The Interdepartmental Group
On Health Risks from Chemicals

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Chemical Mixtures: A framework for assessing risks to human health

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IHRC

The Interdepartmental Group on Health Risks from Chemicals aims to stimulate the development of new, improved approaches to the assessment of risks to human health from chemicals.

The Steering Committee of the Interdepartmental Group on Health Risks from Chemicals comprises participants from the Department for Environment, Food and Rural Affairs, the Department of Health, the Department for Business, Enterprise and Regulatory Reform, the Home Office, the Environment Agency, the Health and Safety Executive, the Food Standards Agency, the Medicines and Healthcare Products Regulatory Agency, the Pesticides Safety Directorate, the Veterinary Medicines Directorate, the Biotechnology and Biological Sciences Research Council, the Medical Research Council and the Natural Environment Research Council.

The Secretariat is based at the Institute of Environment and Health, Cranfield University.

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Foreword

This document has been produced by the Interdepartmental Group on Health Risks from Chemicals (IGHRC) as part of its Phase 2 work programme (October 2003-September 2007), and is informed by a *Chemical Mixtures* workshop that took place in Leicester on 23rd February 2005. Following initial drafting, government departments, agencies and their advisory committees were consulted in order to obtain as broad an input and consensus as possible. The following committees reviewed and provided input to the document:

- Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
- Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
- Advisory Committee on Pesticides
- Advisory Committee on Hazardous Substances
- Committee on Mutagenicity
- Veterinary Products Committee
- Working Group on Action to Control Chemicals

Input was also received from other independent experts on an individual basis and from non-governmental organisations. The Chemical Mixtures Steering Committee, consisting of IGHRC members who have overseen the development of this document, also provided expert opinion. While the committees, advisory groups, organisations and individuals provided input, responsibility for the content of the document remains entirely with IGHRC.

This document is intended to provide a general framework to assist those undertaking risk assessments of chemical mixtures. I hope it will be read as a useful introduction and a worthwhile attempt to clarify what is a complicated area of science.

Professor David Harper CBE
Chairman of the IGHRC
Chief Scientist of the Department of Health

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Executive summary

Current risk assessment practices are largely based on evaluating the toxicity of single chemicals. However, humans are simultaneously exposed on a daily basis to a large number of chemicals, both intentionally and unintentionally. There are regular expressions of media and/or public concern that exposure to this “chemical cocktail” could result in adverse health effects unforeseen by current risk assessment practices. This document aims to provide a framework to help risk assessors think about how to address mixture issues. It discusses the types of mixtures for which UK government has to conduct risk assessments and the circumstances in which people might be exposed. It considers different regulatory approaches that may be adopted for different types of mixtures and the circumstances in which these approaches could be used. It is aimed at both the risk assessors who have to consider mixture issues when assessing chemicals and the stakeholders with whom they communicate. It draws on the approaches that have been described in publications from other advisory or regulatory bodies and presents a flow chart that will help risk assessors identify key issues that have to be considered depending on the type of mixture that is being assessed and the types of data that are available.

To keep the scope of the document to a manageable size of practical use, it focuses on generic issues relating to the human health risk assessment of chemical mixtures at levels of exposure that may be encountered by people in their daily activities. It does not consider the physical hazards of chemical mixtures (e.g. flammability or explosivity) or combined exposures to chemicals and physical hazards such as electromagnetic radiation, ultraviolet radiation or noise. The document does not specifically consider medical issues such as exposure to multiple medicines or co-exposure to medicines and other chemicals on the grounds that medicines are given at levels that are intended to have a biological effect, in contrast to lower level exposures to environmental contaminants. However, it is acknowledged that many of the principles that govern assessment of interactions between different medicines as well as between medicines and non-medicinal substances also have potential relevance to other chemical mixtures. The document does not attempt to review mixture literature to identify specific interactions between chemicals. The goal is to provide a general framework to guide a risk assessor when dealing with chemical mixtures.

The document concludes that chemical mixtures are best considered as a series of discrete, precisely defined problems for which clear boundaries can be set. Each discrete, precisely defined risk assessment can then be compared to other, similar risk assessments to enable the larger picture to be assembled over time. A key factor in risk assessments for chemical mixtures is the availability, or absence, of reliable data for the whole mixture or its components. Where mixture risk assessments follow component-based approaches it is particularly important to have reliable data on the identity, toxicokinetics, metabolic pathways, mechanisms of action and levels of exposure for the key components in order to make expert judgements about the potential for interactions between components to affect the overall toxicity of the mixture. Where this information is lacking, regulators may need to make precautionary default assumptions. Where there is no clear information on the potential for interactions to occur, there is no scientific basis from which to consider interactions in either a quantitative or a qualitative sense. Hence, it is most appropriate to use a default approach assuming no interactions as the starting point for a preliminary (Tier 1) risk assessment. It is acknowledged that the “no-interaction” hypothesis may appear to be a less precautionary approach. The

picture that is emerging from robust mixture studies suggests that interactions are not observed at dose levels below thresholds of effect. This document deals with situations where exposures are likely to be low. Conservative assessment factors are generally used to estimate thresholds of effect for data-limited substances. Hence, providing that exposure to each component is below the estimated threshold of effect for that component (or, for groups of components that cause similar functional effects, exposure to all components in the group is below the threshold of effect for the most hazardous chemical in the group), a no-interaction model is likely to be adequate. Dose addition is the most precautionary no-interaction model to use.

If there is evidence to suggest that interactions may occur, the risk assessor should use all available data to make, as far as is possible, a quantitative assessment of the effects of the interaction. For the situation where chemical-chemical reactions are considered important, the document recommends that potential reaction product(s) should be included in the assessment as additional component(s). For the situation where toxicokinetic interactions may increase overall toxicity, in the absence of information to allow the effects of the interaction to be quantified, the document recommends the use of assessment factors to take account of potential increases in the levels of a toxicant at its target site or prolonged exposure if clearance of the toxicant is delayed. The rationale for the selection of assessment factors must be clearly described. Where there is a potential for toxicodynamic interactions, evidence from robust mixture studies suggests that these are only likely to occur where exposures are around and above thresholds of effect. Therefore, providing the exposure level for each component (or group of components producing functionally similar effects) is below its threshold of effect (noting the need to consider the potential for toxicokinetic interactions to influence the relationship between an external dose and the level of the toxicant at its target site), there should be no toxicodynamic interactions.

Finally, if a risk assessment based on default assumptions raises concerns, this does not necessarily mean that there is a risk to health. It may simply reflect the limitations in our understanding about that particular mixture situation. The impact of default assumptions on the robustness of the risk assessment should be reviewed before the assessment is used to support decision-making. Uncertainty analysis techniques are available that allow risk assessors to look systematically at the impacts of each assumption on the outcome of the risk assessment. It is important to describe the uncertainty that each default assumption introduces and, where possible, make judgements about whether the assumption could result in under or overestimation of risk. If there is considerable uncertainty in the assessment, it may be more appropriate to gather additional information to refine the assessment. The uncertainty analysis may highlight the most important areas where information is needed. We can only be certain that we are making appropriate decisions when we properly understand the hazards and pathways of exposure for the materials that we are dealing with.

The approaches outlined in this document are based upon a reasonable interpretation of current science and reflect pragmatic approaches that can be adopted depending on the type and amount of data available. There is increasing activity in the scientific community geared towards improving our understanding of the behaviour of chemicals in mixtures. As our understanding of mixture toxicology develops, it may be necessary to revisit the advice given here.

1. Introduction and Scope

Current risk assessment practices are largely based on the evaluation of single chemical entities. However, humans are exposed on a daily basis to a large number of chemicals simultaneously, both intentionally and unintentionally. The air we breathe and the food we eat are mixtures of chemicals; some occur naturally, others are man-made. Additional exposures arise because of the products we use in our daily lives at home and at work. There are regular expressions of media and/or public concern that exposure to this “chemical cocktail” could result in adverse health effects unforeseen by current risk assessment practices. It is conceivable that risk assessments based on single substance evaluations may underestimate the toxicity of a mixture. This could occur through exposure to multiple chemicals that cause the same effect, with the impacts of each chemical contributing to toxicity in an additive manner. Alternatively, it is possible that interactions between chemicals may change the dose-response relationships observed for chemicals tested in isolation, leading to adverse effects at lower than expected doses or additional toxic effects that would not be predicted based on the toxicity of individual components.

The “mixtures” issue is not new. In 1939, Bliss published a paper in which he described three types of joint action that chemicals in mixtures may show. These were: independent joint action, where chemicals act independently of one another and have different modes of toxic action; similar joint action, where chemicals have similar effects but do not interact (i.e. dose addition); and synergistic/antagonistic action, where the toxicity of a mixture may be greater than/less than the toxicity that would be predicted from the individual constituents. These concepts are used today by mixture scientists to categorise the behaviour of chemicals in mixtures.

There are several difficulties associated with risk assessments of chemical mixtures. One difficulty in assessing the potential effects of exposure to mixtures of chemicals present at low concentrations is that most experimental data on the effects of exposure to chemical mixtures have been obtained from studies using relatively high levels of exposure, i.e. levels at which effects would be likely to occur. For example, early studies by Smyth *et al* (1969, 1970) used lethality as the end point in a series of studies looking at joint actions in chemicals dosed in pairs. While there is evidence from a few robust studies that both synergistic and antagonistic interactions, as well as additive effects, can occur in mixtures, these are usually observed at high experimental exposure levels, much higher than most real-life exposures. The type of combined action or interaction found at clearly toxic effect levels may not predict what will happen at lower levels. Not all interactions are easy to predict, such as those occurring at the transcriptional level of the genome or second-messenger signalling pathways. Hence, there could be many uncertainties in the hazard assessment and estimated dose-response relationships for chemical mixtures.

There are also difficulties in identifying which chemicals should be considered in the assessment. We need to consider not only mixtures of chemicals that occur in the same place at the same time (concurrent exposure) but also mixed exposures that arise as a result of sequential exposure to different agents. This is of particular importance for persistent and bioaccumulative chemicals where there may be prolonged exposure or a gradual build up of the chemical due to successive exposures. We have very little information on prolonged exposure due to environmental persistence (as opposed to the much more readily assessed scenario of repeated exposure occurring over many months/years). Further challenges associated with the assessment of sequential exposures relate to the fact that the sequence of exposures an individual receives will be unique to that individual and it would present an impossible task to try to consider all possible exposure permutations. As it is not possible to formally construct assessments of the risk to health from all potential combinations of chemical exposure, regulators need to

identify and focus on the particular aspects of exposure to chemical mixtures considered to merit the highest priority for attention. Rather than try to tackle chemical mixtures as a single issue, we need to divide “chemical mixtures” into more discrete, precisely defined problems that will allow clear boundaries to be set for each assessment. Each discrete, precisely defined risk assessment can then be considered in the light of other discrete, precisely defined risk assessments to enable the bigger picture to be assembled over time.

The use of “issue definition” to refine the scope of risk assessment for chemical mixtures is exemplified by the risk assessment for exposure to mixtures of pesticides and related substances conducted for the Food Standards Agency by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT, 2002). The COT was asked to critically review what is known about the toxicology of mixtures and consider the implications for risk assessments of dietary exposure to pesticide and veterinary medicine residues. The terms of reference were:

- To assess the potential for multiple residues of pesticides and veterinary medicines in food to modify individual toxicity of chemicals in humans;
- To evaluate what assumptions can be made about the toxicity of pesticides in combination;
- To consider the potential impact of combined exposure to pesticides and veterinary medicines by different routes;
- Formulate advice on the standard risk assessment procedures applicable to the safety evaluation of individual pesticides and veterinary medicines in the light of the above considerations.

