical class or the chemical with the most amount of toxicological information). May also be referred to as a Potency equivalency factor - PEF). Note that toxic equivalency factor - TEF is a special case of the RPF.
Response addition	See additivity.
Risk	The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.
Risk assessment	A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.
Risk characterization	The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions.
Synergism	Chemicals that interact to produce an effect greater than that predicted on the basis of additivity. Can also be referred to as potentiating, supra-additive.
Tolerable daily intake (TDI)	Analogous to acceptable daily intake. The term tolerable is used for agents that are not deliberately added, such as contaminants in food.
Toxic equivalency factor (TEF)	A factor that expresses the toxicity of one congener from a chemical class relative to an index compound.
Toxic equivalence quotient (TEQ)	The TEQ for a mixture of chemically related substances expresses the toxicity of the mixture in terms of an equivalent dose of a key indicator chemical from that category of substances.
Threshold of effect	Dose or exposure concentration below which a chemical does not exert an effect.

Toxicity	Inherent property of an agent to cause an adverse biological effect.
Uncertainty factor (UF)	A product of several single factors by which the point of departure (POD) of the critical effect is divided to derive a tolerable intake. These factors account for adequacy of the pivotal study, interspecies extrapolation, inter-individual variability in humans, adequacy of the overall database and nature of toxicity. The term uncertainty factor is considered to be a more appropriate expression than safety factor since it avoids the notion of absolute safety and because the size of this factor is proportional to the magnitude of uncertainty rather than safety. The choice of uncertainty factor should be based on the available scientific evidence. Typically a default 10x UF is applied to the POD to account for interspecies differences (e.g. when extrapolating from experimental animal data to human) and a further 10x UF to account for intraspecies variability. Note: The concept of chemical-specific adjustment factors (CSAFs)[1] has been introduced to provide a method for the incorporation of quantitative data on interspecies differences or human variability in either toxicokinetics or toxicodynamics (mode of action) into the risk assessment procedure, by replacing the relevant toxicokinetic or toxicodynamic default subfactors of the UFs of 10 with chemical-specific data.

1 More information on application of CSAFs is available from: http://apps.who.int/iris/bitstream/10665/43294/1/9241546786_eng.pdf

LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
Cefic	European Chemical Industry Council
DBP	disinfection by-product
DI	detected index
DWTW	drinking-water treatment works
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMEA	European Medicines Evaluation Agency
EU	European Union
GDWQ	Guidelines for Drinking-water Quality
HAA	haloacetic acid
HPLC-PDA	high performance liquid chromatography with photodiode array
IGHRC	Interdepartmental Group on Health Risks from Chemicals
IPCS	International Programme on Chemical Safety
JDWQS	Japanese drinking-water quality standards
JECFA	Joint FAO/WHO expert committee on food additives
MTD	minimum therapeutic dose
NMC	N-methyl carbamate
NOEC	no observed effect concentration
NSAID	non-steroidal anti-inflammatory drug
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PDE	permitted daily exposure
PE	population equivalent
PFOA	perfluorinated octanoic acid
PFOS	perflurooctane sulfonate
STW	sewage treatment works
THM	trihalomethane
USDA	United States Department of Agriculture
USEPA	United States Environment Protection Agency
VOC	volatile organic compound

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EXECUTIVE SUMMARY

BACKGROUND

Chemicals are present in drinking-water and its sources as a consequence of natural occurrence (constituent) or from a variety of anthropogenic sources (contaminant). As the numbers of chemicals being detected in source water and drinking-water grows, it becomes increasingly difficult to assess and manage risk-based on single contaminants. The two questions that have frequently been asked are:

- What are the implications of the presence of multiple chemicals?; and
- Should they be assessed for risk and management purposes as mixtures rather than individual chemicals?

These questions really relate to how the individual components of chemical mixtures will interact in terms of their toxicity; there may be no change due to non-interaction, however, an interaction may result in either an increase or a decrease in the mixture's overall toxicity. In the great majority of cases the concentrations of chemicals in drinking-water will be very small (even trace) and the potential for interaction is expected to be limited. Modes of action resulting in increased toxicity often require a sufficiently high concentration of one component to interfere biochemically/physiologically with the other component, although this has not been reported in relation to drinking-water.

SCOPE

The key purpose of this document is to provide an overview of available tools and practical recommendations to support the screening and prioritization of mixtures for the assessment and management of risk to human health associated with exposure to chemical mixtures from drinking-water and its sources[2]. Particular focus is given to the World Health Organization's International Programme on Chemical Safety (IPCS) framework on combined exposures to multiple chemicals (WHO, 2009), and its use is illustrated through a number of case studies relevant to source water and drinking-water. The framework contributes to determination of the relative priority of assessing and managing exposure to mixtures in drinking-water. Having identified a mix of substances and made an assessment of the risks to humans from drinking-water, it is then necessary to decide whether it is appropriate to take action and how that will be managed. Some guidance on these aspects is also included in this document.

HUMAN EXPOSURE TO CHEMICAL MIXTURES IN SOURCE WATERS AND DRINKING-WATERS

At the present time, chemicals policy and risk management processes worldwide are primarily focused on the safety of individual chemicals. However, the general population is exposed, concomitantly, to a wide range and number of chemicals in all media and there is growing concern around recognition of the potential for a combined adverse effect when chemicals occur together. Assessing the combined risks to human health from exposure to chemical mixtures is understandably much more complex than for single entities. The main challenges faced by regulators are how to determine the degree to which humans are co-exposed to chemicals, what interactions may occur among these, and what specific human health impacts are associated with the chemical mixtures.

Due to collaborative efforts of scientists and regulators worldwide, there are now frameworks, procedures, and tools which can facilitate the process (e.g. by enabling appropriate grouping of chemicals and addressing the potential combination effects of the chemical substances within these groups). However, as many of these approaches are resource intensive, care is needed to establish that a risk assessment of the group chemicals is actually necessary and/or beneficial for protecting public health.

Specific chemical mixtures have been considered in the WHO Guidelines for Drinking-water quality (GDWQ) for both nitrate/nitrite and the trihalomethanes, where an additive approach is recommended. The GDWQ also mention the need to consider mixtures of pesticides in drinking-water when these have similar structures and modes of action (e.g. atrazine and simazine). The WHO has also considered mixtures of chemicals that are usually present together in the case of accidental spillage (e.g. petroleum products to water).

2 Literature searches related to this document were carried out in October 2013, with additional ad hoc searches performed up to publication.

HUMAN HEALTH RISK ASSESSMENT FOR CHEMICAL MIXTURES

Chemical mixtures are often defined by the modes of action of individual components and three basic types have been identified namely:

- similar action (dose/concentration addition);
- dissimilar action (independent action); and
- interactions (synergism and antagonism).

In the dose/concentration addition and independent action scenarios, the assumption is made that the individual chemicals in a mixture do not interact and therefore the toxicity of each remains unchanged and an additive approach can be utilized. Indeed, the human health risk assessment of chemical mixtures is most often conducted based on a general assumption of additivity. Although guidance on different methods for mixtures risk assessment has been published by a number of authoritative bodies, at the present time there is no 'regulatory-applicable' accepted methodology for carrying out risk assessments for chemical mixtures, particularly in source water and/or drinking-water.

In an attempt to harmonize methodology and associated terminology at an international level, the WHO/IPCS framework for the risk assessment of combined exposures to multiple chemicals was developed (WHO, 2009; Meek et al., 2011; Meek, 2013). The framework includes problem formulation as a basis to consider appropriate groupings of chemicals (based principally on likelihood for co-exposure but taking into account potential hazard) and application is based on a hierarchical (phased) approach that involves integrated and iterative consideration of exposure and hazard at all phases, with each tier being more refined (i.e. less conservative and uncertain) than the previous one. As with all such frameworks, significant expert resources may be required for its application, particularly in the higher tiers. However, there are a number of approaches available to assist the user in application of the framework; these approaches have associated models (including the hazard index, reference point index/point of departure index and relative potency factor/toxicity equivalency factor/potency equivalency factor) and data-filling tools (threshold of toxicological concern, physiologically-based toxicokinetic and toxicodynamic models). Regardless of the particular model used, these methods assume additivity among mixture components, whether by dose/concentration addition (for similarly acting chemicals) or by independent action (for dissimilarly acting chemicals).

The WHO/IPCS framework was designed to have broad applicability where combined chemical exposures are expected, including in source waters and drinking-waters. It is hoped that by using the framework, risk assessors can maximise efficiency by identifying non-priority assessment groups in the early, non-labour and data intensive tier(s), thereby conserving resources. This does not, however, preclude the use of more complex data, when available, where starting at higher tiers may be most efficient; an example of this is shown in the case study for N-methyl carbamate in food. Although use of such a framework for development of standards or guidelines for chemical mixtures in drinking-water has yet to be applied, the process may identify circumstances when such regulation would be prudent.

APPLICATION OF THE WHO/IPCS RISK ASSESSMENT FRAMEWORK FOR CHEMICAL MIXTURES IN SOURCE WATER AND DRINKING-WATER

The WHO/IPCS framework was designed to be further developed, following publication, through pragmatic application in specific case studies. Within this document, the framework is shown to be applicable to the assessment of risk associated with mixtures of pharmaceuticals, microcystins, pesticides and natural and synthetic oestrogens in source waters and drinking-waters.

CONSIDERATIONS WHEN ASSESSING AND MANAGING CHEMICAL MIXTURES IN SOURCE WATER AND DRINKING-WATER

There are several circumstances in which regulators of drinking-water may need to consider the exposure to chemical mixtures and, for each, there may be a different regulatory outcome. Where a need for regulatory action to manage the risks is considered, then it is important to identify the most effective point at which control can be exercised and the most cost effective means of doing so.

Screening for and assessing the risks

Current thinking supports the assumption that substances that have a similar mode of action generally act in an additive fashion when combined in a mixture including, it can be supposed, when present as a mixture in drinking-water. Where chemicals act on the same target organ with a dissimilar mode of action, an additive approach is also usually assumed. The proposed framework provides a means by which to demonstrate whether additional assessment, utilizing additional approaches, is needed. Translation into regulatory values (i.e. whether a group parameter can be developed) depends on whether the chemicals of interest usually occur together and whether the proportions are usually similar. Guidance is often based on the proviso that the sum of the concentration of each component divided by its standard/guideline value should not exceed 1.

The issue of whether an additive approach should be adopted for substances that are considered to cause cancer has been raised. This approach relates to substances for which a risk value has been calculated using low dose extrapolation, most usually from laboratory animal data. However, it is important to note that this approach is only feasible when the target organ is the same. In addition, the considerable uncertainties inherent in the risk calculations should be considered so as not to create practical problems in achieving any standard with unintended consequences that result in other known risks to health, mislead consumers as to the scale of risk in relation to other risks or divert resources to dealing with comparatively minor risks.

Managing the risks

Having identified a chemical mixture and made an assessment of the risks to humans from drinking-water, it is then necessary to decide if, and what, action is appropriate to take. In particular, any regulatory values for drinking-water need to take into consideration the requirement for meaningful monitoring.

In addition to toxicology, pragmatic considerations can be taken in grouping chemicals in source water and drinking-water for management, as well as for risk assessment. This is already practiced, for example, with the disinfection by-products trihalomethanes and haloacetic acids. A number of questions can assist in making decisions about whether to treat a group of substances as a mixture and what management approaches may be considered.

- Do the chemicals always occur as a mixture and, if not, how frequently do they occur together and under what circumstances?
- Does the proportion of substances vary and is there a small number that usually dominate?
- Are the substances of similar water solubility?
- Can one or two substances act as a surrogate for the others (for both risk assessment and management)?
- How stable is the mixture (i.e. is it always similar)?
- Can the components of the mixture be measured by the same method?
- How readily will components of the mixture be removed in the available drinking-water treatment?
- Are there other upstream interventions that can be applied?

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

There are number of data and knowledge gaps that may impede a more systematic and effective application of the methodologies described in this document. There is a need to better understand human and environmental exposures, both through the use of investigative monitoring and modelling. The gap in exposure data is exacerbated by the lack of standardized sampling and analytical methods for many substances, particularly contaminants of emerging concern, and the important associated quality assured procedures that allow proper comparability of data.

For many chemicals, there is a lack of reliable information on mode of action and research is also needed to define criteria that predict the practical potential for interactions within chemical mixtures. There is also a lack of toxicity data for commonly identified chemical mixtures which would enable assessment of the hazard as a whole rather than being based on individual components.

There is also limited experience with assessing and managing chemicals as mixtures in drinking-water and its sources which, when considered with the knowledge gaps, may preclude this type of assessment being widely implemented at the present time. Although the efficiency, efficacy and cost of undertaking risk assessments as well as cost of the control of potential mixtures in relation to the health benefits need to be carefully considered, a number of useful tools and models have been, and continue to be, developed to carry out risk assessments of chemical mixtures.

CONCLUSIONS

Concurrent exposure to a number of chemicals from drinking-water is common, but not all chemicals are found in all drinking-water supplies and the mixture of chemicals can vary in time and concentration. In assessing the possible risks of exposure to combinations of chemicals a number of frameworks are currently in place, but significant knowledge gaps, complexity, limited practical experience and resource intensity, precludes this type of approach being systematically introduced into drinking-water standards at the present time.

From a regulatory perspective, risk assessment can be used to determine whether regulatory action is needed and the urgency for that action. In the event that regulatory action is needed, it is important to understand the objective of the regulation because this may be the over-riding influence. Both risk assessment and any subsequent regulations for groups of chemicals will need to consider the probability of the group components actually occurring together, the frequency that they are found together in different supplies and the concentrations of the differing components. In addition, the ease of monitoring and removal by drinking-water treatment are also important considerations.

For toxicologically similar chemicals, it would be most appropriate to assume dose addition based on the current evidence. A straight forward and pragmatic approach is to base any regulation/guidance on the basis that the sum (hazard index) of the concentration of each component divided by its standard/guideline value (hazard quotient) should not exceed 1.

There are many knowledge gaps in terms of the modes of toxicity of chemicals and, particularly, their dose-response at low doses. In many cases there is likely to be a practical threshold to toxicity but this may be difficult to define. There is a need for greater understanding of the actual, rather than theoretical, consequences of exposure of humans to very low concentrations of chemicals in drinking-water.

1.

HUMAN EXPOSURE TO CHEMICAL MIXTURES IN SOURCE WATER AND DRINKING-WATER

1.1 INTRODUCTION

At the present time, general chemicals policy and risk management processes worldwide are primarily focused on the safety of individual chemicals. Humans are protected by ensuring that, where possible, both natural and man-made chemicals do not exceed levels that are associated with known harmful effects. However, the general population is exposed, concomitantly, to a wide range and number of chemicals in all media, including drinking-water. The World Health Organization (WHO) Guidelines for Drinking-water Quality (GDWQ) (WHO, 2017) emphasise the need to take a more holistic approach to policies around exposure to chemicals and not to focus only on one source when formulating national policy. This is primarily to ensure that resources are not concentrated on media which represent minor sources of exposure, while ignoring others that represent much higher levels of exposure.

It is being increasingly recognised that when chemicals in the environment are combined together, there is potential for a combined adverse effect, even if each individual component is harmless or present at levels at which adverse effects are not expected to occur. In the absence of confirmatory evidence of this happening in the environment, European and International bodies engaged in risk assessment have recognised the need to address this concern. Indeed, combination effects are required to be included for consideration in the European Union (EU) Classification, Labelling and Packaging Regulation, Plant Protection Products Regulation and Biocides Regulation. Increasingly, decision makers and stakeholders are seeking answers to real-life scenarios that conventional risk assessments cannot answer. Sexton (2012) suggests that this can be rectified "by incorporating consideration of combined health effects from exposure to a diverse array of environmental agents such as people encounter during their normal daily routines" into risk assessments, although how this can be practically achieved is less clear. In addition, it is not clear how this could be meaningfully incorporated into regulation of substances that are already in environmental media and food and which may be difficult to control. It is, therefore, important that any approaches provide a means of delivering appropriate regulation or other risk management that does not result in costs which greatly outweigh any benefits.

In view of the almost infinite number of possible combinations of chemicals to which humans are exposed, the European Commission (EU, 2012) has recommended some form of initial filter to prioritize the risk assessment of chemical mixtures of potential concern.

- Human and/or environmental exposure at significant levels.
- Chemicals that are produced and/or marketed as multi-constituent substances or commercial 'mixtures' with several components and/or active ingredients and/or substances of concern.
- Potential serious adverse effects of one or more chemicals at the likely exposure levels.
- Likelihood of frequent or large scale exposure of the human population or the environment.
- Persistence of chemicals in the body and/or in the environment.
- Known information of potential interaction at levels of human and environmental exposure.
- Predictive information that chemicals act similarly.
- Particular attention should be paid to chemical mixtures for which one or more components are assumed to have no threshold of effect.

The relevance of some of these issues for drinking-water is likely to be very much more limited, for example commercial mixtures that enter drinking-water sources are often dispersed and change in nature. However, there remain questions about the need for regulation of chemicals that occur together and also the utility of grouping chemicals for regulatory purposes. In both cases, there may be practical and cost benefit implications and so there is a need to avoid increasing the complexity of drinking-water regulation without proper consideration of these aspects.

Exposure through drinking-water may be short-term, intermittent or long-term and concentrations of different chemicals may vary with time. Such considerations will be important in the decision as to whether a risk assessment of chemical mixtures is necessary and whether regulatory action, based on such a risk assessment, is required. In terms of delivering workable regulatory frameworks it will not be practical to consider each water supply as an individual risk assessment or to take into account all of the substances that could be present.

Assessing the combined risks to health from exposure to chemical mixtures is understandably much more complex than for single entities. The main challenges are how to determine the degree to which humans are co-exposed to chemicals, what interactions may occur between these chemicals and what specific hazards are associated with chemical mixtures.

Due to the collaborative efforts of scientists and regulators worldwide there are now frameworks, procedures and tools which can be applied to enable appropriate grouping of chemicals and to address the potential combination effects of the chemical substances within these groups (these are described in Section 2). However, most of these approaches are resource intensive and careful consideration is needed as to when examination of combined exposure to a group of chemicals is actually necessary or beneficial for protecting public health.

The issue of chemical mixtures has already been recognised in the GDWQ for both nitrate/nitrite and the trihalomethanes (THMs), where a relatively simple additive approach is recommended. The GDWQ also mention the need to consider mixtures of pesticides in drinking-water when these have similar structures and modes of action (e.g. atrazine and simazine). WHO has also considered mixtures of chemicals that are usually found together in the case of accidental spillage (e.g. petroleum products to water). This introduces an additional practical consideration that some groups of chemicals may well have a significant impact on the acceptability of drinking-water (e.g. on taste) at concentrations below those that are likely to cause even minor health effects.

1.2 OCCURRENCE OF CHEMICALS IN SOURCE WATERS AND DRINKING-WATERS

The safety of water supplies is of paramount public health importance as inadequate drinking- water, sanitation and hygiene are important risk factors for ill health (WHO, 2014). While the great majority of water-related health problems are the result of microbial contamination, chemicals in water supplies can also be related to health risks, generally when associated with long-term exposures (Thompson et al., 2007). There are two basic sources from which drinking-water is derived: surface waters (such as rivers, lakes and reservoirs) and groundwater[3]. Although all water contains natural constituents (e.g. inorganic contaminants arising from geological strata) and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals, groundwater is less vulnerable to pollution than surface waters (Fawell & Nieuwenhuijsen, 2003). The great majority of chemicals that may be of concern in drinking-water are associated with point and diffuse sources, as shown in Table 1. Other sources that may occasionally be important (e.g. accidental or intentional contamination of water supplies) generally need to be assessed on a case-by-case basis because they are much less predictable and relatively infrequent in occurrence.

Table 1. Categorisation of sources of chemicals in drinking-water

Source	Examples of sources
Naturally occurring chemicals (including naturally occurring algal toxins)	Rocks and soils (e.g. calcium, magnesium but also arsenic and fluoride, cyanobacteria in surface water)
Chemicals from agricultural activities (including pesticides)	Application of manure, fertiliser and pesticides; intensive animal practices
Chemicals from human settlements (including those used for public health purposes e.g. vector control)	Sewage and waste disposal, urban runoff, fuel leakage
Chemicals from industrial activities	Manufacturing, processing and mining
Chemicals from water treatment and distribution	Water treatment chemicals; corrosion of and leaching from, storage tanks and pipes, by-products of chemical treatment

Modified from Thompson et al. (2007)

Some of these chemicals have been identified in drinking-water (particularly with advances in analytical technologies), including a range of disinfection by-products (DBPs) and perfluorinated chemicals such as perfluorinated octanoic acid (PFOA) in localized incidents of contamination (Krasner et al., 1989, 2006; Plewa et al., 2004, 2008; Ternes et al., 2005; Richardson et al., 2007; Ternes, 2007; Ericson et al., 2008; WHO, 2012). Wastewater from human activities may also contaminate drinking-water sources with pharmaceuticals (including degradation by-products), nanoparticles, consumer products (such as sunscreens) and other contaminants. Some of these chemicals occur in trace amounts and others are present at higher concentrations in localized circumstances. Although a number of these substances may be found in drinking-water sources (including as combinations) many are removed during drinking-water treatment; thus future emphasis should be placed on chemicals not removed during water treatment.

