Environment Agency

UK Health Security Agency

Evaluation of the potential approaches to risk assessment of unintentional chemical mixtures for future UK REACH assessments

Chief Scientist's Group report

August 2022

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Email: research@environment-agency.gov.uk

Author(s):
Claire Massey (EA), Ruth Bevan (IEH Consulting) and Helen Nakeeb (UKHSA)

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Environment Agency's Project Manager:
Claire Massey, Chemicals Assessment Unit

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Dr Jo Nettleton
Chief Scientist

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Executive summary

There is general international agreement that exposure to mixtures of chemicals has the theoretical potential to result in adverse effects in both humans and the environment, where individually the chemicals are below concentrations of concern. However, the current risk assessment approach under UK REACH considers each chemical in isolation. The issue has therefore been raised that the current risk assessment approach for industrial chemicals may not be sufficiently precautionary.

For unintentional mixtures in environmental media, the exact combination of chemicals and their varying concentrations over time and at different spatial scales is not known or generally predictable. Moreover, comprehensive hazard information, including information on toxicological mode of action, is not available for the vast majority of individual substances. A realistic risk assessment for unintentional mixtures is therefore not feasible in most cases.

This report summarises the current risk assessment approach under UK REACH and provides an overview of the methods available to consider mixture risk. When considering potential mixture risk, there is general agreement that use of concentration addition as a first approach (e.g. as a screening tool) will in most instances give a conservative initial estimate of any potential mixture toxicity. Assessment schemes of varying complexity have been suggested to address the question of unintentional mixtures occurring in environmental media, but recently the use of a Mixture Assessment Factor (MAF) has been proposed for to protect against potential mixture risk.

Although the human health and environmental studies reviewed in this report include a varying number and type of chemicals across different geographical areas and human populations, and used a variety of (eco)toxicology data for comparison, the consistent conclusion is that only a relatively small number of substances seem to be responsible for the majority of the potential risk from unintentional mixtures.

It is practically impossible to identify in advance which specific substances have the potential to contribute most to mixture risk, as this varies depending on the specific sites, time points and populations investigated. In the absence of evidence to identify a particular subset of chemicals that are driving the potential mixture risk, the application of a MAF under UK REACH could be a pragmatic and precautionary way forward, if policy makers decide such an approach is necessary. Alternative approaches to mixture risk assessment require additional data and resource before they can be applied, and the use of a MAF would retain the principle that it is the responsibility of the Registrant to demonstrate that risks are adequately controlled.

Based on six environmental studies, four of which included industrial chemicals, a MAF of 5 appears to be appropriate and protective for environmental risk assessment for the majority of surface water situations. This could be restricted to certain sub-categories of substance (e.g. those that are more ecotoxic or are supplied at higher tonnage in wide dispersive uses), if it were decided that a MAF is not appropriate for all substances. No data were identified to allow a MAF value to be recommended for the sediment and soil

compartments. If the UK decides to further consider the use of a MAF for the environmental risk assessment under UK REACH, then additional studies would be required to determine the appropriate value of the MAF (and its application domain) for all compartments, and to conduct an impact assessment.

In contrast, due to the limited availability of studies further investigating the possibility of a mixture risk from the initial screening analysis based on concentration addition, it is not considered appropriate at this time to further derive a MAF for human health risk assessment purposes. Human health risk assessment differs from environmental risk assessment as it based on levels that do not cause an adverse effect in individuals over a lifetime/critical window, whereas environmental risk assessment is based on population level effects. This means that there is more precaution already present in the individual chemical assessment for human health than for environmental risk assessment and in addition, the use of uncertainty/assessment factors in the derivation of Health Based Guideline Values (HBGVs) mean that any potential mixture risk may already be mitigated. An assessment of the limited available evidence systematically and in an unbiased way in order to identify knowledge gaps which can be addressed by targeted research would be beneficial.

Finally, it should be noted that there is no clear evidence that industrial chemicals contribute to the potential mixture toxicity risk more than chemicals regulated under other regimes. Policy makers will therefore need to consider whether applying a MAF to industrial chemicals alone can be justified.

1 The need to consider unintentional chemical mixtures

Chemical regulation is generally based on an assessment of the hazards and risks posed by single substances whilst, in reality, both people and wildlife are continually exposed to combinations of chemicals both at a single time point and across their lifetimes. It was recently estimated that there are over 350,000 chemicals in current use across the globe (Wang *et al*., 2020), which suggests the range of potential mixtures is extremely large, increasing further if transformation products and naturally occurring substances are included. While a risk assessment may demonstrate that exposure to a single particular chemical is below an acceptable concentration, it remains possible that exposure to the combined concentration of all the chemicals that an organism is exposed to could cause adverse effects.

There is broad international agreement that exposure to mixtures of chemicals has the potential to result in adverse effects in both humans and the environment. The Council of the European Union (2009) acknowledged that exposure to combinations of chemicals can have “serious negative implications for human health and the environment” and a recent paper from the Hazardous Substances Advisory Committee (Matthiesen and Depledge, 2020) concluded that there is “compelling evidence that mixtures of environmental chemicals make a significant contribution to the continuing loss of biodiversity”. The European Council paper *Sustainable Chemicals Policy Strategy of the Union,* adopted by EU Member States (including the UK) in June 2019, committed the European Commission to address combination effects of chemicals (Council of the European Union, 2019). It is therefore necessary to determine the level of risk from unintentional mixtures of chemicals to ensure that any additional risk management measures are sufficient to provide protection.

The exact combination of chemicals and their varying concentrations over time and at different spatial scales is not known or generally predictable. Although environmental regulatory authorities can monitor a much larger group of substances than ever before, this effort entails a cost, tends to focus on water only, and is still limited in terms of sampling locations and the number of chemicals monitored compared with the numbers of substances potentially in the environment. Work to detect larger numbers of chemicals in human samples has only recently begun. Moreover, comprehensive hazard information is not available for the vast majority of individual substances. A realistic risk assessment for unintentional mixtures is therefore not feasible in most cases.

Assessment schemes of varying complexity have been proposed to address the mixture question in the past (e.g. IGHRC, 2008; Meek *et al*., 2011; Price *et al*. 2012a; WHO, 2017; OECD, 2018) and the European Union (EU) has recently been debating the potential use of a Mixture Assessment Factor (MAF) as part of the risk assessments of industrial and consumer chemicals under the Restriction, Evaluation, Authorisation and restriction of CHemicals (REACH) Regulation. Now that the UK has left the EU, the UK Government may need to develop its own view on this topic.

This initial scoping report includes:

- An overview of the current approach to the risk assessment of chemicals under UK REACH;
- The methods available to consider mixture risk;
- A critical review of the evidence on the level of risk from unintentional mixtures;
- An exploration of the pros and cons of using a MAF, including implications for testing and practical and legal consequences;
- A critical evaluation of other possible approaches, based on existing EU guidance (e.g. from the European Food Safety Authority (EFSA)) or under development in other parts of the world;
- A scientific recommendation on the most appropriate way forward to address mixture risks under UK REACH.

2 The current approach to mixture risk under UK REACH

2.1 Registration and Evaluation

Under EU and UK REACH, the hazard and risk from each registered substance is assessed individually. Registrants of the same chemical are required to work together to produce a single joint registration dossier containing the relevant hazard data. If a substance is registered at an annual supply level above 10 tonnes and there is a hazard identified, an exposure assessment is also needed to demonstrate that the substance does not pose an unacceptable risk to human health and the environment. A Registrant is only responsible for demonstrating safe use for the amount of substance they place on the market, although the total amount of substance used by all actors in the supply chain is considered if a Substance Evaluation is performed by the regulator. There is no assessment of the potential risk to human health or the environment from mixtures of chemicals under REACH, although the hazard classification of chemicals placed on the market as mixtures does need to be considered (Section 2.4).

In order to conduct the risk assessment for single substances, levels at which adverse effects would not be expected, or would be minimal, are usually calculated. For human health, such levels are termed health-based guidance values (HBGVs) and under REACH specifically Derived No Effect Levels (DNEL) for threshold endpoints or Derived Minimal Effect Levels (DMELs) for non-threshold endpoints. For the environment, Predicted No Effect Concentrations (PNEC) are used.

For human health, DNEL/DMEL(s) should be established reflecting the substance's likely route(s), duration and frequency of exposure. In addition, it may be necessary to derive DNEL/DMEL(s) for each relevant human population (e.g. consumers or workers) and vulnerable groups (e.g. pregnant women or children). Results derived from toxicity studies (such as the 'no observed adverse effect level' or NOAEL) are divided by assessment factors to calculate the DNEL or DMEL. Assessment factors are numerical values used to account for some of the uncertainties that are inherent in the assessment process; ECHA (2012) lists these as:

- Interspecies differences
- Intraspecies differences
- Differences in duration of exposure
- Issues related to dose-response
- Quality of the whole database

More than one assessment factor can be applied when deriving a DNEL to reflect the uncertainty in the available dataset for a substance. ECHA's guidance on deriving DNELs states that the value for each individual assessment factor should preferably be based on

substance-specific information. However, in practice the available data are often limited (especially toxicodynamic data, and human data); therefore, default assessment factors usually need to be applied. The default assessment factors for deriving a DNEL from animal data are summarised in Table 2.1.

Table 2.1 Default assessment factors for deriving DNELs from animal data

Assessment factor accounting for differences in:		Default value for systemic effects	Default value for local effects
Interspecies	Correction for differences in metabolic weight per body weight	Allometric scaling value	-
	Remaining differences	2.5	1 or 2.5 depending on endpoint
Intraspecies	Worker	5	5
	General population	10	10
Exposure duration	Sub-acute to sub-chronic	3	3
	Sub-chronic to chronic	2	2
	Sub-acute to chronic	6	6
Dose response	issues related to reliability of the dose-response, incl. LOAEL/NOAEL extrapolation and severity of effect	1	1
Quality of the whole database	issues related to completeness and consistency of the available data	1	1
	issues related to reliability of the alternative data	1	1

For the environment, results derived from ecotoxicity studies (such as the median lethal concentration, LC_{50}) are divided by an assessment factor to calculate the PNEC. The size of the assessment factor depends on the environmental compartment, the amount of ecotoxicity data available and whether the results are from acute or chronic studies. For the freshwater environment, assessment factors can range from 1000 (when only acute data are available) to 1 (when a sufficiently large number of chronic endpoints are available that allow a statistical method to be used to calculate the PNEC). The assessment factors are used to account for some of the uncertainties that are inherent in the assessment process, listed by ECHA (2008) as:

- Intra- and inter-laboratory variation of toxicity data
- Intra- and inter-species variations (biological variance)
- Short-term to long-term toxicity extrapolation
- Laboratory data to field impact extrapolation

The assessment factor allocated to each uncertainty is not specified as for the human health default assessment factor values. For the consideration of risk to both human health and the environment, the use of assessment factors accounts for some of the uncertainties in the approach but is not explicitly intended to address the possibility of unintentional mixture effects.

To conduct the risk assessment, the DNEL and PNEC are compared to predicted or measured exposure concentrations for the various use scenarios. If the exposure concentration is lower than the DNEL or PNEC then the risks are considered acceptable (i.e. the risk characterisation ratio (RCR) is below 1). Exposure modelling works on a tiered approach, with initial exposure estimates based on conservative assumptions that can be refined with additional information iteratively. As for the hazard assessment, the assumptions are intended to account for some of the uncertainties in the exposure modelling (in particular, they typically represent a ‘reasonable worst case’ situation), but are not intended to account for possible effects of mixtures.

2.2 Restrictions

Although REACH applies to individual chemicals, there are several examples where restrictions have been applied to groups of chemicals. Restrictions that apply to groups can be used to address risks resulting from combined exposure to members of that group, but have the additional benefit that they reduce the likelihood of regrettable substitution, where one substance is replaced on the market by another with similar (or worse) health or environmental concerns.

Some restriction groupings are based on a common transformation product (e.g. metals or formaldehyde and formaldehyde releasers) or limits that apply to all substances with a particular hazard (e.g. skin sensitisers, irritants and/or corrosive substances). As these are not specifically relevant to the assessment of mixtures of different substances they are not considered further here.

A restriction has been applied to a group of four phthalates (di (2-ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP), dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP)) limiting their concentration both individually and combined in toys and childcare articles and indoor articles. As part of the published European Chemical Agency (ECHA) opinion, individual DNEL and exposure estimates were derived for each phthalate and the risk from each assessed separately before the individual risks were summed to provide a total risk (ECHA, 2017a).

The ECHA opinion on the Annex XV dossier proposing restrictions on substances used in tattoo inks and permanent make-up has explicitly included an additional assessment factor when deriving a DNEL for a mixture of reprotoxic substances (ECHA, 2019). When considering how to set an appropriate concentration limit for reprotoxic substances in these products, the lowest reliable effect concentration was selected from the datasets for chemicals known to be detected in tattoo inks and standard assessment factors applied to derive the DNEL. An additional factor of 10 was then applied to account for "*mixture/cumulative effects and uncertainties, possibility of combined effects of several reprotoxicants present in tattoo inks with the same mode of action, including ED* [endocrine disruption] *effects and the possibility that more potent substances may be present in tattoo inks*".

2.3 Substances of Very High Concern

The possibility of mixture effects has been included as one line of justification for identifying Substances of Very High Concern (SVHCs) under EU REACH, especially for substances that are extremely persistent. For example, Annex XV reports for 1,4-dioxane (ECHA, 2021) and several per- and polyfluoroalkyl substances (PFAS) including perfluorohexanoic acid (PFHxA) (ECHA, 2018) include the potential for mixture toxicity effects to argue that the long-term effects of these substances are unknown. The arguments made around mixture toxicity are qualitative and could similarly apply to any substance(s) to which an organism is exposed.

In addition, the majority of substances identified as SVHCs, such as those with persistent, bioaccumulative and toxic (PBT) properties, are assumed to have no acceptable exposure threshold. In this situation, a risk is assumed to exist if there is any exposure, and a mixture risk assessment would not alter this conclusion.

2.4 Classification and Labelling

Both individual substances and mixtures need to be classified appropriately under Regulation 1272/2008 on Classification, Labelling and Packaging (CLP) and its UK equivalent. A tiered approach to mixture classification is used (ECHA, 2017b):

- If reliable and relevant data are available on the mixture itself then this is used for classification.

- If not, then data on similar mixtures or individual components could be used by applying the bridging principles.
- Finally, the concentration of individual components together with their classification or hazard data can be used to classify the mixture.

For the purposes of this report, how mixtures are classified based on the composition of the mixture and the toxicity of each component is most relevant. This is also the approach most frequently used to classify mixtures because test data on the mixture itself or similar mixtures is often not available.

For human health, the method used to classify a mixture depends on the hazard class being considered and usually involves a calculation approach or concentration thresholds referring to the classified substances present in the mixture.

For the majority of human health hazard classes, classification is based on concentration thresholds. Specific concentration limits (SCL) and generic concentration limits (GCL) are limits assigned to a substance which specify a threshold at or above which the presence of that substance in a mixture leads to the classification of the mixture for the hazard class being considered. SCLs are established for some individual substances based on the available data for the substance, whereas GCLs are generic for a hazard class, differentiation or category.