Although there are many chemicals present in foods that would be worthy of consideration, the task of the COT was made manageable by focusing on a particular group of chemicals that are subject to regulatory controls based on single substance risk assessments (pesticides and veterinary medicines), and by considering a single route of exposure (diet). Having completed an assessment on this basis, subsequent work might then be considered to address additional sources of exposure to these chemicals (e.g. occupational exposure) or the influence of other types of chemicals in the diet (e.g. food additives).

Although the 2002 COT report focused its attention on mixtures of pesticide and veterinary medicine residues in the diet, many of the findings could be applied to other mixtures including food contaminants and additives (COT, 2004). The key conclusions reached by COT (2002) regarding the risk assessment of mixtures were:

- Direct chemical reactions can occur between components of a mixture, though relatively few studies have been conducted to investigate such reactions;
- Mixtures of chemicals that affect the same target organ and have the same mode of action will show additivity (dose addition), which results from simple similar action, over the whole dose range;
- Where components of a mixture have different modes of action and exposures to each component are below any threshold of effect, no additivity and no potentiating interactions are generally found, suggesting that adverse reactions to the mixture at this level of exposure would be unlikely;
- A few studies have found evidence for potentiation when exposure to the mixture exceeded the threshold of effect for some or all of the components. However, it is not scientifically valid to extrapolate these findings to much lower dose levels;

- The probability of any health hazard due to additivity or potentiating interactions of mixtures at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides in food to which humans are exposed is generally much lower than the NOAEL, at least through food.

1.1 Scope of This Document

Mixtures of chemical additives and residues in food are just one type of "mixture situation" for which UK government needs to conduct risk assessments. There are many more mixture exposure situations that fall within the scope of UK government regulatory activities. This document aims to provide a framework to help risk assessors address defined mixture issues, and outlines possible approaches for different situations. It draws on the approaches that have been described in publications from other regulatory bodies. It is aimed both at risk assessors who have to consider mixture issues when assessing chemicals and at the stakeholders with whom they communicate. Hopefully the document will facilitate debate about the most appropriate approaches to take for the assessment of chemical mixtures in different situations.

The principal objectives of this guidance document are to:

- Describe the types of mixtures for which UK government has to conduct risk assessments and the circumstances in which people may be exposed;
- Consider different regulatory approaches that may be adopted for different types of mixtures and the circumstances in which these approaches could be used;
- Outline current risk assessment processes for mixtures in UK agencies and government departments;
- Provide a framework, in the form of a decision tree, to guide regulators when assessing risks from chemical mixtures;
- Aid understanding and promote consistent use of terminology, including the development of a glossary for use by regulators.

To keep the scope of the document to a manageable size it will focus on generic issues relating to the human health risk assessment of chemical mixtures at levels of exposure that may be encountered by people in their daily activities. It will not consider the physical hazards of chemical mixtures (e.g. flammability or explosivity) or combined exposures to chemicals and physical hazards such as electromagnetic radiation, ultraviolet radiation or noise. The document will not specifically consider medical issues such as exposure to multiple medicines or co-exposure to medicines and other chemicals on the grounds that medicines are given at levels that are intended to have a biological effect, in contrast to lower level exposures to environmental contaminants. However, it is acknowledged that many of the principles that govern assessment of interactions between different medicines and between medicines and dietary constituents also have relevance to other chemical mixtures. This aspect has been further expanded upon by the COT (COT, 2006).

This IGHRC document does not attempt to review the literature examining specific interactions between chemicals. It does, however, attempt to summarise current thinking in a developing area of science. The goal is to provide a general framework to guide risk assessors when dealing with chemical mixtures.

2. What Constitutes a Mixture?

It is not possible to provide a single definition of what constitutes a mixture that will be applicable in all situations. In its simplest form, a mixture is a combination of two single chemical entities. However, individual chemicals are rarely pure. Manufactured chemicals such as industrial chemicals, pesticides or food additives will generally contain traces of other substances (impurities) derived from the starting materials and/or production process. Chemical manufacturers will commonly supply different grades of various chemicals (e.g. technical grade, analytical grade, and pure grade) that have different levels of purity depending on their intended use. The European Inventory of Existing Chemical Substances (EINECS), which lists all substances supplied onto the EU market between 1 January 1971 and 18 September 1981, allows a substance to be listed as an individual chemical providing it contains at least 80% of the listed chemical (Vollmer *et al*, 1998). This means that for the purposes of this inventory, a chemical that contains up to 20% process-derived impurities can be regarded as a single chemical entity. Thus, an apparently simple combination of two single chemical entities could contain several additional chemical impurities. Depending on the hazardous properties of these impurities and the intended use for this two component mixture, it may be necessary to take account of these impurities in a risk assessment.

In some cases, mixtures will be produced by mixing together defined chemicals in defined amounts to create a specific product. However, other mixtures are produced as reaction products from a particular set of starting materials or are refined from crude starting materials. These mixtures may not have a well-defined composition and may be selected for a particular set of technical properties rather than on the basis of a particular chemical composition (e.g. lubricating oil). In the situation where specific chemical substances are formulated together to produce a mixture, it is clear which elements of the mixture are the components (the intended chemical substances in the formulation) and which are the impurities (those substances that are present unintentionally as a result of the manufacturing process). The dividing line between component and impurity becomes less apparent with mixtures that are intentionally formed as products of a chemical reaction, mixtures that derive from a refining process (including poorly defined extracts from biological sources), mixtures that are unintentionally produced or released from manufacturing or refining processes (i.e. by-products and wastes), mixtures of chemicals that are present in the environment, and mixtures of chemicals that are present in the diet. With these types of mixture, every substance in the mixture could be considered to be a component rather than an impurity, and it may not be readily apparent which components of the mixture need to be taken into consideration in the assessment and which can be excluded on the grounds that they do not make a meaningful contribution to the overall hazardous properties of the mixture. It will not be possible to define rigid criteria for including and excluding particular components that can be applied in all contexts. Depending on the context for which the mixture is being assessed and the potential for human exposure, the criteria for excluding certain components could be more or less stringent. It may also be necessary to consider sequential exposures, particularly for substances that have relatively long residence times in the body. This document offers suggestions to help risk assessors decide which components should be considered and which can be excluded (see Section 4).

For convenience, mixtures are often referred to in the scientific literature as simple or complex and these descriptions can be useful when considering which assessment approaches are suitable for different types of mixtures (Table 1).

Simple mixtures: Simple mixtures have few components; 10 or less is often used as an arbitrary limit although this should not be regarded as an absolute constraint. It is also helpful to consider the amount of information available on the composition of the mixture and the variability of different components in the definition of a simple mixture. Hence, a simple mixture can be considered to be any mixture for which all components are known and which has a fairly constant composition. Most formulated products would be regarded as simple mixtures because consistent starting materials are blended in consistent amounts to produce a particular product. The concepts of components and impurities apply to simple mixtures. Intentionally included components are likely to be the main determinants of the hazardous properties of the mixture. Impurities that are present at low concentrations will not, in most cases, need to be considered in the risk assessment. An exception might be the case where impurities are present that cause adverse effects at potentially very low doses, such as genotoxicity or allergenicity.

Complex mixtures: Complex mixtures generally have many components; the identity of all components may or may not be known and the composition may be very variable. Complex mixtures can be produced intentionally from manufacturing or refining processes; process emissions, by-products and wastes are also often-complex mixtures. Furthermore, mixtures of contaminants in the environment and mixtures of residues in foods and drinking water can be considered to be complex mixtures. It is generally not possible to apply the concepts of components and impurities to complex mixtures. The context in which the mixture is being assessed, and the particular concerns that are being addressed, will determine which components need to be taken into consideration. When UK regulators deal with mixtures, the mixtures that they need to consider are primarily complex mixtures.

Table 1. Allocation of mixtures by type of mixture and type of data available

Type of data that may be available	Type of mixture	
	Simple	***Complex***
Component data	Formulated products Pesticide/biocide formulations	Chemical residues in food and drinking water Soil contaminants at old industrial sites
Whole mixture data	Pesticide/biocide formulations (usually restricted to information on single dose toxicity, irritancy and skin sensitisation)	Mixtures manufactured as reaction products for sale (complex substances)* Mixtures produced from a refining process* Process emissions, by-products and wastes* Air pollution*

* In some cases, additional data may be available for certain components and sub-fractions of these mixtures

For the purposes of this document, chemical mixtures can be considered to fall into one of four categories, as listed in Table 1. These are:

- Intentionally manufactured mixtures;
- Mixtures that are incidental to specific industrial/chemical processes such as process emissions, process by-products and wastes;
- Mixtures present in the diet and drinking water;
- Mixtures of contaminants at former industrial sites.

These categories have been identified based on the amount and type of information available on the composition and hazardous properties of the mixture, the circumstances in which people might be exposed, and the scope for remedial action if concerns are identified. So, for example, there are regulations in place that require suppliers to identify the hazardous properties of mixtures supplied for sale and to provide appropriate warning labels on packages. If the risks to health from using such mixtures are deemed to be unacceptable, there is scope to change the mixture or change the way the mixture is used to reduce the risks. In contrast, land at an old industrial site might be contaminated by a number of different substances and the combination of chemicals that can be present at such sites will vary from site to site. Regulators have to assess the risks to health from proposed future use of the land and to determine whether or not potentially costly clean-up operations are needed.

2.1 Intentionally manufactured mixtures

Intentionally manufactured mixtures are those that are deliberately produced for a specific purpose. Both simple and complex mixtures are manufactured. Simple mixtures include formulated products, e.g. shampoos, paints, household cleaning products and pesticide formulations. In such cases, known amounts of defined components are combined to form a specific product and there will be little change in the composition of the product between batches. Knowledge of the hazardous properties of such mixtures will generally be based on hazard data for the components, though in certain cases data pertaining to the whole mixture may be available. For example, some whole mixture testing is carried out to support the approval process for pesticide and biocide products. In the case of many consumer products, it is not realistic to obtain toxicity test data for the large number of formulated products available and regulations are in place that prohibit animal testing for certain formulated mixtures such as cosmetics. Whole mixture testing for pesticide and biocide formulations is useful because such mixtures contain biologically active components, and it is possible that co-formulants may alter the toxicological behaviour of the active components. Hence the availability of whole mixture test data is useful for risk assessment purposes and can help to determine the appropriate classification and labelling for the formulation. Such whole mixture testing usually covers a limited range of acute toxicity endpoints.

Combinations of pesticide products created to enable multiple pesticides to be applied simultaneously to a crop can also be regarded as simple mixtures because specific pesticide products are being combined. In this situation, information will be available on the composition and hazardous properties of the individual products in the mixture. There may be some uncertainty about the effects of combined exposure to multiple products, but knowledge of the components and modes of action of the individual active ingredients will allow a judgement of the potential for effects from combined exposure.

Complex mixtures, referred to as complex substances or substances of varying composition in the context of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation, can be intentionally created as a product of a manufacturing process or a refining process. Defined starting materials are used in defined amounts but the chemical reaction

process leads to a product that is a mixture of substances. This could be a mixture of different isomeric forms of a chemical, e.g. methylstyrene, mixed isomers. It could be a mixture of substances from the same chemical class but with, for instance, differing carbon chain lengths and differing amounts of a particular substituent along the carbon chain, e.g. short chain chlorinated paraffins or alcohols, C8-20. It could contain chemicals from more than one chemical class. Often such mixtures are defined by the starting products and not by the components of the final mixture, e.g. 2-naphthalenesulfonic acid, reaction products with formaldehyde and toluene. Mixtures produced by a refining process, such as petroleum distillates or plant extracts, also fall into the category of intentionally manufactured mixtures.