The identification and measurement of low concentrations of numerous chemicals present in water supplies can lead to uncertainties in exposure (and risk) assessments. While concurrent exposure to a number of chemicals from drinking-water is common, the mixture of chemicals can vary over time and in concentration. Uncertainties in the risk assessment of chemical mixtures can be introduced since most toxicology data are for individual chemicals and from animal studies in which very high doses are used. In some cases new approaches are required for risk assessment (Schwarzenbach et

3 The use of recycled water for drinking is less common, largely due to public perception. However a few countries including Singapore, Australia and Namibia, and states such as California, Virginia and New Mexico are already drinking recycled water.

al., 2006). DBPs are chemicals that occur as chemical mixtures, however, as there are several hundred potential DBPs that could be present in public water supplies, a few are used as surrogates for the whole (Richardson et al., 2007). These include the THMs and haloacetic acids (HAAs) which are almost invariably present in chlorinated drinking-water, albeit in varying concentrations (Hinckley et al., 2005; Hoffman et al., 2008; Righi et al., 2012). The correlation between DBP constituents is complex and strongly depends on raw water quality and the type of treatment. Consequently, the use of a specific group of DBPs, such as THMs, may result in the misclassification of exposure to the relevant chemicals for health outcomes (Villanueva et al., 2014). However, for the purpose of controlling DBPs in drinking-water a small number of indicator substances is practical, particularly combined with the approach of reducing the natural precursors in drinking-water treatment.

1.3 CONCLUSIONS

Although microbial contamination of drinking-water remains the highest priority, there is increasing pressure that in assessing the risks associated with chemical hazards in drinking-water and their sources, regulators and water suppliers should consider the implications of chemical mixtures. Chronic exposure to chemical mixtures could potentially, but not necessarily, be associated with increased health risks in the general population (Thompson et al., 2007). Although consideration of chemical mixtures is required by some recent regulations, these relate almost exclusively to the introduction of new chemicals and products and are not targeted at drinking-water. In addition, current risk assessment, risk management and chemical-related policy, in all spheres, predominately consider the safety of individual chemicals.

Reasons for the focus on individual chemicals may include the complexity of assessing the combined risks to health from exposure to multiple chemicals, and that the management options are likely to be both difficult and expensive in relation to the benefits gained. In addition, it is not feasible to provide a single definition for a chemical mixture, which means that any risk assessment needs to consider the degree of co-exposure to the chemicals present, any possible interactions between chemicals and any specific hazards resulting from these.

In an attempt to overcome some of these difficulties, there has been collaboration between risk assessors worldwide resulting in a number of frameworks, procedures and tools that are being utilized to identify appropriate groupings of chemicals for assessment and to evaluate potential interactions between these chemicals. The utility and application of one such framework (WHO, 2009), is explored in the following sections for scenarios relevant to drinking-water.

2.

APPROACHES FOR ASSESSING THE RISK OF CHEMICAL MIXTURES

2.1 INTRODUCTION

Guidance for conducting risk assessments of chemical mixtures has been published by a number of authoritative bodies (USEPA, 1986, 2002; COT, 2002; CVUA, 2007; VKM, 2008; IGHRC, 2009) and attempts have also been made to harmonize this methodology and associated terminology at an international level, particularly within the WHO International Programme on Chemical Safety (WHO/IPCS) Framework (WHO, 2009; Meek et al., 2011; Meek, 2013).

This section explores the approaches available (based on the WHO/IPCS framework) for assessing risks from chemical mixtures to allow regulators and water suppliers to determine if there is likely to be a problem with combinations of chemicals that may be found in the waters for which they are responsible. It is recognised, however, that any approach must be essentially practical and that while a relatively small difference in the concentration of a chemical or group of chemicals will usually be of no significant impact toxicologically, it can have a very significant impact on the water supplier in terms of achieving the value. It is also the case that if extreme conservatism is applied during the risk assessment process, the outcome may result in a high level of costs (to address the perceived problem) in relation to a small (often theoretical) benefit to consumers.

2.2 CHEMICAL INTERACTIONS AND THE ROLE OF MODES OF ACTION

It is not possible to provide a single definition of what constitutes a chemical mixture that will be applicable in all situations. To date, chemical mixtures have often been defined by the modes of action of individual components (Meek et al., 2011; Meek 2013) and three basic types of action have been identified, namely:

- similar action (dose/concentration addition);
- dissimilar action (independent action); and
- interactions (synergism and antagonism).

In the dose/concentration addition and independent action scenarios, the assumption is made that the individual chemicals in a mixture do not interact and therefore the toxicity of each remains unchanged and, thus, an additive approach can be utilized. Indeed, both scenarios are suggested as default approaches to be used by regulators in the risk assessment of chemical mixtures. Accordingly, human health risk assessment of chemical mixtures is generally conducted based on the general assumption of additivity.

2.2.1 Chemicals with similar modes of action

For mixtures in which the chemicals present have a similar mode of action, then a simple additive approach is appropriate. In many circumstances, the chemicals present in a mixture have similar modes of action, but may differ in potency (i.e. the impact of similar concentrations/doses will differ). Where this is the case the effects of the mixture can be estimated directly by summing the doses/concentrations, scaled for the relative toxicity/potency of the individual substances.

To achieve greatest accuracy, the dose/concentration addition approach relies on correctly identifying chemicals with similar modes of action. Guidance on this has been issued by the European Chemicals Agency (ECHA), the Organisation for Economic Co-operation and Development (OECD) and European Food Safety Authority (EFSA) and is discussed in more detail in Appendix A2 (A2.3); there remains uncertainty, however, as to the most reliable approach to use and the process is often done using expert judgement. Evidence is available from a range of disciplines that shows the dose/concentration addition approach gives accurate estimates of mixture effects, if individual components have similar modes of action (Faust et al., 1994; Altenburger et al., 1996; Payne et al., 2001; Charles et al., 2002; Feron & Groten 2002; Walker et al., 2005; Kortenkamp & Haas, 2009; Wolansky et al., 2009). This approach is also considered to be useful in conjunction with the margin of exposure (MOE) as a screening tool for earlier tiers of risk assessment (Section 3). One of the biggest problems, however, associated with applying this approach is a lack of chronic toxicity data at doses/concentrations applicable to human exposure levels (including in drinking-water).

To date, default assumptions of dose additivity (as well as response additivity; see Section 2.3.4.2) have been used to characterize the toxicity of chemical mixtures (Boobis et al., 2011) (see Appendix A2) and a number of models are currently available for considering dose/concentration addition (see Section 2.3.4.1).

2.2.2 Chemicals with dissimilar modes of action

The independent action model is often applied to chemicals with dissimilar modes of action and assumes that the joint action of mixture components can be calculated from the response of individual mixture components. A more detailed description of the approach can be found in a review by Kortenkamp & Haas (2009).

Evidence is available from a number of applications whereby the assumption of independent action gives an accurate estimate of combined effects (Hermens et al., 1985; Payne et al., 2001; Walter et al., 2002; Faust et al., 2003). Because independent action considers the individual effects of the mixture components to calculate the expected mixture effect, it is generally considered that no health concern is posed if each component is present at concentrations below their zero-effect levels[4] (COT, 2002); however, this view has been challenged (Kortenkamp & Haas, 2009).

A number of models are currently available for considering independent action, and these are described in Section 2.3.4.2.

2.2.3 Interactive chemicals

If interaction between two or more individual chemicals in a mixture occurs, the combined effect can be greater (synergistic, potentiating, supra-additive) than that predicted by dose/concentration addition or response addition, or weaker (antagonistic, inhibitive, sub-additive, infra-additive) (USEPA, 2000). The extent of interaction may vary for the following reasons:

- relative dose levels (e.g. too low to have such an effect);
- route of exposure (e.g. absorption too low or different metabolism);
- duration of exposure (including bio-persistence of components); and
- biological target.

Examples of interactions include:

- Toxicokinetic interactions. These result in alterations in the metabolism (enzyme induction or inhibition) or disposition of a chemical. Examples include chemicals modifying the absorption of others (e.g. skin penetration enhancing substances in cosmetics) or chemicals competing for active transport mechanisms (uptake, clearance).
- Toxicodynamic interactions. These are interactions between the biological responses resulting from exposure to the individual chemicals, for example resulting from similar targets (e.g. ligand-receptor binding).

Boobis and colleagues reported that an intensive literature search for evidence of low dose synergy located only a small number of studies with useful quantitative estimates. Within these, synergy at low doses resulted in only modest increases in toxicity, being within four times those predicted using dose addition. In view of the substantial uncertainty factors usually applied in deriving individual regulatory values, this difference is very small (Boobis et al., 2011).

2.3 THE WHO/IPCS RISK ASSESSMENT FRAMEWORK

As in all risk assessment methods, the application of mixture risk assessment methods requires clarity about the goal of the assessment. For example, the aim can be:

- to arrive at a risk estimate;
- an estimate of safe levels;
- determine MOEs; or
- can consist of ways to prioritize certain mixtures.

Recently, there has been an international effort to develop an efficient, harmonized methodology for assessing the impact of combined exposures to multiple chemicals in a project led by the WHO/IPCS. Building on previous expertise and published frameworks/decision trees from the USEPA (2007) and UK government (IGHRC, 2009) this project has developed a proposed framework for the risk assessment of combined exposures to multiple chemicals and associated terminology (WHO 2009; Meek et al., 2011; Meek, 2013). The framework is considered to be relevant for a wide variety of applications; these range from priority setting for testing of chemicals to consideration of the adequacy of risk management for public health protection. General support for this framework and associated terminology was expressed at an international workshop in 2011 (OECD, 2011). However, the use of such a framework for development of standards or guidelines for chemicals in drinking-water has yet to be shown to be practical, although the process may identify circumstances when such regulation would be prudent.

Since its publication, an iteration of the framework has been developed by the European Chemical Industry Council (Cefic). However, the Cefic tool is fairly restrictive, being mainly applicable to data rich cases, including focus on grouping by mode of action. It will not be described further in this document (see footnote for further information[5]).

4 It is important to note that this zero-effect level (concentration) is not identical to the no observed effect level or no observed effect concentration observed in an experimental study.

5 http://www.cefic.org/Documents/PolicyCentre/Identifying_and_assessing_chemical_combinations_of_concern_decision_tree_tool_explained.pdf

The WHO/IPCS framework on combined exposures is a high level organizing structure (Fig. 1), within which specific assessment tools and methods are employed. Meek et al. (2011) and Meek (2013) describe the framework in detail, and much of the text presented in this section is derived from those references. The framework includes problem formulation as a basis to consider appropriate grouping, followed by stepwise integrated and iterative consideration of both exposure and hazard in several tiers of, increasingly data informed, analyses. Each tier is more refined (i.e. less conservative and uncertain) than the previous one, but also more labour, modelling and data intensive. At any tier, the outcome can be risk management, no further action, generation of additional data or further assessment (i.e. additional refinement in a higher tier), based on context specific evaluation of the adequacy of MOEs[6] (Meek, 2013). It is important to note that the exposure and hazard assessment tiers are independent and different tiers can be used (e.g. hazard tier 2 with exposure tier 0, etc.) in a risk assessment, depending on available data.

The framework is predicated on the consideration of MOEs, which are quantitative measures of risk. More specifically, the MOE is the ratio of the point of departure (POD) and the estimated human exposure level. The POD can be the no observed (adverse) effect levels (NO(A)EL), lowest observed (adverse) effect levels (LO(A)EL) and/or benchmark doses (BMD) or benchmark concentrations (BMC) associated with a specified increase in the incidence of an effect (e.g. $[BMD]_{10}$ or $[BMC]_{10}$) (Meek et al., 2011). The calculated MOE at each tier is used to determine whether the mixture proceeds to the next tier of testing.

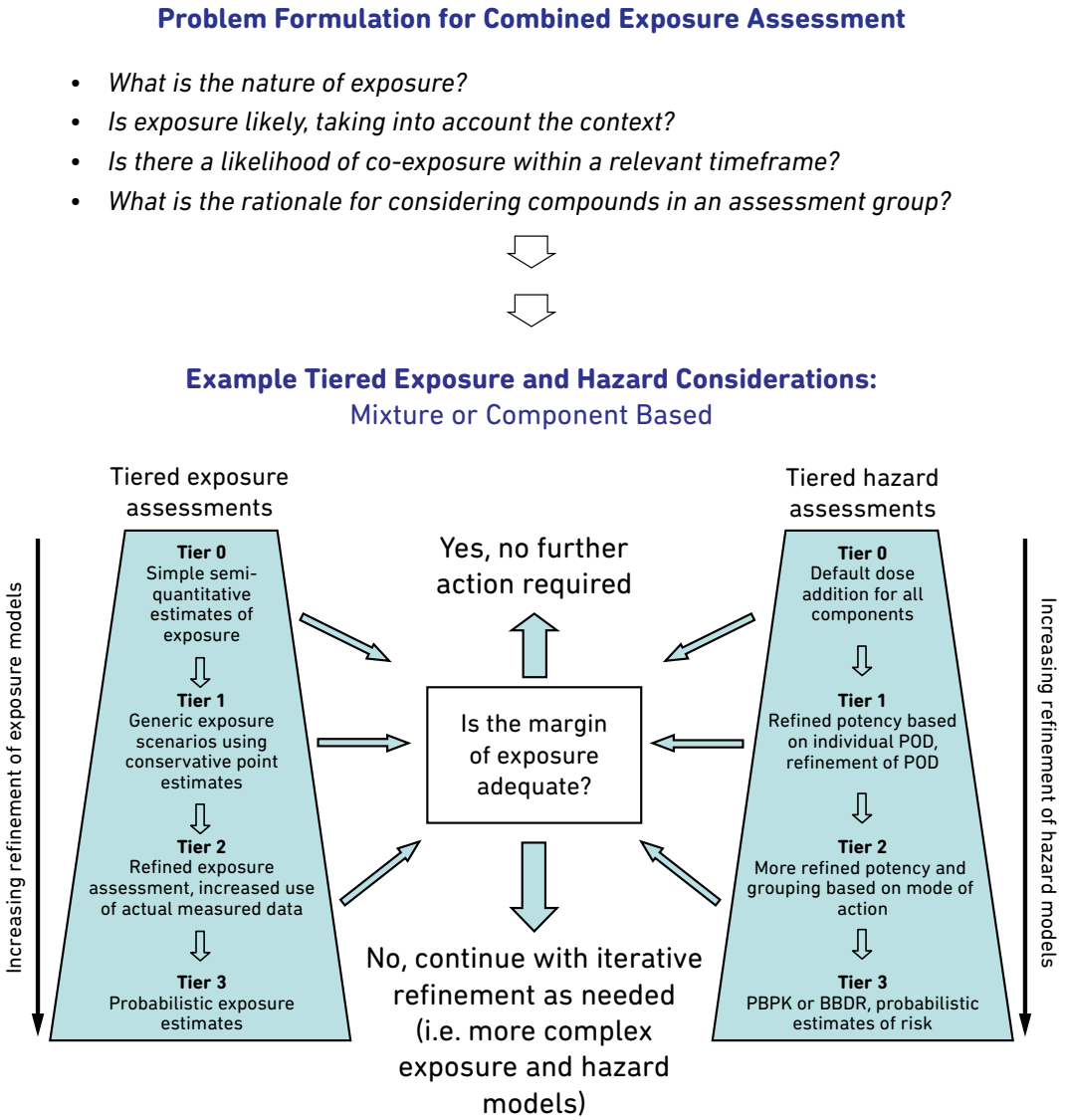

Fig. 1. A conceptual representation of the WHO/IPCS framework (Meek et al., 2011)[7]

6 The evaluation of the adequacy of the MOE is dependent on the actual purpose and/or (legal) framework for which the assessment is performed. Factors such as inter-individual variation (including susceptible groups), interspecies differences, quality and robustness of the database, nature of the hazard and temporal aspects should be taken into account (see IPCS, 2009b, for additional guidance). Approaches to consideration of the adequacy of the MOE should be conservative, but commensurate with the degree of uncertainty at each tier (see IPCS, 2008, for guidance on characterizing uncertainty in exposure assessment).

7 Reprinted from Regulatory Toxicology and Pharmacology, 60(2), Meek, M.E., Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework, S1-S14, Copyright (2011), with permission from Elsevier.

2.3.1 Problem formulation

During development and testing of the WHO/IPCS framework, it became clear that the problem formulation step is of critical importance, that is:

- what is the nature of the problem being addressed;
- what are the circumstances; and
- what is the expected/desired outcome?

This initial step in the framework acts as a basis to "systematically consider the appropriate grouping of chemicals for combined assessment and the approach to and extent of evaluation required to most meaningfully inform potential risk management options" (Meek, 2013).

In general, problem formulation is incorporated as an initial step during the risk assessment process for combined exposure to multiple chemicals by different agencies worldwide. Although some differences in requirements exist typically, if the problem formulation step demonstrates that the likelihood of co-exposure to multiple chemicals is low, a risk assessment may not be considered necessary (USEPA, 2003, 2007; ATSDR, 2004; WHO, 2009; EFSA, 2013). Finalisation of the problem formulation step usually requires close dialogue between risk managers and risk assessors to clarify the context of the requested risk assessment so that the questions formulated have a sound scientific basis to optimise support to decision making, risk management and to communicate to stakeholders (EFSA, 2013).

2.3.1.1 Key problem formulation questions

Four questions are posed in the problem formulation step; three of these relate to exposure, emphasising the importance of considering exposure early in the process as a basis on which to focus priorities (Meek et al., 2011).

What is the nature of exposure? Are the key components known?

Do the substances of interest occur in water? Do they reach drinking-water? Limited information on these aspects precludes a framework analysis. This does not, however, obviate the potential need to introduce risk management measures to reduce exposure, although recognisably in the absence of a robust science basis to inform the process.

Is co-exposure likely, taking into account the context?

Do the substances occur together and, if so, is this occasional or usual? Are they diluted or degraded in the environment or in water treatment, or is absorption precluded because their molecular weights are large? If the response to these questions suggests that exposure is unlikely, then further assessment in a framework analysis for combined exposure is not required.

Is there a likelihood of co-exposure within a relevant timeframe?

Do the temporal aspects of external exposure, toxicokinetics and toxicodynamics preclude co-exposure to the compounds of interest? For example, do the compounds in the assessment group have short half-lives (kinetics) or effects of short duration (dynamics)? Is the time between initial and subsequent exposures for such compounds sufficient so as to preclude co-exposures? If, based on consideration of these aspects, the likelihood of co-exposure is low, a framework analysis of an assessment group is unnecessary.

What is the rationale for considering compounds in an assessment group?

A risk assessment for mixtures begins with the identification of a group of chemicals; an assessment group. The decision to consider compounds in an assessment group is commonly based on information indicating that the components co-occur and/or are believed to act similarly or interact.

Chemicals can be grouped based on different things, such as similar uses or applications, chemicals managed together and common target organs. Although mode of action information is not available for the majority of chemicals, when some data are available, it may be determined using this approach that the chemical grouping does not pose a risk.

Category and analogue approaches are techniques that can be used to group chemicals:

- The category approach groups chemicals whose physicochemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic). Where data are unavailable, a number of data-filling tools can be used; normally, grouping would be based on predictive information on chemical structure, such as structure-activity relationships (SARs), quantitative structure-activity relationship (QSAR) modelling,

structural alerts or, alternatively, data on hazard or other biological data (toxicity or efficacy) that lead to the conclusion that effects are likely to be similar (read-across, trend analysis).
- The analogue approach is used when the grouping is based on a very limited number of chemicals. Empirical data from one or more similar chemical(s) (i.e. the analogue) can be used to predict the endpoint for the target chemical(s). Analogues are selected based on the hypothesis that the properties of a series of chemicals with common structural features will show coherent trends in their physicochemical properties and, more importantly, in their toxicological (human health/ecotoxicity) effects or environmental fate properties (OECD, 2014).

Similarities used for grouping include (but are not limited to) the following:

- a common functional group (e.g. aldehyde, epoxide, ester, specific metal ion);
- common constituents or chemical classes, (e.g. similar carbon range numbers);
- an incremental and constant change across the category (e.g. a chain-length category);
- the likelihood of common precursors and/or breakdown products/metabolites, via physical or biological processes, which result in structurally similar chemicals (e.g. the metabolic pathway approach of examining related chemicals such as acid/ester/salt); and
- a common mode of action.

For further guidance on grouping rationales, readers are referred to Appendix 1, where these are discussed in more detail.