An additivity approach is applied for some, but not all hazard classes under CLP. For the following human health hazard classes an additivity approach is usually not applicable:

- Skin and respiratory sensitisation
- Germ cell mutagenicity
- Carcinogenicity
- Reproductive toxicity
- Specific target organ toxicity, single and repeated exposure, categories 1 and 2
- Skin corrosion/irritation (in certain cases)
- Serious eye damage/eye irritation (in certain cases)

In these cases, if a mixture contains two substances which are each present at a concentration below the GCL defined for the hazard class or differentiation being considered, the mixture will not be classified, even if the sum of the substances' concentrations is above the GCL. However, in certain cases expert judgement can be used to identify when an additivity approach may be scientifically justified for these hazard classes (e.g. if the mode of action is the same for more than one ingredient in the mixture).

An additivity approach is usually applied for the following human health hazard classes:

- Acute toxicity (using calculation method)
- Skin corrosion/irritation
- Serious eye damage/eye irritation

- Specific target organ toxicity, single exposure Category 3 (respiratory tract irritation or narcotic effects)
- Aspiration hazard

For these hazard classes/categories, the mixture should be classified for the hazard if the sum of the concentrations of one or several substances classified for the same hazard class/category in the mixture equals or exceeds the GCL set out for this hazard class/category.

For the acute toxicity hazard class an additivity approach using a calculation is applied. This is based on acute toxicity estimates (ATE) (which reflect the potency of the substance and are derived from acute toxicity studies) and concentrations of the ingredients. There is also a modified formula for determining the classification of a mixture containing substances of unknown acute toxicity. Further details on the acute toxicity additivity formula and worked examples are given in ECHA (2017b).

For the environment, the CLP Regulation assumes that each component in a mixture present above a cut-off value will contribute to the aquatic toxicity in an additive manner. The 'relevant components' are those classified as Aquatic Acute 1 or Aquatic Chronic 1 (i.e. the most toxic categories) which are present at a concentration above 0.1% divided by their respective acute or chronic "multiplying (M) factor" where one has been applied to the component, plus those classified as Aquatic Chronic 2, Chronic 3 or Chronic 4 which are present above 1% by weight. M factors are defined based on the toxicity of a substance and their use gives increased weight to the concentration of highly toxic components in the mixture. Components that do not have an aquatic classification are assumed to not contribute to the toxicity of the mixture. The appropriate mixture classification can be derived either by using the composition of the mixture and the classification of each component (the summation approach), or the composition of the mixture and the raw ecotoxicity data for each component (the additivity approach). Worked examples of both methods are given in ECHA (2017b).

3 Methods to assess the risk from mixtures

Other than in some specific cases, such as those described in Section 2, REACH generally assesses the risk due to each individual substance separately. Intuitively there is a concern that the risk to either the environment or human health may be greater when mixtures are considered. Even in a situation where all individual substances are present below their thresholds of concern, the mixture risk can potentially result in adverse effects (Carvalho *et al.*, 2014). Although the assessment of mixture risk is not required under REACH, UK scientific expert committees have kept up to date with the available approaches and published on this topic (e.g. UK COT, 2002; UK COC, 2020; Matthiesen and Depledge, 2020).

Several methods have been proposed and used to estimate the risks posed by mixtures of chemicals but are generally based on two main approaches; concentration addition (CA) or independent action (IA).

3.1 Concentration Addition

Concentration Addition (CA), also referred to as Dose Addition, is based on an assumption that the chemicals in a mixture have the same mode of action but may have different levels of potency. The mixture effect can be predicted by summing the exposure concentration of the chemicals after adjusting for their potency as necessary. Potency is based on a single common (eco)toxicity endpoint and CA assumes that effects are linearly related to exposure. CA can be calculated as (COT, 2002):

R(x) is the dose-response function of two similarly acting compounds A and B, the response for a mixture standardised dose x_A of A and x_B of B is $R(x_A + x_B)$.

CA is the basis of mixture toxicity concepts such as Toxic Units (TU), the Hazard Index (HI), Risk Quotients (RQ), Relative Potency Factors (RPF) and Toxic Equivalency Factors (TEF). It is also the basis for the approach used under CLP for those endpoints that are considered to have additive toxicity (Section 2.4).

When CA is assumed mixture toxicity can be calculated as:

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = mixture\ toxicity$$

Where for a mixture of n substances, substance i contributes to the mixture toxicity based on its concentration (c_i) and its effect concentration (ECx_i). The sum gives the toxicity of the mixture relative to the mixture effect concentration x, such that a value of 1 indicates that exposure to the mixture would result in an effect of x%.

For environmental assessments, the ratio of c_i:ECx_i is often referred to as a Toxic Unit (TU) and is generally calculated using the median effect concentration (EC_{50}). It allows easy comparison of the relative contribution that each chemical makes to total toxicity.

When calculating TUs, Backhaus and Faust (2012) highlight the importance of using the same biological endpoint and taxon (fish, invertebrate or algae) for each substance in the mixture. They propose that separate TUs should be calculated for each biological endpoint and taxon before selecting the highest sum of TUs to assess the mixture toxicity. However, this requires a basic set of standard acute toxicity data for all components of the mixture as a minimum.

The derivation of a HI or RQ follows a similar approach except these metrics use levels at which adverse effects are not expected (e.g. PNEC) instead of EC_x values for each component. Thus the ecotoxicity threshold used already includes an assessment factor, and therefore HI and RQ provide more conservative estimates of mixture risk than TU. Although HI values are regularly reported (e.g. Price *et al*., 2012b), it is difficult to interpret the output as the PNECs for the individual mixture components may be based on different taxa and endpoints and have different assessment factors applied. However, they have been suggested for use as an initial assessment step, as if the total value is below 1 then no effects would be expected (Posthuma *et al*., 2019b).

Risk characterisation methodologies using the component-based dose or concentration addition assumption commonly applied to human health include the HI, Target Organ Toxicity Dose (TTD), the Reference Point Index (RPI; also known as the Point of Departure Index (PODI)), the combined (or total) Margin of Exposure (MOET) (EFSA, 2019a), RPF and TEF.

The HI is calculated from the sum of hazard quotients (HQ) for individual components. HQ is defined as the ratio between exposure to a chemical and the respective health-based guidance values (HBGV) (e.g. acceptable daily intake (ADI), tolerable daily intake (TDI)) and, where reference values are not available, a potency equal to that of the most potent component is assumed. The HI approach is easily applied but provides a conservative estimate of risk as uncertainty (assessment) factors used in the derivation of HBGVs are combined when the HI is calculated (Kortenkamp *et al*., 2009; Meek *et al*., 2011; SCHER, SCCS and SCENIHR, 2012). In addition, HBGVs may have been derived from different study types, with differing endpoints and differing quality. The TTD is a refinement of the HI approach in which end-point specific HIs are calculated, taking into account that different components may have different adverse effects and target organs. Combined risk is considered acceptable when the HI/TTD is lower than a value of 1 (EFSA, 2013a; Kienzler *et al*., 2014).

The RPI (PODI) is calculated as a sum of the exposures to each component expressed as a fraction of their respective reference point (RP) or point of departure (POD) for effects of toxicological relevance (i.e. NOAEL, lowest observed adverse effect level (LOAEL), benchmark dose level (BMDL)) rather than as a fraction of the health-based guidance value (HBGV) (ADI/TDI). An assessment factor is applied (either a default assessment factor or a chemical-specific adjustment factor) to the RPI to account for potential interpretation bias introduced by a combination of individual but different assessment factors. Combined risk is considered acceptable when the RPI (PODI) is lower than a value of 1 (EFSA, 2013a; EFSA, 2013b; EFSA, 2019a).

MOET is derived from individual MOEs, determined as the ratio of the RP or POD to human exposure, with MOET calculated as the reciprocal of the sum of the reciprocals of the individual MOEs (EFSA, 2008; EFSA, 2019a). Although no acceptable MOE has been defined for mixtures of chemicals with a threshold effect, it is widely accepted that for MOEs above 100, the combined risk is acceptable (EFSA, 2019a). Similarly, for mixtures of chemicals that are both genotoxic and carcinogenic no acceptable MOE has been defined (EFSA, 2019b); however, for a single substance, an MOE of ≥10,000 is considered to represent low concern (EFSA, 2005a; EFSA, 2008; Sarigiannis and Hansen, 2012; SCCS, SCHER, SCENIHR, 2012; UK COC, 2012).

Relative Potency Factors and TEFs adjust the concentration of a particular component in a mixture of similar substances based on its relative toxicity to an 'index chemical', which is generally the most potent member of the group. The concentrations of all the components in the mixture can then be summed and interpreted by comparison to the toxicity of the index chemical. This method has been used for dioxins and dioxin-like substances to set World Health Organisation (WHO) thresholds for human health (van den Berg *et al*., 2006) and when considering these compounds as persistent organic pollutants (POPs) (UNEP, 2019).

Price and Han (2011) introduced the concept of Maximum Cumulative Ratio (MCR), which can be applied to any of the CA approaches described above. The MCR is the ratio of the total toxicity to the highest toxicity of any individual component, for example $\Sigma TU/TU_{max}$. An MCR near to 1 indicates that a single substance is driving the mixture risk, whilst a higher MCR indicates that more substances are contributing. The maximum MCR value for any mixture is equal to the number of mixture components, and would indicate that all components contribute equally. Backhaus and Karlsson (2014) note that calculating the true MCR is only possible if all components in the mixture are included in the calculation, so the MCR should be viewed as the minimum ratio when there is incomplete information.

3.2 Independent Action

Independent Action (IA), also referred to as Response Addition, assumes that each chemical acts independently of any other via different modes of action. The mixture toxicity can be calculated based on the toxicity observed when the test organism is exposed to the components individually. The same taxon and biological endpoint should be used for all components, and information on the full dose response is required for each component of the mixture. IA can be calculated as (COT, 2002):

Effect addition is defined by the summation of the effects of each compound in the mixture, i.e. for the previous mixture of A and B, by $R(x_A) + R(x_B) – R(x_A)R(x_B)$.

A full dose response is rarely available for each component, so it is not usually possible to calculate the IA. However, as CA typically gives a higher, more conservative estimate of mixture toxicity than IA, CA can be used as 'worst case' even when components are not thought to share a common mode of action (Kortenkamp *et al*., 2009).

Backhaus and Karlsson (2014) note that the equation for calculating the MCR also gives the maximum difference in the ratio of the IA-predicted EC_{50} for the mixture and the CA-predicted EC_{50} for the mixture. This allows an estimate to be made of the largest factor by which the CA-predicted EC_{50} may overestimate the IA-predicted EC_{50}, although the actual difference may be lower (Junghans *et al*., 2006).

3.3 Combined models

A two-step model that uses both CA and IA has been proposed by De Zwart and Posthuma (2005). Initially, CA is used to estimate the mixture toxicity of the components thought to have the same mode of action, before the mixture toxicity of constituents with different modes of action is estimated using IA.

In order to increase the relevance of this technique to species assemblages, rather than individual species, ecotoxicity data from various species are combined into a Species Sensitivity Distribution (SSD) and this is used as the dose response. The SSD can be based on acute or chronic data and may be calculated using a smaller number of data points and less diversity in taxonomic groups than would be recommended in the REACH guidance when setting a PNEC (ECHA, 2008). Instead of an estimate of an EC_x, effects are described as the Potentially Affected Fraction (PAF), which is the proportion of species expected to be affected at a particular concentration based on the SSD for each substance. When the mixture toxicity is calculated the results are expressed as a Multi-Substance Potentially Affected Fraction (msPAF).

Various studies have investigated the relationship between msPAF and ecological effects observed in the field. It has been found that msPAF based on acute data provides the best prediction of observed effects (see citations in Munz *et al.*, 2017). Similar to the use of the Hazardous Concentration for 5% of species (HC_5) in SSDs derived under REACH (ECHA, 2008), a threshold of 5% msPAF based on chronic data is generally seen as a level at which unacceptable effects would not be expected to be observed in the environment (Posthuma *et al*., 2019a).

3.4 Remaining uncertainty

When studying the effects of mixtures in laboratory tests, exposure to some combinations of chemicals can result in interactions that lead to greater (synergistic) or lesser (antagonistic) effects than would be anticipated based on either CA or IA. A joint European scientific committee report (EU, 2012) considering human health endpoints concluded that such mixture interactions are unlikely to occur or will be toxicologically insignificant at low exposure levels, but may occur at medium or high dose levels.

Martin *et al*. (2021) conducted a systematic literature review to determine whether interactions occur frequently enough to indicate that CA should not be used as an initial assumption when considering mixture toxicity. The review included studies published between 2007 and 2017 and related to both mammalian toxicology and ecotoxicology.

Most of the toxicological studies reported *in vitro* endpoints, with a few more complex *in vivo* studies investigating the effects of mixtures on carcinogenicity, genotoxicity and mutagenicity. The majority of the ecotoxicity studies were *in vivo*, and focussed on aquatic exposures. Nearly two-thirds of the studies investigated binary mixtures, and fewer than 20% considered mixtures with more than three components.

A total of 1220 mixture experiments were identified, of which 557 reported potential non-additive effects. Martin *et al*. (2021) concluded that the majority of these did not demonstrate mixture toxicity more than two-fold higher or lower than that which would be expected based on CA, with 78 studies reporting synergism and 58 studies reporting antagonism exceeding this threshold. Those reporting synergism typically included triazine, azole and pyrethroid pesticides, chromium and nickel in combination with cadmium and some endocrine disruptors. Overall, Martin *et al*. (2021) concluded that based on the studies they reviewed, CA can be assumed in most cases but regulators should consider the possible synergistic effects of some chemical classes. The lack of frequently identified synergistic effects means that it is possible for regulators to use CA to provide a reasonable estimate of mixture toxicity.

Whichever of the above methods is used to estimate mixture toxicity, all rely on having reliable estimates of the concentrations of each substance that the organism is exposed to and data on the (eco)toxicity of each substance. It would be preferable where possible to have information on internal exposures at the site of action, however in the majority of this information in not available. Although it may be feasible to measure exposure concentrations under controlled laboratory conditions, complete measured data will not be available for real world exposure situations. Every substance present cannot be measured continuously at every site, and so exposure data will either need to be modelled or the available measured data accepted as incomplete – both in terms of the number of chemicals analysed and the regularity of sampling.

In addition, even well-studied chemicals usually have only a small number of (eco)toxicity studies for a limited number of species which are assumed to be representative of all untested species. For less well studied chemicals, the data set will be even smaller or may not exist at all. Similarly to the exposure estimates, the toxicity data will therefore need to be modelled or accepted as incomplete, with methods developed to account for this and the additional uncertainty.

3.5 Conclusions on mixture toxicity assessment

The lack of frequently identified synergistic effects in studies conducted to date means that it is possible for regulators to use CA to provide a reasonable estimate of mixture toxicity in most cases. The possibility of synergistic effects in some chemical classes should be considered on a case-by-case basis. CA provides a more conservative assessment of mixture toxicity than IA, and requires less data to calculate. It is therefore useful as an initial assessment of potential mixture toxicity. CA is already used in regulatory assessments, for example under CLP (as discussed in Section 2.4).

Toxic Units, HI and RQ are frequently used metrics when estimating mixture toxicity based on CA for ecological risk. TUs compare exposure concentrations to the raw ecotoxicity data, whilst HI and RQ compare exposure concentrations to thresholds that include an assessment factor. Risk characterisation methodologies commonly applied to human health include the HI, TTD, RPI (PODI) and MOET.