Complex manufactured mixtures (complex substances) tend to be produced for a specific purpose and are selected for their physical and chemical properties. Although such mixtures must meet defined technical or purity specifications, there is scope for batch-to-batch variation in composition. The composition may vary because of variations in the amount/purity of starting materials or the process conditions, or to meet different technical specifications for different applications. It may be the case that complete chemical analyses to identify all components have not been performed. The identity of minor components may well not be known. Information on the hazardous properties of such mixtures will usually be based on whole mixture data and on data for similar whole mixtures. Occasionally there may also be data on individual components or subsets of components, in which case there would be a question about the relevance of the component data to the whole mixture.

Exposure to intentionally manufactured mixtures may occur during manufacture, use and disposal; exposures arising from these scenarios can be quantified by either measurement or modelling, and risk assessments can be performed. If concerns are identified, risk management options generally focus on changing the composition of the mixture or the way the mixture is used and its disposal.

For the purposes of this document, chemical mixtures can be considered to fall into one of four categories, as listed in Table 1. These are:

- Intentionally manufactured mixtures;
- Mixtures that are incidental to specific industrial/chemical processes such as process emissions, process by-products and wastes;
- Mixtures present in the diet and drinking water;
- Mixtures of contaminants at former industrial sites.

These categories have been identified based on the amount and type of information available on the composition and hazardous properties of the mixture, the circumstances in which people might be exposed, and the scope for remedial action if concerns are identified. So, for example, there are regulations in place that require suppliers to identify the hazardous properties of mixtures supplied for sale and to provide appropriate warning labels on packages. If the risks to health from using such mixtures are deemed to be unacceptable, there is scope to change the mixture or change the way the mixture is used to reduce the risks. In contrast, land at an old industrial site might be contaminated by a number of different substances and the combination of chemicals that can be present at such sites will vary from site to site. Regulators have to assess the risks to health from proposed future use of the land and to determine whether or not potentially costly clean-up operations are needed.

2.2 Process emissions, by-products, wastes and air pollution

This is a diverse category covering mixtures that are incidentally released from specific industrial or chemical processes rather than being deliberately manufactured. Air pollution is included in this category because it is generated by a combination of different anthropogenic and geological processes in different geographical locations. Process emissions, by-products, wastes and air

pollution are complex mixtures. If there is variability in the starting materials for the process or the conditions under which the process takes place (e.g. temperature and pressure variations) there will be variability in the composition of emissions, by-products and wastes. The category includes mixtures arising from both point and diffuse sources. Examples of mixtures that fall into this category are emissions from specific manufacturing processes, e.g. welding fumes, or emissions from combustion processes, e.g. waste incineration or vehicle exhaust fumes. Releases to air or water from particular industrial sites are also considered to fall into this category.

Depending on the nature of the process, any emissions, by-products and wastes that arise may contain many hundreds of components and it may not be possible to identify all of them. There may be considerable day-to-day variability in the composition of these mixtures, although a process using consistent starting materials under consistent conditions is likely to generate broadly consistent emissions. Our understanding of the hazardous properties of process emissions, by products and wastes may be based on studies with whole mixtures. This is most likely in the case of widely occurring process generated emissions in the workplace where studies have been done to support industrial hygiene investigations. Whole mixture studies have also been conducted to investigate the effects of exposure to air pollution. However, if there is considerable variability in the composition of the mixture, as is the case for welding fumes, then it may not be possible to draw general conclusions about the hazards of particular emissions based on information from one or two sources. Where there are no data on the hazardous properties of the whole mixture, as is likely to be the case for emissions and wastes released from industrial premises to the environment (particularly where the release contains material from several processes), it may be necessary to rely on data for key components. In this situation there can be more uncertainty in assessing the risks from a mixture, particularly if the composition is poorly understood.

Exposure to process emissions, by-products and wastes depends on proximity to the source of the emission and on patterns of activity. It is usually possible to quantify exposure by measuring the levels of emissions produced by various sources and estimating the amount of time an individual will spend in proximity to the emission or the amount that is released to the environment. Risk assessments can be performed to examine the risks associated with the release of particular emissions, by-products and wastes. If concerns are identified, risk management options will seek to modify the process and/or the starting materials to change the nature of emissions, by-products and wastes.

For air pollution, factors such as geographical location, changing weather conditions, industrial activities and traffic levels will influence exposure, and it will be necessary to monitor what is present in the air to determine the risks at any particular time. Risk management options tend to focus on the identification and reduction of pollution from contributing sources.

2.3 Mixtures present in the diet and drinking water

Food and drink represent complex mixtures of chemicals. In addition to macronutrients from foods, such as proteins, carbohydrates and fats, there are other naturally occurring constituents. There may also be a wide range of other substances that do not occur naturally, including additives used to enhance flavour, shelf-life etc., as well as incidental contaminants arising from processing and packaging materials and also, if applicable, chemicals formed during cooking. In addition, there may be pesticide and veterinary medicine residues present in foods, traces of environmental pollutants and sometimes mycotoxins and other microbial toxins from fungal contaminants. Micronutrients either added to foods or taken by individuals to supplement their diet further add to the dietary mixture. The composition of dietary substances to which an individual will be exposed will vary depending on the

type of diet that they eat, the extent to which food items have been processed before consumption, and whether they choose to ingest additional nutritional supplements.

In the case of food additives, the composition of the additive is known and rates of ingestion can be estimated. Less is known about nutritional supplements, trace contaminants and chemicals formed during cooking. Periodic analyses are performed to determine the levels of known residues and contaminants in foodstuffs, including, where applicable, veterinary medicine residues. Details of pesticide and veterinary medicine surveillance programmes and non-statutory residue surveillance schemes in the UK are described within the COT report (COT, 2002). In addition to surveys such as the periodic Total Diet Studies, which aim to identify general levels of exposure to particular residues across a range of food groups, there are a number of, smaller and more targeted studies examining specific contaminants in specific food groups. Details of these studies are available on the Food Standards Agency (FSA) website at http://www.food.gov.uk/science/surveillance/.

Over time, these studies will enable a general picture to be assembled of the levels of various different substances in the diet. There is still work to be done in this area.

Drinking water, both tap and bottled, also contain a diverse range of residues and contaminants. Any water-soluble chemical present in the environment that is not completely removed by the treatment process may potentially be present in the water supply. In addition, the treatment process itself can introduce residues and by-products into the supply. Which contaminants are present in the supply depends on a number of factors including whether the water is obtained from a groundwater or surface water source, and factors such as the local geology and land-use. Potential contaminants include naturally occurring metal salts; chemicals used in agriculture, substances discharged from industrial processes and other environmental contaminants as well as drinking water treatment chemicals and disinfection by-products. Regular and frequent analyses are performed to ensure that levels of regulated contaminants do not exceed permitted levels.

In the case of both food and drinking water, although the identity and quantity of the natural constituents, residues and contaminants present is likely to be highly variable, in general, these substances will be present at very low concentrations. In the case of pesticide and veterinary medicine residues, specific residues are likely to be associated with particular foods but there may be variations in the levels of these residues depending on the season and the geographic region from which the food was obtained. Understanding of the hazardous properties of residues and contaminants in food and drinking water is based on information for individual substances. It will not be possible to obtain meaningful whole mixture data because of the variable nature of these mixtures. However, data may be available for certain commonly occurring groups of components.

Everyone will be exposed to mixtures present in the diet and drinking water. Owing to the variable intakes of different dietary components across the population and the nature of residue and contaminant surveillance schemes, there is uncertainty about the precise levels of human intake. Exposures are usually estimated using modelling approaches. Given the paucity of whole mixture data, it is necessary to use component-based approaches for risk assessments. Generally these approaches involve a comparison of measured levels of particular chemical residues or contaminants against acceptable or tolerable daily intakes (ADIs/TDIs) and maximum residue levels (MRLs) that have been derived for specific individual substances or sometimes for groups of similarly acting substances. Group ADIs are generally developed for groups of chemicals that have similar chemical structures or toxic effects and are based on the assumption that the effects of each member of the group will show simple dose addition. The group ADI may be derived from an average of the NOAELs for all of the

compounds, but more usually is derived from the lowest NOAEL of any member. Alternatively the NOAEL may be based on the toxicity of a common metabolite. For example, the assessment of allyl esters is based on the toxicity of the hydrolysis product, allyl alcohol, and its metabolites.

2.4 Mixtures of contaminants present at old industrial sites

Mixtures of contaminants at old industrial sites may contain a large number of components. The composition of these mixtures is usually specific to a particular location and will depend on the nature of the industrial activities that have been carried out at the site and the extent to which the mixture has been modified by photochemical and weathering processes. The chief concerns for mixtures of contaminants at old industrial sites relate to the pollution of local ecosystems caused by leaching of contaminants into watercourses and human exposure arising from possible future land use. The composition of pollutants being transported into watercourses by leaching and other geological processes may be different to the composition of pollutants remaining at the site. Owing to the site-specific nature of such mixtures it is unlikely that there will be any whole mixture hazard data to inform a risk assessment. Our understanding of the hazardous properties will be based on information for components and possibly commonly occurring groups of components. It may be necessary to carry out chemical analyses of the materials leaching into watercourses and remaining at the site to determine which components are present and the levels at which they are present. Exposures can be assessed by measuring or modelling. Hence, it will generally be necessary to use component-based approaches for risk assessment of mixtures of contaminants at old industrial sites.

3. How Might Mixtures in Chemical Act?

There are a number of different ways in which chemicals in a mixture may act in combination to produce an effect. This issue has been addressed by many individuals over the years and a rather confused terminology has developed. Some of the concepts/terms that have been used are provided in Table 2. The COT has followed the terminology of Cassee *et al* (1998) and divided the joint actions of chemicals in mixtures into non-interactive behaviour (where the presence of one chemical does not directly influence the toxicological effects of other chemicals in the mixture) and interactive behaviour (where one chemical does have a direct influence on the toxicological effects of others); this terminology is used here (COT, 2002).

However, the potential modes of interaction between different components within any one "real world" mixture will rarely be restricted to only one of these categories.

As the number of different chemicals in a mixture increases, the joint actions that might occur between components in the mixture become more complicated. A mixture may potentially exhibit the characteristics of some or all of the categories described below and the level of interaction between components may change at different doses, or potentially differ between different individuals and at different sites in the body in the same individual exposed to the mixture. Moreover, there does not have to be simultaneous exposure to chemicals for those chemicals to act in combination. In particular, chemicals that are persistent and bioaccumulative may gradually accumulate over time to levels at which they impact on the toxicological effects of other chemicals.

Table 2. Some terms used to describe the combined actions of components of mixtures (based on COT, 2002 and Seed *et al*, 1995)

Concept	Term used in this report	Synonym(s)	Effects observed
Non-interaction	Dose addition	Simple similar action Additivity Concentration addition Simple joint action Summation Loewe additivity	Chemicals have the same effect on the body and differ only in potency; hence the combined effect of two agents can be estimated from the total dose of both agents together.
	Independent action	Simple dissimilar action Simple independent action Independent joint action Effect/response addition Bliss independence	Chemicals have differing effects on the body and hence the combined effect of two agents is equal to the separate effects of each agent given alone.
Interaction	Synergism	Augmentation Potentiation Supra-additivity	The combined effect of two agents is greater than would be seen if no interaction had occurred.
	Antagonism	Depotentiation Sub-additivity Inhibition Infra-additivity Negative synergy Masking	The combined effect of two agents is less than would be seen if no interaction had occurred.

3.1 Non-interactive behaviour

Non-interactive behaviour occurs where chemicals in a mixture do not directly influence the toxicological effects of other chemicals in the mixture. Non-interactive behaviour can be divided into dose addition and independent action (Table 2).