2.3.1.2 Relevance for drinking-water and source water

For drinking-water and their sources, the problem formulation questions require knowledge of what substances are likely to be present remembering that, in many circumstances (particularly with larger supplies), there will be water treatment that can have a major impact on the substances present in final drinking-water. For example, the range of pharmaceutical residues observed in drinking-water is considerably less than that seen in untreated and sewage-impacted surface water. However, it might be appropriate to look at groups of substances in source water to determine whether there is any need for further action or, at least, whether action is needed urgently. Before considering these questions, however, it is also important to determine what the purpose of the assessment is and whether it relates to a single supply (in which case the purpose is to determine the necessity of remedial action) or a large number of supplies (with the objective of taking regulatory action).

It is important to determine whether the substances in the identified group do actually occur together or, in some cases, whether all of the possible substances in the group occur together. There then needs to be consideration of the length of exposure and also whether exposure is continuous or intermittent. Many pesticides, for example, appear in surface water but they will only be present at certain times of the year and, unless used in the same crops or at the same times of the crop cycle, related pesticides may or may not be present together.

It is only then that decisions can be made as to whether a risk assessment of the chemical mixture in drinking-water is necessary or appropriate. The decision may also be modified by consideration of types of source. For example, trichloroethene and tetrachloroethene have been widely used in degreasing/dry cleaning operations and are sometimes found in groundwater. Because they are so volatile, they are rarely (if ever) seen in surface water derived supplies. In addition, they and other related substances of historical use, such as carbon tetrachloride, may not be identified together in the same groundwater sources.

Groups that have been proposed/used for drinking-water and/or source water-related risk assessments have been based on similarities including:

- similar use (pharmaceutical families);
- common origin (DBPs);
- common analysis (volatile organic compounds - VOCs; THMs);
- treatment technology (DBPs);
- indicators (THMs, N-nitrosodimethylamine);
- efficiency to be risk managed together (pesticides, DBPs); and
- toxicology (nitrate/nitrite; cholinesterase inhibiting pesticides, statins).

These groupings fall into one of two broad logistics; practical operations (e.g. analytical and technological) and common toxicology. Regulatory implementation is simplified if a group of substances is analysed by a single method, derived from a common source or is treatable by a single technology. For example, THM regulations group by common origin, common analytical method and common treatment. VOCs can also be grouped as to common analytical methods and treatments. Grouping by common toxicology is a more complex issue, with regulations of groups based on common modes and common organ toxicology. For example, some pesticide programmes have considered groups of cholinesterase inhibiting

pesticides in drinking-water. Statins (a pharmaceutical family) have a known common mode of action which may allow grouping, should they need to be considered for regulation.

2.3.2 Framework tiered structure

The WHO/IPCS framework utilizes a tiered approach for both exposure and hazard which provides a systematic way of determining the level of consideration that is most appropriate for the chemicals of concern, minimising unnecessary investigations, and allowing more efficient use of resources. The tiers range from predictive methodologies and conservative assumptions in early tiers (tiers 0 and 1) to more refined approaches based on increasingly data informed and probabilistic approaches (tiers 2 and 3), but only if necessary. Communication of the outcomes at each tier is an important aspect of the framework, even within early tiers; being able to 'set-aside' chemicals in a particular tier (as they have shown a sufficiently large MOE) is a valuable message to communicate to the general public for assurance.

It is important to note that by proceeding from tier 0 to tiers 1, 2 and 3, there is a decreasing degree of conservatism and an increasing degree of certainty that the values reached approximate the true values. For exposure assessment, approaches may range from simple semi-quantitative estimates in tier 0 based on surrogates (e.g. sales, percentage composition of compound in a product and the number of users - see, for example, Douglass et al., 1997) to probabilistic estimates in tier 3 based on considerable representative monitoring (including biomonitoring) data. For the hazard assessment, approaches may range from default dose addition for all components based on very conservative (sometimes predicted) PODs in tier 0 to full biologically-based case specific models incorporating toxicokinetic and toxicodynamic data in tier 3. As previously described, dose addition is used as a default assumption in estimating risk in all tiers for chemicals placed into assessment groups. At each tier within the framework, risk characterization can then be carried out by estimating the adequacy of the MOE. If the outcome of risk characterization in tier 0, using conservative default values, concludes that there is no cause for concern, then no further resources are invested. However, if the outcome indicates potential for excess risk, the assessment is taken to the next tier which utilizes more refined data and accurate models.

The WHO has emphasised that when using this approach, the judgement of the adequacy of the MOE will depend on the actual purpose and/or (legal) framework for which the assessment is performed. Factors such as inter-individual variation (including susceptible groups), interspecies differences, quality and robustness of the database, nature of the hazard and temporal aspects should be taken into account (WHO, 2009). Meek and colleagues also consider that the adequacy of the MOE should be conservative, but commensurate with the degree of uncertainty at each tier (Meek et al., 2011). The extent of assessment to be undertaken and nature of recommendations for the generation of additional data are based on a weight of evidence approach, dependent upon the extent of the knowledge base, the magnitude of public health concern (i.e. taking into account margins between exposure, frequency of exposure and effect) and the objective of the risk assessment (e.g. implications of potential risk management decisions) (WHO, 2009; Meek et al., 2011).

The tiered model does not preclude the requirement for iterations within each tier where necessary. In the context of the WHO/IPCS framework, iterations may be needed for cases requiring a greater level of scrutiny where it is necessary to dedicate additional resources to allow a greater level of discernment.

The need for uncertainty analysis in the risk assessment of chemicals is now well recognised. However, while some guidance exists (e.g. EFSA, 2006; WHO, 2008; NRC, 2009) the design and conduct of such analysis is still under discussion. When determining the toxicity of chemical mixtures, an assessment of uncertainty needs to be considered for both the individual chemicals and the mixture as a whole. For the mixture there will be additional sources of uncertainty, particularly relating to the assumptions relating to the combined risk.

Uncertainties in the exposure assessment include, but are not limited to, the following (IGHRC, 2009):

- the level of accuracy with which exposure to the chemical mixture has been characterized;
- the extent and profile of co-exposure to different chemicals (different chemicals have different persistence in the environment and in the body, so duration of exposure will vary and it may be episodic for one chemical and continuous for another); and
- the determination of the identity of the chemicals involved.

Uncertainties in the hazard assessment of chemical mixtures include (IGHRC, 2009):

- the adequacy of the toxicological database;
- the lack of knowledge on human relevance;
- the lack of an agreed definition of criteria for similar modes of action and of grouping criteria for chemicals into assessment groups;

- chemicals may need to be considered in the same assessment group even if the effect does not drive the individual risk assessment;
- assumptions on the consequences of the combined effect of co-exposure (i.e. dose addition, independent action / response addition, synergy, antagonism);
- for some methods of dose/concentration addition (e.g. the method of Finney, 1971), assumptions regarding similarity in the shape of the dose-response curves;
- nature and identification of PODs for use in combined risk assessments; and
- assumptions about departures (or absence of departure) from additivity at human relevant exposures to chemicals in an assessment group.

2.3.3 Data-filling tools for use with the framework

For a group member that lacks data for an endpoint, the data gap can be filled in a number of ways, including by read-across, trend analysis, QSARs, threshold of toxicological concern (TTC) and physiologically-based modelling (OECD, 2014; Bopp et al., 2015). Read-across is a technique used to predict endpoint information for one chemical by using data for the same endpoint from another chemical which is considered to be similar in some way (on the basis of structural similarity and similar properties and/or activities and/or uses). For a given category endpoint, the category members are often related by a trend (e.g. increasing, decreasing or constant) in an effect, and a trend analysis can be carried out using a model based on the data for the members of the category. Data gaps can also be filled by an external QSAR model, where the category under examination is a subcategory of the wider QSAR. The TTC concept was originally developed as an approach to help prioritize which chemicals, present at very low levels in food, should be included in a risk assessment. However, the technique can be applied to source water and drinking-water scenarios. The TTC approach is based on the premise that chemicals will have a 'practical human threshold value', below which there will be no significant risk to health. Physiologically-based approaches are a highly refined methodology to estimate the concentration of the compound at the target site for a toxicological effect.

Further information on these tools is provided in Appendix 1.

2.3.4 Models for use with the framework

In principle, the hazard of chemical mixtures can be assessed as a whole or based on the individual components. Principal limitations of the 'whole-mixture' approach relate to sample collection and the availability of analytical standards. 'Sufficiently similar' chemical mixtures can also be utilized; these, however, are limited due to the variable nature of each mixture. If toxicity data on specific chemical mixtures are available that should be used, however, availability of such data are mostly very limited. In essence, any guidance value for one mixture is unlikely to be applicable to others.

For further information on whole-mixture approaches see Appendix 2.

If the components of a chemical mixture are known then a 'component approach' is usually performed, and there are a number of models that can be used to achieve this (see the following sub-sections and Appendix 2). The optimal approach for a component-based risk assessment of chemical mixtures is dependent on:

- knowledge of the modes of action of the individual components; or
- information regarding their association with groups of chemicals demonstrating identical, similar or dissimilar modes of action (assessment groups).

In the absence of sufficient information on the mode of action of the individual components, the dose/concentration addition method is often used as a default in human toxicology chemical mixture assessments. This includes a general assumption that interactions either do not occur at all or are small enough to be insignificant to the risk estimate (EU, 2012).

2.3.4.1 Models for chemicals with similar modes of action

The most frequently used approach currently for chemicals with similar modes of action is dose/concentration addition (IGHRC, 2009). Appropriate models are shown below (with additional information in Appendix 2).

Hazard Index (HI): this is the sum of the hazard quotients (HQ) for each chemical, where the HQ is the exposure of each of the individual component compounds divided by its individual reference value (e.g. acceptable daily intake – ADI or tolerable daily intake - TDI). The HI approach is widely applicable to component-based risk assessments of toxicologically similar chemicals. Ideally this should be used for groups of toxicologically similar chemicals for which dose-response

data are available, but can be used for chemicals that affect a common target organ even where there are no additional mechanistic data.

Reference Point Index (RfPI) / Point of Departure Index (PODI): this approach is similar to the HI approach but differs in that it uses each chemical's POD (e.g. NOAEL, LOAEL, BMDL) instead of a reference value. As in HI, this should be used for groups of toxicologically similar chemicals for which dose-response data are available, but can be used for chemicals that affect a common target organ even where there are no additional mechanistic data.

Relative Potency Factor (RPF), Toxic Equivalency Factor (TEF) and Potency Equivalency Factor (PEF): these approaches have been applied to mixtures that consist of a single class of structurally similar chemicals, where extensive information is available for one member of the chemical class but less is known about other members. They rely on the use of scaling factors (RPFs) or equivalency factors (TEF, PEF) to express the toxicity of the chemicals with less available toxicity data in terms of an equivalent dose of the index chemical (usually the most extensively studied) in order to estimate what the overall toxicity of the mixture will be.

2.3.4.2 Models for chemicals with dissimilar modes of action

The consequences of a chemical mixture showing independent action will be described by the effects of the individual components when administered alone at their respective concentrations in the mixture. This assumes that any biological stress or perturbation induced by a chemical has no effect on the dose-response relationships for the other chemicals in the mixture (IGHRC, 2009).

Currently, most risk assessment methods for mixtures in use are applications of the concept of dose addition, and methods to explicitly assess independent action have not been developed to date (EFSA, 2013).However, in the absence of specific methods, it is considered that the following approaches are sufficiently conservative to serve as a default for evaluating mixtures of dissimilarly acting chemicals:

Modified HI Approach: an adaptation of the HI approach, in which an additional uncertainty factor (UF) (between 1 and 100) is added to the conventional HI calculation to reflect the degree of confidence in the available information on the interactions and the concentrations of the mixture components.

Binary Weight of Evidence Approach: enables the direction of interactions in a chemical mixture to be evaluated when information about the toxicity of the mixture as a whole is unavailable. This approach systematically evaluates the potential for interaction for each possible pair of chemicals in the mixture.

Response Addition: a probabilistic approach to determining the effects of exposure to a combination of independently acting substances. In the response addition model, combination effects can be estimated directly by summing the probability that individual components will affect the exposed organisms; chemicals present at levels below NOAELs are not expected to contribute to the total effect.

Relative Potency Factors: this is an adaptation of the RPF approach whereby components are grouped into sub-classes based on their mode of action and RPFs derived against an index chemical in each sub-class. Risk estimates for each sub-class are then added, as for dose and response addition, giving risk estimate for whole mixture.

2.3.4.3 Models for interactive chemicals

For chemicals that are interactive (i.e. those that display non-additive behaviour), an interaction-based HI approach has been proposed. This approach applies a UF relating to the potential for interactions to the calculated HI, based on an evaluation of the weight of evidence (EFSA, 2006; Sarigiannis and Hansen, 2012).

2.4 **CONCLUSIONS**

The choice of risk assessment method for chemical mixtures is governed by the modes of action of the individual mixture components. Although human health risk assessment of chemical mixtures is often conducted based on the general assumption of additivity, at the present time there is no 'regulatory-applicable' accepted methodology for carrying out risk assessments for chemical mixtures in source water and drinking-water. Guidance has been published by a number of authoritative bodies and, in an attempt to harmonize methodology at an international level, a framework for the risk assessment of combined exposures to multiple chemicals and associated terminology has been developed (WHO 2009; Meek et al., 2011; Meek, 2013). The framework includes problem formulation as a basis to consider appropriate chemical groupings. For chemicals relating specifically to drinking-water and source water, grouping can be based on

similar uses or applications, common origin, common analysis or treatment technology chemicals managed together and common toxicology.

Application of the framework is based on a hierarchical (phased) approach that involves integrated and iterative consideration of exposure and hazard at all phases, with each tier being more refined (i.e. less conservative and uncertain) but more labour and data intensive than the previous one (Meek, 2013). The tiered framework allows the assessment to be tailored to the magnitude of potential risks, the objective (e.g. priority setting, screening or risk management) and scope (local or national) of the perceived problem. In common with all such frameworks, the assessments may require significant and expert resources, particularly in the higher tiers and often relate to much wider control scenarios. However, a number of tools and models are available which can be utilized within the framework.

It has not yet been shown that the use of such a framework for development of standards or guidelines for chemicals in drinking-water is practical, although the process may identify circumstances when such regulation would be prudent. It is hoped that by using the framework, risk assessors can maximise efficiency by identifying non-priority assessment groups in the early, non-labour and data intensive tier(s), thereby conserving resources.

The framework was designed to be additionally developed through pragmatic application in specific case studies (Meek, 2013), and some examples of the use of the framework with a focus on source water and drinking-water are detailed in Section 3 of this document.

3.

CONSIDERATIONS FOR THE APPLICATION OF THE WHO/IPCS RISK ASSESSMENT FRAMEWORK FOR CHEMICAL MIXTURES IN SOURCE WATER AND DRINKING-WATER

3.1 INTRODUCTION

This section provides more detailed information on the WHO/IPCS framework, including the types of data that can be considered within each tier of the framework. It also includes several case studies illustrating use of the framework at lower tiers for exposure to chemical mixtures specifically in drinking-water; the examples use a step-by-step approach. Should more complex exposure or hazard data be available, however, it may be most efficient to start at a higher tier that is more appropriate to the data. At the time of writing, illustration of a higher tier assessment relating to chemical mixtures in water was not feasible and therefore this is demonstrated for exposure to chemical mixtures in food. The methods used to illustrate the framework are included as examples only.

Following on from the problem formulation step (Section 2.3.1) three key tasks are undertaken at each tier of the framework to provide information and to allow a decision to be taken on risk management, or whether to proceed to the next level of detail.

- Exposure assessment (is there credible evidence of exposure to some or all of the chemicals in the selected group and how frequently do they occur together?).
- Hazard assessment (is there credible evidence of adverse effects in humans and do the chemicals in the selected group cause toxicity in a similar way or affect the same organs?).
- Risk characterization (is there a credible risk to health and is that risk likely to be significantly increased by the combined exposure?).

The *WHO Human Health Risk Assessment Toolkit: Chemical Hazards*[8] (WHO, 2010) may prove a useful tool for these three tasks. Although not specifically designed for chemical mixtures, the toolkit provides guidance to identify, acquire and use the information needed to assess chemical hazards, exposures and the corresponding health risks in their given health risk assessment contexts at local and/or national levels. However, this approach also requires significant resources and expertise.

In the following sections, each of these three steps is described in principle for each tier of the framework. The use of the framework is then illustrated for a number of drinking-water related case studies.

3.2 TIER 0 RISK ASSESSMENT

This section provides a summary of information and methodological guidance for a tier 0 risk assessment of combined exposure to multiple chemicals in source waters and drinking-waters (for a more detailed description of each process see WHO, 2009 and Meek et al., 2011).

3.2.1 Exposure assessment

The objective of exposure assessment is to answer the question: **what concentration of contaminants might receptors be exposed to?**

Exposure assessment at tier 0 provides a simple semi-quantitative estimation of summed exposure of the contaminants of concern at the target receptor, often derived using default assumptions. In determining exposure, all routes (oral, inhalation and dermal) and potentially exposed sub-groups should be considered.

For source waters and drinking-waters the primary pathways will be directly through drinking and, potentially, from inhalation of water vapour and dermal contact during showering/bathing. Indirect exposure is also possible through consumption of food cooked in the water where there is capacity for uptake by the food (e.g. rice or pasta). One potential high risk sub-group are infants exposed to contaminants through formula made up with tap water, which is an infant's only source of nourishment. The risk of exposure from contaminants in food may be heightened if the contaminants are lipophilic, preferentially partitioning and accumulating in breast milk; this is not likely to occur in treated drinking-water as lipophilic substances partition out of the water phase or are readily removed during treatment.

The importance of the route of exposure will depend on both the chemical and the target organ. For liver toxicity, it is reasonable to assume that the oral route will be of primary concern. However, for other target organs, inhalation or dermal exposure may be of greater importance. For example, dermal and inhalation exposures have been highlighted to be of greater importance than ingestion for bromodichloromethane and chloroform in drinking-water (Jo et al., 1990; Leavens et al., 2007; Kenyon et al., 2015).

8 Available at: http://www.who.int/ipcs/publications/methods/harmonization/toolkit.pdf?ua=1

3.2.2 Hazard assessment

Hazard assessment has the objective to determine: **what potential adverse effects might the mixture of chemicals cause and at what concentration?**

For a tier 0 risk assessment, assuming that accurate assessment groups have been formed, it is assumed that the rules of dose addition will apply and that all components are as toxic as the most toxic compound present (i.e. the same potency). If reference or guidance values[9] are available for components of the chemical mixture, the HI approach (Appendix 2) can be used as a basis for comparison of potencies. However, the TTC concept can also be utilized when values are unavailable. Risk is determined from the magnitude of the HI, with an HI of < 1[10] (i.e. the sum of HQs for each chemical present does not exceed 1) indicating that there is no need for further assessment.

3.2.3 Risk characterization

The risk characterization step combines the results of the exposure assessment and hazard assessment to develop MOEs to determine whether a more robust assessment or risk management is required. It should be noted that risk characterization needs to take into account the limitations of the data collected, and the assumptions and uncertainties inherent in the data and models used. If risk characterization at tier 0 indicates an inadequate MOE, this does not automatically indicate significant risk, rather, that further risk assessment at a higher tier of the framework is needed (Meek et al., 2011). The approach has been used in assessing the potential risks from pharmaceuticals and groups of related pharmaceuticals in drinking-water (WHO, 2012) and where the MOE was less than the arbitrary MOE chosen, the risk assessment was further refined as illustrated in Box 1 and covered in more detail in Appendix 3.

3.3 TIER 1 RISK ASSESSMENT

If the MOE for a tier 0 assessment is considered to be insufficient, the assessment is iteratively refined using more complex exposure and hazard models.

3.3.1 Exposure assessment

Tier 1 exposure assessments are refined from tier 0 by the use of deterministic estimates of exposure data for individual components, including measured and/or modelled data (e.g. upper bound daily use or intake). Individual estimates are then summed for use in risk characterization (Meek et al., 2011).

3.3.2 Hazard assessment

Within tier 1, hazard assessment is refined through the use of individual component potency data for the common critical effects within the assessment group (Meek et al., 2011). PODs are used as an indicator of potency and include LOAEL, NOAEL, BMD or BMC (Meek et al., 2011).

3.3.3 Risk characterization

As for tier 0, risk characterization can be undertaken by calculation of the HI. However, for tier 1 this is refined by using individual component reference values or reference values adjusted for the common effect (Lambert and Lipscomb, 2007). Alternatively, a PODI can be calculated by dividing the sum of exposures by the POD for each individual component of the assessment group (i.e. Exposure 1/POD1 + Exposure 2/POD2, etc.). This represents a risk-based summation of exposures to individual components and overcomes the higher levels of uncertainty associated with using the HI approach (Meek et al., 2011).

As previously for the HI approach, values of ≤ 1 indicate there is no need for further assessment; for the PODI (assuming the same uncertainty factor of 100 has been used on experimental data), values of ≤ 0.01 suggest there is no requirement for further assessment. Risk is assessed by consideration of the adequacy of the MOE, taking all associated uncertainties into account, as a basis to determine if a higher-level tier risk assessment is needed.