Another useful summary statistic for environmental and human toxicity is the MCR. This is the ratio of the total toxicity to the highest toxicity of any individual component and provides an indication of the number of substances driving the toxicity risk in a particular mixture. A value near to 1 indicates that a single substance is driving the risk. The highest MCR for a mixture of substances is equal to the number of substances present, and would indicate that all substances contribute equally to the risk.

Whichever method is used to estimate mixture toxicity, all rely on having reliable estimates of the concentrations of each substance that the organism is exposed to and data on the (eco)toxicity of each substance. As it is not possible to measure for all chemicals continuously, and (eco)toxicity data is often limited, both the exposure and toxicity data will be incomplete, adding uncertainty to the assessment.

4 Estimated risk based on field exposure from unintentional mixtures

We have identified and reviewed published studies that have attempted to quantify the level of risk due to chemical mixtures based on field monitoring data of chemical concentrations in Europe. European data was used as it is expected that the levels of mixture exposure will be similar in the UK due to similarities in chemical use and regulation. Generally, these are observational monitoring studies that have attempted to interpret the measured concentrations of chemical contaminants in terms of their possible mixture effects. The studies each involve monitoring a range of different substances and use a variety of methods to assess the potential for mixture toxicity. For the environment, all the studies identified were in the aquatic compartment. For human health, biomonitoring and epidemiology studies were predominant. It would be unrealistic to expect any of these studies to have measured every chemical present, and most rely on single spot samples so can only identify a sub-set of the chemicals present at one specific time point. Therefore, while these studies give an incomplete view of the possible mixture toxicity at these sites, they do provide an indication of the level of risk, bearing these uncertainties in mind.

This report does not attempt to summarise the available evidence linking biological effects in the field to individual chemicals or chemical mixtures (as opposed to other potential stressors). For the environment, these types of 'eco-epidemiological' studies were reviewed by Hutchinson and Dungey (2011) and there is on-going research in this area (e.g. the EU PROTECT project, https://www.solutions-project.eu/). For human health, a leading research study is the European HBM4EU project (https://www.hbm4eu.eu/the-project/).

4.1 Environment

4.1.1 Evidence discussed at a joint Dutch/Swedish workshop, March 2020

A joint Dutch/Swedish *Workshop on a pragmatic approach to address the risk from combined exposure to non-intentional mixtures of chemicals – REACH as an example*, was held in March 2020 (Anon., 2020). Several published studies were discussed at this workshop and these are reviewed below. Studies relating to human health are reviewed separately in Section 4.2.1.

Bopp *et al*. (2016) carried out a literature search to identify studies that conducted risk assessments for chemical mixtures published between 2014 and 2016, although some earlier studies were also included. Seven case studies were identified for the environment. Three used predicted exposure data only (Junghans *et al*., 2006; Marx *et al*., 2015) or data from outside of Europe (Nowell *et al*., 2014), so are not reviewed further here. The

remaining four studies (Price *et al*., 2012b; Malaj *et al*., 2014; Backhaus and Karlsson, 2014; Ccanccapa *et al*., 2016) are summarised below.

- Price *et al*. (2012b) reported the application of a decision tree developed by Cefic to assess the risks posed by combined chemical exposure. Monitoring data for a wide range of chemicals from surface water and waste water treatment plant (WWTP) effluents (adjusted for dilution) were collated from seven data sets collected across Europe (including the UK). In total, 559 samples (362 surface water, 197 WWTP) were included, with between 21 and 123 substances analysed for in each. As the sources of the monitoring data varied, so did the number of analytes, the sampling frequency and the limits of detection. The potential mixture toxicity was calculated for both the environment and human health (see Section 4.1.2). For the environment, a HI approach was used, with the measured concentrations compared to an environmental quality standard (EQS) or PNEC (calculated following ECB, 2003) to protect from acute or intermittent exposures, or if these were not available an EQS or PNEC based on chronic exposures. The endpoint and taxa upon which the threshold used was based therefore varied between chemicals, and as the threshold already includes an assessment factor and is based on the most sensitive taxon across species for a substance, the estimated mixture toxicity is considered to be 'worst case'. Non-detects were either assumed to be zero or set at half the limit of detection and the effect of this on the analysis was determined.

  For the environment and when non-detects were set to zero, 81% of samples had a HI value above 1, indicating the potential for mixture effects. A marginally higher proportion (82%) was indicated as posing a potential mixture risk when non-detects were set at half the limit of detection. Individual substances had a HI value above 1 in 68% of samples when non-detects were set to zero, or 73% when set to half the limit of detection.

  The average MCR across all environmental samples was 1.8, with 72% of samples having an MCR value below 2. Price *et al*. (2012b) noted that although the overall environmental risks were higher than those calculated for human health based on the higher HI, the environmental risk was more likely to be driven by a single substance, based on the MCR.

  Based on the HI, the largest potential risks were observed at sites that also had potential risks based on individual substances. Sites at which at least one individual chemical exceeded its environmental threshold had a mean HI of 15. By comparison, sites at which no individual substance exceeded its environmental threshold had mean total HI values of 0.4 when mixture risk was not predicted and 1.3-1.8 (maximum 3.4) when potential mixture risk was predicted. The authors concluded that for the relatively small proportion of sites where unacceptable risks would have been missed by single substance assessment, the data indicate that the risk is being driven by a relatively small number of chemicals. These were listed as diclofenac, clarithromycin, tramadol, terbuthylazine desethyl, terbuthylazine, and polybrominated diphenyl ethers (PBDEs).

- Malaj *et al*. (2014) analysed collated surface water monitoring data collected between 2006 and 2010 from across Europe (including the UK), covering 4001 sampling sites from 91 European river basins and up to 223 organic chemicals. As the sources of the monitoring data varied, so did the number of analytes, the sampling frequency and the limits of detection. Acute experimental ecotoxicity data for algae, daphnids and fish were collected, and data gaps were filled by predicted values. The acute ecotoxicity data were used to calculate acute and chronic thresholds for each taxon, using an assessment factor of 10 for the acute threshold and an assessment factor of 1000, 100 and 50 for the chronic thresholds for invertebrates, fish and algae, respectively. The assessment factors were selected based on acute to chronic ratios from laboratory data and field evidence that indicated changes in invertebrate communities when exposed at 1000th of the acute endpoint. The chemical risk was expressed as the proportion of sites for which at least one chemical exceeded the relevant threshold for each taxon separately or combined. Overall, 14% of sites were predicted to be acutely affected, and 42% chronically affected. Pesticides were found to be the major driver for acute risk, producing 81, 87 and 96% of exceedances of the acute thresholds for fish, invertebrates and algae, respectively. The substances driving the chronic risk are not stated by Malaj *et al*. (2014) and the mixture risk was not assessed. When looking at UK data alone, between 0 to 10% of sites exceeded the acute threshold for at least one chemical for either algae, invertebrates or fish, and exceeded the chronic threshold for algae or fish. Up to 100% of sites in each UK river basin studied exceeded the chronic threshold for invertebrates, but this assessment was conducted using acute data with an assessment factor of 1000, so this approach may be more conservative than when using chronic ecotoxicity data.

- Backhaus and Karlsson (2014) analysed a data set of measured concentrations of 26 pharmaceuticals in WWTP effluent from France, Greece, Italy and Sweden. Dilution of the effluents was not taken into account, and if a substance was not detected the concentration was assumed to be zero. Acute experimental data for algae, daphnids and fish were collected, and data gaps were filled by predicted values. Two approaches were used to determine the mixture risk. In the first, a PNEC was derived by applying an assessment factor of 1000 to the lowest acute endpoint. This was then compared to the measured exposure concentration (MEC) and summed across substances to provide an RQ (termed RQ MEC/PNEC). In the second approach, TUs were calculated separately for each taxon and summed to give the total TU for each sample for each taxon. The highest TU had an assessment factor of 1000 applied to convert this to an RQ (termed RQ TU).

  Whichever method was used, RQs above 1 were calculated for all seven WWTP. The RQ MEC/PNEC ranged from 19.9 to 49.2 and the RQ TU ranged from 16.1 to 48.0. Using an RQ MEC/PNEC gives a more conservative result, as the most sensitive taxon is used for every substance, but the authors note that the small differences in this case study are due to the fact that for most substances algae are the most sensitive taxon, so the two methods provide similar results. All sites also had an RQ above 1 when considering the individual pharmaceuticals separately.

The MCR ranged from 1.2 to 4.4 across all taxa, indicating that the potential mixture risks were driven by a small number of the pharmaceutical substances measured.

- Ccanccapa *et al*. (2016) studied the potential for mixture effects in aquatic organisms due to pesticide exposure in the Ebro River, Spain. Up to 50 pesticide active substances or pesticide transformation products were analysed in water and sediment grab samples taken from 24 sampling points in 2010 and 2011. Acute and chronic ecotoxicity data for algae, invertebrates and fish were gathered from the University of Hertfordshire's Pesticide Property Database, which collates key hazard data from published regulatory risk assessments. If experimental ecotoxicity data were not available from this source, predicted values were generated.

  Ccanccapa *et al*. (2016) calculated the sum of TUs for each site for each taxon separately based on acute data. The sum of TUs for each taxon was below 1 at all sites, up to a maximum of 0.26 for invertebrates at one site in 2010. TUs based on chronic ecotoxicity data were not calculated. The authors noted that the time of the year at which samples were taken is a period of lower pesticide discharge, so it cannot be concluded that the sum of TUs would be below 1 throughout the year based on this study. However, for this sampling period the EC_{50} for the mixture of pesticides measured was not reached at any site.

  RQs were calculated for each substance individually, based on PNECs derived from chronic ecotoxicity data using an assessment factor of 1000. As RQs above 1 were observed for individual pesticides, a mixture assessment was not considered by the authors. However, an assessment factor of 1000 would not generally be applied to chronic data in a UK regulatory context, so this approach is likely to have overestimated the chronic risk. If an assessment factor of 10 or 100 had been used instead, individual RQs above 1 would still have been calculated.

4.1.2 Additional evidence sources

A number of additional case studies were identified that have been published after Bopp *et al*. (2016). A literature search was carried out in April 2021 using Science Direct and Pubmed and the search terms *"Mixture risk" AND chemical AND environment AND monitoring* for studies published since 2016. The results were screened to identify studies conducted in Europe that investigated mixture toxicity using measured exposure concentrations. Citations of relevant studies within the identified publications were also reviewed.

- Rico *et al*. (2016) used monitoring data on a range of substances (235 organics and 8 metals) from 55 surface water sites along the length of the Danube River. The samples were collected in August and September 2013. Acute ecotoxicity data for daphnids was obtained from the E-Tox database (De Zwart, 2002) for 27% of the chemicals, with predicted data used for the remaining 73%. TUs were calculated for each substance and summed for each site. TUs were also summed for different classes of substances (metals, industrial chemicals, insecticides, herbicides,

fungicides, pharmaceuticals, home and personal care products (HPCPs) and 'miscellaneous').

The total TU ranged from 0.012 to 0.16 across the 55 sites. The main contribution to the total risk was from heavy metals and industrial substances, but the number of substances contributing to the total TU at each site was not stated. Herbicides, fungicides and miscellaneous substances were not found to contribute significantly to the TU, although as the ecotoxicity endpoint used invertebrate data this is not surprising for herbicides and fungicides. The authors also note that the timing of the sampling in late summer may not have coincided with the peak use of pesticides in early spring. The calculated TU was correlated with various biological metrics based on benthic macroinvertebrates and habitat and physico-chemical parameters measured at the same sites. Habitat and physico-chemical parameters were found to have the strongest correlations to the biological metrics, with limited correlation between the biological metrics and TU for either the total contaminants or the contaminant groups.

- Munz *et al*. (2017) measured the concentrations of a range of chemicals upstream, downstream and in the effluent of WWTPs in Switzerland in 2013 and 2014. For two of the sampling dates, the upstream and downstream samples were analysed for 389 substances using a non-targeted analytical method. For all other samples, 57 chemicals identified as being a priority for these water bodies had a targeted analysis conducted. The analytes included pesticides, biocides, pharmaceuticals, personal care products, industrial substances and metals. The mixture risk was calculated using the msPAF approach based on SSDs constructed with EC_{50} data from various taxa, as the authors state that the use of msPAF based on this endpoint has been found to provide the best estimate of environmental effects. Ecotoxicity data of sufficient quality was available for 124 of the substances in the screening and for 36 of the targeted analytes.

  The calculated msPAF ranged from 0 to 2.1%, indicating that no sites had values above the generally accepted 5% affected threshold. In general, only the top 5 chemicals at each site and timepoint drove the (low) risk, with the main risk drivers across all sites being diclofenac, diazinon and clothianidin. The substances with above average ecotoxicity were found to contribute more to the total risk than those with above average measured concentrations (in both cases, the average was based on the data for those compounds included in this study). For the sites monitored in this study, pesticides were the main drivers of the potential risk.

  Munz *et al*. (2017) also compared the measured exposure concentrations to the acute EQS for the subset of chemicals for which these were available. It was found that 2% of upstream and 35% of downstream sites had RQs greater than 1. The authors note that the RQ is also driven by a few substances (again, pesticides), with the maximum RQ being around 9 (read from a graph). It is not stated whether any individual chemical had an RQ above 1. The large difference in exceedances at sites upstream and downstream of a WWTP indicates that the WWTP effluent is the

source of the chemical contamination. The authors also note that the WWTP was a source of pesticides into the river.

- Gustavsson *et al*. (2017a) monitored a range of chemicals in Swedish marine waters. Samples were collected from five sites in June 2012 and 172 organic substances were analysed for. Non-detects were accounted for using three different methods. They were either assumed to be present at the limit of detection, to be zero, or were estimated using a non-parametric estimation method. A variety of environmental thresholds were collated to compare to the measured concentrations in the priority order: Water Framework Directive (WFD) EQS, regulatory PNEC, or PNEC derived from ecotoxicity data as part of this study. Freshwater thresholds had an additional assessment factor of 10 applied for use with these marine samples.

  Four of the five sites had individual chemicals (triclosan, irgarol and tributyl tin (TBT)) at concentrations above their environmental thresholds. All five sites had RQs above 1, whichever method was used to account for non-detects. The method that replaced non-detects with the limits of detection resulted in very high total mixture RQ values (97612 to 97617) due to some chemicals having environmental thresholds below their limits of detection. This method was therefore not considered appropriate for considering mixture risk. The total mixture RQ ranged from 2 to 9 when using zero or the non-parametric estimates for non-detects.

  The MCR ranged from 1 to 4.3 across all methods and sites, and was below 2 at three of the five sites, indicating that the potential risk is largely driven by a small number of components.

- Gustavsson *et al.* (2017b) collated monitoring data for a range of pesticides in Swedish streams collected between 2002 and 2013. The data set included 1308 samples, with 141 different pesticides detected at least once and up to a maximum of 53 chemicals detected in a single sample. Non-detects were accounted for using three different methods, as in Gustavsson *et al*. (2017a). Environmental thresholds were defined using two different approaches; the first used Swedish Water Quality Objectives (WQO, which are environmental standards that already incorporate an assessment factor) to calculate RQs, and the second used TUs calculated for each taxon separately based on acute ecotoxicity data.

  The method that replaced non-detects with the limits of detection resulted in higher median mixture RQs (220 to 270) due to some substances (mainly pyrethroids) having environmental thresholds below their limits of detection. Replacing non-detects with zero resulted in median RQs ranging from 0.7 to 3.9, with 70.5% of samples having RQs greater than 1 based on the WQO.