Dose addition: This behaviour is expected where chemicals in a mixture act at the same site and by the same molecular mechanism, differing only in potency. There are no interactions between components. In effect, the chemicals are behaving as if they were concentrations or dilutions of each other. This is the basis for the Toxic Equivalency Factor (TEF) approach that has been used for dioxins and dioxin-like substances (see Section 5.3.1 for details). This type of joint action is generally what is meant when people use the term "additive behaviour". It should not be confused with the terms "effect addition" or "response addition" which refer to an assessment approach for independently-acting chemicals. It may also be appropriate to assume dose addition for chemicals that produce similar effects but by different molecular mechanisms. Kortenkamp (2007) reviewed the joint actions of chemicals that have endocrine modulating effects and found that among this class of chemicals a key determinant of dose additive behaviour is similarity of effect rather than molecular mechanism. Where there is doubt about the type of joint action likely to occur between chemicals in a mixture, it is more precautionary to assume that non-interacting components show dose addition.

Independent action: This is the situation where chemicals in a mixture act by different mechanisms/modes of action and possibly also at different sites. Individual components will not modulate the effects of other components of the mixture hence, the health effects of exposure to the mixture are expected to be qualitatively and quantitatively similar to those produced by individual components when administered alone. Where doses/exposures are below the biologically active doses of each component, no effects would be expected. Another term used to describe this approach to mixtures of chemicals that show independent action is "effect" or "response" addition, because the toxicological effects of each chemical rather than the dose of each chemical is combined to determine the overall toxicity of the mixture. Statistical methods that take account of differing susceptibilities to different chemicals are available to help assess the effects of exposure to a mixture of independently acting chemicals. These are discussed in more detail in Section 5.3.2.

3.2 Interactive behaviour

Any situation that deviates from the concepts of dose addition or independent action may be defined as an interaction but, more specifically, may be considered to be the toxicological influence one chemical exerts on another. Interactions may occur because of:

- direct reactions between the chemicals in the mixture (chemical-chemical reactions);
- one chemical altering the toxicokinetics of another;
- competition between chemicals for binding sites on receptors or for conjugates such as glutathione;
- one chemical affecting the physiology which alters the cellular or tissue responsiveness to another chemical;
- the effects of one chemical masking or compensating for the effects of another.

Types of interactive behaviour, the likely effect of the interaction on the overall toxicity of a mixture and examples of chemicals that show interactions are discussed in detail in Chapters 7 and 8 of the COT report (2002) and in a report by the Danish Veterinary and Food Administration (2003). Other documents that discuss mechanisms of interaction include Calabrese (1991), Ogata *et al*, (1993) and Alessio *et al*, (1994).

Different terms are used to describe the consequences of particular interactions on the overall toxicity of chemicals in mixtures (see Table 2 for some commonly used terms). Potentiation and inhibition describe the situation where one chemical acts to enhance or reduce the toxicity of another but is itself unaffected. Synergism and antagonism describe the situations where two or more chemicals affect the toxicity of each other and the toxicity of both chemicals is either enhanced (synergism) or reduced (antagonism). Masking is the situation where components produce functionally competing effects on the same organ system or the effects of one override the effects of another. In the case of potentiation and synergism, the combined effect is greater than would be predicted if no interactions are assumed. In the case of inhibition, antagonism and masking, the combined effect is less than would be predicted if no interactions are assumed. It is important to remember that the nature of chemical interactions in a mixture may change at different dose levels and that the interactions that are reported at high dose levels may not occur at lower concentrations. It is also important to consider that the critical target tissue (i.e. the target tissue which is most sensitive to the effects of the chemical) for one or more components may change as a result of an interaction. Changes in tissue dosimetry as a result of toxicokinetic interactions or competition for a key receptor may either reduce or enhance the effects of a chemical in particular target tissues compared to the effects seen by the chemical alone. If the effects in the critical target tissue are reduced and/or the effects in another tissue are enhanced, the effect in the alternative tissue may become the most sensitive endpoint for the risk assessment.

The following sub-sections provide examples of types of interactions, an indication of whether the interaction is most likely to occur at dose levels above or below those causing observable toxic effects, and whether the interaction has the potential to enhance or reduce the toxicity of components.

3.2.1 Chemical-chemical interactions

Chemical-chemical interactions occur where components react together to form another compound or chemical complex. Where new compounds form, these may possess different toxicity to the starting components, e.g. the reaction between chlorine and organic matter in water creating so-called disinfection by-products such as trihalomethanes and other halogenated organic compounds (WHO, 2000). Alternatively, where components react together to form a complex, the complex may enhance the toxicity of the starting materials, e.g. the reaction of lead with dithiocarbamates to form a lead-dithiocarbamate complex that, compared with inorganic lead, is retained to a greater extent in the body and has a higher capacity to penetrate the blood-brain barrier and bind to lipid rich brain tissue components (Oskarsson and Lind, 1985). Chemical-chemical interactions can also act to reduce toxicity, such as the reaction between cobalt edetate and cyanide to produce a complex that is less toxic than the starting materials (Paulet, 1960). Chemical-chemical interactions that produce new components with different or greater toxicity are of particular concern because the hazardous properties of the mixture may include a health effect or threshold of effect that differs significantly from that predicted using the known toxicity of the starting materials. It is therefore advisable, if a component-based risk assessment is being conducted, to consider what is known about the chemical reactivity of components and whether the conditions are right for reactions that lead to the formation of toxicologically active products or complexes. If this potential is identified then the reaction product or complex should be included as a component in the risk assessment.

3.2.2 Toxicokinetic interactions

Toxicokinetic interactions can occur when chemicals share or influence aspects of their absorption, distribution, metabolism or elimination. Toxicokinetic interactions occur independently of a common mechanism of toxicodynamic action, and can lead to an increase or decrease in the internal dose of

the active form (parent compound or metabolite) of a substance compared to the levels that would arise if no interactions occurred. One consequence of toxicokinetic interactions is the potentiation or inhibition of the toxicity of one chemical by another. For example, a non-mutagen may potentiate the effects of a mutagenic compound as a result of enhanced metabolic activation of the mutagenic compound.

3.2.2.1 Interactions affecting absorption

Most chemicals are absorbed by passive diffusion. This is true for absorption across the gastrointestinal (GI) tract, skin and respiratory tract. Anything that changes the conditions under which passive diffusion takes place to facilitate the process will increase the amount of a substance that is absorbed. This can happen if: a co-administered substance acts as a carrier, for example a fat soluble substance may be preferentially absorbed from a vegetable oil vehicle compared with an aqueous vehicle (the vehicle effect); if a co-administered substance alters the pH allowing a greater proportion to exist in the more readily absorbed non-ionized form, or; if a co-administered substance modifies the barrier properties of epithelial membranes to make them more permeable. The latter example will have most relevance in high exposure situations. In addition, certain nutrients and essential minerals may be absorbed from the GI tract by active uptake systems. Interactions affecting absorption are most likely to arise where an active transport process or a specific transporter is involved (Feron *et al*, 1995). For example, uptake of cadmium and cobalt from the GI tract may be enhanced by iron deficiency because iron competes with these metals for sites on transporter proteins (Groten *et al*, 1991).

The rate-limiting step for absorption across the skin for the majority of chemicals is passage across the stratum corneum, the lipid rich outer layer of the skin. When this layer is damaged, for example by exposure to an irritant substance, the barrier properties are impaired allowing a range of chemicals to cross more easily. The use of skin penetration enhancers is common where increased bioavailability of topically applied pharmaceuticals is required.

For the respiratory tract, it is more likely that interactions will affect the site at which materials deposit rather than bioavailability per se. Changes to the site of deposition can permit materials to contact an area of the respiratory tract that would not normally be exposed, perhaps leading to site-of-contact effects.

3.2.2.2 Interactions affecting distribution

Distribution is the process whereby a chemical, once absorbed, is carried around the body and into tissues and organs. It includes transport in the blood (either free in plasma or bound to plasma proteins) and diffusion from the blood and into tissues and organs. Substances that are not bound to plasma proteins have the greatest potential to diffuse into tissues and organs. The most likely interaction affecting distribution concerns competition for binding sites on proteins in blood and tissues, leading to an increase in the amount of the active form available at the target site. This situation is a well-known cause of drug-drug interactions (Feron *et al,* 1995). Given that there is generally a large capacity for binding sites on plasma and tissue proteins, these interactions are most likely to occur when exposure levels are relatively high, i.e. close to or exceeding thresholds of saturation of available binding sites (such a state often coinciding with the onset of toxicity).

Distribution can also be affected where one substance has an effect on internal barrier properties, e.g. bradykinin B2 receptor agonists have been found to increase the permeability of the blood brain barrier by disengaging the tight junctions of the epithelial cells that form the blood brain barrier (Emerich *et al*, 2001). This particular effect is likely to be relatively rare.

3.2.2.3 Interactions affecting metabolism

Interactions affecting metabolism are probably the most frequently studied form of toxicokinetic interaction. Altered chemical metabolism can occur as a result of enzyme induction, enzyme inhibition or saturation of

an enzyme by the presence of two or more substrates. Metabolism of most non-nutrient chemicals is usually undertaken by enzymes and processes that show low specificity (i.e. are capable of acting on a broad range of substrates) but high capacity (i.e. have high throughput). The high capacity of these systems means that a low level of induction or inhibition will probably not have any noticeable effect on the observable toxicity. Humans are continually exposed to a multitude of chemicals at low levels. This will contribute to a normal day-to-day fluctuation in the activity of different enzymes within an individual. Therefore, to have a toxicologically relevant effect, exposure to an enzyme inducing or inhibiting agent will have to be sufficiently large to take enzyme activity to a level beyond this normal variability. Interactions that affect enzyme induction are a particular issue for sequential exposure situations and where persistent and bioaccumulative chemicals are present, because the change in enzyme activity is not an immediate response.

Where enzyme saturation is a concern, this implies that relatively high levels of exposure have been achieved. The potential for interactions to affect metabolism should be considered wherever chemicals are known to share common metabolic pathways, particularly those involved in activation and in deactivation of the active form.

3.2.2.4 Interactions affecting elimination

Clearance via the urine or bile is generally a passive process. A substance that alters the conditions under which elimination processes take place may affect the rate at which other chemicals are cleared from the body. For example, a chemical that raises or lowers the pH of urine can increase or decrease renal clearance of ionisable substances by making it more or less likely that they will be in the more readily excreted ionised form. This is illustrated by the situation where ingested sodium bicarbonate raises urinary pH, increasing the rate at which salicylic acid is cleared. If an active secretory process is involved, competition between chemicals for binding sites on carrier proteins can delay elimination.

3.2.2.5 Summary of toxicokinetic interactions

Toxicokinetic interactions can occur at all dose levels, but the effects may not be measurable or toxicologically relevant at low doses. The most likely effect of toxicokinetic interactions is to alter the relationship between the external dose and the corresponding level of a toxicant at its target site, leading to an alteration in the threshold for effects. If toxicokinetic interactions are anticipated that could lead to an alteration in toxicity, the data should be examined to try to quantify the effect of the interaction. Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modelling (also referred to as physiologically based toxicokinetic (PBTK) modelling) is a useful mathematical tool that can help to quantify the effects of toxicokinetic interactions between chemicals over a range of dose levels and under different physiological conditions. El-Masri (2007) has published an overview of the use of mathematical approaches to investigate toxicological interactions and the ways in which such approaches complement traditional experimental studies. The use of PBPK/PD modelling in a tiered approach to mixture risk assessment is discussed in more detail in Section 6. If there is insufficient information to enable the effects of toxicokinetic interactions to be quantified, a possible default position would be to apply an additional assessment factor. The size of the assessment factor required to take account of the possibility of toxicokinetic interactions between components will depend on the nature of the toxic effects that are anticipated, i.e. a larger factor would be considered for a more serious effect. The rationale for selecting a particular factor should be clearly explained.