9 conservative numerical values designed to help risk assessors determine if chemicals are present at sufficient levels to pose a potential risk to human health. Examples include RfD, ADI and TDI.

10 the 'acceptable' values used here are generic guidance which can be modified on a case-specific basis.

3.4 TIER 2 RISK ASSESSMENT

Should the outcome of a tier 1 risk assessment show the MOE to be insufficient, the framework allows for further iterative refinements using more complex exposure and hazard models.

3.4.1 Exposure assessment

In tier 2 assessments, the deterministic estimation of exposure is refined by incorporation of increasing numbers of measured values for more realistic exposure scenarios (i.e. tailored to the specific situation under consideration) or inclusion of a greater number of parameters in models; multiple sources are still taken into account by summation. Although these estimates of exposure are more realistic, they are still considered conservative (Meek et al., 2011).

3.4.2 Hazard assessment

Hazard assessment at tier 2 is refined through additional definition of the assessment group, for example, through consideration of more specific information on mode of action or other factors on which the group was based (e.g. molecular modelling). If measures of potency are available for individual components, RPFs can be calculated (as percentage) against that of an index compound, usually selected on the basis of the most robust and reliable database (Meek et al., 2011).

3.4.3 Risk characterization

As with tier 1, the margin between estimated exposure and hazard is considered in the context of associated uncertainties as a basis to determine whether or not a higher tier assessment is required (Meek et al., 2011). Using RPFs, if combined exposure is 100% or less of the reference value, exposure would be considered acceptable, by analogy with that of a single substance. The nature of the considerations that constituted the basis for determining that a higher tier assessment is required (i.e. adequacy of the MOE in the context of uncertainty associated with both estimated exposure and hazard) should be explicitly stated (Meek et al., 2011).

3.5 TIER 3 RISK ASSESSMENT

The WHO/IPCS framework allows one further tier, for iterative refinements (tier 3) should the outcome of a tier 2 risk assessment show the MOE to be insufficient.

3.5.1 Exposure assessment

In tier 3 assessments, probabilistic exposure assessments are utilized with distributions of exposure factors and parameters. Where possible, measured data from relevant populations are used; if these are unavailable, data can be obtained from models using input from multiple sources of exposure (Meek et al., 2011).

3.5.2 Hazard assessment

Tier 3 assessments of hazard are refined through consideration of mode of actions, including PBPK and biologically-based dose-response models, where available. These types of models may provide probabilistic estimates of hazard and be used as a basis for extrapolation across species and among humans (Meek et al., 2011).

3.5.3 Risk characterization

In probabilistic assessments, risk can be estimated as:

- the percentile of the population exceeding the reference value;
- the maximum exceedance of the reference value; or
- the percentage of the population at or below the reference value for a given percentile of the distribution (e.g. 99.9th percentile).

When considering risk at any tier, the margin between the estimated exposure and hazard is considered in the context of associated uncertainties as a basis to determine whether or not clear recommendations can be made to risk managers (Meek et al., 2011). WHO (2008) states that "an adequate characterization of the uncertainties is essential to the

transparency of risk assessment and characterization of relevant data gaps to improve defensibility; it is also a critical basis for informed decision making regarding the need for action to reduce risk and the nature of appropriate measures. Uncertainties should be considered explicitly in each step of the analysis and communicated throughout the process."

3.6 CASE STUDIES ILLUSTRATING USE OF THE WHO/IPCS FRAMEWORK

In the following sections (3.6.1 to 3.6.5) a number of case studies relevant to drinking-water (3.6.1 to 3.6.4) and food (3.6.5) are presented, with detailed use of the WHO/IPCS framework and available tools given for each in Appendices 3 – 7.

3.6.1 Assessment of risk to human health from exposure to pharmaceuticals in drinking-water

This case study described in Box 1 illustrates the grouping of pharmaceuticals based on similar structure and use, together with an HI type approach to assess hazard. The HI type approach consists of comparing the measured or modelled environmental concentrations of pharmaceuticals in drinking-water with a health screening level.

BOX 1. PHARMACEUTICALS (TIER 0 AND TIER 1)

Pharmaceuticals and their break down products are primarily found in sewage following excretion by humans taking the medicines. Not all pharmaceuticals will reach sewage and some will be completely or partially broken down during sewage treatment. Some, however, will reach surface water in the effluent. In order to prioritise those pharmaceuticals that should be considered for either further research on occurrence or to determine whether drinking-water treatment is adequate to remove them a number of risk assessments have been carried out.

Some pharmaceuticals are very similar, e.g. non-steroidal anti-inflammatory drugs (NSAIDs) and statins. The tier 0 approach used was to compare the estimated reasonable worst case concentration (dose) of individual substances and groups of substances (assuming dose addition in groups) to the lowest dose giving rise to a pharmacological effect (minimum therapeutic dose, MTD). Those that showed an MOE of less than 1000 were subjected to a tier 1 evaluation. The tier 0 assessments showed that for statins the MOE was sufficiently protective, while for NSAIDs the MOE was not adequate.

Therefore for NSAIDs, a more realistic tier 1 evaluation of probable exposure was used taking into account removal in sewage treatment and natural attenuation in surface water, as well as probable removal during drinking-water treatment. The MOE for all of the individuals and groups in the tier 1 assessment was greater than 1000.

The risk management recommendation was that there was no need for urgent action and this was confirmed by studies of occurrence in drinking-water. This assessment did not mean that pharmaceuticals could be ignored but that a more long-term holistic view could be taken with regard to actions for the future. The consideration of impacts on aquatic life, however, may result in a different conclusion.

3.6.2 Assessment of risk to human health from exposure to microcystins in drinking-water

This case study described in Box 2 details the use of the WHO/IPCS framework for assessment of combined exposures to a group of microbial toxins.

BOX 2. MICROCYSTINS (TIER 0 AND TIER 1)

Microcystins are toxins often produced by cyanobacteria (blue-green algae) that occur in still and slow flowing freshwater. There are > 80 different variants of microcystin, with microcystin-LR being the most toxic. Cyanobacteria can form massive blooms that normally appear on the surface of water bodies and it is these blooms that pose a potential risk to drinking-water. While microcystins can be removed in drinking-water treatment it is important to establish whether the drinking-water treatment is adequate or whether additional management actions are necessary.

In this example, a concentration of 55 µg/L of microcystin-LR has been used which is at the upper end of concentrations detected in Lake Taihu in China (which has been subject to severe cyanobacterial blooms). Tier 0 assumes that all microcystins are of equal toxicity. As the provisional WHO guideline value for microcystin-LR is 1 µg/L (WHO, 2017) the exposure is clearly in excess of this level, meaning that a tier 1 risk assessment is necessary.

In the tier 1 risk assessment a more refined exposure assessment was conducted, taking into account the manipulation of the drinking-water depth intake (to a level well below the bloom) and toxin removal during various stages of treatment. This gave a concentration in drinking-water of 0.06 µg/L, which is well below the WHO guideline value, even assuming that all microcystins are of the same toxicity.

A tier 2 risk assessment, if that had been required, would have needed to consider the HI derived from the proportion of microcystins present with lower toxicity than microcystin-LR.

3.6.3 Selecting pesticides for consideration in HIs on the basis of risk ranking and detection frequency

This case study summarised in Box 3 addresses a tiered approach to priority setting for monitoring of pesticides in drinking-water for which analysis is recommended in line with the Japanese Drinking-water Quality Standards (JDWQS). It outlines an increasingly data informed (tiered) approach to potential refinement of a list of pesticides selected to be considered in an HI for assessment of combined exposures.

BOX 3. ASSESSMENT OF PESTICIDES FOR MONITORING (TIER 0 AND TIER 1)

Pesticides in surface waters can vary significantly with the time of year, the crops grown in the catchment, pest attacks and rainfall patterns. Japan has addressed the problem of prioritization using a tiered risk assessment. The pesticides considered are known to occur in Japanese drinking-water supplies.

In the period since the primary group of pesticides was introduced into the JDWQS (approximately 10 years), regulatory authorities have collected additional monitoring data for these and other pesticides suspected to be present in drinking-water. An approach has been proposed for refining the list of highest priority pesticides, through ranking based on scoring for risk indicators, which have been verified based on comparison with the more recent monitoring data.

As a basis for potential refinement, 236 of 530 pesticides registered in Japan in 2011 were considered, including the primary (102), secondary (26) and tertiary (77) groups specified in the JDWQS; an additional 31 were selected from among three categories including top sales and sales in comparison to the ADI for each pesticide.

Twenty-four different risk indicators were created, ranging from those based solely on quantity of sales to those incorporating additional parameters including quantities of sales for specific applications in more localized areas (e.g. rice farming in regional versus national land areas), regional precipitation, physical/chemical properties and guidance values).

The suitability of the risk indicators for prioritization of pesticides included in the JDWQS was considered on the basis of their detection rate. Detection rate was based on identified monitoring data which included two samples a year (average sampling frequency) for a four year period for raw water samples from water treatment plants and additional information. Based on the analysis, data were considered sufficient to determine 'detected' and 'undetected' for 105 index pesticides; monitoring data for the remaining 131 were considered insufficient. Detection rates were highest for the combination of two indicators. Based on application of this combined indicator, for pesticides for which monitoring data were sufficient, 44 of 134 pesticides that are currently unregulated were selected as potential priorities to be added to the standards primary group, while 17 of the 102 pesticides in the primary group were deprioritised.

3.6.4 Assessment of risk to human health from exposure to natural and synthetic oestrogens in drinking-water

This case study, summarised in Box 4, is designed to assess risk from combined exposure to natural and synthetic oestrogens. These are commonly detected in surface waters (although there are no routine monitoring programmes for drinking-water).

BOX 4. NATURAL AND SYNTHETIC OESTROGENS IN DRINKING-WATER (TIER 0 AND TIER 1)

Natural and synthetic oestrogens enter the environment through excretion by humans and through intentional disposal of pharmaceuticals to sewage and household waste. Routine monitoring programmes to test drinking-water for natural and synthetic oestrogens have not been implemented and incomplete removal by wastewater treatment leads to occurrence in surface waters which may impact on drinking-water sources.

There are a number of studies that have assessed the risk to human health from the presence of natural and synthetic oestrogens as individual chemicals. However, similarities in structure and mode of action allow for grouping of these chemicals for risk assessment purposes.

The tier 0 approach estimated worst-case exposure of adults and children to the group of oestrogens, using a freely available model. As the constituents of the group varied in oestrogenic potency, RPFs were calculated for each relative to 17β-oestradiol (E2, index chemical) and summed to give an overall potency (E2 equivalents) for the combined exposure. When compared to the lowest guideline value for E2 exposure, an MOE <1000 was obtained for adults and children.

In tier 1, a more realistic assessment of exposure was used taking into account metabolism in the human body and removal during drinking-water treatment. The resulting MOE was >1000.

The risk management recommendation was that there was no need for urgent action. The findings could also be confirmed by monitoring studies of occurrence in drinking-water.

3.6.5 Assessment of risk to human health from exposure N-methyl carbamate (NMC) insecticide residues in foods

This case study, summarised in Box 5, is designed to assess risk from combined exposure to NMC insecticide residues which are commonly detected in a number of food stuffs. A food example was chosen to illustrate application of the higher tier of the framework as no drinking-water specific examples were available. It should be noted that although exposure to NMC insecticides may also occur through drinking-water this route of exposure will be very small in comparison to that for food and therefore the drinking-water route is not specifically addressed in this example.

BOX 5. NMCS IN FOODS (TIER 0, TIER 1, TIER 3)

NMC insecticides are a group of substances with a well-understood mode of action and a relatively complete toxicity database, which allows grouping of these chemicals for risk assessment purposes. They are used on a number of food crops, and the possibility may exist of dietary exposure to more than one NMC insecticide during one day. In combination, the potential for co-exposure, and the high potency of these pesticides, raises concerns about potential health effects from exceeding margins of safety.

The tier 0 approach utilised assessment of dietary consumption and agricultural commodity residues to estimate worst-case exposure of children to individual NMCs. Hazard was assessed using the TTC approach to produce a combined HI. As the HI was >1 at both the 95th and 99th percentile of exposure, further refinement of exposure and hazard data was needed.

In tier 1, a more realistic assessment of exposure was obtained by comparing estimates of intakes to the acute reference dose for each NMC. As a set of refined hazard assessment data (in the form of RPFs for children and adults, calculated using BMD modelling) was available for the NMCs, this was utilised for the tier 1 hazard assessment. However, in general, this represents a more sophisticated approach than would be necessary at this tier.

The exposure assessment at tier 1 showed that a varied diet could result in significant exposure to residues of multiple NMCs in one day, even if the consumption of each individual compound is below the acute reference dose. It was, therefore, considered that a refined exposure assessment should be carried out at a higher tier. This would generally be achieved at tier 2 using actual measured data of dietary residues and food consumption. However, as an exposure data set (using probabilistic modelling) was available for the NMCs, this was utilised in a tier 3 exposure assessment and compared with the refined hazard data set identified in tier 1.

The highly refined analysis at tier 3 demonstrated that there is no concern for combined dietary exposure to N-methylcarbamate insecticides.

3.7 CONCLUSIONS

As stated by Meek (2013), the regulatory risk assessment community has been challenged for some time to consider the impact of combined exposures to multiple chemicals. However, to date, there are limited numbers of such assessments at an international level. Where such assessments have been performed, they have tended to be more complex than necessary, equivalent to a tier 3 assessment in the WHO/IPCS framework (where data permit); an unnecessary use of resources. The identification of priority compounds for assessment using screening methods has also been limited.

The case studies presented in this section were developed to illustrate use of the framework for exposure to chemical mixtures specifically in source water and drinking-water; they were designed to provide context as a basis to increase understanding of the framework, associated terminology and tools, and are not to be considered as actual risk assessments.

The first case study was based on two assessment groups of pharmaceuticals commonly detected in water sources, i.e. NSAIDs and statins. As pharmaceuticals, these groups have well defined toxicity data, allowing use of the minimal therapeutic dose for calculating the MOE at tier 0. Exposure was estimated for both groups using an HI type approach with 'worst-case' assumptions for tier 0. Although a very conservative approach, the tier 0 risk characterization was able to show that, for the statins, the MOE was sufficiently protective and further evaluation at higher tiers was unnecessary. This illustrates the value of the framework in being able to communicate to stakeholders and the public that an issue has been considered and further assessment is not considered necessary, with only limited resources having been used.

For the NSAIDs, assessment at tier 0 did not show an adequate MOE, however, following a refined exposure assessment at tier 1, incorporating more realistic exposure scenarios (derived from probabilistic or deterministic modelling) the MOE was shown to be adequate (and further evaluation using higher tiers unnecessary).

The further case studies presented show the utility of the framework in being applied to a range of assessment groups:

- contaminants from cyanobacteria (microcystins) to prioritize the need for further evaluation;
- pesticides to prioritize monitoring in drinking-water;
- natural and synthetic oestrogens to prioritize further evaluation and the need for monitoring in drinking-water; and
- insecticide residues in food.

Meek[11] (2013) describes the WHO/IPCS framework as a "unifying construct for transparency for increasingly data informed, value-of-information-driven, 'fit for purpose' priority setting and assessment of combined exposures". The problem formulation step is considered to be of particular importance from a communication perspective, "as it provides the rationale in those cases where it has been determined that an assessment of combined exposures is unwarranted." Such a rationale allows for transparent communication with stakeholders and the public. However, the approach can be highly resource intensive and may require the availability of significant expertise, particularly at the higher tiers. Furthermore, the use of such a framework for development of standards or guidelines for chemicals in drinking-water has yet to be shown to be practical, although the process may identify circumstances when such regulation would be prudent.

11 Reprinted from Regulatory Toxicology and Pharmacology, 60(2), Meek, M.E., Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework, S1-S14, Copyright (2011), with permission from Elsevier.

4.

CONSIDERATIONS WHEN ASSESSING AND MANAGING CHEMICAL MIXTURES IN SOURCE WATER AND DRINKING-WATER

There are several circumstances (including the examples given below) in which regulators of drinking-water may need to consider the exposure to chemical mixtures.

1. When several substances may be present at the same time in water supplies and there is a requirement to provide reassurance to consumers that the water is safe (e.g. pharmaceutical residues).
2. Substances which are frequently found together in some drinking-water sources (e.g. tri- and tetrachloroethene in groundwater, THMs and other DBPs in chlorinated drinking-water).
3. An emergency in which a number of chemicals are spilt into a water source.

In each case there may be a different regulatory outcome. In the first, the question is: **is there a need to introduce regulations for concentrations in drinking-water?** and, if so, **how can this be achieved without requiring excessive monitoring costs and taking into account that not all substances will be present in all supplies?** An additional objective may be to determine the need for trigger values against which to judge the efficacy of drinking-water treatment.

In the second, the objective is to develop regulatory values for drinking-water but again this can be complicated, particularly if the grouping of the chemicals includes substances with very different modes of action.

The third is much more straight forward and relates to determining the threat to health from drinking-water and the remedial actions to be taken, but such actions may themselves introduce other risks, such as issuing 'do not use' notices that then leave a problem of alternative water supplies.

Where it is considered that there is a need for regulatory action to manage the risks then it is important to identify the most effective point at which control can be exercised and the most cost effective means of doing so. Often it will require time for actions to be introduced and where a group of substances are to be restricted then there may be a need to identify alternatives which will not simply create further problems in the future.

4.1 CONSIDERATIONS WHEN ASSESSING THE RISKS

While there is currently no 'regulatory-applicable' accepted risk assessment methodology for conducting risk assessments for chemical mixtures in source water and drinking-water, current thinking supports the assumption that substances usually act in an additive fashion when present as a mixture in drinking-water. At the very low concentrations normally encountered in drinking-water the potential for interactive effects, such as synergism or antagonism, appears to be relatively small. Only where there is sound evidence that chemicals interact should a non-additive approach be utilized.

A component-based MOE approach can be used to demonstrate whether regulatory control is needed. In this case it is possible to develop a group parameter based on total concentration allowable in drinking-water. Where this is not the case, the approach suggested by WHO for nitrate/nitrite and the THMs would be a viable way forward that is both transparent and relatively simple to implement. This approach is to determine that the sum of the concentrations of the substances divided by their respective WHO guideline values is less than or equal to 1 (HI approach). The advantage is that, for example, nitrite is much less frequently found than nitrate and the formula allows the regulation to be applied on a case-by-case basis when they do occur together. This does not introduce a costly and potentially unachievable value for one where both are not present.

For carcinogens, where risk is calculated using mathematically derived low dose extrapolation methods, the issue of whether an additive approach could be applied should also be considered. While, initially, this seems an attractive proposal it must be remembered that for compounds considered to be genotoxic carcinogens, WHO guideline values are normally determined using a mathematical model, where the guideline value represents the maximum potential risk, taking into account large uncertainties. Guideline values in the GDWQ represent an estimated upper bound excess lifetime cancer risk of 10^{-5} or one additional cancer per 100 000 of the population following a lifetime exposure (70 years) to 2 litres of water per day containing the chemical at that concentration (WHO, 2017). In developing standards, the most common values are based on a risk level of 10^{-5} or 10^{-6} (i.e. one possible additional cancer per 100 000 or 1 000 000). This assumes that the chemical operates through a direct genotoxic mode of action and that the dose-response at low doses is linear. The risks are, therefore, very small and since the lower confidence interval on the risk calculation is sometimes below zero the risk may also be much closer to zero than the value represented by the WHO guideline value.

In view of the significant uncertainties in the risk calculations, which err on the side of caution, considerable care would be needed not to:

- create practical problems in achieving any standard with unintended consequences that result in other risks to health;
- mislead consumers as to the scale of risk in relation to other risks; or
- divert resources to dealing with minor risks compared to greater risks.

While it may seem attractive to take an extremely conservative approach this may be very costly (taking resources away from other more important requirements), may result in no discernible benefits and could lead to unintended adverse consequences. It is therefore, appropriate to take a holistic view of risk assessment and contamination of drinking-water by chemicals with consideration of the benefits of control in water compared to other sources of human exposure. This also requires consideration of how practical controls are, where they can most effectively be applied and the time frame under which action can be introduced. This is particularly important for chemical mixtures because the process is usually more complex than in dealing with individual chemicals.

4.2 CONSIDERATIONS WHEN MANAGING THE RISKS

Having identified a mix of substances and made an assessment of the risks to human health from drinking-water (bearing in mind the fact that standards and WHO guideline values for chemical substances are often very conservative) it is then necessary to decide whether it is appropriate to take action and what action to take. Any regulatory values for drinking-water need to take into consideration the requirement for monitoring and particularly the need to ensure that the substances in the mixture of interest can be measured; preferably by the same analytical method and at the same time.

There are a number of questions that can assist in making decisions about whether to treat a group of substances as a mixture and consideration of management approaches:

- Do the chemicals always occur as a mixture, e.g. petroleum products?
- If not, how frequently do they occur together and under what circumstances?
- Does the proportion of substances vary and is there a small number that usually dominate (e.g. chloroform in THMs)?
- Are they of similar water solubility?
- Do one or two substances act as a surrogate for the others?
- How stable is the mixture (i.e. is it always similar)?
- Can the components of the mixture be measured by the same method?
- How readily will the substances be removed in the available drinking-water treatment?
- Are there other upstream interventions that can be applied?