  When considering the TU for individual taxa and replacing non-detects with zero, median algal TUs ranged from 0.00078 to 0.0098, median crustacean TUs ranged from 0.000079 to 0.00044, and median fish TUs ranged from 0.000032 to 0.0001. Gustavsson *et al.* (2017b) applied assessment factors of 10 for algae and 100 for fish and crustaceans based on EFSA (2013c) to determine a critical TU threshold.

None of the median TUs exceed these thresholds. However, 0.4 to 3.4% of samples did exceed these thresholds based on individual taxa.

The authors do not state whether the concentration of any individual chemical exceeded the WQO at any site or resulted in a TU that exceeded the critical TU for each taxa. However, the median MCR for each taxa ranged from 1 to 3, with the majority of MCR around 2, indicating that the potential risk at each site was driven by a small number of chemicals. Gustavsson *et al*. (2017b) found that although only a small number of the pesticides measured drove the risk in each sample, the pesticide driving the risk varied across sites and time.

Gustavsson *et al*. (2017b) imagined a scenario whereby substance-specific risk management measures are implemented that reduce the concentrations of all chemicals with RQs above 1 to a concentration equivalent to an RQ of 0.95. In this scenario, and when replacing non-detects with zero, the potential mixture risk is reduced from a median RQ of 2.1 to 1.8, but 70% of sites still have RQs above 1. The MCR increases, indicating that the risk is more evenly spread across all substances in the mixture.

- Papadakis *et al*. (2018) monitored the concentrations of 103 pesticides over a period of two years in the Strymonas and Nestos river basins in Greece. A total of 631 samples were collected from Strymonas and 386 samples from Nestos. The authors calculated PNECs for each substance based on chronic ecotoxicity data reported in the University of Hertfordshire's Pesticide Property Database, using an assessment factor of 100, 50 or 10 depending on whether 1, 2 or 3 pieces of chronic ecotoxicity data were available. RQs were calculated for each sample. An RQ above 1 was identified for a single chemical in 19% and 20% of the samples from Strymonas and Nestos, respectively. When the RQs were summed to give the mixture RQ, the percentage of samples with RQs above 1 only increased slightly, to 22% and 21%, respectively. When the pesticides were grouped based on type it was found that more than 60% of RQs above 1 were due to insecticides; the authors noted that the potential risk appeared to be driven more by chemicals with higher ecotoxicity than those measured at higher concentrations. Across all samples, the 75th percentile RQ was below 2 (read from a graph).

- Freeling *et al*. (2019) analysed composite effluent samples collected across seven days from 33 WWTP treating predominantly domestic waste in Germany in 2018. Samples were analysed for linear alkylbenzene sulfonates (LAS) and alkylethoxysulfates (AES) and also screened for 1564 surfactants and transformation products. Concentrations were divided by the dilution factor at each discharge point to provide an estimate of the environmental concentration. Individual homologs were grouped based on their weighted carbon number into 22 chemical groups. Concentrations were compared to PNECs derived by applying an assessment factor of 10 to experimental chronic data or an assessment factor of 1000 to predicted acute data. RQs were reported for chemical groups, and for the total sample based on CA. RQs for each chemical group and the mixture RQ were all below 1, except for one WWTP which had a mixture RQ of 1.065. At all sites the

majority of the risk was due to LAS and its by product di-alkyl tetralin sulfonates (DAT). MCR ranged from 1.4-2.7 indicating that the risk was driven by a low number of the chemical groups at each site.

- Riva *et al*. (2019) collected surface water samples from seven sites on three Italian rivers in 2011. Samples were analysed for a range of pharmaceuticals, illegal drugs, industrial chemicals and personal care products, 39 of which were detected above the limits of quantification. PNECs were derived by applying an assessment factor of 1000 to acute ecotoxicity data collected from literature sources. When acute data were not available, predicted data were used to fill the gaps or chronic data were used with a lower assessment factor, and substances for which no ecotoxicity data could be found were excluded from the analysis. Two methods were used to estimate the mixture risk. In the first the RQ was calculated for each substance and summed for each sample (termed RQ MEC). In the second approach, TUs were calculated separately for each taxon and summed to give the total TU for each sample for each taxon (STU; Sum of Toxic Units). The highest TU had an assessment factor of 1000 applied to convert this to an RQ (termed RQ STU).

  Ten individual chemicals had an RQ MEC above 1. These included pharmaceuticals, disinfectants, personal care products, anthropogenic markers (caffeine and nicotine) and industrial chemicals (bisphenol A (BPA), 4-nonylphenol and 4-ter-octylphenol). Estrone and 17β-estradiol had the highest RQ (up to 1457) and these substances were excluded from the subsequent analysis. At each site, the potential mixture risk was calculated separately for those chemicals with MEC/PNEC ratios above 1 and those below 1. RQ MEC values ranged from 14.3 to 66 and 0.89 to 2.9, respectively. The sum of the TUs for each taxon ranged from 0.0013 to 0.0352 and 0.0002 to 0.0016, respectively. The RQ STU ranged from 10.3 to 35.2 and 0.44 to 1.6, respectively. This demonstrates that even when excluding individual substances with RQs above 1, the potential mixture risk of the remaining constituents can be above 1, whichever method is used.

- Posthuma *et al.* (2019a) collated aquatic ecotoxicity data to generate acute and chronic SSDs for 12386 chemicals as part of the EU SOLUTIONS project. Although the data collation and evaluation of the quality of the data and the resulting SSDs constituted the main focus of this paper, an example case study was also reported that used the SSDs to estimate mixture risk. SSDs and predicted exposure data were generated for 1760 chemicals (REACH-registered industrial chemicals, pesticides and pharmaceuticals) in over 22000 European waterbodies, including the UK. The SSDs and predicted exposure concentrations were used to calculate the potential acute and chronic mixture risk in each waterbody based on the 95th percentile exposure concentration and assuming CA, with outputs expressed as an msPAF.

  When using the chronic SSD, around 65% of waterbodies had an msPAF greater than 5%, indicating that mixture effects could not be ruled out. Based on the acute SSD, a subset of 15 chemicals was found to explain nearly 99.5% of the expected mixture effects. These were a range of industrial substances and pesticides, but all

were high tonnage, with wide use and high hazard. The industrial chemicals were BPA, N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine, anthracene, octamethylcyclotetrasiloxane, cumene hydroperoxide, difenylamine, 1-dodecanol and p-phenylenediamine). The subset of 15 chemicals also included two pesticides that are no longer approved in Europe (terbufos and phorate). Posthuma *et al.* (2019a) noted that as they used the 95th percentile predicted concentration, their method may have underestimated risks of chemicals that could be expected to have peak exposures, for example pesticides. The equivalent list of substances driving the mixture risk is not provided for the chronic SSD and no information is provided on whether the predicted concentration of individual chemicals was above the thresholds derived.

- Posthuma *et al*. (2020) estimated the potential mixture toxicity for 24 WFD Priority Substances (including pesticides and industrial chemicals) and linked this to ecological quality as part of the EU SOLUTIONS project. As measured exposure concentrations were not available, the 50th percentile concentration was predicted for each waterbody in Europe, including the UK. The potential mixture toxicity was assessed by assuming CA and using three different methods: i) calculating the RQ based on WFD EQS; ii) calculating the RQ based on a HC_{50} from an acute SSD; and iii) calculating the msPAF based on an acute SSD.

  When considering the chemicals separately, Posthuma *et al*. (2020) estimate that 67% of sites would have exceedances of individual annual average EQS. When the mixture RQ is calculated for each site, 74% of sites have an RQ above 1; this includes sites at which individual substances are all below their respective EQS. The authors state that although an RQ below 1 has a clear meaning (i.e. a low potential mixture risk based on the substances included in the assessment), an RQ above 1 has no valid scientific interpretation due to the use of different species, endpoints, assessment factors, etc.

  When the mixture risk was estimated using the HC_{50}, 15% of waterbodies had a value above 1, meaning that these waterbodies would be expected to have mixture concentrations that could result in at least a 50% effect on 50% of the species present. The HC_{50} is a much higher threshold than an EQS, which is designed to be a precautionary threshold. Posthuma *et al*. (2020) argue that the acute HC_{50} can be used as an estimate of ecological impacts.

  The same data were recalculated to provide an output expressed as msPAF and this was correlated with WFD ecological status. This analysis suggested that chemical exposure (expressed as msPAF) may limit the ecological status of a site, i.e. sites with better ecological status did not have high potential mixture toxicity. However, sites with poor ecological quality had a wide range of potential mixture toxicity, suggesting that other pressures could also be adversely affecting the biology at those sites.

- Markert *et al*. (2020) analysed samples collected from 39 sites along the Erft River, Germany in 2016/2017. Pesticides and pharmaceuticals constituted 141 of the 153

chemicals analysed for, and 98 of the chemicals were detected in at least one sample. The measured concentrations were compared to acute and chronic ecotoxicity data collated from several online databases. Mixture risk was calculated in several ways. Firstly, acute and chronic TU were calculated for each substance and summed for each sample and taxon; then the RQ was calculated for each sample by taking the highest TU and applying an assessment factor of 100 to the acute TU ($RQ_{mix, acute}$), an assessment factor of 1000 to the acute TU ($RQ_{mix, 1000}$), or an assessment factor of 10 to the chronic TU ($RQ_{mix, 10}$). Secondly, measured concentrations were compared to published EQS or PNEC (RQ).

Across all samples, 90% had single substances that exceeded environmental thresholds. Mixture risks were only predicted for up to an additional 1% of sites, whichever measure was used.

Acute mixture toxicity was predicted for 32% of the samples, with RQs ranging from 1 to 10. In 93% of samples 1 to 3 chemicals accounted for 90% of the acute TU. Chronic mixture toxicity was predicted for 60% ($RQ_{mix, 1000}$), 90% ($RQ_{mix, 10}$) and 91% (RQ) of samples. Maximum chronic toxicity RQ were 100 ($RQ_{mix, 10}$), 509 (RQ) and 2588 ($RQ_{mix, 1000}$). In 76 to 80% of samples 1 to 3 chemicals accounted for 90% of the chronic TU.

For algae and macrophytes, the substances contributing significantly to the acute and chronic mixture risk were herbicides and fungicides, triclosan, clarithromycin and sulfamethoxazole. No acute risk was identified for fish, but the chronic risk was driven by diclofenac and ibuprofen. For invertebrates, acute mixture risk was predicted at 0.6% in samples and chronic risk in up to 34% samples. Although the authors state that insecticides were under-represented in the analytical suite, the main drivers were found to be insecticides, galaxolide, benzotriazole, carbamazepine and clarithromycin.

- Gosset *et al*. (2021) measured the concentrations of pharmaceuticals and pesticides in the effluent of 10 WWTP in France. Up to 41 chemicals (37 pharmaceuticals and 4 pesticides) were detected across all the samples. The measured concentrations in the effluent were adjusted for the dilution rate of the receiving water and compared to PNECs gathered from regulatory risk assessments and published reports, or calculated from published ecotoxicity data or predicted values using the assessment factor defined in the EU Technical Guidance Document (TGD) (ECB, 2003). Seven WWTP had RQs above 1 due to single chemicals. When the mixture RQ was calculated, nine WWTP had RQs above 1, up to a maximum RQ of 5304. Across all samples and sites, 7 substances (methocarbamol, venlafaxine, terbutryn, atorvastatin, lidocaine, atenolol and diclofenac) contributed nearly 98% of the potential risk, with all the other constituents contributing <1% individually.
- Spurgeon *et al*. (2021) conducted an analysis of Environment Agency monitoring data to prioritise organic chemicals of concern in freshwater and groundwater, and as part of the assessment also considered the potential for mixture toxicity. The Environment Agency uses two analytical semi-quantitative scan methods to identify

the chemicals present in samples, allowing a much larger number of substances to be identified than when using targeted analysis. In total, 1144 substances can be detected in the analytical suite. The environmental monitoring data were for samples collected and analysed by Gas Chromatography Mass Spectrometry (GCMS) between 2009 and 2019 and by Liquid Chromatography Mass Spectrometry (LCMS) between 2014 and 2019 (these methods detect different substance types). The measured semi-quantitative concentrations in each sample were compared to the HC_{50} from a chronic SSD (data provided in Posthuma *et al*., 2019a) and the mixture toxicity calculated assuming CA. Non-detects were assumed to be zero.

For groundwater, 64 out of approximately 10800 samples (0.6%) had a potential for mixture toxicity based on the GCMS data, but none of the LCMS samples had a potential for mixture toxicity. Spurgeon *et al*. (2021) noted that samples had higher risk when considering mixtures rather than individual chemicals. For surface waters, 876 samples out of approximately 23000 (3.8%) had a potential for mixture toxicity based on the GCMS data, as did a small number of samples (number not stated) out of approximately 2800 LCMS samples. No individual substance exceeded its HC_{50} in any LCMS sample. Analysis to identify the specific substances driving the risk in these samples was not conducted.

Spurgeon *et al*. (2021) also calculated the MCR across all the samples. The MCR was less than 5 in over 98% of samples, and less than 2 in over half of all samples, indicating that potential mixture risks were driven by a small number of the substances measured.

4.1.3 Summary of evidence for the level of risk from unintentional mixtures - environment

A major limitation of the evidence base is that all of the reviewed environmental studies focus on the assessment of mixture toxicity in surface waters. The possibility of mixture effects is just as relevant in the sediment and soil compartments, but no data were identified to address this. The majority of the studies were performed in mainland Europe, although some also included the UK (Price *et al*., 2012b; Posthuma *et al*., 2019a; Posthuma *et al*., 2020). A single study used only data from England (Spurgeon *et al*., 2021), and this was also the largest dataset in terms of both sample numbers and number of chemicals analysed for. In all the studies, monitoring locations were not selected to provide a representative dataset across the geographical area (i.e. rural versus urban, headwaters versus estuaries) but instead often used data collected for other purposes or focussed on particular emission sources.

Bopp *et al*. (2016) identified a number of limitations in the case studies they reviewed. All mixture assessments require knowledge of the identity and concentration of all the components of the mixture, and effects data for each of these, and for all the case studies this information was incomplete. In addition, many of the case studies focussed on a sub-set of chemicals or chemical groups regulated under specific pieces of legislation. For both

these reasons, all the case studies in Bopp *et al*. (2016) will have underestimated the total potential mixture risk at a site.

These same limitations apply to all the studies reviewed in this report, as no study can measure every chemical present in a sample or measure all the substances an organism is exposed to over its lifetime. The study by Spurgeon *et al*. (2021) using Environment Agency sampling data analysed using non-targeted GCMS and LCMS offers the potential to identify more of the chemicals present in each sample, but even this method cannot identify all chemicals present. In addition, the potential mixture toxicity is still assessed on a sample-by-sample basis and so only considers a single point in time and place. Other studies have used modelled exposure data (e.g. Posthuma *et al*., 2019a; Posthuma *et al*., 2020) and have been able to calculate estimates of exposure over time for a large number of substances. However, using modelled data introduces its own uncertainties, which can be significant.