3.2.3 Toxicodynamic interactions

The toxicodynamic phase covers all events that follow the delivery of the ultimate toxicant to the target tissues and lead to the toxic effect. This includes interaction of the substance or metabolite with the target

tissues (comprising cells, organelles and biological macromolecules such as enzymes, receptors and DNA) and any resulting patho-physiological consequences, ultimately leading to the expression of toxicity. Occurrence of toxicodynamic interactions implies that a sufficient amount of a toxicant has been delivered to its target site to cause some perturbation of normal physiology and that a sufficient amount of a second toxicant has been delivered to its target site to cause a second perturbation that either exacerbates (potentiates) or compensates for (inhibits/masks) the effects of the first toxicant. If the chemicals are acting in a dose additive manner or independently then, by definition, there is no interaction.

3.2.3.1 Consequences of toxicodynamic interactions

Where toxicodynamic interactions occur there is the potential for synergistic (potentiating) or antagonistic (inhibiting) effects. An example of potentiation is the amplification of the hepatotoxicity of halomethanes such as carbon tetrachloride by prior exposure to the pesticide Kepone (chlordecone), which is thought to be due to an inhibitory effect of Kepone on the ability of the liver to repair damage caused by exposure to halomethanes (Mehendele, 1989). Examples of antagonistic effects include the effect of histamine and noradrenaline on vasodilation and blood pressure, and the anticonvulsive effects of barbiturates in relation to compounds that cause convulsions. With such toxicodynamic interactions, the key requirement is that biologically effective dose levels for each component are achieved at the target site. In the example of potentiation, this interaction would not arise if either the dose of Kepone was insufficient to have an effect on the repair mechanisms in the liver or if the dose of carbon tetrachloride was insufficient to cause liver damage. Hence, it is reasonable to conclude that where dose levels are below thresholds of effect for each component, no toxicodynamic interactions will arise. This is the conclusion reached by the COT based on its examination of toxicodynamic interactions between pesticides and other chemicals (COT, 2002). This was also the conclusion reached by Yang and Dennison (2007) in a study that compared thresholds of effect for three mixtures with the thresholds for individual components in those mixtures. The data sets examined included benchmark dose data for two sets of *in vitro* data, one looking at the cytotoxicity of the metals arsenic, chromium, cadmium and lead or a mixture of these four metals on human keratinocytes, and the other at induction of the oestrogen receptor alpha reporter gene in MCF-7 human breast cancer cells by oestrogenic xenobiotics. Yang and Dennison (2007) also looked at venous blood levels, under hypothetical exposure conditions, of gasoline and six component markers using PBPK modelling.

Their work suggested that the thresholds of effect seen with each mixture were generally in the range of thresholds of effect for the individual mixture components. Yang and Dennison emphasized that additional data sets needed to be examined before definitive conclusions could be drawn on the relationships between thresholds of effect for mixtures and their components.

When considering the potential for interactions to occur in relation to thresholds of effect, it is important that the influence of any potential toxicokinetic interaction in the relationship between external dose and the level of the toxicant at its target site is taken into account.

3.2.3.2 Dealing with data poor substances

In many cases, the substances that UK government deals with are data poor. Robust data to indicate thresholds of effect may not be available and the mechanisms or modes of action that underlie the effects may not be fully understood. In such situations, it may not be known whether interactions occur between mixture components at relevant levels of exposure. If we are not confident that exposure levels are below the thresholds of effect then there is the possibility that toxicodynamic interactions may occur and these interactions may result in synergistic effects. The advice from

regulatory bodies is that, in this situation, it is most appropriate to assume dose addition. This advice was based initially on evidence from an early series of studies looking at effects on LD_{50} values when various randomly selected organic chemical liquids were given as pairs in either equivolume or equitoxic doses (Smyth *et al*, 1969; 1970). The LD_{50} values obtained experimentally for each pair were compared with the LD_{50} values that would be predicted if dose addition was assumed. In many cases the LD_{50} of the mixture corresponded to the predicted value. Where deviations either greater than or less than dose additivity were found, the difference was usually within a factor of two.

The greatest deviation from dose additivity (in this case, an increase in toxicity over that predicted assuming dose addition) was by a factor of five. Periodically, regulatory bodies and others have reviewed mixture literature to determine how frequently synergistic interactions occur that markedly enhance toxicity beyond a level that would be predicted if dose addition was assumed. These issues were considered in reviews by Carpy *et al*, (2000), COT (2002) and the Danish Veterinary and Food Administration (2003). One theme to emerge from the literature is the inappropriate design of many mixture studies that prevents the drawing of conclusions about the joint actions that may have occurred. Based on the limited data from robust studies that are available for various toxicological endpoints, the evidence suggests that, at low doses, either no effect or dose addition occurs. This supports the assumption of dose addition as the most appropriate joint action where data are sparse.

A lack of adequate data from which to quantify the effects of toxicodynamic interactions will be a source of uncertainty in the risk assessment. If there are no reliable data from which to quantify the potential effects of toxicodynamic interactions between components, and where adequate exposure control is the goal (as opposed to a determination of the level of risk at a particular level of exposure), then it is suggested that the assessor should advocate the application of risk management approaches that will reduce exposure below levels judged to be "true threshold" levels. This may include the use of conservative assessment factors for components of the mixture, considered further in Section 5.35 and Box 7. Where there is information to suggest that groups of components in a mixture have similar effects, even if the underlying mechanisms may be different, it is precautionary to assume that chemicals in the group will show dose additive behaviour and that the threshold of effect for the most toxic chemical within the group applies to all components in the group. All default assumptions should be re-examined as understanding of the nature of joint actions between chemicals develops.

3.2.3.3 Dealing with toxicodynamic interactions where a clear threshold cannot be identified

Current understanding and measurement methods suggest that a threshold will exist for most toxicological effects. However, gaps in data and incomplete understanding of certain mechanisms of toxicity mean that thresholds of effect cannot always be identified. An example of this is provided by chemicals that are direct-acting genotoxicants. Such chemicals have traditionally been regulated on the assumption that there is no identifiable clear threshold of effect. Where clear thresholds of effect cannot be identified, risk management strategies have focused on eliminating exposure or reducing exposures as low as is reasonably practicable (ALARP) and it is recommended that this approach should continue until understanding of the underlying mechanisms has advanced to the point where it is possible to reliably identify thresholds of effect. The ALARP approach for genotoxic carcinogens was outlined in an IGHRC document (IGHRC, 2002) and is supported in the current guidelines on risk assessments for chemical carcinogens published by the UK Committee on Carcinogenicity (COC, 2004). In the situation where contaminants are present at very low levels and for which exposure is unavoidable, the COC indicate that a pragmatic minimal risk level may be

identified (COC, 2004). The minimal risk level would be identified on the basis of an evaluation of all available dose-response data for carcinogenicity and would include the application of an appropriate assessment factor to a dose without discernible carcinogenic effect, or the lowest dose tested if effects are apparent at all doses. Further details are available in the COC publication.

If there is evidence for a synergistic interaction between components and no clear threshold of effect can be identified, this may be a trigger to raise the priority of this mixture for risk management activities. The UK Committee on Mutagenicity is evaluating the published literature to determine the types of joint action shown by mixtures of mutagenic compounds and whether there is evidence of synergy between mutagenic compounds. The picture that is emerging is one of uncertainty owing to limitations in the studies that have been conducted. At present, the COM advocates a case-by-case approach to the assessment of potential interactions between genotoxic chemicals. More information can be obtained from: http://www.iacom.org.uk.

The greatest challenge to risk assessments for chemical mixtures is the lack of detailed information on the toxicokinetics, metabolic pathways, mechanisms of action and levels of exposure for the majority of chemicals in use and present in the environment. In order to make science-based judgements about interactions between components it is necessary to have a good understanding of the chemical reactivity, the toxicokinetics including metabolic pathways, and the mechanisms of action of each component. For the majority of non-pharmaceutical compounds, this information may be limited or absent. It may be necessary to make predictions based on information on similar chemicals or to use default precautionary assumptions to ensure that a component-based risk assessment will not underestimate the true toxicity of a mixture.

Table 3. Interactions that could increase[1] the overall toxicity of chemicals in a mixture and how they might be dealt with in a risk assessment

Interaction	Effect	How it could be taken into account
Chemical - Chemical		
Produce a new component	Additional toxicity may occur that would not be seen from the individual components.	Consider whether the conditions exist for the new component or complex to form and, if so, include this as an additional component in the risk assessment.
Produce a complex between components	This could affect the bioavailability of components. If the complex allowed a component to be transported to a target site that was not normally accessible, additional toxicity may occur.	
Toxicokinetic		
Absorption	Increased bioavailability leading to higher levels of the toxicant at the target site.	Ideally, sufficient data will be available to make a quantitative assessment of the impact of the interaction. If this is not possible, apply an additional assessment factor to take account of potential increases in the levels of a toxicant at its target site or to take account of prolonged exposure if clearance of the toxicant is delayed. The potential for toxicokinetic interactions to influence the relationship between external dose and the level of a toxicant at its target site should be taken into account before the potential for toxicodynamic interactions is considered.
Distribution	Competition for binding sites on plasma and intracellular proteins leading to higher levels of the toxicant at the target site. Owing to excess protein binding capacity in the body, this is most likely to occur at higher levels of exposure, except where high affinity transport mechanisms are involved.	
Metabolism	Saturation, induction or inhibition may produce greater levels of the toxicant or slower detoxification. Given the large capacity of the body to metabolize xenobiotic substances, these effects are most likely to occur at higher levels of exposure.	
Elimination	Slowed elimination could prolong the time the toxicant is available to act at its target site.	
Toxicodynamic		
	Toxicodynamic interactions are only likely to occur where components are at or above thresholds of effect but it will be difficult to predict the nature of any toxicodynamic interactions in the absence of information on mechanisms of toxicity.	Providing exposures to all components are below thresholds of effect (for groups of components that have similar effects, it may be necessary to derive thresholds on a group basis) there should be no toxicodynamic interactions. Where there are concerns for toxicodynamic interactions it may be necessary to adjust assessment factors for individual components to increase confidence that the amount of that component in a mixture is below its threshold of effect.

[1] Interactions that could decrease the overall toxicity of chemicals in a mixture have not been specifically considered in this table because they do not carry the same health concerns.

4. When is a Mixture Assessment Necessary?

Although we are continually exposed to mixtures of chemicals, it is clearly neither feasible nor scientifically appropriate to consider every possible combination of chemicals to which the population or the wider environment might be exposed. The circumstances that will trigger the need to conduct a risk assessment for a mixture will depend on the context in which exposure to the mixture occurs. Certain mixtures, usually those that are commercially supplied, fall within the scope of regulatory risk management schemes. For these mixtures, the data reporting schemes generally dictate the framework in which the risk assessment is conducted.

In other situations, for mixtures that are not commercially supplied, there may be no clear guidelines to indicate the circumstances when a mixture risk assessment is required. Traditionally, substances that are present in such mixtures have been regulated based on single substance evaluations such as occupational exposure limits (OELs), acceptable daily intakes (ADIs) and maximum residue levels (MRLs). These evaluations generally do not include an assessment of the effects of co-exposure to other chemicals. However, in cases where groups of similarly acting chemicals are known to occur together, e.g. similarly acting food additives, group evaluations have been performed and group ADIs assigned.

Where mixtures fall outside the scope of reporting schemes and there is no existing requirement to conduct a mixture risk assessment, the need for mixture assessments should be considered in any situation where:

- There is the potential for significant human exposure to occur, and;
- There is direct evidence for toxicity of the mixture; or
- There is evidence for a synergistic interaction between substances that are known to occur together; or
- For individual components in the mixture, the margins between measured/predicted levels of exposure and thresholds of toxicological effect are narrow or there is a concern that exposures may exceed thresholds of effect; or
- There is the likely presence of other similarly acting substances; or
- Chemicals are present together that share aspects of their absorption, distribution, metabolism and elimination and there is reason to believe that this may affect the levels of a toxicant at its target site.