As noted in Section 2 and indicated above, in addition to toxicology, pragmatic considerations can be taken in grouping chemicals in source water and drinking-water for risk assessment as well as management. An example of the pragmatic approach is that of THMs and also HAAs. These are by-products resulting from the reaction between chlorine and natural organic precursors in the water. They represent the most abundant of a much larger number of DBPs found at the same time but at much lower concentrations. The values chosen for regulatory purposes should be informed by the toxicity data and provide a balance between the need to provide microbially safe water while protecting against adverse effects of contaminants formed as a result of disinfection. Minimising DBP formation can be achieved, for example, by changing some aspects of water treatment practice (such as optimising treatment to remove the natural precursor organics before chlorination, thereby reducing the formation of all of the chlorination by-products).

Another example is the microcystins which are toxins sometimes produced by blooms of cyanobacteria, or blue-green algae, in still or slow flowing bodies of water. There are many microcystins but a WHO provisional guideline value for only one, microcystin-LR. This is one of the most toxic and is usually the dominant congener so using this value for total microcystins is a very conservative approach. Normally the value will be used to assess whether treatment is effective when a bloom occurs, rather than be a basis for routine monitoring (as there are more practical ways of identifying the start of a bloom, such as cell numbers or chlorophyll a).

On occasion, a group of contaminants which occur together may be found in groundwater usually as a consequence of poor handling in industrial or other facilities. The contamination will persist even after the input of polluting chemicals has been stopped and so decisions need to be taken as to whether treatment will be necessary. One such example is the group of perfluorinated compounds that include PFOA and perflurooctane sulfonate (PFOS). Treatment is difficult and losing an otherwise viable water source is often not an option. These substances often occur as a mixture and now that more data on potential toxicity are emerging it may be possible to consider them as a mixture based on PFOS being the most toxic

substance in the group and using a toxicity equivalent approach. This will allow a reasoned decision to be made regarding whether there is a need for an intervention and the actions to be taken.

4.3 KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Attention should be drawn to a number of data and knowledge gaps that may impede a more systematic and effective application of the methodologies described in this document.

- A major gap, at the present time, is the lack of systematic data on where, how often and to what extent humans and the environment are exposed to certain chemical mixtures and how exposure may change over time. There is a need to better understand human and environmental exposures, both through the use of investigative monitoring and modelling.
- The gap in exposure data is exacerbated by the lack of standardized sampling and analytical methods for many substances, particularly contaminants of emerging concern, and the important associated quality assured procedures that allow proper comparability of data.
- For many chemicals, there is a lack of reliable information on mode of action, although knowledge bases are currently being developed for adverse outcome pathways (Vinken, 2013; OECD, 2016[12]) and transcriptomic data (Thomas et al., 2012a, b).
- Interactions within chemical mixtures are difficult to foresee, particularly for long-term effects at very low levels of exposure. Research is needed to define criteria that predict the practical potential for potentiation, synergy or antagonism.
- There is a lack of toxicity data for commonly identified chemical mixtures which would enable assessment of the hazard as a whole, and not based on individual components.

Further, there is limited experience with assessing and managing chemicals as mixtures in drinking-water and its sources which, when considered with the knowledge gaps, may preclude this type of assessment being widely implemented at the present time. Although a number of useful tools and models have been developed to carry out risk assessment of chemical mixtures, the cost of undertaking risk assessments as well as the expense of the control of potential mixtures in relation to the health benefits need to be carefully considered. For example, the use of bioanalytical tools (bioassays) in relation to screening chemical mixtures as part of potable reuse is receiving increasing attention (WHO, in press) to assess the potential interactions of trace levels of chemicals during mixture exposures. However, there are substantial challenges in translating data from such tools, particularly for regulatory purposes and their application to combined exposure in potable water has not, to date, been assessed.

4.4 CONCLUSIONS

In summary, the key conclusions are:

- Exposure to multiple chemicals in drinking-water at the same time is common; however, variability exists in each mixture with respect to composition and changes in concentrations of individual components.
- Many approaches are available to assess the possible risks of exposure to combinations of chemicals and these are outlined in this document. However, in many circumstances these require significant resources and expertise.
- In terms of regulatory action, the assessments can be used to determine whether action is needed and the urgency for any regulatory action. As a means of developing standards it is perhaps of less value unless a flexible approach can be developed that takes into account the variability in composition and changes in individual component concentrations (as outlined above).
- In the event that regulatory action is needed, it is important to understand the objective of the action, as this may be the over-riding influence. For example, the standards for THMs and HAAs are set to drive changes in water supply practice that will reduce the overall load of chlorination by-products.
- Any decision to introduce regulations for groups of chemicals needs to take into account the probability of them actually occurring together, the frequency that they are found together in different supplies and the concentrations of the differing components. It also needs to consider the ease of monitoring (i.e. can they be monitored together or is a range of methods required or can one parameter be used as a surrogate for the mixture) and how they respond in drinking-water treatment (e.g. will all components respond to one treatment or will several treatments be required). It is also important to consider exposure from other sources. If drinking-water is predominately a minor source, then there is the possibility that there will be significant cost implications with very little benefit.
- For toxicologically similar chemicals, it would be most appropriate to assume dose addition based on the current evidence. A straight forward and pragmatic approach is to base the regulation/guidance on the basis that the sum

12 http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

(HI) of the concentration of each component divided by its reference value (HQ) should not exceed 1 (it should be noted that although straightforward, this approach remains data intensive).

- In the absence of sufficient information on the mode of action of the individual components, the dose/concentration addition method is often used as a default in human toxicology chemical mixture assessments. Due to the limited evidence available, this includes a general assumption that interactions either do not occur at all or are small enough to be insignificant to the risk estimate.
- There are many knowledge gaps in terms of the modes of toxicity of chemicals and, particularly, their dose-response at low concentrations. In many cases, there is likely to be a practical threshold to toxicity but this may be difficult to define. There is a need for greater understanding of the actual, rather than theoretical, consequences of exposure of humans to very low concentrations of chemicals in drinking-water.

In assessing the possible risks of exposure to combinations of chemicals a number of frameworks are currently in place, but significant knowledge gaps, complexity, limited practical experience and resource intensity, precludes this type of approach being more widely introduced into drinking-water standards at the present time. However, it is hoped that this document presents a pragmatic approach to priority setting for the assessment and management of risk to human health associated with exposure to chemical mixtures from drinking-water and its sources.

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APPENDIX 1

RATIONALES FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP AND DATA-FILLING TOOLS

The OECD QSAR Toolbox[13] is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical work flow, and a key part of this is grouping chemicals into chemical categories. A description of the approaches taken by the OECD for grouping chemicals is given below[14] and, as such, much of the text is derived and/or extracted from this reference. For more detailed information see OECD, 2014.

A1.1 CATEGORY APPROACH

The OECD define a chemical category as "a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity".

Similarities used for grouping include (but are not limited to) the following:

- a common functional group (e.g. aldehyde, epoxide, ester, specific metal ion);
- common constituents or chemical classes, similar carbon range numbers;
- an incremental and constant change across the category (e.g. a chain-length category);
- the likelihood of common precursors and/or breakdown products, via physical or biological processes, which result in structurally similar chemicals (e.g. the metabolic pathway approach of examining related chemicals such as acid/ester/salt); and
- a common mode of action.

Groups that have been used/proposed for drinking-water and/or source water-related risk assessments have been based on similarities including:

- similar use (pharmaceutical families);
- toxicology (nitrate/nitrite);
- common origin (disinfection by-products - DBPs);
- common analysis (volatile organic compounds - VOCs);
- treatment technology (DBPs);
- indicators (trihalomethanes - THMs, N-nitrosodimethylamine); and
- efficiency to be risk managed together.

The approach assesses the effects of individual chemicals within a category, based on the category as a whole. The approach is strengthened as the number of chemicals within the category increases, as it becomes possible to develop hypotheses for specific endpoints and subsequently make generalisations about trends within the category. As a result, measured data can be extrapolated to similar 'untested' chemicals, providing estimates that are adequate for some regulatory purposes (e.g. the Classification, Labelling and Packaging Regulations) or to allow risk assessment without the need for further testing. Indeed, the category approach is an important tool in the drive to decrease animal testing of chemicals (OECD, 2014).

A1.2 ANALOGUE APPROACH

The analogue approach is utilized when risk assessment is being carried out for a specific chemical with limited data. Gaps can be filled using data from one or more similar chemical(s) – called the analogue(s) or source chemical – which are used to predict endpoints for the chemical under consideration (target chemical). The approach is most useful for chemicals with a shared, evaluated mode of action and for which the adverse effects have been identified. The choice of analogue is usually governed by being data rich. For single substances (or a dominant constituent(s) in a mixture) read-across can be utilized to identify a common chemical structure, with the following assumptions being made:

- In the case of qualitative read-across, the presence (or absence) of a property/activity for the target chemical can be inferred from the presence (or absence) of the same property/activity for the analogue.
- In the case of quantitative read-across, the known value of a property for the analogue can be used to estimate the unknown value of the same property for the target chemical. In the case of a toxicological effect (human health or ecotoxicological), this assumption implies that the potency of an effect shared by the two chemicals is similar or follow a regular pattern.

13 (available at: http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm)

14 Adapted and/or extracted from Guidance on grouping of chemicals, second edition: Series on testing & assessment No. 194, Copyright OECD (2014). Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282014%294&doclanguage=en

A1.3 DATA-FILLING TOOLS

As both the category and analogue approaches are based on similarities between grouped chemicals, the OECD recommends data gap filling in a chemical category by applying one or more tools from read-across, trend analysis, (external) (Q)SARs, threshold of toxicological concern (TTC) and physiologically-based models (OECD, 2014; Bopp et al., 2015).

A1.3.1 Read-across

In this technique, endpoint information for one chemical is used to predict the same endpoint for another chemical, which is considered to be similar in some way (usually on the basis of structural similarity) (OECD, 2014). Read-across is generally recommended for predicting environmental fate, human health effects and ecotoxicity; although it is feasible to apply read-across to predict basic physicochemical properties, this is not recommended as more reliable data are usually obtainable.

Within a group of chemicals, read-across can be performed in the following ways to fill data gaps:

- one-to-one (one analogue used to make an estimation for a single target chemical);
- many-to-one (two or more analogues used to make an estimation for a single target chemical);
- one-to-many (one analogue used to make estimations for two or more target chemicals); and
- many-to-many (two or more analogues used to make estimations for two or more target chemicals).

Read-across can be qualitative or quantitative. Qualitative read-across gives a 'yes/no' answer for the presence (or absence) of a property/activity for the target chemical which is inferred from the presence (or absence) of the same property/activity for one or more analogue chemicals. Quantitative read-across is used to obtain a quantitative value for an endpoint (e.g. a dose-response relationship) using the known value(s) for one, or more, analogue chemical(s) to estimate the unknown value for the same endpoint for the target chemical (OECD, 2014).

A1.3.2 Trend analysis

For a given category endpoint, the category members are often related by a trend (e.g. increasing, decreasing or constant related to molecular mass, carbon chain-length, or to some other physicochemical property). Such trends can be used as a basis for estimating values for a member of the category through interpolation (using values from category members adjacent to the member of interest in the defined spectrum) and possibly also extrapolation (using values from category members to estimate a value for a member that is near or at the category boundary) (OECD, 2014).

Interpolation between measured analogues may give a more reliable result depending on the reliability of the measured data and is generally preferred to extrapolation. However, where data gaps exist for the boundary chemical, extrapolation will be necessary and the robustness of this will be closely related to the general evaluation of the whole category.

A1.3.3 QSARs

Assessment groups can be formed by grouping mixture components and/or their metabolites based on structural similarities. If toxicological data are lacking on the individual components of a mixture and on the mixture as a whole, a (Q)SAR-based approach could be used as a first approach. If such models are used to fill data gaps in a category, they should be based on experimental data that are obtained from a wider range of chemicals than those used in the category.

A1.3.4 TTC approach

The TTC concept has evolved from a lengthy history of attempts by scientists over the years, in regulatory authorities and elsewhere, to develop generic approaches to the safety assessment of large groups of chemicals or of individual chemicals of unknown toxicity (Barlow, 2005; EFSA/WHO, 2016). Cramer et al. (1978) proposed three class categories based on potential for toxicological risk, within which most chemicals (excluding polymers) would fall. Cramer calculated a protection index that could be used to establish priorities and the extent of appropriate toxicity testing (permitted daily exposure - PDE).

Initial applications of TTC data are from the mid-1990s and were limited to use in the Food and Drug Administration's risk assessment of migrants from food packing materials (threshold of regulation) and an assessment of flavours (Munro, 1996). In 2004, Kroes et al. published a proposed methodology for applying the TTC approach for more general use and

included additional TTC toxicity thresholds. After passing some exclusion criteria, a chemical can be placed in one of five tiers based on chemical structure:

- Cramer Class I substances have simple chemical structures and predictable and efficient modes of metabolism that suggest a low order of toxicity (e.g. L-glutamic acid, mannitol or propylene glycol) – PDE of 1800 µg/d.
- Cramer Class II substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III (e.g. β-carotene, diallyl phthalate or maltol) - PDE of 540 µg/d.
- Cramer Class III substances permit no strong initial presumptions of safety, and may suggest significant toxicity, because their chemical structure has similarities to those of known toxins (e.g. acetonitrile,2,4-dinitrotoluene, chlorobenzene or p-aminophenol) – PDE of 90 µg/d.
- Organophosphates – PDE of 18 µg/d.
- Chemicals with structural alerts for DNA reactivity – PDE of 0.15 µg/d.

It should be noted that as a probability-based screening tool, some additional uncertainty is inherent in the TTC approach, which should be considered by risk assessors and risk managers. For further information on the derivation of TTC values see Kroes et al. (2000, 2004).

A1.3.5 Physiologically-based toxicokinetics (PBTK) and toxicodynamics (PBTD)

Should a higher tier assessment be considered necessary, physiologically-based modelling may be appropriate. Modelling of both toxicokinetics (PBTK) and toxicodynamics (PBTD) is feasible and the two can be linked. PBTK permits an estimate of the concentration of the compound at the target site of a toxicological effect. In the risk assessment of combined exposures, the concentrations of each component of the mixture would need to be corrected for potency (e.g. by using potency equivalency factors). PBTK and PBTD approaches can also be used to investigate other types of combined effect and the general approach has been described by Teuschler et al. (2004), for the assessment of combinations of DBP. Physiologically-based approaches have also been outlined for the assessment of mixtures of pesticides (Conolly et al., 2005; Lowit et al., 2004). Although these approaches provide a highly refined methodology, they are resource intensive and demanding of specialised expertise and therefore are unlikely to be routinely used in the near future.

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APPENDIX 2

AVAILABLE TOOLS FOR USE WITH THE WHO/IPCS FRAMEWORK

In principle, the human health hazard of chemical mixtures can be assessed as a whole or based on the individual components. The tools described below can be used within the WHO/IPCS framework and align with increasingly data informed approaches to characterization of exposure and hazard for individual chemicals.

A2.1 WHOLE-MIXTURE APPROACHES

If toxicity data on the chemical mixture itself are available, a quantitative assessment can be done directly from these data. Whole-mixture approaches have the advantage of accounting for any unidentified materials in the chemical mixture and for any interactions among components (Boobis et al., 2011). However, the major limitations of the whole-mixture approach relate to sample collection and the availability of analytical standards. 'Sufficiently similar' chemical mixtures can also be utilized; however, these are limited due to the variable nature of each mixture. If toxicity data on specific chemical mixtures are available they should be used, although these data tend to be sparse.

In the absence of data on the mixture of interest, assessment can be based on data for a 'surrogate mixture', which is close in composition (components and proportions) to the mixture under investigation. This technique has been used for poorly characterized but stable mixtures and for specially designed mixtures (EU, 2012). In addition, it may be possible to separate the whole-mixture into fractions and evaluate these. This approach has been used for diesel exhaust separated into gaseous and particulate matter fractions.

A2.2 COMPONENT-BASED APPROACHES

If the components of a mixture are known, a component-based approach is usually performed. Information on the mode of action should be used to assess the type of combined action (dissimilar action, similar action) applicable. The optimal approach for a component-based risk assessment of chemical mixtures is therefore dependent on:

- knowledge of the modes of action of the individual components, including dose-response information; or
- information regarding their association with groups of chemicals demonstrating similar or identical modes of action (assessment groups). Such information may be based on chemical structure and structure-activity relationship - SAR (either qualitatively or quantitatively), molecular modelling, structural alerts or on toxicological responses or effects.

In the absence of sufficient information on the mode of action of the mixture components, dose addition is often used as the default in human toxicology mixture assessments, with the general assumption that interactions either do not occur at all or are small enough to be insignificant to the risk estimate.

The available methods are described in the sub-sections below and summarised in Table A2.1.

A2.2.1 Models for chemicals with similar modes of action

The most frequently used approaches currently for chemicals with similar modes of action (with increasing levels of complexity and refinement) are:

- the hazard index (HI);
- the reference point index (RfPI, also known as the point of departure index - PODI); and
- the relative potency factor (RPF), toxic equivalency factor (TEF) and potency equivalency factor (PEF).

These methods assume that the chemicals act in an additive fashion according to dose/concentration addition. They are described below with much of the text being derived and/or extracted from EFSA[15] (2008), IGHRC[16] (2009) and EU[17] (2012). For additional information the reader is also directed to the review by Boobis et al. (2008).

15 Adapted and/or extracted from Opinion of the Scientific Panel on Plant Protection Products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005, EFSA Journal, 704, 1–84, Copyright EFSA (2008).

16 Adapted and/or extracted from Chemical mixtures: A framework for assessing risk to human health (CR14), Institute of Environment and Health, Cranfield University, U.K., Copyright Crown Copyright (2009).

17 Adapted and/or extracted from Opinion on the toxicity and assessment of chemical mixtures, SCHER, SCCS, SCENIHR. Copyright European Union (2012).

A2.2.1.1 HI approach

This is the most widely applicable approach for component-based risk assessment of toxicologically similar chemicals. Ideally, it should be used for groups of toxicologically similar chemicals for which dose-response data are available, but can be used for chemicals that affect a common target organ even where there are no additional mechanistic data.

The HI is the sum of the hazard quotients (HQ) for each chemical, i.e. the ratios between exposure (E) and the reference level (RL) for each component to be evaluated. To determine the HI for a mixture, a HQ is calculated for each component by dividing the dose or exposure level for each component with a suitable RL. The individual HQs are then summed to give an overall HI according to the following equation:

$$HI = \frac{E_1}{RL_1} + \frac{E_2}{RL_2} + ... + \frac{E_n}{RL_n} \quad \text{or} \quad HL : \sum_{i=1}^{n} \frac{E_i}{RL_i}$$

Where: HI is the hazard index; E represents the exposure level of each individual component; and RL represents a reference level for each individual component.

The RL of exposure could be a derived level such as acceptable daily intake (ADI), tolerable daily intake (TDI) or minimum risk level. In order to interpret the HI outcome, it is essential to use the same type of reference level throughout the calculation (e.g. ADI). The HI value provides a qualitative assessment of the hazardous properties of a mixture and is interpreted according to whether or not it exceeds unity. An HI value of less than 1 indicates that exposure is below the chosen reference level (i.e. low risk). When the HI value is 1 or above, exposures are at or above the reference level, signalling greater concern. The HI approach is used widely for assessing the effects of exposure to chemical mixtures that are considered to show dose addition.

BOX 2.1. HI APPROACH FOR CHEMICALS THAT INTERACT

When interaction data are available, an interaction-based HI approach can also be used, whereby, the available information on the interactions is converted into a numerical score on an expert judgement basis or a weight of evidence evaluation. Information on this approach can be found in US EPA (2007).

A2.2.1.2 RfPI approach

This differs from the HI as it represents the sum of the exposures to each chemical component expressed as a fraction of their respective reference points (RfPs - also known as point of departure POD) for the relevant effect (e.g. the dose that causes a 10% effect [10% benchmark dose - BMD_{10}] or the no observed adverse effect level - NOAEL). When the RfPI multiplied by the chosen group uncertainty factor (UF) is lower than 1, the combined risk is considered acceptable.

$$RfPI = \frac{Exp_1}{RfP_1} + \frac{Exp_2}{RfP_2} + \frac{Exp_3}{RfP_3} \text{ etc.}$$

The reciprocal of the RfPI is the combined margin of exposure (MOET), where the individual margin of exposure (MOE) is the ratio of the RfP to the level of exposure in humans (measured or estimated). MOET is calculated as the reciprocal of the sum of the reciprocals of the individual MOEs. If the MOET is greater than 100 or other alternative value specified for the MOE by the risk manager, the combined risk is considered acceptable.