Although all the studies will have underestimated the potential total mixture risk when using CA, as not all chemicals have been analysed for, it may be that the difference between the estimated and 'true' value is not significant. Several of the studies reported that the chemicals identified as driving the risk are those with higher production tonnages (Posthuma *et al*., 2019a), higher toxicity (Munz *et al*., 2017; Papadakis *et al*., 2018; Posthuma *et al*., 2019a) and wide use (Posthuma *et al*., 2019a). These type of chemicals are more likely to already be identified as of potential concern and be included in monitoring programmes.

The studies reviewed here have dealt with non-detects in a variety of ways, from assuming them to be zero, replacing them with the detection limit, or replacement with derived values using various methods. Difficulties have been identified when chemicals have ecotoxic effects below their limits of detection (e.g. Gustavsson *et al*., 2017a). This issue (which also applies to the assessment of single substances) can lead to high mixture risk estimates if non-detects are replaced with the detection limit.

The availability of ecotoxicity data also varies between case studies. If ecotoxicity data or an agreed threshold are not available then a substance cannot be included in the mixture assessment. For many of the studies reviewed, this meant that substances without ecotoxicity data were excluded from the mixture analysis or that data were replaced with predicted values, which may be uncertain. Data were collated from a variety of sources, quality assessed using different methods, and data gaps dealt with in different ways, resulting in considerable variation across the different studies. The choice of ecotoxicity threshold will also have a large effect on the assessment of potential mixture risk and the interpretation of the output. The studies reviewed here used either agreed water quality standards (e.g. WFD EQS), thresholds derived from acute or chronic data with various different assessment factors applied (e.g. PNEC), thresholds derived statistically (e.g. HC_5 from an SSD), or raw acute or chronic ecotoxicity data.

All the environmental case studies that calculated mixture risk used CA methods. CA is more conservative than IA, and is easier to calculate. As more chemicals are included in the mixture assessment then higher risk will be estimated using CA, as each additional

substance can only increase the risk. CA has been found to provide a reasonable estimate of mixture toxicity in the absence of information on specific modes of action or synergisms (Martin *et al*., 2021).

Nearly all of the studies reviewed reported that the ecotoxicity thresholds selected were exceeded when considering chemicals individually. While this does not necessarily mean that adverse effects would have occurred in reality, it does indicate the potential for effects due to individual substances. The studies used a variety of thresholds which are based on acute or chronic ecotoxicity data, with or without an assessment factor. TUs are based on the ecotoxicity data without an assessment factor applied, so are not directly comparable to RQ or HI approaches. In addition, as different thresholds were used in each study, the absolute values reported are not directly comparable to each other, even when the same metric is reported.

Three studies (Ccanccapa *et al*., 2016; Rico *et al*., 2016; Riva *et al*., 2019) based their assessment of mixture toxicity on comparison of measured environmental concentrations and acute toxicity data. The maximum sum of TUs reported were 0.26, 0.16 and 0.0352 respectively. This indicates that the EC_{50} of the most sensitive organisms were not exceeded, but that the mixture toxicity was within 1 to 2 orders of magnitude of this. If we assume that an assessment factor of 1000 could be applied to the acute ecotoxicity data used to calculate TU in these three studies, then the maximum RQ would be 260, 160 and 35.2 respectively. RQ or HI values reported in other studies ranged from the 75^{th} percentile being below 2 (Papadakis *et al*., 2018) to a maximum of 5304 (Gosset *et al*., 2021). This suggests that the potential for mixture toxicity effects is observed across all the studies.

Several studies also calculated the MCR. The average MCR for all sites reported by Price *et al*. (2012b) was 1.8. The MCR for each taxon ranged from 1.2 to 4.4 in Backhaus and Karlsson (2014), but the authors noted that for the taxon driving the risk (algae), the MCRs were all below 2. Gustavsson *et al*. (2017a) calculated MCRs ranging from 1 to 4.3 across all methods and sites, but the MCR was below 2 at three of the five sites. Gustavsson *et al.* (2017b) calculated the median MCR for each taxon as ranging from 1 to 3. Freeling *et al*. (2019) calculated the MCR to be between 1.4 and 2.7. Spurgeon *et al*. (2021) also calculated the MCR for groundwater and surface water samples combined. The MCR was less than 5 in over 98% of samples, and less than 2 in over half of all samples.

These six studies included a broad range of different chemicals (including industrial chemicals) (Price *et al*., 2012b; Gustavsson *et al*., 2017a; Freeling *et al*., 2019; Spurgeon *et al*., 2021), pharmaceuticals (Backhaus and Karlsson, 2014) and pesticides (Gustavsson *et al.,* 2017b). The results from these studies, focussing on different locations, numbers and types of chemicals and using different methods and ecotoxicity thresholds, all report similar MCR ranges. This suggests that typically mixture risk is driven by a small number of chemicals at each site, up to 5 but generally lower than this, although the specific substances driving the risk at each site will vary.

In addition, four studies identified a subset of substances that were responsible for the majority of risk. Munz *et al*. (2017) noted that in general only 5 chemicals at each site

drove the risk and Markert *et al*. (2020) found that 90% of the total TU was generally driven by up to 3 chemicals. Gosset *et al*. (2021) reported that across all samples and sites, 7 chemicals contributed nearly 98% of the risk, with all the other constituents contributing <1% individually. Based on modelled exposure data Posthuma *et al.* (2019a) reported that a subset of 15 substances were found to explain nearly 99.5% of the expected mixture effects across all sites, all of which were high tonnage, with wide use and high hazard.

In many of the studies, unacceptable risks would already have been identified using a chemical-by-chemical approach at all or the majority of sites that a mixture assessment would also have identified as having unacceptable risk (e.g. Price *et al*., 2012b, Gustavsson *et al*., 2017a, Markert et *al*., 2020), suggesting that a mixture risk assessment would not have added any new information in these cases. Although many of the studies did not use formal regulatory thresholds (e.g. EQS) but instead derived their own thresholds, large numbers of exceedances for individual substances across many monitoring programmes suggests that current risk assessment may not be sufficiently precautionary for individual chemicals. It may be that existing individual substance assessment and management needs to be strengthened to ensure that single chemicals are not posing unacceptable risks, before consideration of potential mixture effects.

Some authors have argued that, as the MCRs indicate that mixture risk is generally driven by a low number of chemicals, mixture risk may not always need to be considered (Price and Han, 2011). However, in contrast, Gustavsson *et al*. (2017b) concluded that even when the concentrations of all substances with an RQ above 1 are reduced to a concentration equivalent to an RQ of 0.95, the majority of sites investigated still had RQs above 1, suggesting that there was still a potential mixture risk, with contributions more evenly spread across all substances in the mixture.

Several of the studies reviewed focus on a specific type of substance (e.g. pesticides) rather than looking across all potential contaminants. The specific chemicals driving the potential mixture risk varied both between sites and over time; they were often pesticides and pharmaceuticals, with some industrial chemicals identified as important in some studies. However, there is no evidence that industrial chemicals are the main driver for mixture toxicity.

Two of the studies reviewed here attempted to link the estimated mixture toxicity to biological endpoints. Posthuma *et al*. (2020) correlated their estimate of mixture toxicity based on predicted environmental concentrations with the WFD ecological classification status of each waterbody. Their analysis suggested that increased mixture toxicity might limit the ecological status of a site; i.e. sites with better ecological status did not have indicators of high potential mixture toxicity. Nevertheless, potential mixture toxicity was low for some sites with poor ecological quality, suggesting that other pressures were more important. Rico *et al*. (2016) correlated TU, habitat and physico-chemical parameters with biological metrics based on benthic macroinvertebrates. Habitat and physico-chemical parameters were found to have the strongest correlations to the biological metrics, with limited correlation between the biological metrics and TU for either total contaminants or the contaminant groups.

4.2 Human health

4.2.1 Evidence discussed at the March 2020 workshop

The literature search reported by Bopp *et al*. (2016), detailed in Section 4.1, identified 14 studies related to human health and one joint study related to both human health and the environment. Those meeting the inclusion criteria of measured data and within Europe are summarised below.

- The HI approach (Section 3.1) was used to conduct a cumulative risk assessment for exposure to a mixture of 15 anti-androgenic chemicals via multiple routes and pathways (Kortenkamp and Faust, 2010). Human exposure data, defined as intake (amount per day and kilogram (kg) body weight) from all known routes and pathways, was sourced from the peer-reviewed literature or from publicly available reports of European scientific committees or international regulatory authorities. Where possible, median and highly exposed (95th percentile of exposure level) population groups were defined or, where data were not available, intake figures from WHO/FAO (Joint Meeting on Pesticide Residues; JMPR) for Europe were used. The authors reported that the cumulative risks from anti-androgen exposures exceed acceptable levels for people on the upper end of exposure levels. The value obtained for median exposures to the 15 substances were judged adequate, however significant gaps in toxicity prevented any definitive conclusions being drawn. Kortenkamp and Faust (2010) considered that cumulative risk assessment can be used to select chemicals that should be subjected to greater scientific scrutiny to clarify concerns about their possible impact on toxicological risks.
- As discussed in Section 4.1.1, Price *et al*. (2012b) used the decision tree developed by Cefic to assess the risks posed by combined exposure to 559 mixtures of chemicals in European surface and waste waters. The number of chemicals present at detectable levels (i.e. within analytical limits) within these mixtures ranged from 2 to 49 chemicals (median 20.4). With regard to human health, a HI approach was used with measured concentrations of chemicals being compared to a human health reference value (RV). These were available for around half of the analytes (100 of 222) and Cramer classes were used to conservatively estimate RVs for the majority of the remainder; however this was not possible for five polycyclic aromatic hydrocarbons (PAHs). Non-detects were either assumed to be zero or set at half the limit of detection to determine the effect of this on the analysis.

  The authors reported that of the 559 mixtures evaluated using the decision tree, nine had a predicted HI > 1, with a single substance that was a concern under the

exposure assessment assumptions (i.e. Group I[1] as defined by the Cefic-MIAT (Mixtures Industry Ad-Hoc Team) decision tree (Price *et al*., 2012a)). The nine mixtures were from surface water samples, and chromium, estrone and ethinylestradiol levels exceeded the human health RV in one or more of these mixtures. The remaining mixtures had a HI < 1 indicating low concern for the mixture as well as the individual chemicals (i.e. Group II) and no mixture fell into Groups IIIA or IIIB. There were no differences between the groupings when using non-detect data set at zero or half the limit of detection.

MCRs were also calculated for the different datasets and the mixtures fell into distinct groups when plotted against the HI for combined exposures, reflecting the differences in the number, nature and levels of the contaminants in the waters being surveyed. Price *et al*. (2012b) reported that the average MCR was 2.4, with 44% of values being < 2.0. As this was much lower than the theoretical limit of 20 or higher (median number of substances detected), it was concluded that only a few compounds made significant contributions to the HI values for individuals exposed to the mixtures. However, as no mixture exposures fell into Group III, the authors commented that a chemical-by-chemical approach would have identified every mixture exposure that has an HI > 1 and therefore would have been sufficient to address risk from the combined exposures.

- In a further study, Price *et al*. (2014) utilised the Cefic-MIAT decision tree to assess the potential for human health effects following exposure to multiple chemical migrants (intentionally or non-intentionally added) from food contact-grade plastic

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[1]Group I “Single substance concern”: mixtures containing at least one substance in a concentration that poses a health risk; the risk would have been identified also in a substance-by-substance assessment.

Group II “Low concern”: mixtures of low concern with regard to individual substances and their combined effects.

Group IIIA “Concern for combined effect dominated by one substance”: mixtures with low concern for the individual substances, but with concern for combined effects where one substance is responsible for most of the mixture's toxicity; further cumulative risk assessment is required; a substance-by-substance assessment would not have identified this mixture as of concern, since the maximum HQi is < 1

Group IIIB “Concern for combined effect by several substances”: mixtures with low concern for the individual substances, but with concern for combined effects where several substances are responsible for the mixture's toxicity; further cumulative risk assessment is required; a substance-by-substance assessment would not have identified this mixture as of concern, since the maximum HQi is < 1.

(organic), polypropylene water bottles (organic), and plastic and glass water containers (inorganic). MCRs were calculated based on HQs of the individual substances and the cumulative HI. As previously described (Price *et al*., 2012b), the authors applied toxicological data (when available) or structural information on the specific components to set corresponding toxicological limits (RV or Threshold of Toxicological Concern (TTC), respectively). Exposure was determined from analysis of organic or inorganic migrants and fell into Group II (low toxicological concern) for all samples. MCR values were reported as 1.3 and 2.4 for food contact-grade plastic and polypropylene water bottles, respectively, indicating that the mixture's toxicity was driven by one or two components. For inorganic ions released from the glass containers, MCR values of between 1.1 and 3.8 were determined and from plastic containers, between 1.1 and 5.0. The average MCR value was 2.0, suggesting that one to two compounds drove toxicity. Price *et al*. (2014) concluded that the approach described was shown to be a helpful screening tool.

- De Brouwere *et al*. (2014) described the application of the MCR approach (Section 3.1) to evaluate whether health risks due to indoor air pollution are dominated by one substance or are due to concurrent exposure to a number of substances. Exposure data were collated from four European indoor air studies for which indoor air or personal exposure (dosimeter) measurements were available at the individual level. This included the EXPOLIS study (201 participants; 1996 to 1998), the Flemish homes study (360 homes; 2008 to 2011), the Flemish schools study (90 classrooms from 30 schools; 2008 to 2009) and the French Indoor air quality survey (OQAI) which measured indoor air quality in 567 homes across France (2003 to 2005). Single substance health-based reference values (RVs) were selected through a structured review process.

  The authors classified mixture exposures into the four groups defined by the CEFIC-MIAT decision tree (Price *et al*., 2012a; 2012b). MCR values ranged between 1 and 5.8, with an average of 1.8, with a tendency to be small relative to the number of components, indicating that the toxicity of the mixtures was driven generally by only a few of the chemicals. In addition, a statistically significant decline of MCR was seen with increasing HI value ($p < 0.0001$), with the largest values of HI occurring in the Group I classification. A high variability (2% and 77%, respectively) was noted across the different surveys with regard to the proportion of mixtures of concern for combined effects (Groups IIIA and IIIB); however, in 4 out of the 5 datasets, a considerable proportion of cases were found where a chemical-by-chemical approach failed to identify the need for the investigation of combined risk assessment. De Brouwere *et al*. (2014) concluded that the MCR methodology provides a tool for discrimination between those mixtures requiring further combined risk assessment and those for which a single-substance assessment is sufficient.

- A case study illustrating use and refinement within the hazard portion of the WHO/IPCS Framework (Meek *et al*., 2011) was reported by Evans *et al*. (2015). A large regulatory dataset of international estimated daily intake (IEDI) values was compiled from evaluations of 67 pesticides by the Joint FAO/WHO Meeting on

Pesticide Residues (JMPR) between 2006 and 2010. This dataset was applied to each tier of the proposed Framework. The authors concluded that single chemical data are not currently sufficient for use in the risk assessment of mixtures; however, there is a major knowledge gap regarding the numbers of chemicals likely to be present in a mixture scenario. Guidance was recommended regarding the decisions resulting from HI calculation at each tier, including risk management action in the case of the number of tiers being exceeded, or where higher tiers cannot be evaluated due to a lack of data.