5. Risk Assessment Approaches for Mixtures

The assessment should describe the reasons for choosing a particular mixture or particular components from a mixture. The following chapter discusses issues that should be considered when conducting mixture risk assessments based on whole mixture data and component data, starting with considerations for risk assessments using whole mixture data.

Ideally, the risk assessment for a mixture will be informed by hazard data gathered for the whole mixture across the exposure range and encompassing the likely variability in composition of the mixture. Whole mixture data of this type will provide a clear understanding of the hazardous properties of the mixture and the dose-response relationships across relevant exposure ranges and composition ranges. Any interactions that occur between components will be taken into account within these data. It may be possible to identify a threshold of effect for the mixture. With such data it will be possible to base a risk assessment on a comparison of measured or predicted exposure levels with no effect levels, or some other point of departure, using the same approaches used for single chemical entities. Unfortunately, sufficient data of this type for whole mixtures are usually lacking and even where whole mixture data are available, it is often necessary to supplement this with information on components. The following section provides a step-by-step overview primarily of the hazard assessment process for mixtures based on whole mixture data and indicates how component data may be used to supplement this information where necessary. The section also touches upon some of the issues to be considered when assessing exposure to mixtures. It is noted that the circumstances under which exposure occurs will influence the exposure assessment approach that needs to be taken. A review of specific exposure assessment approaches that could be considered for specific exposure situations is outside the scope of this document.

5.1 Defining the mixture

The first consideration in any hazard/risk assessment of a chemical mixture is the precise definition of the mixture being assessed. The definition should encompass the extent of knowledge about the exposure situations including the existence of susceptible groups, exposure levels and circumstances of exposure. Hazard characterisation involves assessing the type of hazard data available (i.e. data for the whole mixture or components or both) and whether the available hazard data cover the predicted/measured exposure range and possible variability in composition of the mixture. The questions that will help define the mixture are:

5.1.1 Is the mixture simple or complex?

If the mixture has few components and these are present in consistent proportions, the mixture can be considered to be simple. If the mixture has many components and/or the composition is variable, the mixture is a complex mixture (see Section 2 for definitions of simple and complex mixtures).

5.1.2 What is the predicted/measured range of exposure?

Exposure assessment for mixtures is challenging and is probably the area requiring most work. The approaches taken to assess exposure will depend on the context of the risk assessment; for example, different approaches will be needed to assess workplace exposures to process emissions compared with exposures to emissions from industrial premises via the environment. The latter will have much more complex transport characteristics and partitioning behaviour and it is highly likely that the composition of industrial emissions in the environment will change as a result of various degradation processes.

Exposure assessments within the occupational setting tend to focus on the exposures that an individual worker will receive as a result of the conditions in a particular workplace and the task(s) being undertaken. In contrast, exposure assessments for mixtures present in the environment will focus on population groups

and will need to identify the relevant target populations (susceptible groups). Exposure assessments for mixtures in the occupational setting and those in the general environment need to consider the form in which the mixture and its components are present (e.g. whether they are present in a form that is readily bioavailable), as well as the pathways by which exposure will occur, whether exposure is to the whole mixture or to a sub-fraction, and, if it is a sub-fraction, what determines the composition of the relevant sub-fraction.

When deciding which exposures need to be included in the risk assessment, the risk assessor should not only consider chemicals that are present concurrently but should also give thought to sequential exposure issues. If there is a possibility that effects from an earlier exposure could influence the response to a second later exposure, and it is a commonly occurring sequence of exposures such as. an industrial process in which one worker may be expected to carry out a particular sequence of tasks involving exposure to different chemicals for each task, it may be necessary to consider the combined effects of both exposures. The rationale for selecting particular chemicals to evaluate in a mixture risk assessment should be explained when defining the mixture to be assessed.

The most straightforward exposure assessments are those for mixtures that are supplied onto the market for a specific purpose. Assessing exposure to process emissions and wastes once they have been released into the environment, and to contaminants and residues present in food and drinking water, is a much more complicated process. Different sub-fractions of these mixtures will have different transport pathways through the environment and degradation processes will work at different rates for different components within these mixtures. Understanding of many of the factors that affect the persistence and transport of chemicals in the environment is at an early stage and data gaps in this area will be a key source of uncertainty in a risk assessment. General issues relating to exposure assessment of chemicals in the environment are outlined in the US EPA guidelines for conducting health risk assessments for chemical mixtures (US EPA, 2000).

5.1.3 Is knowledge of the hazardous properties of the mixture based on data for the whole mixture or on data for components or both?

Hazard assessments for mixtures should be based on data obtained for the whole mixture. If whole mixture data are available for all relevant endpoints, the data can be used to derive dose-response relationships for the whole mixture in the same way that dose-response relationships are derived for single substances. A threshold of effect may also be identified. However, whole mixture data are often not available or are incomplete.

Should whole mixture data be available, the data will, more frequently, relate to complex mixtures rather than simple mixtures. Data, however, may not be available for every endpoint. It may be necessary to supplement information on the mixture itself with information on a sufficiently similar mixture(s). Criteria to help decide whether two complex mixtures are sufficiently similar are given in Section 5.2 below. In a situation requiring the use of hazard data for mixture components to supplement whole mixture hazard data, it will be necessary to determine whether the hazards that have been identified for components and groups of components can be extrapolated to the whole mixture. Toxicological hazards for minor components or minor groups of components for which there is an identifiable threshold may not be relevant for the whole mixture because the dose-levels needed for the effect to occur may not be achieved in the mixture. Where component hazards can be extrapolated to the whole mixture it is also necessary to consider whether thresholds for components or groups of components are applicable to the whole mixture. This is determined on a case-by-case basis and will depend on the proportion of the whole mixture represented by the

component or group of components, and whether there is any reason to suspect that interactions with other components in the mixture could alter the threshold. For many complex mixtures, there will be no whole mixture data and the risk assessment will have to rely on hazard data for components. Where component data are used, the context in which the data were obtained (e.g. whether the data derive from a controlled laboratory study or from an epidemiological study involving a mixed exposure situation) needs to be taken into account. Component-based assessments for complex mixtures are discussed in more detail in Section 5.3 below.

With the exception of certain pesticide formulations, simple mixtures are generally not tested as whole mixtures. Hazard assessments for simple mixtures usually need to rely on data for components. In order to judge possible dose-response relationships and define thresholds of effect, it will be necessary to consider whether the components in the mixture act independently of each other or show dose addition or the occurrence of interactions. Component-based approaches for hazard assessment of simple mixtures are discussed in more detail in Section 5.3 below.

5.1.4 If the mixture is complex, does the available whole mixture hazard data cover the potential variability in composition?

The composition of many complex mixtures is variable. This is because in many cases the starting materials and the processes by which the mixture is formed are variable. In some cases, the variability will be slight and will not have an impact on the hazardous properties of the mixture. In other cases, e.g. welding fume, the variability may be so great that it will not be possible to extrapolate hazards of mixtures obtained from one source to mixtures obtained from another source. Before a risk assessment of the mixture can be performed it will be necessary to determine the extent to which changes in composition will influence the hazardous properties and whether the mixtures that have been tested are sufficiently similar to the mixtures to which the particular populations are exposed.

Complex mixtures can be defined either in terms of the components of the mixture, in terms of the starting materials and process conditions, or in terms of its technical properties. Where the composition of a mixture is defined in terms of its components it will be possible to compare the ranges of various components in the tested mixtures with the ranges in the mixtures to which particular populations are exposed. If there is a high degree of overlap in component ranges then it may be appropriate to apply the hazards and dose-response relationships that have been determined for the tested mixtures to the mixtures to which the particular populations are exposed. In the situation where hazard data include the extremes of component ranges and the hazards that have been identified follow predictable trends across the range, it may be possible to predict the likely hazards and dose-response relationships for mixtures within the range by interpolation.

If the data suggest that the hazards across the mixture do not follow a trend across the range, it will be necessary to define smaller component ranges where consistency of toxicological effects can be expected. Interpolation/extrapolation of hazards and dose-response relationships may be considered within these smaller ranges. It will not be possible to extrapolate hazards to mixtures that fall outside these reduced ranges, and it may be necessary to consider component-based approaches for hazard assessment. This may also be the case where hazard data are only available for a narrow range of composition and particular populations are exposed to compositions outside the range.

For mixtures that are defined on the basis of starting materials and process conditions, the range in variability will be defined by the most extreme starting materials and most extreme process conditions. In order to determine the extent to which the tested range covers the possible range in variability, the starting materials and process conditions for the mixtures that have been tested should be evaluated and compared to

the starting materials and process conditions for the mixtures to which the particular populations are exposed.

If there is a high degree of similarity, it can be assumed that the hazards and possibly the dose-response relationships identified are applicable to the mixtures to which the populations are exposed. If there are differences then it may be necessary to determine, by chemical analysis, the effect that different starting materials and process conditions have on composition. Judgements of similarity on the basis of components of the mixture can then be made. If the identification of key components indicates that the available hazard data cannot be applied to the mixture to which particular populations are exposed, component-based approaches for hazard assessment can be considered.

For mixtures defined in terms of their technical properties, where the same technical properties may be achieved despite variations in composition or where there is no clear basis on which to define composition, it may be necessary to use the data that are currently available as a means to define the limits of knowledge about the mixture. If particular populations are exposed to compositions that fall within or close to the range for which data are available, it may be possible to perform a risk assessment based on interpolation or extrapolation from existing data. For mixtures that fall considerably outside the observed data range, it may be necessary to consider component-based approaches.

The following sections look in more detail at risk assessment methods that might be applied to different types of data.

5.2 Mixture risk assessments using whole mixture data

Whole mixture data may be available for manufactured mixtures and for process derived emissions in the workplace. Whole mixture data may also be available for other specific mixtures such as tobacco smoke and vehicle exhaust emissions. If sufficient whole mixture data are available, it may be possible to identify critical target tissues and thresholds of effect for the whole mixture within the boundaries of observed data (i.e. for the composition range that has been studied and for the exposure conditions that have been studied). Whole mixture data will not generally provide information on interactions between components, and this will increase uncertainty where there is a need to extrapolate beyond the boundaries of observed data. In addition, where there is degradation of the mixture following its release, the degradation products may not have been present in the tested material. If there is extensive degradation, data from tests using “fresh” mixture may not be applicable to “aged” mixture. Where whole mixture data are available, it should be possible to apply approaches as for single substance risk assessments. If data gaps are identified, it may be possible to fill these with information on a sufficiently similar mixture.

5.2.1 What criteria can be used to determine whether two complex mixtures are sufficiently similar?

Criteria to determine whether two mixtures can be regarded as sufficiently similar are being developed to assist the grouping of mixtures (complex substances) for category based risk assessments under the OECD High Production Volume (HPV) scheme and also in the Reference Technical Guidance Document for registrants preparing a chemical safety report under REACH (ECHA, 2008). The key requirement for grouping mixtures together to enable a read across approach to be adopted is mixture similarity. Mixtures can be considered to be similar if they share a common mode of toxicological action, there is consistency of results in short-term tests, there are similarities in chemical class or structure, share common components in similar proportions, or have a common source of formation or emission. It is useful to have an understanding of the way in which any differences between the surrogate mixture and the mixture of concern will affect toxicity. The greater the dissimilarity between the mixture for which data are available and the mixture of concern, then the less confidence there will be that dose-response estimates for the tested mixture will apply to the

mixture of concern. The Agency for Toxic Substances and Disease Registry (ATSDR) in its guidance manual for the assessment of joint toxic action of chemical mixtures gives two examples to illustrate the meaning of 'sufficiently similar' (ATSDR, 2004). JP-5 (a jet engine fuel) derived from one source will be 'sufficiently similar' to JP-5 derived from any other source because it is produced to meet uniform specifications with minor differences between one source and another. Gasoline from different sources is not considered to be sufficiently similar because formulations can vary widely. As with assessments based on data for the mixture itself, sources of uncertainty should be elucidated. In particular, care must be taken to ensure that uncertainties regarding the composition of the tested mixture compared with the mixture of concern are fully described.