For instance, if the BMD_{10} is used as RfP:

$$MOE = \frac{BMD_{10}}{Exposure}$$

$$MOET = \frac{1}{(1/MOE_1) + (1/MOE_2) + (1/MOE_3)} \text{ etc.}$$

The RfPI has the advantage that it sums the exposures to the different components in relation to their relative potencies, expressed as the RfP. A single group UF can be applied as the last step in the process or, alternatively, chemical-specific adjustment factors (CSAFs) can be applied earlier in the process, if needed.

A2.2.1.3 RPF/PEF approach

These approaches have been applied to mixtures that consist of a single class of chemicals where extensive information is available for one member of the chemical class (index chemical - IC) but less is known about other members. They rely on the use of scaling factors (RPFs or PEFs) to express the toxicity of the lesser known chemicals in terms of an equivalent dose of the IC in order to determine the overall toxicity of the mixture. These approaches rely on the assumption that the chemicals in the mixture act via the same mode of action.

Usually the potencies are derived from dose-response curves, using the same benchmark response (e.g. 10%) for each compound, but NOAELs have also been used. The activity of the mixture is then determined by the sum of the potency-normalised doses to yield a total equivalent exposure expressed as IC equivalents. This total equivalent exposure is then compared to the reference value (RV) of the IC. If the total equivalent exposure is lower than the RV of the IC, the combined risk is considered acceptable. Alternatively, the MOE of the total equivalent exposure is calculated from the RfP of the IC. In this case, if needed, additional UFs are applied to the individual RPFs before calculating the MOE.

The RPF and PEF approaches were adapted from the toxic equivalency factor (TEF) method that was developed initially for halogenated aromatic hydrocarbons (e.g. dioxins and other Ah receptor agonists) (Haws et al., 2006; van den Berg et al., 2006).

Surrogate marker approach

If the toxicokinetic and toxicological data are inadequate to establish TEFs, the risk assessment for the mixture may be based on a single component, or surrogate. For example, there are insufficient data to derive TEFs for oral exposure to polycyclic aromatic hydrocarbons (PAHs) and PEFs and RPFs are more commonly used. However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that benzo[a]pyrene could be used as a marker of exposure to, and the carcinogenicity of, the PAHs present in food. The risk assessment compares dietary exposure to benzo[a]pyrene with the benzo[a]pyrene content of a relevant PAH mixture that has been tested for carcinogenicity (WHO, 2006).

Another type of surrogate approach, used when fewer data are available, is the conservative assumption that the toxicity of the mixture is equivalent to the toxicity of its most potent component. Thus, for the polybrominated diphenyl ethers (PBDEs), the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment compared the total dietary exposure to PBDEs with the NOAEL for the most potent congener (COT, 2004).

A2.2.2 **Models for chemicals with dissimilar modes of action**

The consequences of a chemical mixture showing independent action will be described by the effects of the individual components when administered alone at their respective concentrations. This assumes that any biological stress or perturbation induced by a chemical has no effect on the dose-response relationships for the other chemicals present. If this assumption is not true, the actual risks to health posed by the mixture may be underestimated (IGHRC, 2009).

A2.2.2.1 Modified HI approach

The simplest approach for chemical mixtures showing independent action is to consider each component of the mixture in isolation, as a modified HI approach. An additional UF (between 1 and 100) is added to the conventional HI calculation to reflect the degree of confidence in the available information on interactions and the concentration of the mixture components (since there is a greater likelihood of interactions with increasing dose) (Seed et al., 1995). If the HQ resulting from addition of the modified HI for each component is less than one, this indicates an unlikely potential for adverse health effects. This approach is straightforward, and may be the only one that can be used if data are scarce. However, it is limited in that it does not take into account varying sensitivities to components of the mixture within a population and, as such, should only be considered for a preliminary risk assessment (tier 0).

A2.2.2.2 Binary weight of evidence approach

A more systematic adaptation is the binary weight of evidence approach developed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2004). This approach allows for a more systematic consideration of the nature of interactions, however, it is again qualitative and a lengthy process. Details of the procedure and the values of the numeric scores that are assigned in various circumstances can be found in the following references (Mumtaz and Durkin, 1992; De Rosa et al., 1996; Pohl et al., 1999; ATSDR, 2004).

A2.2.2.3 Response addition

Response addition is a probabilistic approach to determining the effects of exposure to a mixture of independently acting substances (IGHRC, 2009). The toxic response (rate, incidence, risk or probability of effects) of the mixture is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities. As dictated by the theory of independent action, this approach assumes that, for two or more chemicals, the body's response to the first chemical is the same whether or not the second chemical is present. The USEPA has described approaches that can be used to calculate the probability of an adverse effect occurring in an individual, and to calculate the percentage of individuals in a population that may respond (USEPA, 2000). Response addition is succinctly described by Cassee et al. (1998) and Könemann and Pieters (1996).

For an individual, the probabilities that adverse effects will occur as a result of exposure to each of the components of a mixture (usually expressed as a risk estimate) are multiplied together, using the formula below, to define a probability (or risk estimate) for an adverse effect to arise for the mixture as a whole.

$$p_m = 1 - (1-p_1)*(1-p_2)*(1-p_3) \ldots \quad \text{or} \quad p_m = 1 \prod_{i=1}^{n} (1-p_i)$$

Where p_m is the probability of an adverse effect from the mixture and p_1, p_2, p_i, etc. are the probabilities of an adverse effect from the individual components.

When chemical mixtures containing large numbers of components are being considered, the outcome of response addition calculations can be heavily influenced by data for single components. If data for one component are of poor quality, this will markedly affect the reliability of the response addition calculation. It is, therefore, important to describe uncertainties in the individual component response estimates when deriving an overall response estimate for the population (IGHRC, 2009).

A2.2.2.4 RPFs

An adaptation to response addition has been used by the USEPA for a risk assessment of drinking-water DBP mixtures. These mixtures contain sub-groups of components that produce similar effects. Components were grouped into sub-classes based on their mode of action. For each sub-class an IC was identified and RPFs derived for all other chemicals in each sub-class. Risk estimates were then made for each sub-class based on the dose-response relationship for the IC. Finally, the sub-class risk estimates were added, using methods for response addition, to arrive at a risk estimate for the whole mixture (Teuschler et al., 2004).

Table A2.1. Comparison of component-based models

Method	Advantages	Limitations	Use in framework
Models for chemicals with similar modes of action			
Hazard Index (HI)	Sums exposure in relation to RVs. Transparent, rapid and simple to apply.	RVs often not directly comparable, incorporating varying policy and scientific judgements, periods of review and methodologies. Development of the basis of RVs for individual compounds may be needed during refinements of the risk assessment. Difficult to apply for new and data-poor chemicals for which RVs have not been established.	Suitable as an initial screening method.
Reference Point Index (RfPI)	Sums exposure in relation to RfP. Straightforward and mathematically simpler than other methods. UFs are not used prior to calculating RfP thereby increasing transparency. PODs are directly comparable (e.g. selected benchmark response). Easy to apply for chemicals for which RVs have not been established.	Refer to limitations for HI method.	Can be used in lower tier screens.
Relative Potency Factor (RPF) (including surrogate marker approach)	Separates potency correction from exposure considerations, providing better basis for standardizing dose metrics for each chemical. If scaling/potency factors already exist, very straightforward method. Can potentially be used for assessments of risk at or above the RV by utilizing BMDs. Can also be used to calculate POD for a mixture in its entirety.	Reliance on the quality of the toxicological database of the IC. Labour intensive in the case of chemicals for which scaling/potency factors have not been defined yet.	

Method	Advantages	Limitations	Use in framework
Models for chemicals with dissimilar modes of action			
Response addition	Uses single substance effects for predicting mixture effects. Straightforward summation.	Data reliability at low dose levels increases exponentially as number of mixture components rises. This is often unavailable. NOAELs unsuitable for use within the model.	Hazard screening and lower tier use.
Combined RPF	Advantages of RPF method apply. Accounts for the potency of different groups of chemicals present in mixture.	Disadvantages of RPF method apply. Relies on the existence of RPF scaling/potency factors for different groups of chemicals.	
Modified HI	Advantages of HI apply. Can be used if data are scarce.	Does not take into account varying sensitivities to components of the mixture within a population.	
Binary weight of evidence	Systematic evaluation of the types of interactions.	Qualitative approach.	Lengthy process.
Models for chemicals that interact			
Interaction-based HI	Advantages of HI apply.	Disadvantages of HI apply. UFs rely on scientific judgement and may not represent a true measure of the possibility for interaction.	

RV – reference value; RfP – reference point; POD – point of departure; BMD – bench mark dose; IC – index chemical; UF – uncertainty factor.

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APPENDIX 3

DETAILED CASE STUDY FOR THE USE OF THE WHO/ IPCS FRAMEWORK TO ASSESS RISK TO HUMAN HEALTH FROM EXPOSURE TO PHARMACEUTICALS IN DRINKING-WATER

This case study (originally reported by Watts et al., 2007[18]) illustrates the grouping of pharmaceuticals based on similar structure and use. The hazard index (HI) type approach consists of comparing the measured or modelled environmental concentrations of pharmaceuticals in drinking-water with a health screening level. The smaller the environmental concentration in comparison to the health screening level, the lower the risk.

A3.1 PROBLEM FORMULATION

The problem formulation step for the example of pharmaceuticals in drinking-water is detailed in Box A3.1 below.

BOX A3.1. PROBLEM FORMULATION

What is the nature of exposure? Are the key components known?

Pharmaceuticals are synthetic or natural chemicals that can be found in prescription medicines, over-the-counter therapeutic drugs and veterinary drugs, containing active ingredients designed to have pharmacological effects.

Pharmaceuticals enter the environment as trace pollutants largely by way of their intended use in human and veterinary medical practices, agriculture and personal care. Unwanted exposure to medications occurs primarily through unintentional and largely unavoidable dissemination via excretion and bathing. A secondary route of unwanted exposure can occur following their purposeful disposal to sewerage and household rubbish (Ternes, 1998).

The occurrence of pharmaceuticals in the environment and the water cycle at trace levels (in the range of nanograms to low micrograms per litre) has been widely discussed and published worldwide in literature in the past decade (e.g. WHO, 2012). Many surveys and studies have confirmed the presence of pharmaceuticals in municipal wastewater and effluents, and these have been identified as a major source of pharmaceuticals in drinking-water sources (e.g. Watts et al., 2007; WHO, 2012).

Is co-exposure likely taking into account the context?

Routine monitoring programmes to test drinking-water for pharmaceuticals have not been implemented. Available studies have, however, reported that concentrations of pharmaceuticals in surface waters, groundwater and partially treated water are typically less than 0.1 µg/L, and concentrations in treated water are generally below 0.05 µg/L (Ternes et al., 2005; Watts et al., 2007).

Is there a likelihood of co-exposure within a relevant timeframe?

Yes. Humans can be unintentionally exposed to trace residues of pharmaceuticals from the environment by ingesting drinking-water (when the source water contains municipal or domestic animal waste inputs).

What is the rationale for considering compounds in a common assessment group?

Some groups of similar substances do occur and it is appropriate to consider them both individually and as a group since the structure and applications are similar. These groups include the non-steroidal anti-inflammatory compounds (NSAIDs) that include such compounds as ibuprofen, and the statins that are widely, and increasingly, used as lipid lowering drugs.

18 Adapted or extracted from Desk based review of current knowledge on pharmaceuticals in drinking water and estimation of potential levels, Watts C., Maycock D., Crane M., Fawell J., Goslan E., Copyright Crown Copyright (2007).

A3.2 EXPOSURE ASSESSMENT

The objective of exposure assessment for this specific example is to answer the question: **what concentration of NSAIDs and statins might consumers be exposed to through drinking-water?** It should be remembered that initial exposure assessments at tier 0 of the framework are designed to provide a semi-quantitative estimate of summed exposure from all potential routes using default values, including consideration of susceptible populations as a worst-case scenario. If risk assessment at this tier shows acceptable margins of exposure, i.e. MOE > 1000, then further assessment at higher tiers will not be necessary.

An exposure assessment at tier 0 for NSAIDs and statins in drinking-water is detailed in Box A3.2 below.

BOX A3.2. TIER 0 EXPOSURE ASSESSMENT

Concentrations of NSAIDS and statins in drinking-water can be predicted using a modified model for risk assessment of pharmaceuticals in the environment (EMEA, 2005).

$$PEC_{dw} = \frac{A \times (100-R) \times (100-M) \times (100-W)}{365 \times P \times V \times D \times 100 \times 100 \times 100}$$

Where:

PEC_{dw} is the predicted concentration in drinking-water (mg/L);

M is the percentage metabolised in humans;

A is the amount of active ingredient used per year in the catchment (mg/y);

R is the removal rate in sewage treatment works – STW (set as a percentage, see below);

P is the population under consideration (i.e. for the UK; 59 600 000 or the population equivalent [PE] for each catchment scenario);

V is the volume of wastewater produced per capita per day (assumed to be 200 L);

W is the removal rate in the appropriate drinking water treatment works (DWTW) scenario; and

D is the dilution factor in the environment (derived from the 5th percentile flow rate).

To reflect a worst-case situation, conservative assumptions are made for each of the model inputs, as detailed by Watts et al. (2007) and summarised below:

- total usage per year [A] is twice that estimated from published statistics (i.e. large overestimate).
- there is no metabolism [M = 0%] after taking the drug, i.e. all of the amount of NSAIDs and statins used are excreted unchanged (large over-estimate).
- there is no loss of NSAIDs or statins in STWs [R = 0%] (i.e. over-estimate)
- very low river flow rate resulting in low dilution factor [D] (i.e. under-estimate)
- no further dilution or loss of NSAIDs or statins during transport between STW discharge point and DWTW intake point (i.e. under-estimate)
- removal rate in DWTWs is zero [W =0%](i.e. over-estimate)

As a worst case, it is assumed that the population of greatest concern are infants <1 year. Using the predicted worst case assumptions, total predicted drinking-water concentration for NSAIDs is 0.0975 mg/L and 0.00447 mg/L for statins.

A3.3 HAZARD ASSESSMENT

For this case study, the objective of the hazard assessment is to answer the question: **what potential adverse effects might the combined exposure to multiple NSAIDs and statins cause, and at what concentration?** For a tier 0 risk assessment it should be remembered that it is assumed that the rules of dose addition will apply and that all components are equipotent (i.e. they have the same potency as the most toxic compound present - a scaling factor can be applied if available). The hazard characterization step for NSAIDs and statins is detailed in Box A3.3 below (Watts et al., 2007).

BOX A3.3. TIER 0 HAZARD ASSESSMENT

Pharmaceuticals are an example of chemicals where toxicity data are readily available. Adverse effects of NSAID overdose include nausea, vomiting, headache, drowsiness, blurred vision and dizziness. For statins, adverse effects are mainly focused on muscle toxicity and range from myopathy causing pain, tenderness or weakness, to the potentially life-threatening condition of rhabdomyolysis which is associated with renal failure[1].

Two equally valid approaches, that have previously been used to assess hazard for a group of pharmaceuticals in drinking-water, include use of the median dose, or the minimum therapeutic dose (MTD; which is the lowest clinically effective dose).

The NSAIDs, in this example, comprise 19 anti-inflammatory compounds and, at this tier of risk assessment, all are assumed to have the same mode of action and MTD (7.5 mg/day) as the most active NSAID (Meloxicam). This is a conservative approach as the range of MTDs for the group of NSAIDs is 7.5 – 3000 mg/day[2].

The statin group, in this example, comprises four lipid-lowering compounds. For this tier of risk assessment, all are assumed to have the same mode of action and MTD (5 mg/day) of the two most active compounds (Rosuvastatin and Simvastatin). This is a precautionary approach as the range of MTDs for the group of statins is 5 – 20 mg/day[3].

1 source - http://www.globalrph.com/druglist.htm

2 source - http://www.globalrph.com/nsaids.htm;

3 source - http://www.globalrph.com/statins_comparisons.htm

A3.4 RISK CHARACTERIZATION

The risk characterization step combines the results of the exposure assessment and hazard assessment to develop MOEs to determine whether a more robust assessment or risk management is required. It is important to remember that all assumptions made, limitations and uncertainties of the data used should be taken into account at this stage. The risk characterization step for NSAIDs and statins is detailed in Box A3.4 below (WHO, 2012).

BOX A3.4. TIER 0 RISK CHARACTERISATION

An MOE can be determined by dividing the MTD, derived in the hazard assessment, by the theoretical maximum level present in drinking-water, derived in the exposure assessment.

- For the NSAID group the worst case MOE is equivalent to 77 (i.e. 0.0975:7.5)
- For the statin group the worst case MOE is equivalent to 1118 (i.e. 0.00447:5)

Is the MOE sufficient for each group?

When assessing the adequacy of the MOE for all pharmaceuticals, the starting point is a safety factor of 100 which is applied to account for differences in response between humans (x10) and the use of the MTD (x10) which is not a no-effect level. Additional safety factors could also be included if the pharmaceuticals being assessed were cytotoxic (additional x10) or hormonally active (additional x10).

At this low tier of the risk assessment framework, it would be precautionary to raise the MOE to 1000 when determining which pharmaceuticals may need to undergo further assessment at higher tiers. This approach has been used previously in assessing the risks of individual pharmaceuticals in drinking-water in the UK and has been accepted as being conservative by the medical profession. It also provides an additional reassurance with regard to infants and young children.

Under worst-case assumptions, the MOE for the NSAID group is insufficient and further evaluation at higher tiers should be carried out.

Under worst-case assumptions, the MOE for the statin group is sufficient and further evaluation at higher tiers does not need to be carried out.

A3.5 HIGHER TIER RISK ASSESSMENT OF THE NSAID GROUP

As previously described, should the outcome of a tier 0 risk assessment show the MOE to be insufficient, as in this worked example, the framework allows continuation with iterative refinement using more complex exposure and hazard models. This process is described in Box A3.5 below (WHO, 2012).

Refined exposure assessments can be carried out by the use of probabilistic or deterministic realistic worst-case estimates of exposure for individual NSAIDs. These may include measured and/or modelled data, with individual estimates then summed for use in risk characterization; this achieves a more realistic worst-case scenario. An evaluation of the MOE can then be made using the refined exposure estimates against the values from the lower tier hazard assessment. If the MOE is found to be sufficient then further refinement of the hazard assessment would not be needed.

BOX A3.5. HIGHER TIER (TIER 1) RISK ASSESSMENT

Exposure assessment

The initial modelling assumptions outlined in Box A3.2 can be revised as follows using more realistic (less conservative) data:

- Total usage per year [A] is that estimated from published statistics[1].
- There is metabolism [M = X%] after taking the drug, with values for individual NSAIDs taken from literature (e.g. Bound & Voulvoulis, 2005; Alder et al., 2006).
- There is loss of NSAIDs in STWs [R = X%] with values for individual NSAIDs taken from literature or estimated using QSAR (Yu et al., 2006).
- Very low river flow rate[2] resulting in low dilution factor [D] (i.e. under-estimate).
- No further dilution or loss of NSAIDs during transport between STW discharge point and DWTW intake point (i.e. under-estimate).
- There is removal in DWTWs [W =X%] with values for individual NSAIDs taken from literature or using a default range of 50-100% (Watts et al., 2007).

The probabilistic modelling has the advantage that selected combinations of input values can be chosen at random from the ranges of values provided and estimated drinking-water concentrations obtained for each combination. At this tier of exposure assessment, it is likely that the model would be run around 10 000 times for each NSAID to produce a range of concentrations that are likely to be seen in a realistic worst case situation.

Using the refined model, a more realistic worst-case estimate of exposure for the NSAID group is 0.00274 mg/L (compared to 0.0975 mg/L previously estimated).

Hazard assessment

As for tier 0, the NSAIDs are assumed to have the same mode of action and MTD (7.5 mg/day) of the most active NSAID (Meloxicam).

Risk characterization

For the NSAID group the realistic worst case MOE using the refined exposure estimates is equivalent to 2737 (i.e. 0.00274:7.5). Thus, the MOE is sufficient (i.e. >1000) and further iterations of exposure and/or hazard assessments are not required.

1 for example, http://www.imshealth.com

2 5th percentile value from data covering several years of flow measurements

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APPENDIX 4

DETAILED CASE STUDY FOR THE USE OF THE WHO/IPCS FRAMEWORK TO ASSESS RISK TO HUMAN HEALTH FROM EXPOSURE TO MICROCYSTINS IN DRINKING-WATER

This case study details the use of the WHO/IPCS framework for assessment of combined exposures to a group of microbial toxins.

A4.1 PROBLEM FORMULATION

The problem formulation step is carried out to determine the likelihood of co-exposure to multiple chemicals; the outcome of this step determines whether risk assessment is necessary (Box A4.1).

BOX A4.1. PROBLEM FORMULATION

What is the nature of exposure? Are the key components known?