- Dewlaque *et al*. (2014) used a modified HI cumulative assessment approach to assess human health risks of exposure to phthalates in the Belgian population. Daily intakes were estimated for diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), DiBP, BBP and DEHP from the levels of metabolites in spot urine samples from 261 individuals aged from 1 to 85 years. The chosen RV for determining the HI for DEHP, DnBP and BBP was the TDI determined by EFSA (EFSA, 2005a; EFSA 2005b), and for DEHP, DnBP, DiBP and BBP it was the Reference Dose for Anti-Androgenicity (RfD AA) (Kortenkamp and Faust, 2010). Cumulative risk assessment was carried out through calculation of an individual's HI, based on their phthalate profile, as the sum of the HQs based on similar toxicological endpoints, and not the most sensitive endpoint. The authors reported that for the HIs calculated using the TDI, 6.2% (13 of 209) of adults and 25% (13 of 52) of children had an HI > 1. The HI calculated using the RfD AA was 3- to 4-fold lower than that calculated with the TDI, which demonstrated the impact of the RV on the outcome of cumulative exposure assessments.

- A cumulative assessment for phthalate exposure of the Austrian general population has been carried out (Hartmann *et al*., 2015). Daily intakes were derived from the levels of 14 phthalate metabolites in spontaneous urine samples of 595 individuals aged from 6 to 81 years, taken as part of the Austrian Study of Nutritional Status (2010/2012). Acceptable levels (AL) for the individual phthalates DEHP, DnBP and DiBP were identified from authoritative reviews (e.g. EFSA, TDI; US EPA, RfD) and for the anti-androgen phthalates DEHP, DnBP, DiBP and BBP the RfD AA (Kortenkamp and Faust, 2010) to allow calculation of the HI. The authors reported that median HIs based on all ALs used were less than 1. However, individual exceedances were seen in all age groups. The highest exceedances (13.3%) of the HI based on TDIs were in children aged 6 to 8 years, with 4.2% in children aged 7 to 15 years. It was also noted that, based on calculations related to RfD AA, no exceedances were identified as a result of the underlying RfD AA values being up to 20 times higher (except for DEHP) than the underlying TDI values.

- Kennedy *et al*. (2015) described a new aggregate model that could be used with varying levels of data to provide outputs ranging from simple deterministic values through to probabilistic analyses, including characterisations of variability and uncertainty. The model was designed to be used with EU-specific databases, guidance and regulatory frameworks relevant to the exposure of humans to plant protection products (PPP) through dietary and non-dietary sources, and is implemented in the web-based software tool MCRA (Monte Carlo Risk Assessment)

(mcra.rivm.nl). The authors described 6 case studies for the conazole group which are commonly used in PPPs. Of these, 5 (Dutch amateur user, acute exposure; Italy worker, chronic exposure; UK/Dutch bystander/resident, acute exposure; UK/Dutch resident, chronic exposure; UK cumulative, acute exposure, from multiple sources) used modelled/predicted exposures and are not detailed further here; one case study using measured exposure data is detailed below.

The aggregate model was applied to evaluate operator exposure from arable boom spraying and the associated activities of mixing and loading of pesticides in the UK. Dietary exposure to conazoles was determined from the National Diet and Nutrition Survey (NDNS) for the study group of male operators (766) aged 19 to 64 years. Non-dietary exposure was assessed as actual dermal exposures (ADE) on the body, actual hand exposures (AHE), and actual inhalation exposure (AIE), with values simulated for each operator from a EUROPOEM dataset. Usage data, including typical triazole combinations (between one and 4 triazoles) sprayed and total amounts applied on different days, were extracted from the UK Pesticide Usage Survey. Aggregate exposures were calculated using RPF. Kennedy *et al*. (2015) reported that, as a proportion of total exposure, the relative contributions were dermal (96.6%), oral (0%), inhalation (2.6%), and dietary (0.5%) for an acute assessment. Median, 90th and 95th percentile aggregate exposures were estimated as 0.65, 14.7 and 35.1 µg/kg bw/day, respectively, although these may be subject to certain limitations due to the approximations used. All aggregated exposure values were below the threshold of 800 µg/kg bw/day for this scenario.

4.2.2 Additional evidence sources

A number of additional case studies that have been published since Bopp *et al*. (2016) were identified, together with outputs from several large European projects on mixtures.

- Larsen *et al*. (2017) evaluated cumulative exposure of children under 3 years and pregnant women/unborn children to 56 endocrine disrupting chemicals (EDCs) and suspected EDCs. An RCR was calculated based on the ratio between the overall exposure to the substance from all sources and the tolerable exposure level (DNEL). Simultaneous exposure routes were considered to be mainly from food, drinking water, soil/dust and cosmetic/personal care products. Overall risk was estimated by the addition of RCR values for the substances having the same mode of action, although it was cautioned that this approach also added the uncertainties associated with individual RCR values. Both “medium” exposure, representing a typical exposure using average values or median values, and "high" exposure, as an expression of realistic worst-case or 95th percentile exposures, were evaluated. Where a number of exposure routes were relevant, the internal dose was calculated using absorption data for each route and then concentrations added. DNELs were based on tolerable exposure levels calculated by authoritative bodies relating to endocrine and chronic neurotoxic effects. In cases where DNELs were unavailable, specific DNELs were calculated. As with the exposure calculations, the DNEL value could be adjusted with

the relevant absorption factor with respect to the route of exposure forming the basis of the DNEL value.

The authors commented that due to a lack of exposure and/or dose-response data for adverse health effects, it was not possible to assess risk for all the substances identified. Among the evaluated EDCs, exposure of children under 3 years and pregnant women/unborn children was identified for dioxins/polychlorinated biphenyl (PCBs), phthalates (DEHP, DBP, DiBP), BPA, tert-butylhydroxyanisole (BHA) and butylated hydroxytoluene (BHT) via foods, leading to total RCRs above 1. For medium exposure of children under 3 the RCR for PCBs and dioxins alone was above 1, and for high exposure of children under 3 the RCR for PCBs and dioxins, DEHP, DBP, DiBP, BPA (depending on the DNEL used) and BHT individually were above 1. For high exposure of pregnant women/unborn children, the RCR for PCBs and dioxins, BHA and BHT were individually above 1. Cosmetics were reported to be a concern for exposure to butyl and propyl paraben (assessed together) and octyl methoxycinnamate (OMC) in high use individuals, particularly during sensitive periods of development, due to potential endocrine disrupting effects. Both individually had RCRs above 1 for children under 3 in the high exposure scenario. Among the evaluated chronic neurotoxins, exposure to lead, dioxins/PCBs, mercury/methyl mercury, BPA and acrylamide in children under 3 years and pregnant women/unborn children were identified, with lead having an RCR of 50 at medium exposure levels for children under 3. Some of the other substances also individually had RCRs above 1. Food was a significant source for all the chronic neurotoxins evaluated, with drinking water, soil and metal objects (such as jewellery and other consumer articles) that can be mouthed by small children also significant for lead, and breast milk for dioxin/PCB exposure.

Papers related to the risk assessment of chemical mixtures have been published as outputs from the EDC-MixRisk research programme, funded by the European Union's Horizon 2020 Research and Innovation Programme between 2015 and 2019. EDC-MixRisk focused on the effects of mixtures of EDCs on children by developing appropriate methods for risk assessment, and was aimed at promoting the safer use of chemicals for future generations.

- Bornehag *et al*. (2019) proposed a ‘whole mixture’ strategy for the risk assessment of chemical mixtures, with the aim of linking epidemiological and experimental evidence. As a ‘proof of concept’, the authors identified mixtures of EDCs associated with anogenital distance (AGD) reduction in baby boys, measured prenatally in a human pregnancy cohort (Swedish Environmental Longitudinal, Mother and Child, Asthma and allergy; SELMA) study. A ‘typical mixture’ was constructed from these findings and tested in a mouse model *in vivo* to determine a POD to be used for risk evaluation. Sufficient similarity was then tested for between the experimentally observed typical, or reference, mixture and those from SELMA. Where sufficient similarity was determined, a risk quotient was calculated for each individual by comparing the experimental POD to human exposure data and subsequently a risk index (the “similar mixture risk indicator”; SMRI) calculated. Using the whole mixture approach the authors reported greater risk (13%) for

pregnant women than was obtained using either the HI approach (3%) or a compound-by-compound strategy (1.6%).

- The desirability function (DF) approach has been developed as a means to allow the simultaneous estimation of PODs for risk assessment of combinations of individual substances that are components of chemical mixtures detected in humans (Costa *et al*., 2011). This approach has been incorporated into a new class of nonlinear statistical models (acceptable concentration range (ACR) models) that incorporate and evaluate regulatory guideline values into analyses of health effects of exposure to chemical mixtures. The strategy uses a Mixture Desirability Function (MDF) calculated from individual DFs for each chemical present in the mixture towards a specific endpoint, which determines an 'acceptable region' of exposure. The models were evaluated for maternal levels of mixtures of 11 suspected EDCs, measured as part of the SELMA pregnancy cohort, against adverse effects on birth weight and language delay at 2.5 years in the offspring (Gennings *et al*., 2018). The authors concluded that when chemicals are present in combination, to achieve a similar level of protection the guidance value needs to be lower than for the same chemicals in isolation. The chemical-by-chemical approach was found to under-estimate the risk by a factor that ranged from 1 to 100 for different chemicals.

A further research programme funded by the European Union's Horizon 2020 Research and Innovation Programme is the European Test and Risk Assessment Strategies for Mixtures (EuroMix) project.

- A tiered strategy for the risk assessment of mixtures of multiple chemicals derived from multiple sources across different life stages has been developed that integrates hazard, exposure, toxicokinetic and toxicodynamic modelling approaches. The system is able to assess quantifiable uncertainties and their influence on the results of cumulative and aggregated risk assessment using a 2D Monte Carlo approach.

  The primary modelling used by the EuroMix toolbox uses dose addition and RPFs with potency-scaled exposures derived against the index substance. PODs take the form of a BMD(L) or NOAEL and can be based on the critical effect of the substance that is the basis for setting the ADI/TDI or the POD for the specific effect that is the focus of the mixture risk assessment, where data allow. A EuroMix toolbox has been developed as version 9 of the MCRA tool (van der Voet *et al*., 2020), which is a web-based platform that employs a high-performance computation cluster to run simulations.

  The utility of the EuroMix approach has been illustrated for human exposure to BPA and its analogues bisphenol S (BPS) and bisphenol F (BPF) which are increasingly used in many consumer products in place of BPA. Measured biomonitoring data were collected from adult volunteers in Norway and compared with exposures modelled using the EuroMix toolbox (Karrer et al., 2020). The authors reported that individual-based medians of modelled BPA exposures were in good agreement with the measurements, but individual-specific correlation was lacking. The good agreement between the ranges of modelled BPA exposure and measured BPA

amounts indicates that available concentrations, especially from the main exposure source food, mirror the exposure situation realistically, and suggests that the exposure model considers the relevant exposure sources. Where detected, modelled exposures mostly underestimated BPS and BPF levels in participants, meaning that not all relevant sources may have been included in the respective exposure models (Karrer *et al*., 2020). Using the EFSA temporary TDI of 4 µg BPA/kg bw and assuming CA and that the same threshold could also be used for BPS and BPF, the highest mixture risk estimated for any individual was 10 fold lower than the threshold (Karrer *et al*., 2020).

One of the primary aims of the Human Biomonitoring for EU project (HBM4EU) is to develop summary indicators to describe the exposure and body burden of chemical mixtures, with an emphasis on defining priority mixtures and identifying the drivers of mixture toxicity. Existing human biomonitoring data on mixtures will be re-evaluated, as well as collecting new human biomonitoring data on mixtures, with the aim of identifying real-life exposure patterns to mixtures. Practical approaches to identify and assess the potential health risks and impacts of mixtures will be further developed and applied. Policy makers, stakeholders and the public at large will be kept informed about the exposure of the European population to mixture and the associated health risks.

4.2.3 Summary of the evidence for the level of risk from unintentional mixtures – human health

The human health studies reviewed here assessed mixture toxicity from a wide range of sources including food, drinking water, indoor air/dust and, in a more limited way, cosmetics/personal care products and pesticides. Exposure was determined mainly from European published data, although the study reported by Kennedy *et al*. (2015) did include UK data. Existing human biomonitoring and exposure data were most frequently used to assess exposure, although the EuroMix project did include a new biomonitoring study to collect data in a specified way (Karrer *et al*., 2020).

All human health applications included an assessment of the utility of the approach proposed. Some common limitations to performing mixture risk assessments for human health include the absence of data for the identity and concentrations of all mixture components and also incomplete toxicological data, including that for the mode of action, for all components, which could lead to either an underestimation or overestimation of potential risk (Kortenkamp and Faust, 2010; Bopp *et al*., 2016).

A further common issue is that a large proportion of human data is left censored, and non-detects can be dealt with in a number of ways, which can impact on the overall assessment (KEMI, 2021). For example, some studies assumed the chemical to be present at the limit of detection/$2^{0.5}$ whilst others treated non-detects as zero. The use of zero is considered likely to lead to an underestimation of risk, whilst the use of a fraction of the limit of detection can lead to overestimations (e.g. De Brouwere *et al*., 2014; Han and Price, 2011, 2013; Price *et al*., 2012b). However, a comparison of the two approaches to

account for non-detects carried out by Price *et al*. (2012b) found no difference in the findings of the risk assessments using the two approaches.

In the same way as for the environmental case studies (Section 4.1) the human health studies calculated mixture risk using CA methods. As previously stated, CA is more conservative than IA and is easier to calculate.

The HI approach was used in several studies. Of these, Kortenkamp and Faust (2010) reported exceedances of defined acceptable levels (i.e. HI >1) at the highest exposure levels to a mixture of anti-androgenic chemicals; however, interpretation was limited due to significant gaps in availability of adequate toxicity data. Exceedances of the HI were reported by Price *et al*. (2012a) for exposure to chemical mixtures in surface and waste water in Europe. Dewlaque *et al*. (2014) reported exceedances to a mixture of phthalates in 6% of adults and 25% of children, in a modified HI cumulative assessment approach. Similarly, exceedances related to phthalate exposure were noted in 13% of children aged 6-8 years and 4 % of children aged 7-15 years in Austria (Hartmann *et al*., 2015). The type of threshold value used was shown by Dewlaque *et al*. (2014) to have an impact on the overall risk assessment, where use of the TDI (covering all endpoints) resulted in an HI 3 to 4 times higher than that calculated using the RfD (for the specific endpoint of concern). Limitations of the HI approach include the multiplication of assessment factors used to derive individual HBGVs, resulting in a conservative assessment of risk.

It should be noted that CA, and HI, should be considered as an initial approach to assessing potential mixture risks. Not all chemicals assessed in these studies will necessarily have the same modes of action, and even where they do, the uncertainty factors included in the HBGVs mean that these approaches can at most demonstrate a potential for risk, rather than suggest there will be a risk. This is because the uncertainty factors in the HBGVs provide a degree of conservatism in the assessment of each individual chemical considered in these unintentional mixtures. Where such a potential for risk is found, further consideration should be made of the modes of action of the substances in question, and the assumptions and uncertainties in the assessment before any evaluation of the degree of potential risk can be made, to date limited studies that do this have been conducted.

The MCR approach was also used to assess the risk associated with combined exposures to a broad range of chemicals, including those present in surface and waste water in Europe (Price *et al*., 2012b), multiple chemical migrants from food contact-grade plastics (Price *et al*., 2014) and indoor air pollutants (De Brouwere *et al*., 2014). The average MCRs reported by these studies were 2.4, 2.0 and 1.8 respectively, suggesting that in all cases only a few compounds make significant contributions to overall potential toxicity. This is also supported by the European Food Safety Authority's (EFSA) work on cumulative effects from dietary exposure to pesticide residues, which found that combination effects of pesticides were driven by a small number of active substances (EFSA, 2020a; EFSA 2020b, EFSA, 2021b).