5.3 Mixture risk assessments using component data

Owing to the lack of good experimental data for whole mixtures, regulatory bodies have developed component-based (bottom-up) approaches to evaluate the hazards and risks of chemical mixtures. These approaches can be divided into the relatively straightforward approaches that have been developed for simple mixtures and the more involved approaches that have been developed for complex mixtures. Overviews of various approaches have been published by the US EPA, the ATSDR and the Danish Food and Veterinary Administration (US EPA, 1986; US EPA, 1988; US EPA, 2000; ATSDR, 2004; Danish Food and Veterinary Administration, 2003). The EPA documents - guidelines for conducting risk assessment of chemical mixtures (US EPA, 1986; US EPA, 2000) and a technical support document providing background information (EPA, 1990) - provide reference manuals for conducting chemical mixture risk assessments. The ATSDR document has been drafted to provide background information on the approaches used by the Agency to develop "interactions profiles", currently available for 11 different mixtures. More information on interactions profiles can be found at: http://www.atsdr.cdc.gov/interactionprofiles/#bookmark09. The following section discusses the approaches outlined in these documents and other sources.

Component-based approaches to mixture risk assessment can be divided into those based on the assumption of dose addition, those based on the assumption of independent action, and those that include some adjustment to take account of interactions between components. Component-based approaches contain many uncertainties. Sources of uncertainty include the composition of the mixture of concern – have all the chemicals in the mixture been identified? How comprehensive and reliable is the data set for each component? How much is known about potential interactions between components? Do we know the relative concentrations of each component in the mixture and whether humans will be exposed to these same relative concentrations? How much evidence is available to support an assumption of dose addition or independent action?

Before a regulatory authority can use component-based approaches to predict the hazards of a mixture, a decision must be taken about the type of joint action that the components are expected to show, and hence which is the most appropriate model to use. As a first step, the risk assessor should look for evidence of potential interactions between components and, if such evidence exists, see whether the effect of the interaction can be quantified. In many cases, there will be no clear information on potential interactions and hence no scientific basis to account for the effects of potential interactions. In this situation, the risk assessor should select a non-interaction model as the starting point for a preliminary (Tier 1) risk assessment.

The generally held view is that dose addition should be assumed for groups of chemicals that produce the same toxic effect in the same target organ via the same mechanism. The US EPA has published guidance for identifying substances that have a common mechanism of action (US EPA, 1999). This

was produced with the primary aim of identifying pesticides that can be considered as candidates for cumulative risk assessment, but the principles can equally be applied to the identification of components in a mixture that might act by dose addition. The US EPA working group concluded that two chemicals act via a common mechanism of toxicity if they:

a) cause the same critical effect;

b) act on the same molecular target or the same tissue;

c) act on the same biochemical mechanism of action, possibly sharing a common toxic intermediate.

However, data on the molecular targets, biochemical mechanisms of action and the identity of toxic intermediates will not necessarily be available for the majority of chemicals that are present in the workplace and wider environment. Where chemicals affect the same target organ and there is uncertainty about the mechanism of action, it is more precautionary to assume that the effects of co-exposure to these chemicals will be additive rather than independent.

The US EPA uses an example of two substances that cause hypothyroidism, one by inhibiting the catalytic activity of the peroxidase enzyme within the thyroid and the other by preventing the synthesis of thyroid-stimulating hormone within the anterior pituitary gland. Although the toxic effects are the same, the underlying mechanisms are different and the two substances could be regarded as acting independently. However, if the function of an organ is compromised twice, or if the chemicals in a mixture target different elements of one homeostatic process, the overall effects may well be more severe than would be predicted by the assumption of independent activity, i.e. more like those of dose addition.

This has been observed with endocrine modulating chemicals that have similar effects even where the underlying mechanisms are different (Kortenkamp, 2007). For this reason, the US EPA recommends that when it is not clear whether or not chemicals are acting independently, the more conservative assumption of dose addition is preferable to the assumption of independent action (US EPA, 2002). This stance is consistent with the views of a working group convened to discuss risk assessment and standard setting as part of a European Conference on Combination Toxicology held in Veldhoven in 1995 (Bolt and Mumtaz, 1996). The group concluded that where two components of a mixture share the same target organ(s), models assuming dose addition should be used unless there is clear scientific data to justify the use of models assuming independent action.

5.3.1 Models for dose addition

When chemicals are considered to show dose addition, each chemical can be considered a concentration or dilution of another. Models for dose addition can be applied to chemicals that affect the same target tissues and have the same molecular mechanism of toxicity, but models for dose addition may also be applied in the situation where chemicals produce functionally similar effects in a target tissue by different molecular mechanisms. Models for dose addition work on the principle that the dose of chemical ‘A’ required to elicit an effect is a fixed multiple of the dose of chemical ‘B’ required to elicit the same effect. Where there is extensive information about the toxicological properties of components of a mixture and data that allow the relative potencies of each component to be judged, it may be possible to derive Relative Potency Factors (RPFs) for a quantitative estimate of the toxicity of a mixture (see Box 1).

Box 1. ***Relative Potency Factor/ Toxic Equivalency Factor Methods:***

These approaches have been applied to mixtures that consist of a single class of chemicals where extensive information is available for one member of the chemical class but less is known about other members. They rely on the use of scaling factors called Relative Potency Factors (RPFs) or Potency Equivalency Factors (PEFs) to express the toxicity of the lesser known chemicals in terms of an equivalent dose of the index chemical (usually the most extensively studied) in order to determine what the overall toxicity of the mixture will be. The scaling factors are derived using the assumption that the potency ratios between each member of the chemical class remain constant at all dose levels. Robust RPFs can only be derived where there is an extensive body of good experimental data showing the relative potencies of the chemicals in different assay systems and demonstrating their underlying mechanisms of toxicity. If there are insufficient data to confirm common modes of action for all endpoints, or the data suggest that different modes of action operate at different target organs or under different exposure conditions, then end-point specific RPFs could be derived.

The Toxic Equivalency Factor (TEF) method is a special case of the RPF method. Here the assumption is made that all chemicals in the mixture act at the same target sites and by the same underlying mechanisms, i.e. there is toxicological equivalence across all endpoints. A single scaling factor or TEF can be derived for each chemical. This is the approach that is used to calculate the toxicological potency of mixtures of dioxins and dioxin–like PCBs, with the index chemical being 2,3,7,8-TCDD (Van den Berg *et al,* 1998). The concentrations of each congener in a mixture are multiplied by their TEF value and summed to give the Toxic Equivalent (TEQ) of the mixture using the following formula:

$$TEQ = \sum_{i-1}^{n} C_I \times TEF_i$$

Where:

TEQ is the concentration of the index chemical that would give equivalent toxicity to that of the mixture;

C is the concentration of each congener; and,

TEF is the toxic equivalency factor for each congener.

The advantage of the RPF and TEF methods is that they provide a numerical estimate of the toxicological potency of the mixture of concern. However, in order to judge the toxicological potency of specific mixtures, the amount of each chemical for which a RPF or TEF has been derived must be determined. This can be a resource intensive task. Also, in order to derive robust RPFs or TEFs, extensive experimental information is required on the individual components and their relative potencies in a variety of test systems.

The most extensive application of this methodology is the use of TEFs to express the toxicological potency of mixtures of dioxins and dioxin-like polychlorinated biphenyls (PCBs). For these substances, there is evidence that a subset of 17 dioxin and 12 PCB congeners bind to a specific receptor, the Aryl hydrocarbon (or Ah) receptor, and produce a characteristic spectrum of toxicological effects. The strength of binding to this receptor determines the toxicological potency of the different dioxins and PCB congeners, and can be used as a basis for deriving TEFs

for this group of chemicals (Van den Berg *et al,* 1998). However, the reliability of an assessment based on the use of TEFs or any other potency-ranking scheme is only as good as the data from which the TEFs were derived. For example, at the Dioxin 2003 conference, concerns were raised that the samples of the less potent congeners used for the studies from which TEFs were derived were contaminated with small amounts of highly potent congeners (Phibbs, 2003). This was suggested as one explanation for the wide variations in potency of individual congeners that have been observed between studies (Phibbs, 2003). The net result of such contamination is that overly high TEFs could have been assigned to low potency congeners, leading to an overestimation of the toxicological potency of mixtures containing predominantly low potency congeners. In June 2005, the WHO convened a meeting of experts to re-evaluate the TEFs assigned to certain congeners using the refined TEF database. The outcomes from the meeting were published by Haws *et al*, (2006). The expert group confirmed that the concept of dose additivity was applicable to mixtures of dioxins and dioxin-like PCBs and identified certain individual and groups of compounds for possible future inclusion in a TEF scheme, including 3,4,4'-triclorobiphenyl (PCB 37), polybrominated dibenzo-p-dioxins and dibenzofurans, mixed polyhalogenated dibenzo-p-dioxins and dibenzofurans, polyhalogenated naphthalenes and polybrominated biphenyls (Van den Berg *et al*, 2006). The group also identified possible future approaches for the determination of TEFs, including probabilistic approaches that better describe the uncertainties that are present in the data.

Other classes of chemicals for which potency-ranking schemes have been developed include organophosphate (OP) pesticides and PAHs. For both classes of chemical, end-point specific rankings have been developed. In the case of OP pesticides, schemes are based on cholinesterase inhibiting potency. Seed *et al*, (1995) refer to the use of chlorpyrifos as an index compound to predict the cholinesterase inhibiting potency of mixtures of anticholinesterase compounds in the context of evaluating pesticide residues in foods. Work is being undertaken by the Pesticides Safety Directorate in the UK to develop TEFs for 22 cholinesterase inhibitors (including both organophosphate and carbamate pesticides) based on their ability to inhibit rat or dog red blood cell or brain acetylcholinesterase. The US EPA has derived TEFs for 33 organophosphate compounds based on their ability to inhibit brain acetylcholinesterase activity and using methamidophos as the index compound (US EPA, 2002).

For PAHs, carcinogenic potency has been used as the basis on which individual compounds are ranked, using benz[a]pyrene (BaP) as the index compound. The various equivalency factors that have been proposed, and the uncertainties that are associated with each proposal, are discussed in EHC 202, Appendix I (WHO, 1998). One major criticism is the limited availability of data to indicate the carcinogenic potency of BaP in humans (Pufulete *et al*, 2004). An examination of this approach to assess the risks of oral exposures to PAHs in contaminated soil, using BaP factors derived by the US EPA over a decade ago, found that the BaP equivalency factors did not adequately describe the potency of many PAH mixtures and, in many cases, underestimated the actual carcinogenic potency (Schneider *et al*, 2002). In particular, the lung carcinogenicity of PAH mixtures following oral or inhalation/lung implantation was underestimated by factors ranging from under 13 to 60 fold; for skin tumours after dermal application, the underestimation ranged from 2 to 11 fold. The authors considered that similar results would have been obtained had the analyses been carried out with any of the other BaP equivalency factors that have been put forward. It is thought that the underestimation occurred because it was assumed that no PAH would have greater carcinogenic potency than BaP. This assumption is incorrect. Pufulete *et al* (2004) identified several PAHs that have greater

carcinogenic potency than BaP, including dibenzo[a,l]pyrene and dibenzo[a,h]anthracene. This illustrates the need for robust data and a comprehensive description of uncertainties in the data underpinning any scheme relying on relative potency ranking. An alternative marker based approach for PAHs is discussed in Section 5.3.4.