Microcystins are a group of hepatotoxins produced by the cyanobacterium Microcystis and a range of other species including Dolichospermum (Anabaena), Planktothrix, Nostoc and Anabaenopsis. The basic structure consists of seven amino acids in a ring (cyclic heptapeptide) with molecular weights in the range 800–1100. Within this structure there can be modifications of all seven amino acids resulting in over 80 structural variants. Microcystin-LR is the best characterized and among the most toxic of the congeners (WHO, 2017).

The primary route of exposure to microcystins is ingestion from drinking-water or use of recreational waters containing toxin-producing cyanobacteria. Exposure through fish and shellfish harvested from heavily contaminated water may also occur. In rare cases, people may be exposed through consumption of contaminated health food supplements.

The Guidelines for Drinking-Water Quality (WHO, 2017) recommend that the best option to reduce exposure is to prevent the formation of blooms, for which there are a number of approaches but which may be difficult in some circumstances.

There is no single technique that provides an accurate measure of the toxin concentration in microcystin-LR toxicity equivalents where complex mixtures of microcystins occur in a water sample. Assays that measure overall toxicity exist but the precision of these methods is variable. The most precise method currently in use is that of liquid chromatography (to separate mixtures of microcystins) combined with photodiode array detection (HPLC-PDA). Confirmation of variant identity is usually possible by subsequent mass spectrometry. The HPLC-PDA method can be used to calculate microcystin-LR equivalents from detected microcystin variants based on the characteristic UV absorbance peak at 238 nm (WHO, 2003). This method will typically result in an overestimation of toxicity as most variants are less toxic than microcystin-LR.

Is exposure likely taking into account the context?

Microcystin-producing cyanobacteria are common environmental organisms and are widely distributed in freshwaters used as sources of drinking-water and for recreational purposes. When environmental conditions support growth, high density blooms of microcystin-producing cyanobacteria can occur.

Reported concentrations of microcystins in untreated water containing Microcystis and other toxic cyanobacteria are variable and can be influenced by the uneven distribution of cyanobacteria in the water column. In addition, Microcystis species can form surface scums containing very high concentrations of the organism. It is considered that appropriate treatment (e.g. filtration and chlorination) is likely to reduce concentrations of microcystin-LR to less than the WHO provisional guideline value for drinking-water of 1 µg/L (WHO, 2017). However, not all water supplies have adequate treatment.

Is there a likelihood of co-exposure within a relevant timeframe?

Yes. Blooms of microcystin-producing cyanobacteria in water bodies can contain more than one cyanobacterial strain or species and multiple variants of toxins (Chorus and Bartram, 1999).

What is the rationale for considering compounds in a common assessment group?

Microcystins include more than 80 variants of a cyclic heptapeptide with a common mode of action. A provisional WHO guideline value has been derived for microcystin-LR based on limited data. There is insufficient data to develop WHO guideline values for other variants.

A4.2 EXPOSURE ASSESSMENT

The objective of exposure assessment for this specific example is to answer the question: **what concentration of microcystins might consumers of drinking-water be exposed to?** It should be remembered that initial exposure assessments at tier 0 of the framework are designed to provide a semi-quantitative estimate of summed exposure from all potential routes using default values, including consideration of susceptible populations as a worst-case scenario. If risk assessment at this tier shows acceptable margins of exposure (MOE) (i.e. indicates that concentrations of microcystins are equal to or below the WHO provisional guideline value), then further assessment at higher tiers will not be necessary.

An exposure assessment at tier 0 for a mixture of microcystins in drinking-water is detailed in Box A4.2 below.

BOX A4.2. TIER 0 EXPOSURE ASSESSMENT

Concentrations of microcystins in drinking-water can be predicted from those found in untreated sources of drinking-water by applying expected reductions achieved by treatment processes and manipulation of water intakes.

In some circumstances the depths of intakes into treatment plants or distribution systems can be varied to reduce toxin concentrations. This can be effective for species such as Microcystis and Dolichospermum (Anabaena) which produce surface blooms but less so for Planktothrix which can be more evenly spread in the water. Coagulation and filtration are effective in removing cyanobacterial cells and intracellular toxins while chlorine and other oxidising disinfectants are effective in reducing concentrations of extracellular toxins.

The concentration in drinking-water PECdw can be calculated using the following formula:

$$PEC_{dw} = \frac{RW \times (100-DR) \times (100-TR1) \times (100-TR2)}{100 \times 100 \times 100}$$

Where:

PEC_{dw}	is the predicted concentration in drinking-water (µg/L)
RW	is the concentration in untreated water
DR	is the reduction in concentration as a percentage by manipulating the intake depth
TR1	is the reduction in concentration as a percentage by treatment process 1 (e.g. filtration)
TR2	is the reduction in concentration as a percentage by treatment process 2 (e.g. chlorination)

To reflect a worst-case situation, conservative assumptions are made to determine maximum risk without variable depth intakes and inadequate or poorly managed treatment. In this example, a concentration of 55 µg/L of microcystin-LR has been used which is at the upper end of concentrations detected in Lake Taihu in China (which has been subject to severe cyanobacterial blooms - Jia et al., 2003; Sakai et al., 2013).

A4.3 HAZARD ASSESSMENT

The objective of hazard assessment in this case is to answer the question: **what potential adverse effects might the combined exposure to multiple microcystins cause and at what concentration?** For a tier 0 risk assessment it should be remembered that it is assumed that the rules of dose addition will apply and that all components are equipotent (i.e. they have the same potency as the most toxic compound present). The hazard assessment step for microcystins is detailed in Box A4.3.

BOX A4.3. TIER 0 HAZARD ASSESSMENT

Microcystins are primarily hepatotoxins. The mode of action involves inhibition of protein phosphatase enzymes. In animals, acute exposure to high doses causes severe liver damage characterized by disruption of liver cell structure, a loss of sinusoidal structure and increases in liver weight due to intrahepatic haemorrhage (WHO, 2017). In 1996, exposure through dialysis to water containing microcystins in Brazil caused a range of symptoms including headache, eye pain, blurred vision, nausea and vomiting (Jochimsen et al., 1998). At autopsy, the patients' livers were enlarged and damaged.

As discussed earlier, one of the most common and toxic of the microcystins is microcystin-LR. A provisional WHO guideline value of 1 µg/L has been established for microcystin-LR and HPLC-PDA can be used to determine variants and convert concentrations into microcystin-LR equivalents by assuming that all variants are as toxic as microcystin-LR.

A4.4 RISK CHARACTERIZATION

The risk characterization step combines the results of the exposure assessment and hazard assessment to determine whether a more robust assessment or risk management is required. It is important to remember that all assumptions, limitations and uncertainty should be characterized and justified at this stage. The risk characterization step for microcystins is detailed in Box A4.4 below.

BOX A4.4. TIER 0 RISK CHARACTERIZATION

As there is a provisional WHO drinking water guideline value available for microcystin-LR, this can be utilised for risk characterisation. The estimated concentration of microcystin-LR assuming no removal by treatment processes, 55 µg/L, is clearly in excess of the provisional WHO guideline value for microcystin-LR of 1 µg/L. Therefore, further evaluation considering impacts of treatment on exposure assessment should be undertaken.

A4.5 HIGHER TIER RISK ASSESSMENT OF THE MICROCYSTINS GROUP

As previously described, should the outcome of a tier 0 risk assessment show the MOE to be insufficient or, as shown in this worked example, is expected to significantly exceed a WHO guideline value (or value as specified in national or regional drinking-water regulations or standards), the framework allows continuation with iterative refinement using more complex exposure and hazard models. This process is described in Box A4.5 below.

Refined exposure assessments can be carried out in tier 1 by incorporating removal frequencies into the initial exposure estimates to achieve a more realistic worst-case scenario. The refined exposure estimates would again be compared against the values from the lower tier hazard assessment. If the estimated concentration of microcystin-LR is equal to or less than the WHO provisional guideline value, as shown, then further refinement of the hazard assessment would not be needed. It must be remembered that exposure to microcystins through drinking-water is usually, but not always, intermittent and may be for varying periods of time.

BOX A4.5 REFINED EXPOSURE ASSESSMENT (TIER 1)

Exposure assessment

The initial modelling assumptions outlined in Box A4.2 can be revised as follows where surface water is subject to coagulation, filtration and disinfection:

- The reduction achieved by manipulation of the intake depth is 90%.
- The reduction achieved by coagulation and filtration is 99.5% of whole cells (Westrick et al., 2010).
- In healthy blooms 90-95% of toxin is intracellular; based on this filtration can remove 90% of toxins.
- In the latter stages of a bloom 50% of the toxin may be intracellular; based on this filtration can remove 50% of toxins.
- Providing that a chlorine contact time of at least 30 mg.min/L is achieved, chlorination can remove at least 98% of toxins remaining after filtration (Ho et al., 2006).

Using a deterministic approach and based on a conservative estimate of 50% of the toxin being intracellular the predicted concentration PECdw can be calculated as:

$$PEC_{dw} = \frac{55 \times (100-90) \times (100-50) \times (100-98)}{100 \times 100 \times 100} = 0.06\ \mu g/L$$

Hazard assessment

As for tier 0, the microcystins are assumed to have the same mode of action and the most toxic is microcystin-LR which has a provisional WHO guideline value of 1 µg/L.

Risk characterization

The estimated drinking-water concentration of 0.06 µg/L is well below the provisional WHO guideline value. Even if intake depth data was not available, further assessment would not be necessary as the estimated concentration of microcystins-LR in that case would be 0.6 µg/L, which is still below the WHO provisional guideline value.

As detailed in Box A4.5, following refinement of the modelling assumptions, the estimated exposure from drinking-water is considered acceptable. However, if that had not been the case the risk assessment could be further refined by taking into account relative toxicities of the microcystin variants. These have been summarised by Zurawell et al. (2005) based on median lethal dose (LD50) values in mice. Values vary from 50 µg/kg for microcystin-LR to >1000 µg/kg for some variants. However, in practice the lack of reliable analytical standards and the need for sophisticated instruments for congener identification may render this option impractical.

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APPENDIX 5

DETAILED CASE STUDY FOR THE USE OF THE WHO/ IPCS FRAMEWORK TO SELECT PESTICIDES FOR CONSIDERATION IN HAZARD INDICES ON THE BASIS OF RISK RANKING AND DETECTION FREQUENCY

This case study addresses a tiered approach to priority setting for monitoring of pesticides in drinking-water for which analysis is recommended in line with the Japanese Drinking-water Quality Standards (JDWQS) in Japan. It outlines an increasingly data informed (tiered) approach to potential refinement of a list of pesticides selected to be considered in a hazard index (HI) for assessment of chemical mixtures.

These pesticides are those designated in the JDWQS for monitoring to enable calculation of an HI otherwise known as the detected index (DI) value, in order to take into account risk associated with chemical mixtures (MHLWJ, 2003). The DI is defined as:

$$DI = \sum_i \frac{DV_i}{GV_i} \leq 1$$

where DVi is the observed concentration of pesticide i, and GVi is the reference concentration of pesticide i, which is determined in the JDWQS based on the acceptable daily intake (ADI).

It is further specified that pesticide monitoring is to be conducted with the minimum detection limit equal to 1% of each GVi value, the summation should include the monitored pesticides, and the DI should be 1 or less. Where the sum for the DI is greater than 1, additional assessment and/or management is considered.

A5.1 PROBLEM FORMULATION

The problem formulation step for this case study is detailed in Box A5.1 below.

BOX A5.1. PROBLEM FORMULATION

What is the nature of exposure? Are the key components known?

Prioritized pesticides for inclusion are proposed based on sales and use profiles, physical chemical properties and monitoring data.

Is exposure likely taking into account the context?

Yes. The pesticides considered here have been prioritized based on comparison of risk indicators with monitoring data on their presence in Japanese water supplies.

Is there a likelihood of co-exposure within a relevant timeframe?

Yes. Available data indicate that substances from the selected groups of pesticides can co-occur based on both use profiles and monitoring data.

What is the rationale for considering compounds in a common assessment group?

These pesticides have been selected as those appropriate for consideration in an HI for assessment of combined exposures and for which analysis is recommended in line with the JDWQS. Their selection was based on the annual sales and ADI values; proposed additional refinement takes into consideration additional information on sales, use, precipitation, etc.

A5.2 TIER 0 ASSESSMENT

The tier 0 assessment for the pesticides group is described in Box A5.2 below.

BOX A5.2. TIER 0 PRIORITY SETTING

For inclusion in the primary group of pesticides regulated by the JDWQS, the Ministry of Health, Labour and Welfare selected 102 from approximately 550 registered pesticides (MHLWJ, 2003) based on the annual sales and ADI values. Although there were limited monitoring data at the time of the selection, the selected 102 pesticides were suspected to be present in water sources at concentrations greater than 1% of each GVi value.

A5.3 TIER 1 ASSESSMENT

The tier 1 assessment for the pesticides group is described in Box A5.3 below.

BOX A5.3. TIER 1 PRIORITY SETTING

In the period since the primary group of pesticides was introduced into the JDWQS (approximately 10 years), regulatory authorities have collected additional monitoring data for these and other pesticides suspected to be present in drinking-water. Narita et al. (2014) have also proposed an approach for refining the list of highest priority pesticides, through ranking based on scoring for risk indicators, the most predictive of which have been verified based on comparison with the more recent monitoring data.

As a basis for potential refinement, 236 of 530 pesticides registered in Japan in 2011 were considered including the primary (102), secondary (26) and tertiary (77) groups specified in the JDWQS; an additional 31 were selected from among three categories including top sales and sales in comparison to the ADI.

Twenty-four different risk indicators were created, ranging from those based solely on quantity of sales to those incorporating additional parameters including quantities of sales for specific applications in more localized areas (e.g. rice farming in regional versus national land areas), regional precipitation, physical/chemical properties and guidance values.

The suitability of the risk indicators for prioritization of pesticides included in the JDWQS was considered on the basis of their detection rate. Detection rate was based on identified monitoring data which included two samples a year (average sampling frequency) for a four year period for raw water samples from water treatment plants and additional information obtained directly from nine water supply authorities that conducted frequent measurements. For each pesticide, data were pooled for the four year period as a single data point to determine whether the pesticide could be classed as 'detected' or 'undetected'. Based on the analysis, data were considered sufficient to determine detected/undetected status for 105 index pesticides; monitoring data for the remaining 131 were deemed insufficient.

Detection rates were highest for the combination of two indicators (local sales of a pesticide for the purposes of either rice farming or other farming divided by the guideline value and annual precipitation; amended with the scores from the physical and chemical properties of the pesticide). Based on application of this combined indicator for pesticides for which monitoring data were sufficient, 134 pesticides that are currently unregulated in the JDWQS were considered for regulation. Of these, 44 were selected as potential priorities to be added to the primary group in the guidelines. The detection probability of the 44 pesticides was more than 72%. Among the 102 pesticides currently in the primary group, 17 were deprioritized, based on application of the most predictive (combined) indicator.

A5.4 HIGHER TIER ASSESSMENT

Higher tier assessment for the pesticides group is described in Box A5.4 below.

BOX A5.4. POTENTIAL HIGHER TIERS OF PRIORITY SETTING AND ASSESSMENT

Additional tiers of priority setting can be envisaged, beyond the development and refinement of the national prioritized list of pesticides for monitoring as a basis for consideration within an HI. This list could be additionally refined, for example, for regional and local areas (e.g. individual water supplies), through refinement of risk ranking based on more area-specific informed risk indicators and local monitoring data. In addition, groups for chemical mixtures could be additionally refined through information on nature of the adverse outcome and/or mode of action (e.g. carbamates, organophosphates).

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APPENDIX 6

DETAILED CASE STUDY FOR THE USE OF THE WHO/IPCS FRAMEWORK TO ASSESS RISK TO HUMAN HEALTH FROM EXPOSURE TO NATURAL AND SYNTHETIC OESTROGENS IN DRINKING-WATER

This case study is designed to assess risk from combined exposure to natural and synthetic oestrogens. These are commonly detected in surface waters, however no routine monitoring programme for drinking-water has been implemented.

A6.1 PROBLEM FORMULATION

The problem formulation step for this case study is detailed in Box A6.1 below.

BOX A6.1. PROBLEM FORMULATION

What is the nature of exposure? Are the key components known?

This assessment refers to the natural hormones 17β-Oestradiol (E2), 17β-Oestriol (E3) and Oestrone (E1) and the synthetic hormone 17α-Ethinyloestradiol (EE2), which are considered to contribute the greatest potential oestrogenic potency to drinking-water.

Naturally occurring endogenous oestrogens (E1, E2 and E3) enter the environment through unavoidable and unintentional excretion. Prescribed endogenous oestrogens (used to treat menopausal symptoms, in veterinary medicine for growth enhancement, and in athletic performance enhancement) and prescribed synthetic oestrogen (EE2), found in oral contraceptives, will also primarily enter the environment through unavoidable and unintentional excretion. However, a second route occurs through intentional disposal of pharmaceuticals to sewerage and household waste.

Is exposure likely taking into account the context?

Routine monitoring programmes to test drinking-water for synthetic and natural oestrogens have not been implemented, but the available evidence indicates that they are readily removed in water treatment where they are not adequately removed by natural processes in rivers. However, this may not always be the case.

Incomplete removal of excreted oestrogens (natural and/or prescribed) at wastewater treatment works leads to occurrence in surface waters and, potentially, in drinking-water sources that rely on surface water. E1, E2, E3 and EE2 have been widely reported in the literature to be present in wastewater influents and effluents and surface waters in the USA and Europe. In addition, E1, E3 and EE2 were measured at levels of 17, 0.3 – 0.9 and 1.4 ng/L respectively in source waters of drinking-water treatment plants in the USA. While they were not detected in finished water, it is feasible that they were present at levels below current limits of detection (Caldwell et al., 2010).

Is there a likelihood of co-exposure within a relevant timeframe?

Yes; through unintentional exposure from trace residues of synthetic and natural oestrogens from the environment, and through consuming animal and/or plant tissue that has been similarly exposed. However, exposure from drinking-water is likely to be a very minor source.

What is the rationale for considering compounds in a common assessment group?

The structures of synthetic and natural oestrogens are similar and have a similar mode of action. All have been shown to be agonists of the human ERα signalling pathway, antagonists of the androgen receptor, antagonists of glucocorticoid receptor and thyroid receptor signalling pathways.

It is appropriate to consider them both individually and as a group.

A6.2 **EXPOSURE ASSESSMENT**

An exposure assessment at tier 0 for a combined exposure of natural or synthetic oestrogens in drinking-water is detailed in Box A6.2 below.

BOX A6.2. TIER 0 EXPOSURE ASSESSMENT

Alternative models to that described in Box A3.2 for estimating predicted concentrations in drinking-water are available. These include the PhATE (Pharmaceutical Assessment and Transport Evaluation) model (latest version 2.1.1) (Anderson et al., 2004) for selected North American watersheds and drinking-water treatment plants and the GREAT-ER (Geo-referenced Regional Exposure Assessment Tool for European Rivers) model (latest version 3.0) for selected watersheds and drinking-water treatment plants in the EU (France, Belgium, Germany, Italy, Netherlands and the UK) (Feijtel et al., 1997).

The use of the 90th percentile value of the low flow predicted concentrations from PhATE and GREAT-ER is considered to provide a conservative estimate of exposure for risk assessment purposes (USEPA, 1992; Fox et al., 2000; European Commission, 2003; Versteeg et al., 2005). Uses of the GREAT-ER model include prediction of endocrine disruption risk across the UK (Williams et al., 2009) and Switzerland (Ort et al., 2009). Anderson et al. (2012) have reported use of the PhATE model to characterize the potential endocrine disruption risk to USA surface waters.

However, these models can be complex, requiring large amounts of information on each substance and scenario that is to be modelled. If the required data are not available, default values would be utilised, adding to the overall level of uncertainty for the risk assessment.

For a tier 0 exposure assessment for the oestrogens group, it is considered adequate to utilise the model proposed by the European Medicines Evaluation Agency (EMEA, 2005) for risk assessment of pharmaceuticals in the environment, as for the example described in Box A3.2, and detailed below utilising some of the input data described by Anderson and colleagues (2012).

$$PEC_{dw} = \frac{A \times (100-R) \times (100-M) \times (100-W)}{365 \times P \times V \times D \times 100 \times 100 \times 100}$$

Where:

PEC_{dw}	is the predicted concentration in drinking-water (mg/L);
M	is the percentage metabolised in humans;
A	is the amount of active ingredient used per year in the catchment (mg/y);
R	is the removal rate in sewage treatment works – STW (set as a percentage, see below);
P	is the population under consideration (i.e. for the UK; 59 600 000 or the population equivalent [PE] for each catchment scenario);
V	is the volume of wastewater produced per capita per day (assumed to be 200L);
W	is the removal rate in the appropriate drinking water treatment works (DWTW) scenario; and
D	is the dilution factor in the environment (derived from the 5th percentile flow rate).