As with the environmental case studies, a number of the same limitations apply to the studies that have been reviewed here, as no study can measure every chemical present in

a sample or measure all the substances a person is exposed to over their lifetime. In addition, the potential mixture toxicity is still assessed on a sample-by-sample basis and so only considers a single point in time. It is possible to use modelled exposure data (for example see Junghans *et al*., 2006; Marx *et al*., 2015) to increase the number of chemicals assessed; however, this can introduce additional uncertainties, which can be significant.

Additional approaches used for the risk assessment of cumulative exposures include use of the RCR to evaluate exposure to EDCs and suspected EDCs and chronic neurotoxins in pregnant women/unborn child and children under 3 years. Significant exposure, mainly via food, was identified for a number of chemicals individually and then as a combined exposure (Larsen *et al*., 2017). The DF approach has been developed to allow simultaneous estimation of PODs for the risk assessment of chemical mixtures and has been incorporated into ACR models. Calculation of the MDF determines an ‘acceptable region’ of exposure for a specific endpoint (Gennings *et al*., 2018).

5 Methods to address mixture toxicity risk in the context of UK REACH

Currently, chemical regulations consider each substance on a chemical-by-chemical basis. However, we know that in the real-world, people and wildlife are exposed to numerous chemicals (and other stressors) simultaneously. The theoretical basis for mixture risk has been demonstrated (Section 3), and numerous studies have attempted to estimate the mixture risk for the environment and human health in Europe (Section 4). We therefore need to consider methods that could be used to account for mixture toxicity in a pragmatic and proportionate way.

5.1 Mixture Assessment Factors (MAFs)

5.1.1 Introduction

A MAF is an additional assessment factor that is applied to the risk assessment specifically to account for the possibility of mixture toxicity effects (KEMI, 2015).

Under the current EU and UK REACH system, the Chemical Safety Assessment (CSA) for a substance registered above 10 tonnes per year must demonstrate that risk is adequately controlled. RCRs must therefore be below 1. There is no incentive for Registrants to refine risk assessments if the RCRs are only narrowly below 1.

Applying a MAF lowers the acceptable concentration of a substance, providing a greater chemical 'safe space' such that mixture effects – if they were to occur – are less likely to lead to a risk.

Use of MAFs was proposed as an intermediate and precautionary step in a joint statement from a workshop in 2018 that brought together stakeholders from several major EU research programmes on mixtures (Drakvik *et al*., 2020). A joint Dutch/Swedish workshop held in March 2020 (Anon., 2020) also recommended the application of a MAF under EU REACH as the most practical and pragmatic way for EU regulatory authorities to address risks resulting from unintentional mixtures. In the absence of data to the contrary, it was suggested that the same value of MAF should be applied for both human health and environmental risk assessments. The possibility of including a MAF under EU REACH was included in the 2021 European Commission consultation "Chemicals Legislation - Revision of Reach Regulation to Help Achieve a Toxic-free Environment" (EC, 2021).

A summary of the 2020 joint Dutch/Swedish workshop was presented in the open session of the 34th Meeting of the Competent Authorities for REACH and CLP (CARACAL) in March 2020 (https://ec.europa.eu/environment/chemicals/reach/competent_authorities_en.htm). The comments and issues raised by European member states, industry and non-governmental organisations are summarised within the following sections.

5.1.2 Implications of using a MAF

The impact of a MAF will largely depend on the value chosen, but in all cases would make the outcome of a risk assessment more conservative than current practice. For chemicals with RCRs an order of magnitude below 1, the application of a MAF would increase the RCR but would unlikely alter the outcome of the assessment. However, for chemicals with RCRs close to 1 the application of a MAF could increase the RCR to above 1. The implication is that such a chemical could make a potentially significant contribution to mixture toxicity.

If the resulting RCR is above 1, it would need to be addressed in the usual way by either:

- refining the exposure assessment (e.g. by performing higher tier degradation tests, using specific environmental release categories for modelling, carrying out a monitoring programme, etc.);
- refining the effects assessment to justify use of a lower assessment factor when deriving acceptable concentrations (e.g. by providing new chronic toxicity data); or
- reducing emissions by applying additional risk management measures.

As an exposure assessment is not required for chemicals registered below 10 tonnes per year, the application of a MAF would not apply to those chemicals. In addition, for substances which are considered to be non-threshold, for example those with PBT properties, a MAF would not apply since releases are already required to be minimised.

A MAF would be relatively straightforward for legislators to implement as only Annex I of the REACH Regulation needs to be updated. However, there would be a cost implication for Registrants as they would need to update – and in some cases, refine – their CSAs. Depending on the refinement options available, this could result in additional vertebrate testing, which must be a last resort under REACH.

Since the UK REACH registration database is not yet populated, it is not known what proportion of UK Registrants would need to carry out additional work to address newly identified risks under different MAF size scenarios. An initial assessment by ECHA (ECHA, 2020) examined the possible effect of applying a MAF of 10 to the occupational and environmental industrial exposure scenarios from 24 randomly selected CSAs submitted under EU REACH. A ‘significant impact’ was defined as likely to require additional higher tier studies, modelling or monitoring, or additional risk management by the Registrant to demonstrate safe use; ‘moderate impact’ was likely to require refining emission factors and refining the exposure assessment; and ‘no impact’ indicated that safe use was still demonstrated. For occupational human health scenarios, ECHA estimated a significant impact on 30% of scenarios, a moderate impact on 60%, and no impact on 10%. For the environment, ECHA estimated a significant impact on 10%, a moderate impact on 15% and no impact on 75%. ECHA is understood to be presently carrying out an analysis using its REACH database as part of a European impact assessment.

It is debateable whether the MAF should be applied to the DNEL and PNEC or the RCR. The overall conclusion on the level of risk is the same whichever method is used, but the practical implications may vary.

The benefit of applying the MAF to the DNEL and PNEC is that this value is communicated throughout the supply chain, so downstream users can easily account for potential mixture effects as well. However, applying a MAF to a DNEL or PNEC may result in a threshold below the analytical limit of detection, making it difficult for companies to demonstrate compliance with the threshold. In addition, as the DNEL or PNEC may also be used for purposes outside of REACH – in the context of standards setting, for example – and some substances may be subject to more than one legislative regime, varying requirements could result if MAFs are not applied on a consistent basis. If a MAF was applied to a REACH PNEC, but not standards derived under other regulatory regimes, then industrial chemicals may appear to be more hazardous than other compounds, simply due to the additional assessment factor. The reasons for this would need to be communicated clearly.

Applying the MAF to the RCR would address these potential problems. There is a precedent for additional assessment factors to be applied to RCRs in this way: for substances with a high octanol-water partition coefficient (K_{OW}), the soil and sediment RCRs based on equilibrium partitioning are increased by a factor of 10 to account for potential enhanced soil and sediment risk (ECHA, 2008). The additional factor is applied to the RCR rather than the PEC or PNEC because the uncertainty applies to both. A MAF could be viewed in the same way. Currently, we favour applying the MAF to the RCR.

Another consideration is whether to apply the same MAF to all parts of the risk assessment. For the environment, the studies summarised in Section 4 only considered the aquatic compartment. However, the theoretical basis for the concerns over potential mixture toxicity apply equally to the terrestrial and sediment compartments. A MAF could be applied to all environmental compartments considered in the risk assessment, or to a subset. For example, applying a MAF solely to the aquatic compartment in the risk assessment may trigger additional risk management measures that could reduce exposure across all compartments. However, chemicals that do not partition to the water phase, for example poorly soluble substances, are unlikely to demonstrate a risk even if an aquatic MAF was applied. Poorly water-soluble chemicals are more likely to pose a mixture risk in the soil or sediment compartments, and only applying a MAF to the aquatic assessment may miss this. In addition, the value selected as the MAF could be different for different environmental compartments and human health scenarios, including, for example, worker and consumer exposures.

Some studies in Section 4 highlight that the chemicals driving the environmental risk appear to have certain characteristics, including higher production tonnage (Posthuma *et al*., 2019a), higher toxicity (Munz *et al*., 2017; Papadakis *et al*., 2018; Posthuma *et al*., 2019a) and wide use (Posthuma *et al*., 2019a). Applying a MAF only to chemicals that meet certain thresholds based on tonnage, (eco)toxicity or use type could be considered, and would follow the precedent set by applying an additional assessment factor to the sediment and soil PNEC if log K_{OW} thresholds are met. An additional MAF could also be

applied to substances known to display synergistic effects (Sarigiannis and Hansen, 2012; KEMI, 2015).

The implications of applying a MAF to only certain types of chemical would need to be considered carefully. For example, a MAF might only be applied to chemicals with certain environmental hazard classifications or M-factors, as this would focus the risk reduction on the most ecotoxic substances, but these chemicals may already be well controlled due to their high hazard (and have low PNECs, which the MAF would push down further). Or a MAF could be applied to chemicals above an agreed tonnage threshold. This could be based on the total registered tonnage or the tonnage of each individual Registrant, although if the MAF is applied to the PNEC the second option could result in different thresholds for Registrants of the same chemical depending on their level of supply. Thirdly, a MAF could be applied to any chemical with an identified use that results in wide, dispersive environmental emissions. Again, the possibility of this leading to differing requirements between Registrants with different uses would need to be considered.

This report focusses on the UK REACH Regulations. However, the studies summarised in Section 4 for the environment and human health suggest that pesticides, pharmaceuticals, personal care products and industrial chemicals may be contributing to mixture risks. Since chemical exposures result from multiple uses, sometimes falling under more than one regulatory regime, regulatory consistency would need attention if a MAF was considered appropriate for the purposes of UK REACH. The implications of any such change to risk assessment methodologies would need to be assessed by the relevant regulatory authority for each regime. However, the overall approach could consider whether any specific regime should be given greater ‘access’ to the chemical ‘safe space’ than others, for example based on perceived socio-economic importance, or the sophistication of the exposure and hazard assessment in comparison to REACH. The application of a MAF might also be relevant for standard setting (e.g. under UK water protection legislation).

The implications of potential divergence from EU regulations should also be considered. Currently, the risk assessment approach is identical under UK and EU REACH. However, if a MAF were applied under EU REACH but not in the UK, or if the UK selected a smaller MAF value than the EU, the standard of protection for human health and the environment could be perceived by some stakeholders as being weaker in the UK. Alternatively, if the UK selected a MAF that is higher than that used in the EU, industry stakeholders might complain that it puts them at a commercial disadvantage compared to their EU competitors, as they might have additional refinements to make to their CSAs, which would involve a cost.

5.1.3 Possible MAF value

As noted above, the value of the MAF applied can have a large impact on the risk assessment. Defining a suitable value for the MAF on a scientific basis is difficult, as it requires some knowledge of the number of constituents, their concentration and their

toxicity in the potential mixtures that humans and the environment may be exposed to (Swedish Government Inquiries, 2019).

Suggested MAF values between 10 and 100 were reported in KEMI (2015), who also calculated MAFs between 2 and 17 for four case studies in which exposure concentrations for individual components were reduced to an RCR of 0.99 (representing a situation where substance-specific risk management was implemented before a mixture assessment was conducted). A MAF between 3 and 100 was suggested by various speakers at the March 2020 workshop (Anon., 2020). As an example, a MAF of 10 would make the risk threshold more conservative by a factor of ten.

KEMI (2021) describe an updated approach to determine the size of a MAF and apply this to environmental and human health case studies. Similarly to KEMI (2015), the first step is to assume that appropriate substance-specific risk management is implemented, so that the maximum RQ of any single substance is 1. Then the MAF required to result in a mixture RQ of 1 is iteratively determined for each sample. For the environment, KEMI (2021) use the data from Markert *et al*. (2020) and from a Swedish pesticide monitoring campaign (Boye *et al*., 2019) to demonstrate this method. Median MAFs were 5.2 and 3.6 for the two datasets respectively. The median number of chemicals whose concentration would need to reduce was 3 in both cases. The maximum MAF in each dataset that would be needed to result in all samples having a mixture RQ below 1 was 27.5 and 31.7, respectively.

With respect to the human health data in KEMI (2021), analysis of data from the indoor air pollutant study reported by de Brouwere *et al*. (2014) determined a MAF of 10.7, with a median number of 6 chemicals whose concentration would need to be reduced to meet this. A MAF of 7 and median number of 3 chemicals was similarly determined for the anti-androgen exposure study reported by Kortenkamp and Faust (2010). A number of scenario-specific MAFs were determined from the study reported by Andersen *et al*. (2012) for mixtures of anti-androgens, oestrogens and thyroid hormones. MAFs for anti-androgen exposure scenarios (3 in total) were between 2.5 and 4.19, with median numbers of chemicals between 2 or 4. The MAFs determined for oestrogen exposure scenarios (3 in total) were between 4.48 and 7.17, with the median number of chemicals between 4 and 6. The MAFs determined for thyroid exposure scenarios (3 in total) were between 2.51 and 4.48, with median numbers of chemicals between 2 and 4.

For the environment, we have considered whether the data summarised in Section 4 can be used to indicate appropriate MAF values for the UK, should this approach be selected for use. The only data available were for the aquatic compartment, so only this compartment can be considered here. All the studies demonstrated that at a particular site a small number of the chemicals detected (often 5 or less) contributed the majority of the potential risk. This finding has been highlighted previously (e.g. van Broekhuizen *et al*., 2016). However, the specific chemicals contributing most to the total mixture risk at each site varies, and it is not possible to identify these in advance. Also it is possible that some important chemicals could have been missed if they were not included in the analytical suite or assessment of mixture toxicity in these studies, in which case the stated number of chemicals may be an underestimate.

Backhaus *et al*. (2010) demonstrated that if all chemicals are present in a mixture below their respective RCR, a MAF value equal to the number of components will ensure that the mixture risk is below 1. However, considering that the number of chemicals that could be present in a mixture is potentially very large, this could result in extremely low PNECs – or high RCRs – that are unnecessarily conservative. An alternative approach is to set the MAF value equal to the number of components thought to be contributing significantly to the mixture risk. Although this would not guarantee a total RCR below 1 in all cases it would lower the overall RCR and provide additional precaution against potential mixture risks.

Assuming that the potential mixture risk is driven by a small subset of chemicals, as noted above, and that the contribution from all other chemicals present is typically negligible, then a MAF of up to 5 may be appropriate. Spurgeon *et al*. (2021) suggest that as a pragmatic approach an additional assessment factor of 5 could be used to account for mixture effects in groundwater and surface water samples. This would mean that the potential mixture risk would be considered acceptable if the current RCR of any individual substance was below 0.2. A MAF of 5 would not account for mixture risk in all samples, as in some cases more than 5 chemicals will contribute to the mixture risk. If a MAF was applied to a subset of compounds based on their eco(toxicity), tonnage or use, a MAF of up to 5 could still be appropriate as researchers have found these types of chemicals to be more likely to drive the mixture risk.