BOX A6.2. CONTINUED

As for the pharmaceutical case study, for a tier 0 assessment, conservative assumptions are applied to provide a worst case scenario as follows:

- The total usage per year [A] for each of the medically used oestrogens was set at twice the value estimated from available statistics (e.g. http://www.imshealth.com) to allow for uncertainties in the data (large over-estimate).
- There was no metabolism [M = 0%] of prescription oestrogens (large over-estimate).
- There was no loss in STWs [R = 0% as a default] unless there was published data providing information on losses in which case the minimum percentage removal value was used (over-estimate).
- River flow rate used to estimate the dilution factor [D] was the 5th percentile value i.e. very low flow conditions experienced for only a short period in most years (under-estimate).
- There was no loss or further dilution during transport in rivers between STW discharge points and DWTW intakes (under-estimate).
- There was no loss in DWTWs [W = 0% as a default] unless there was published data providing information on losses, in which case the minimum percentage removal value was used (over -estimate).

Input data used is shown below

	E1	E2	E3	EE2
Metabolism %[A]	0	0	0	0
Usage per capita (µg/day)	38	15.4	162	0.82
Total usage for USA[B] (kg/y)	3604	1560	16412	82.4
STW loss (%)	67	85	97	84
DWTW loss (%)	0	0	0	0
In-stream dilution (L/d)	0	0	0	0

A – Metabolism only used to estimate excreted mass of prescribed hormone; B - Total mass excreted assumes a USA population of 277 048 382 (based on 2001 US census)

Using these conservative assumptions, predicted drinking-water concentrations for E1, E2, E3 and EE2 were estimated as 0.118, 0.023, 0.049 and 0.001 ng/L respectively.

The group under assessment comprises four oestrogens with varying potencies. A common approach to assess overall potency for oestrogens is to express each member as oestradiol equivalents (E2-eq) and combine concentrations. This approach assumes additivity of the effects of the four oestrogens, as has been demonstrated experimentally (Anderson et al., 2012).

For this group total E2-eq was equal to 0.084 ng/L. Using default water intake values of 2 L (adults) and 1 L per day (children) results in an estimated intake of 0.168 ng E2-eq /person/day) and 0.084 ng E2-eq /person/day respectively.

A6.3 HAZARD ASSESSMENT

The hazard characterization step for natural and synthetic oestrogens is detailed in Box A6.3 below.

BOX A6.3. TIER 0 HAZARD ASSESSMENT

There is abundant animal and human data to support a primary emphasis for adverse effects from exposure to environmental oestrogens on in utero exposure during critical periods of organogenesis (Fisch et al., 2000). Several adverse health effects have been proposed to be linked to in utero exposure to environmental oestrogens including; adverse effects on male reproductive tracts, male and female fertility problems, testicular cancer, prostate cancer, breast cancer, endometriosis, learning disability or delay, alterations of sexual behaviour, immune system effects and thyroid effects (Fisch et al., 2000; Cruz et al., 2014). Other potentially sensitive subpopulations include prepubescent males who have naturally low serum oestrogen levels and a low metabolic clearance rate (Caldwell et al., 2010).

There are four independently derived Acceptable Daily Intake (ADI) values in the literature for E2 (Caldwell et al., 2010).

- 3 µg E2/person/day (from value of 0.05 µg E2/kg bw/day based on changes in hormone-dependent parameters in humans for 60 kg adult - WHO, 2000).
- 0.15 µg E2/person/day based on threshold of toxicological concern - TTC approach (Caldwell et al., 2010).
- 0.02 µg E2/person/day based on occupational exposure limits (Caldwell et al., 2010).
- 3 µg E2/person/day based on Australian water reuse guidelines.

As the lowest value, an ADI of 0.02 µg E2/person/day is the most conservative for use in the risk assessment at tier 0.

A6.4 RISK CHARACTERIZATION

The risk characterization step for natural and synthetic oestrogens is detailed in Box A6.4 below.

BOX A6.4. RISK CHARACTERISATION

A margin of exposure (MOE) can be determined by dividing the ADI, derived in the hazard assessment, by the theoretical maximum level present in drinking-water, derived in the exposure assessment.

For the oestrogens group the worst case MOE is equivalent to 119 for adults and 238 for children.

Is the MOE sufficient for each group?

At this low tier of the risk assessment framework, it would be precautionary to allow an MOE of 1000 when determining whether further assessment at higher tiers is needed. This approach has been used previously in assessing the risks of individual pharmaceuticals in drinking-water in the UK and has been accepted as being precautionary by the medical profession. It also provides an additional reassurance with regard to infants and young children.

Under worst case assumptions, the MOE for the oestrogens group is insufficient for both adults and children and further evaluation at higher tiers needs to be conducted.

A6.5 HIGHER TIER RISK ASSESSMENT OF THE OESTROGENS GROUP

As the outcome of the tier 0 risk assessment has shown the MOE to be insufficient (i.e. <1000), the framework allows continuation with iterative refinement using more complex exposure and hazard models. For example, refined exposure assessments can be carried out for the oestrogens group by incorporating DWTW removal rates, as described in Box A6.5 below, allowing a revised risk characterization.

BOX A6.5. HIGHER TIER EXPOSURE ASSESSMENT (TIER 1) AND REVISED RISK CHARACTERIZATION

The Table shows available data for the oestrogen groups which can be utilised in a higher tier exposure assessment in addition to tier 0 data. As these compounds are lipophilic they will tend to bind to particles so removal in drinking-water treatment with coagulation and filtration would be expected to be high (>80%), further reducing the possible concentrations in drinking-water.

	E1	E2	E3	EE2
DWTW removal (%)*	>80	>80	>80	>80
Metabolism	80	60	0	50

*with chlorine treatment only

Using these less conservative assumptions, PECs for E1, E2, E3 and EE2 in drinking water were estimated as 0.02, 0.005, 0.009 and 0.0003 ng/L respectively. The group total E2-eq was equal to 0.017 ng/L, giving an estimated intake of 0.034 ng E2-eq/person/day for adults and 0.017 ng E2-eq/person/day for children.

Risk characterization

Using the figures obtained from the refined exposure assessment and the precautionary ADI, the MOE is equivalent to 1538 for adults and 3125 for children.

Under the refined assumptions, the MOE for the oestrogens group is sufficient for both adults and children and further evaluation at higher tiers is not required.

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APPENDIX 7

DETAILED CASE STUDY FOR THE USE OF THE WHO/IPCS FRAMEWORK TO ASSESS RISK TO HUMAN HEALTH FROM EXPOSURE TO N-METHYL CARBAMATE INSECTICIDES IN FOODS

This case study is designed to assess risk from the combined exposure to N-methyl carbamate (NMC) insecticides in foods and was presented at an international workshop (OECD[19], 2011). It should be noted that although exposure data appropriate to higher tiers are available for NMCs, lower tiers are included for illustrative purposes.

A7.1 PROBLEM FORMULATION

The problem formulation step for this case study is detailed in Box A7.1 below.

BOX A7.1. PROBLEM FORMULATION

What is the nature of exposure? Are the key components known?

This assessment refers to the NMCs registered for food use in the USA (aldicarb, carbaryl, carbofuran, formetanate HCl, methiocarb, methomyl, oxamyl, pirimicarb, propoxur and thiodicarb).

Exposure may take place in the following ways:

- dietary exposure to carbamate insecticides on food or in drinking water (this may be to only one substance or to multiple carbamates);
- occupational exposure during application of an insecticidal formulation (this may be on crops or in a residential or other setting); or
- residential exposure through uses on lawns and gardens, or on pets.

Is exposure likely taking into account the context?

Yes. Quantifiable residues of some of these NMCs have been measured in food monitoring programmes in the USA (USDA, 2007). NMCs are relatively stable in water, and it is likely that some of the applied amounts will be present in the drinking-water after either agricultural or residential application. As three of the NMCs are registered for residential uses, there is also the potential for exposure to NMC residues. However, the potential for exposure to NMCs through either drinking-water or residential exposure is not considered further here.

Is there a likelihood of co-exposure within a relevant timeframe?

Yes. A wide variety of fruit, vegetable and grain crops are treated each year with NMC insecticides. Given the high use of NMC insecticides, the large number of crops treated, and the frequency with which NMC insecticides are detected in monitoring programmes, the possibility of exposure to multiple carbamate insecticides from multiple foods is feasible.

What is the rationale for considering compounds in a common assessment group?

The NMC pesticides all act, in both target insects and in mammals, by a rapidly reversible inhibition of the acetylcholinesterase enzyme. Although organophosphate pesticides such as chlorpyrifos also inhibit acetylcholinesterase, the NMCs can be differentiated as they cause rapid onset of effects as well as rapid recovery following inhibition (in comparison to the largely non-reversible manner in which organophosphates act).

It is appropriate to consider them both individually and as a group.

19 Adapted from International workshop on risk assessment of combined exposures to multiple chemicals: Workshop report, 15-16 February 2011, Series on Testing & Assessment No. 140, WHO, OECD, ILSI/HESI, Copyright OECD (2011). Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2011)10&doclanguage=en

A7.2 **TIER 0 RISK ASSESSMENT**

A7.2.1 Exposure assessment

An exposure assessment at tier 0 for a combined exposure of NMCs in foodstuffs is detailed in Box A7.2 below.

BOX A7.2. TIER 0 EXPOSURE ASSESSMENT

Semi-quantitative exposure assessment is carried out for the ten pesticides considered to present a potential for exposure in food stuffs. Estimates of dietary intake can be derived for all age groups using the Dietary Exposure Evaluation Model and Food Intake Surveys (e.g. in the USA, Continuing Survey of Food Intakes by Individuals from 1994-1996 and the 1998 Children's Survey). Food consumed can then be converted to amounts of agricultural commodities and residues within the commodities calculated based on the USDA Pesticide Data Program monitoring results.

As a worst case scenario, exposure estimates are presented below for each pesticide for children weighing 10 kg (< 1 year as the population group of greatest concern) and consuming 1 kg of food per day using the 95th and 99th percentiles of exposure.

NMC	Estimated exposure (mg/kg bw/d)	
	95th percentile	99th percentile
Aldicarb	0.000029	0.0000136
Carbaryl	0.0000706	0.0001919
Carbofuran	0.0000041	0.0000091
Formetanate HCl	0.0089488	0.0146534
Methiocarb	0	0
Methomyl	0.0000307	0.0000573
Oxamyl	0.0000229	0.000839
Pirimicarb	0.0002215	0.0003945
Propoxur	0	0
Thiodicarb	0	0

A7.2.2 Hazard assessment

The tier 0 hazard characterization step for NMCs is detailed in Box A7.3 below.

BOX A7.3. TIER 0 HAZARD ASSESSMENT

Although there is abundant animal and human data on the hazards of the NMCs, the threshold of toxicological concern (TTC) concept can be used at lower tiers as a conservative preliminary rapid screen to determine whether further assessments are needed for a common mechanism group. The TTC approach is first used to provide a conservative hazard estimate based on exposure.

For each NMC, the similarity in structure results in a Cramer Class 3 value of 0.15 mg/kg bw/day. Incorporation of a standard 100x safety factor for inter- and intra-species uncertainty results in a TTC of 0.0015 mg/kg bw/day for each of the NMCs.

The TTC value for each NMC and the estimated daily exposure for each compound can be used to determine a cumulative hazard index (HI), as shown below.

NMC	Hazard quotient TTC*	
	95th percentile	99th percentile
Aldicarb	0.0193	0.0091
Carbaryl	0.0471	0.1279
Carbofuran	0.0027	0.0061
Formetanate HCl	5.9659	9.7689
Methiocarb	0	0
Methomyl	0.0205	0.0382
Oxamyl	0.1527	0.5593
Pirimicarb	0.1477	0.2630
Propoxur	0	0
Thiodicarb	0	0
Cumulative HI	**6.36**	**10.77**

* – division of estimated exposure by TTC

A7.2.3 Risk characterization

The tier 0 risk characterization step for NMCs is detailed in Box A7.4 below.

BOX A7.4. TIER 0 RISK CHARACTERIZATION

Under worst case assumptions and using the conservative TTC approach, the cumulative HI clearly exceeds 1 whether the 95th or the 99th percentile value is used. Further evaluation at higher tiers is therefore required.

A7.3 TIER 1 ASSESSMENT

A7.3.1 Exposure assessment

An exposure assessment at tier 1 for a combined exposure of NMCs in foodstuffs is detailed in Box A7.5 below.

BOX A7.5. TIER 1 EXPOSURE ASSESSMENT

At tier 1, the exposure assessment is refined using conservative point estimates of exposure. For the NMCs, the estimated consumption values calculated in tier 0 can be compared with established reference values such as the acute reference dose (ARfD), maximum residue level (MRL) or tolerances, to establish what proportion of the reference is being consumed. Note that only seven NMCs are included in this tier as three NMCs were not found to be present in food stuffs in the tier 0 exposure assessment.

As a worst case scenario, data for children and infants are given below.

NMC	Reference value (mg/kg bw/day) and source study	Age group	% of reference value consumed
Aldicarb	0.001 (human)	Children 1-6 years	19
Carbaryl	0.01 (rat)	Children 1-2 years	68
Formetanate HCl	0.00065 (rat)	Infants < 1 year	56
Methomyl	0.02 (rabbit)	Infants < 1 year	27
		Children 1-6 years	72
Oxamyl	0.001 (rat)	Children 1-6 years	81
Pirimicarb	0.01 (rat)	Children 1-2 years	10
		Children 1-6 years	7
Thiodicarb	0.01 (rat)	Children 1-6 years	31
		Infants < 1 year	60

It is possible for a varied diet to contain significant proportions of the acute reference doses of compounds within the same common mechanism group; for example, children 1-6 years of age could consume 19% of the ARfD for aldicarb by eating citrus, sweet potatoes or potatoes, 81% of the ARfD for oxamyl by eating citrus, sweet potatoes, cucumber or apple and 7% of the ARfD for pirimicarb through consumption of asparagus and leafy petiole crops such as celery.

A7.3.2 Hazard assessment

The tier 1 hazard characterization step for NMCs is detailed in Box A7.6 below.

BOX A7.6. TIER 1 HAZARD ASSESSMENT

In Tier 1, the hazard assessment calls for refining the potency of the individual members of the common mechanism group, as the potency of NMC insecticides is not equivalent. Relative potency factors (RPFs) based on an index compound therefore provide a more refined assessment of hazard.

For this NMC example, RPFs were calculated using benchmark dose modelling data (BMD_{10} and $BMDL_{10}$) reported by the USEPA (2007) and Oxamyl as the index compound as this has the most robust toxicological database of the NMCs under consideration. It should be noted that BMD and BMDL data was used at this level as it was available; however, it may be more complex than is usually required at this tier.

Following calculation of the RPF, additional safety factors are applied to account for inter-species differences; in this example, where human data were unavailable the default value of 10 was applied, however, for some of the NMCs human data was used meaning a lower safety factor could be applied.

Where data existed on the relative susceptibility of juvenile and adult experimental animals, these data were also used in deriving a RPF for children. In the cases where no comparison between juveniles and adults was available, a standard 10-fold safety factor (FQPA safety factor) was applied for children. This additional safety factor is a statutory requirement in the USA and differs from current standard requirements in other markets and from other regulatory agencies. The adjusted inter-species and FQPA safety factors, as well as the resulting RPFs of each compound for children and adults, are shown below.

NMC	Oral RPF	Adjusted RPF Children (IS-SF; FQPA SF)	Adjusted RPF Adult (IS-SF)
Aldicarb	4	16 (2;2)	8 (2)
Carbaryl	0.15	2.7 (10; 1.8)	1.5 (10)
Formetanate HCl	2.18	44 (10; 2.03)	22 (10)
Methomyl	0.67	10 (5; 3.05)	3.3 (5)
Oxamyl	1.0	10 (3; 3.48)	3 (3)
Pirimicarb	0.02	2 (10; 10)	0.2 (10)
Thiodicarb.	0.89	89 (10; 10)	8.9 (10)

IS-SF: inter-species safety factor; FQPA SF: The food quality protection act safety factor (USA specific).

A7.3.3 Risk characterization

The tier 1 risk characterization step for NMCs is detailed in Box A7.7 below.

BOX A7.7. TIER 1 RISK CHARACTERIZATION

There is the possibility for significant exposure to residues of multiple NMCs in one day, even if consumption of each individual compound is below the reference dose.

The tier 1 exposure assessment does not provide sufficient margin of exposure (MOE) and further assessment of exposure is needed. However, as a refined assessment of hazard has been used at tier 1, further hazard assessment may or may not be needed.

A7.4 TIER 2 ASSESSMENT

A7.4.1 Exposure assessment

An exposure assessment at tier 2 for a combined exposure of NMCs in foodstuffs is detailed in Box A7.8 below.

BOX A7.8. TIER 2 EXPOSURE ASSESSMENT

At tier 2, refinement of exposure could include use of actual measured data. For the NMC insecticides, or for any group of food-use pesticides, source data could include that available from the programmes such as the USDA Pesticide Data Program on detection of pesticide residues and metabolites in dietary components.

In an original evaluation of combined risk, this assessment would be conducted before moving on to probabilistic estimates of exposure. However, if a probabilistic exposure assessment (such as the USEPA cumulative risk assessment of the NMC insecticides) is available, this should be used as the data source.

Note - this should not be taken as an indication that such highly refined estimates of exposure are called for in all cases; this is simply a use of existing data in order to test the WHO/IPCS framework.

A7.4.2 Hazard assessment

The tier 2 hazard characterization step for NMCs is detailed in Box A7.9 below.

BOX A7.9. TIER 2 HAZARD ASSESSMENT

As a refined hazard assessment was carried out in tier 1, these data require testing against tier 3 exposure assessments prior to any further refinement. If the tier 1 refinement of hazard assessment proves not to be sufficient when tested in tier 3, additional refinements can be conducted.

A7.4.3 Risk characterization

The tier 2 risk characterization step for NMCs is detailed in Box A7.10 below.

BOX A7.10. TIER 2 RISK CHARACTERIZATION

Ordinarily, a complete tier of both exposure and hazard assessment would not be omitted. However, as the data on which this assessment is based begin with very generic exposure estimates and move directly to probabilistic exposure assessment of dietary intake, the exposure assessment provided for at this tier has been omitted. Similarly, hazard assessment will not be further refined until it is clear whether such refinement is needed or not.

A7.5 TIER 3 ASSESSMENT

A7.5.1 Exposure assessment

An exposure assessment at tier 3 for a combined exposure of NMCs in foodstuffs is detailed in Box A7.11 below.

BOX A7.11. TIER 3 EXPOSURE ASSESSMENT

At tier 3, exposure assessments can be refined using data for food consumption (obtained from surveys such as the USDA food consumption survey), in conjunction with pesticide residue information (obtained from monitoring programmes such as the USDA pesticide data programme). Unlike the data used in tier 0 or 1, this represents a more realistic picture of actual exposure.

The USEPA cumulative risk assessment of the NMC insecticides used such data in combination with Monte-Carlo simulations to produce probabilistic assessments of exposure to NMC insecticides at the 95th, 99th, and 99.9th percentile for each age group, as shown below. The concentrations for each compound were expressed as equivalents to the index chemical, rather than as residues of each specific compound.

Age Group	95th percentile	99th percentile	99.9th percentile
	mg/kg bw/day		
General population	0.0004	0.0023	0.0115
Infants < 1 year	0.0005	0.0024	0.0106
Children 1–2 years	0.0013	0.0051	0.0229
Children 3–5 years	0.0010	0.0044	0.0209
Children 6–12 years	0.0006	0.0028	0.0145
Youth 13–19 years	0.0003	0.0017	0.0098
Adults 20–49 years	0.0001	0.0008	0.0042
Adults 50+ years	0.0002	0.0009	0.0044
Females 13–49 years	0.0004	0.0019	0.0101

A7.5.2 Hazard assessment

The tier 3 hazard characterization step for NMCs is detailed in Box A7.12 below.

BOX A7.12. TIER 3 HAZARD ASSESSMENT

As a refined hazard assessment was carried out in tier 1, these data were tested against tier 3 exposure assessments to derive an MOE for each age group at the 95th, 99th, and 99.9th percentile, as shown below.

Age Group	95th percentile	99th percentile	99.9th percentile
	MOE		
General population	404	79	15
Infants < 1 year	342	74	16
Children 1–2 years	141	35	7.9
Children 3–5 years	185	40	8.6
Children 6–12 years	323	63	12
Youth 13–19 years	576	106	18
Adults 20–49 years	1278	236	42
Adults 50+ years	1035	193	40
Females 13–49 years	505	97	17

A7.5.3 Risk characterization

The tier 3 risk characterization step for NMCs is detailed in Box A7.13 below.

BOX A7.13. TIER 3 RISK CHARACTERIZATION

An MOE of > 10 is considered to be protective. For the majority of age groups and exposure levels, this is achieved. Although predicted exposure of children between 1 and 5 years of age exceeds the MOE of 10 at the 99.9th percentile level of protection, for these age groups the MOE is equal to 10 at the 99.8th percentile (not shown) and is therefore not considered to be of concern.

This highly refined assessment at tier 3 demonstrates that there is no concern for combined dietary exposure to N-methyl carbamate insecticides.

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