The studies reviewed in Section 4 include chemicals from across a range of regulatory regimes, and identified both industrial and other chemicals as driving the mixture risk. It must therefore be considered whether the size of a potential MAF should be altered when we are only considering additional risk management for industrial chemicals registered under UK REACH. At any site, typically up to 5 chemicals drive the risk and of these between 0 and 5 will be industrial chemicals. The MAF would therefore need to be applied unadjusted to account for the potential contribution from industrial chemicals. However, in order to reduce the overall mixture risk to below a RCR of 1, the same MAF should also be applied to chemicals in other regulatory regimes.

It must be noted that in the majority of the environmental studies reviewed here, individual substances were present at concentrations above the thresholds for safe use defined in the study. In this case, the MAF based on the MCR may not reduce the mixture risk sufficiently. However, this is due to the risk posed by individual chemicals which would have to be addressed separately.

In the absence of any datasets to inform the MAF value that may be appropriate for the sediment and soil compartments, no value can be proposed for them. It could be decided to read across the MAF from the aquatic compartment and use the same value. Alternatively, it could be decided that a higher MAF value may be appropriate, due to the greater uncertainty.

The human health studies reviewed in Section 4 similarly identified chemicals from across a range of regulatory regimes, with both industrial and other chemicals driving the mixture risk. It should be noted though that, unlike environmental monitoring, analytical techniques

that measure both target and non-target chemicals are largely missing from these studies. When such approaches have been applied to human samples, large numbers of chemicals have been identified; for example, Wang *et al*. (2021) identified 1440 suspect chemicals (550 unique structures) in 30 matched maternal/cord blood samples, of which 73 were tentatively identified using fragmentation spectra. Of the human health studies discussed in KEMI (2021), between 2 and 6 chemicals were assessed as driving the risk; however, these were targeted analyses and, as suggested by Wang *et al*. (2021), this number could be higher if non-targeted analysis was undertaken. Due to the limited availability of studies further investigating the possibility of a mixture risk from the initial screening analysis based on concentration addition, it is not considered appropriate to derive an indicative MAF for human health risk assessment purposes.

5.2 Maintaining current practice in UK REACH

The UK could choose to make no changes to the current risk assessment approach under UK REACH. Although the theoretical potential for mixture effects has been demonstrated and is thought to occur, it is difficult to attribute biological effects to a specific exposure of a mixture of chemicals, and it is unclear whether industrial chemicals contribute more to the total risk than other types of substances (e.g. biologically active substances such as plant protection products, biocides and pharmaceuticals). Without this evidence it could be argued that although mixture effects are theoretically possible, the existing risk assessment approach is conservative enough, with the uncertainty factors already applied in the DNEL/DMEL and PNEC derivation, to adequately account for mixture risks and that, as such, the costs of including a mixture assessment would be disproportionately high for industry. There is also a risk that applying a MAF to UK REACH chemicals alone may not result in a significant environmental improvement or enhanced protection of human health.

For human health, Herzler et al (2021) argue that for an adverse mixture effect to be elicited a combination of several conditions needs to occur together:

a) the chemicals need to have common or interlinked modes of action to act via dose addition;

b) the hazard posed by the individual chemicals must be of high concern (i.e. classified as a Carcinogen Mutagen and Reproductive Toxicant (CMR) and/or STOT-RE);

c) humans must be exposed to each individual component in the mixture below their individual regulatory thresholds (otherwise the scenario is already covered by existing substance-specific regulatory frameworks) and in combination a toxic level must be reached;

d) these exposure levels have to remain more or less constant (not above the individual thresholds and not below an overall toxic level) over the whole time window required for such chronic effects to occur.

They argue that the number of chemicals meeting these criteria occupy a limited chemical space and estimated that REACH-registered substances with relevant hazards (CMR and/or STOT-RE) and direct consumer exposure represent only 4% of all registered substances. Co-exposure to these substances at levels causing toxicity in combination constantly over a relevant time window will lead to a much smaller fraction. In addition, the requirement for these substances to have common modes of action to act additively would further reduce the likelihood that adverse mixture effects would occur in reality (Herzler et al, 2021).

Applying a MAF to the thousands of chemicals registered at >10 tonnes per year (i.e. those that may require an exposure and risk assessment) may be considered disproportionate if the mixture risk is only being driven by up to five chemicals at any individual site or human exposure scenario. The alternative argument is to invoke the precautionary principle on the basis that the potential for adverse effects due to mixture toxicity, even if these cannot be quantified, may be a sufficient reason to apply additional risk management. This may not be justified given the evidence base available.

Deciding to make no changes to UK REACH would not necessarily mean that the potential risk due to mixtures is not addressed. For example, it may be that regulatory mechanisms other than REACH are more appropriate to address potential mixture toxicity in a more targeted manner (see Section 5.5).

5.3 Grouping chemicals by type for risk assessment

There are various examples of the risk management of chemicals as a group across different pieces of regulation. Generally, the groups are based on similar chemistries that are shown to have similar modes of action. Some examples under REACH are described in Section 2. Under the Stockholm Convention (UNEP, 2019), TEFs were used to assess the toxicity of polychlorinated dibenzo-p-dioxins and dibenzofurans in a chemical grouping approach. Grouping chemicals by using new approach methodologies (NAM) to provide information on their modes of action may allow faster and simpler grouping of chemicals in future.

Some more recent research has attempted to identify combinations of chemicals that are likely to co-occur. For example, Posthuma *et al*. (2018) investigated three “land use–related chemical emission scenarios” that enabled them to identify mixtures that were characteristic of each scenario and which were distinguishable based on the components, concentrations and exposure patterns over time. In the US, researchers have used data on purchases of personal care and home cleaning products to identify groups of chemicals that households are likely to be exposed to, based on the demographics of the household (Tornero-Velez *et al*., 2020).

Although the merits of grouping chemicals likely to have similar toxicity and exposure profiles are clear, reaching an agreement on chemical grouping can take time and resource. Under REACH, the Registrant is required to conduct the risk assessment for

their chemical and level of supply, and may not have access to information on other substances that are in the same chemical group to allow them to conduct a mixture assessment. Having to share information on different substances between Registrants and across supply chains raises potential confidentiality issues, and necessitates regular updates as group membership or formulations or use scenarios change. This option may therefore increase the burden on both industry and the regulator to conduct retrospective mixture assessments. In addition, the chemical groups identified may still contribute to mixture toxicity if they are present in a mixture with chemicals from outside the group, so this only provides a partial answer to the assessment of mixture toxicity.

5.4 Grouping chemicals by adverse effect for risk assessment

EFSA conducts a Cumulative Risk Assessment (CRA) for pesticides acting on a particular effect endpoint for human risk assessment via dietary exposure. Initially, a retrospective analysis is conducted based on monitoring data. To date, effects on the nervous system and the thyroid have been investigated (EFSA, 2021). The results of the retrospective analysis are then used to inform future prospective risk assessment, including product authorisations and the setting of Maximum Residue Levels (MRLs).

Kienzler *et al*. (2014) note that the same approach could also be applied to other groups of chemicals (for example, PAHs and flame retardants). It can also be imagined that a similar approach could be used for the environment, for example environmental endocrine disruptors. Although this approach should give realistic estimates of risk, as it would be focussed on specific effects and based on measured data, the disadvantage is the amount of data required and the time needed to do the analysis. In addition, as for grouping by chemical type, the burden of this analysis falls on the regulator and the groups identified may still contribute to mixture toxicity if they are present in a mixture with chemicals from outside the group. Under REACH, the burden for demonstrating safe use should be on the Registrant.

5.5 Requiring mixture assessments for known mixtures at industrial sites

In order to avoid potential confidentiality issues, but to increase the realism of risk assessment to include mixtures that are known to occur together, risk assessment could be performed for chemicals known to be emitted together at single industrial manufacturing or formulation sites. An assessment of a known mixture would be similar to the requirement under the pesticides legislation to consider co-formulants in the risk assessment of products, although in this case the use scenario would be discharge from manufacture and formulation rather than use.

Although this could be considered under UK REACH, not all formulators would currently be REACH Registrants (if they are considered downstream users), so this may introduce

additional regulation to some sites and not others. The emissions from many (though not all) industrial sites are permitted outside of REACH, and it could be a requirement of the permit that mixture effects are considered. However, the permitting regime does not apply to all industrial sites.

Although this option would not address downstream uses, it might contribute to reducing the level of chemical exposure from these sites. Alternatively, techniques such as direct toxicity assessment (DTA) (Hutchinson and Dungey, 2011) could be required to demonstrate that emissions from a site do not pose unacceptable risks. A disadvantage is that DTA generally involves rapid acute tests rather than chronic ecotoxicity studies, which could potentially miss more subtle effects.

6 Recommendations on how to address mixture risks under UK REACH

There is general international agreement that exposure to mixtures of chemicals has the theoretical potential to result in adverse effects in both humans and the environment, where individually the chemicals are below concentrations of concern. However, the scale and level of effects due to mixture toxicity is difficult to determine, due to our incomplete knowledge of exposure concentrations over an organism's life time, our ability to link this causally to adverse effects, and the confounding influence of other stressors. This report does not attempt to summarise the available evidence linking observed biological effects to chemical mixtures, but instead has reviewed studies that have attempted to estimate the potential mixture toxicity based on measured chemical concentrations.

When considering mixture risk, there is general agreement that use of CA as a first approach (e.g. as a screening tool) will in most instances give a conservative initial estimate of any potential mixture toxicity. For the purposes of prospective risk assessment, where the composition of the potential unintentional mixture is unknown, CA is considered to be a reasonable, albeit generally precautionary, approach to evaluating potential mixture risk. When considering retrospective risk assessment further refinement should be applied where CA indicates a potential risk, to take into account modes of action and the assumptions and uncertainty in the screening approach. In addition, regulators should also consider the possibility of synergistic effects of some chemical classes.

Although the human health and environmental studies reviewed in this report included a varying number and type of chemicals across different geographical areas and human populations, and used a variety of (eco)toxicology data for comparison, the consistent conclusion is that only a relatively small number of substances seem to be responsible for the majority of the potential risk from unintentional mixtures. In addition, nearly all of the studies suggest that some (eco)toxicity thresholds selected were exceeded when considering chemicals individually. This does not necessarily mean that adverse effects would have occurred in reality, but it does indicate the potential for effects due to individual substances. Therefore, there may be the potential for reducing risk by focussing on individual compounds and ensuring that both the regulatory risk assessment and risk management measures in place are sufficient to ensure safe use of single chemicals, before considering mixture risk.

It is practically impossible to identify in advance which specific substances have the potential to contribute most to mixture risk, as this varies depending on the specific sites, time points and populations investigated. In the absence of evidence to identify a particular subset of chemicals that are driving the potential mixture risk, the application of a MAF under UK REACH could be a pragmatic and precautionary way forward if it is considered that further regulatory measures are required. Alternative approaches to mixture risk assessment require additional data and resource before they can be applied, and the use of a MAF would retain the principle that it is the responsibility of the Registrant to demonstrate that risks are adequately controlled.

For the environment, the REACH risk assessment aims to protect all species at a population level using data from a small number of standard laboratory test species and the use of assessment factors to derive a theoretical threshold (PNEC) below which significant adverse effects would not be expected. We do not know whether the current approach to calculating a PNEC is protective of the whole environment, or how much conservatism may already be built in. Due to the high level of biodiversity in the environment, it is unlikely that we would ever have full information on the modes of action of all chemicals in all species, or on critical exposure windows during an organism's life span, in order to refine a mixture risk assessment based on CA. Due to these uncertainties, and as a precautionary approach, the application of a MAF would add an additional layer of conservatism to the current risk assessment approach and mean that the environmental risk assessment would be more protective than it currently is. This may be particularly relevant given the unknown pressures placed on the environment by climate change. Based on six environmental studies, four of which included industrial chemicals, a MAF of 5 appears to be appropriate and protective for the majority of situations for the aquatic risk assessment. This could be restricted to certain sub-categories of substance (e.g. those that are more ecotoxic or are supplied at higher tonnage in wide dispersive uses) if it were decided that a MAF is not appropriate for all substances. No data were identified to allow a MAF value to be recommended for the sediment and soil compartments. If the UK decides to further consider the use of a MAF for the environmental risk assessment under UK REACH, then additional studies would be required to determine the appropriate value of the MAF (and its application domain) for all compartments, and to conduct an impact assessment.

In contrast, due to the limited availability of studies further investigating the possibility of a mixture risk from the initial screening analysis based on concentration addition, it is not considered appropriate at this time to further derive a MAF for human health risk assessment purposes. For a mixture risk to be elicited in humans the chemicals an individual is exposed to need to i) have a common or interlinked mode of action to act via CA, which may or may not be the basis of the individual chemicals HBGV, and ii) exposure must be relatively constant during a life-time or critical window (e.g. in utero) for a chronic effect to occur. Therefore, it is likely that the chemicals that meet these criteria occupy a limited chemical space. Another limitation of the evidence base is that for a number of the screening analyses, there are individual chemicals which exceed their regulatory thresholds, which would trigger substance specific regulatory risk measures; potential mixture risks in these circumstances should not be used as a basis to justify use of a MAF without further evaluation. In addition, the use of uncertainty/assessment factors in the derivation of HBGVs mean that any potential mixture risk may already be mitigated. Human health risk assessment differs from environmental risk assessment as it based on levels that do not cause an adverse effect in individuals over a lifetime/critical window, whereas environmental risk assessment is based on population level effects. This means that there is more precaution already present in the individual chemical assessment for human health than for environmental risk assessment. An assessment of the limited available evidence systematically and in an unbiased way in order to identify knowledge gaps which can be addressed by targeted research would be beneficial. In the first instance we would suggest undertaking further analysis of the studies outlined in Section 4

to investigate the endpoints on which the HBGVs are based, considering the mode of action, to establish the validity of the use of concentration addition in these initial assessments to gain a more refined assessment of the potential mixture risk.

Finally, it should be noted that there is no clear evidence that industrial chemicals contribute to the potential mixture toxicity risk more than chemicals regulated under other regimes. Policy makers will therefore need to consider whether applying a MAF to industrial chemicals alone can be justified. A further exploration of the issues with stakeholders (and especially REACH Registrants' representatives) would be useful to better understand the likely impacts of the use of a MAF in a UK context.

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List of abbreviations

ADI	Acceptable Daily Intake
CA	Concentration Addition
CARACAL	Competent Authorities for REACH and CLP
CLP	Classification, Labelling and Packaging
DMEL	Derived Minimal Effect Level
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
ECx	Effect Concentration at x%
EQS	Environmental Quality Standard
GCL	Generic Concentration Limits
HBGV	Health-Based Guidance Values
HCp	Hazardous Concentration for p% of species
HI	Hazard Index
IA	Independent Action
LCx	Lethal Concentration at x%
MAF	Mixture Assessment Factor
MCR	Maximum Cumulative Ratio
MOE	Margin of Exposure
MOET	Total Margin of Exposure
msPAF	Multi-Substance Potentially Affected Fraction
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
PBT	Persistent, Bioaccumulative and Toxic
PNEC	Predicted No Effect Concentration
PODI	Point of Departure Index
RCR	Risk Characterisation Ratio
RPF	Relative Potency Factors
RPI	Reference Point Index
RQ	Risk Quotient
SCL	Specific Concentration Limits
SSD	Species Sensitivity Distribution
SVHC	Substance of Very High Concern
TDI	Tolerable Daily Intake
TEF	Toxic Equivalence Factor
TTD	Target Organ Toxicity Dose
TU	Toxic Unit
WHO	World Health Organisation

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