For the majority of mixtures, the kind of detailed information that would allow relative potency estimates to be derived will not be available. A more general approach that can be used where there is little or no information about the commonality of mechanisms of action and the relative potency of different components is the Hazard Index (HI) approach (see Box 2).

Box 2. ***Hazard Index approach:***

This is the most widely applicable approach for component based risk assessment of toxicologically similar chemicals. Ideally it should be used for groups of toxicologically similar chemicals for which dose response data are available, but can be used for chemicals that affect a common target organ even where there are no additional mechanistic data. To determine the hazard index for a mixture, a hazard quotient is calculated for each component by dividing the dose or exposure level for each component with a suitable reference level. The individual hazard quotients are then summed to give an overall hazard index according to the following equation:

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

Where:

HI is the hazard index;

E represents the exposure level of each individual component; and,

RL represents a reference level for each individual component.

The reference level of exposure could be a derived level such as an occupational exposure limit (OEL), acceptable daily intake (ADI) or minimum risk level (MRL). In this case, the numerical values of the derived levels will have been arrived at by the application of assessment factors to NOAELs, and there may be inconsistency in the way that the assessment factors have been applied to different chemicals. A more robust approach is to choose a scientifically derived point of departure, e.g. a benchmark dose or a dose producing a specified level of effect (e.g. ED_{10}). These points of departure are obtained directly from the data before assessment factors are applied. In order to interpret the HI outcome, it is essential to use the same reference level throughout the calculation.

The HI is interpreted according to whether or not it exceeds unity. A HI value of less than 1 indicates that exposure is below the chosen reference level. When the HI value is 1 or above, exposures are at or above the reference level, signalling greater concern.

The HI approach is used widely in occupational risk management for assessing the effects of exposure to mixtures of chemicals that are considered to show dose addition (HSE, 2005). It is also used within the EU Preparations Directive and the Globally Harmonised Scheme (GHS), in certain circumstances, to determine the appropriate hazard classification for simple formulated mixtures based on the classification and labelling of components (EC, 2006). The HI approach does not require the use of scaling factors derived from experimental data and can therefore be used where data are sparse. However, it will only provide a qualitative assessment of the hazardous properties of a mixture.

The HI method can also be applied to mixtures containing chemicals that act on different organ systems by various mechanisms and where the critical health effects of components may differ. Strictly speaking, such mixtures do not fit the criteria for dose addition, but the HI method will provide a more precautionary assessment than one based on an assumption of independent action if there is doubt about the most appropriate model for the overall behaviour of the mixture. In this situation, an approach suggested by regulatory agencies in the USA is to calculate separate hazard indices for each endpoint of interest. This is referred to in US EPA and ATSDR documents as the Target Organ Toxicity Dose (TTD) modification (see Box 3). This is likely to be a resource intensive approach

Box 3. ***Target organ toxicity dose (TTD) modification:***

In order to use this method, a TTD is derived for each target organ of concern for each chemical. The ATSDR and US EPA documents indicate that a TTD would be derived by a process analogous to that for deriving an RfD or MRL. The highest NOAEL or lowest LOAEL for the effects of the chemical on each target organ will be identified and assessment factors will be applied to take account of data quality, etc. Usually, TTDs would be derived for the critical target organs for each chemical and target organs that are affected at slightly higher doses. Target organs that are only affected at dose levels producing severe general toxicity or mortality would not be included in these calculations. The TTDs for each chemical for each target organ of concern will then be used as the denominator (reference level) in conventional hazard index calculations to determine target organ specific hazard indices.

As an alternative to the use of TTDs derived from NOAELs or LOAELs, it may be possible to identify the dose for each component that caused a specific level of effect in each organ. The chosen effect would ideally be a marker of toxicity in the organ and not some other change that was secondary to the toxic effect.

In the situation where there are insufficient toxicological data on components of a mixture to derive hazard quotients for each component, a precautionary approach is to consider that each component is of equivalent toxicity to the most potent component. In this case, the numerical value of the reference level chosen for the most potent component would be used for each component. This approach was taken by the COT in its assessment of dietary exposure to polybrominated diphenyl ethers (PBDEs) (Food Standards Agency, 2004).

5.3.2 Models for independent action

Chemicals in a mixture can be considered to show independent action when each chemical has a different mode/mechanism of action and possibly affects different target organs. The consequences of exposure to a mixture of chemicals showing independent action will be described by the effects of the individual components when administered alone at their respective concentrations in the mixture. This assumes that any biological stress or perturbation induced by a chemical has no effect on the dose-response relationships for the other chemicals in the mixture. If this assumption is not true, the actual risks to health posed by the mixture may be underestimated.

The simplest approach for mixtures of chemicals showing independent action is to consider each component of the mixture in isolation. This is the approach that is used in the occupational setting (HSE, 2005). Using the terminology used to describe the HI approach; this simple approach would be written thus:

$$\frac{E_1}{RL_1}; \quad \frac{E_2}{RL_2}; \quad \frac{E_3}{RL_3}; \ldots \quad \frac{E_n}{RL_n}$$

Where:

E represents the exposure level of each component; and,

RL represents a reference level for each component.

In this situation, providing that the hazard quotient for each component is less than 1, there will be less concern about the mixture than would be the case if the hazard quotient for any component was 1 or more.

The simple approach described above does not take into account the fact that individuals within a population may be differently sensitive to different components. To address this issue, the US EPA uses a procedure called response addition (see Box 4) to derive quantitative risk estimates for the health risks arising from environmental exposure to mixtures of chemicals showing independent action.

Box 4. *Response addition:*

Response addition is a probabilistic approach to determining the effects of exposure to a mixture of independently acting substances. It is based on the principle that each individual will have a certain level of susceptibility to each chemical and will only exhibit a response if the threshold of susceptibility is exceeded. The US EPA document describes approaches that can be used to calculate the probability of an adverse effect occurring in an individual, and to calculate the percentage of individuals in a population that may respond (US EPA, 2000). Response addition is succinctly described by Cassee *et al*, (1998) and Könemann and Pieters (1996).

For an individual, the probabilities that adverse effects will occur as a result of exposure to each of the mixture components (usually expressed as a risk estimate) are multiplied together using the formula for statistical independence to arrive at the probability (or risk estimate) for an adverse effect to arise for the whole mixture. The formula for statistical independence is given as:

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

Where:

p_m is the probability for an adverse effect from mixture; and,

p_1, p_2, p_i etc are the probabilities for an adverse effect for individual components

Where the effect of a mixture in a population is being considered, the calculations aim to determine the percentage of the population that may respond. Different equations are used depending on the correlation of susceptibility within the population to the chemicals in the mixture.

In the illustrations below, a two-component mixture containing chemical A and chemical B will be considered. The simplest equations refer to the situations where there is either complete positive correlation of susceptibility (i.e. individuals who are the most susceptible to chemical A are also the most susceptible to chemical B) or complete negative correlation (i.e. individuals who are most susceptible to chemical A are the least susceptible to chemical B).

Where there is complete positive correlation, the chemical which affects the greatest percentage of the population will determine the total percentage of the population susceptible to the mixture. This can be expressed in the following way:

$P_{mixtureA,B} = P_A$ if $P_A \geq P_B$ or

$P_{mixtureA,B} = P_B$ if $P_B \geq P_A$

Where, P is the percentage of individuals in the population who respond.

So, for example, if the amount of chemical A in the mixture was expected to elicit a response in 15% of the population and the amount of chemical B was expected to elicit a response in 10% of the population, the total percentage that would be expected to respond is 15%.

Where there is complete negative correlation, the total percentage of the population responding to the mixture will include all those responding to chemical A plus all those responding to chemical B.

$P_{\text{mixture A,B}} = P_A + P_B$

In this case, if 15% of the population respond to chemical A and 10% to chemical B, the total percentage of the population that will respond to the mixture is 25%. This is the most conservative approach to a mixture of chemicals showing independent action and is the approach recommended by the US EPA for assessing the risks to health posed by mixtures of carcinogens.

More complex statistical equations are required where there is no correlation or partial correlation in susceptibility.

An adaptation to response addition has been used by the US EPA for a risk assessment of drinking water disinfectant by-product mixtures (Teuschler *et al*, 2004). These mixtures contain sub-groups of components that produce similar effects. The approach adopted by the US EPA was to group components into sub-classes based on their mode of action. For each sub-class, they identified an index chemical and derived relative potency factors for all other chemicals in each sub-class. Risk estimates were then made for each sub-class based on the dose response relationship for the index chemical. The sub-class risk estimates were then added, using methods for response addition, to arrive at a risk estimate for the whole mixture. The US EPA refers to this approach as the Cumulative Relative Potency Factors (CRPF) approach. Several research needs were identified to provide additional scientific support for the use of this approach to assess the risks from disinfectant by-products in drinking water.

In order to obtain reliable outcomes from assessments based on response addition, it is necessary to have good data for each component.

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

5.3.3. Models that take account of interactions

Although it is difficult to predict the effects of interactions in the absence of data, attempts have been made to take account of interactions when using component-based approaches. To date, all approaches that have been proposed are modifications of the hazard index method and allow a qualitative rather than quantitative assessment of the possible effects of interactions. If it is possible to quantify the effects of interactions then this information should be used in preference to a qualitative model.

The first approach, suggested in 1989 by the US National Research Council Safe Drinking Water Committee's Subcommittee on Mixtures, was to apply an additional uncertainty factor to the conventional hazard index calculation (see equation) (Seed *et al,* 1995).

$$HI = (UF) \sum_{i=1}^{n} \frac{E_i}{RL_i}$$

Where:

HI is the hazard index;

UF represents an uncertainty factor;

E represents the exposure level of each individual component; and,

RL represents a reference level for each individual component.

The value of the factor, which would lie between 1 and 100, should reflect the degree of confidence in the available information on interactions and the concentration of the mixture components (since the likelihood of interactions increases with increasing dose). This is a very simplistic approach. Since it is based on the hazard index approach, each component is considered to contribute to the overall toxicity of the mixture in proportion to its concentration in the mixture. This will not be the case where toxicokinetic interactions occur that change the dose-response characteristics of components.

This is not a scientifically robust method but it may be the only approach that can be taken where data are scarce. In this situation the uncertainty factor will be large. Such an approach could only be considered for a preliminary (Tier 1) risk assessment.

A more systematic approach developed by the ATDSR is the binary weight of evidence (BINWOE) approach (see Box 5). This approach allows for a more systematic consideration of the nature of interactions, but is again qualitative.

There are many uncertainties inherent in this approach. The method is best applied to mixtures containing few components; the ATDSR has used the approach to assess interactions between groups of 4 or 5 contaminants in soils from old industrial sites. It would not be practicable to use this approach for mixtures containing several components because of the large number of pair-wise interactions that would have to be considered. The method does not take account of the effect of additional chemicals in the mixture on any pair-wise interaction. Also, in many situations there may be insufficient data to categorize all pair-wise interactions. The method also assumes that the pair-wise interaction will remain the same at all dose levels; this assumption is not supported by interactions data which shows that the nature of interactions will change at different dose levels (COT, 2002).

Box 5. ***Binary weight of evidence (BINWOE) approach:***

This approach, sometimes referred to as an Interaction-Based Hazard Index, provides a framework for the systematic assessment of interactions data enabling qualitative judgements to be made about whether interactions between chemicals within a mixture will increase or decrease the toxicity of the mixture compared with predictions assuming dose addition. It relies on the assumption that interactions in a mixture can be adequately represented as departures from dose-addition and that the influence of any interactions can be approximated by looking at pairwise interactions between components in the mixture. The procedure is lengthy. The first step entails a review of all relevant information on the binary interactions in the mixture. For each pair of chemicals two determinations will be made, one for the effect of chemical 'A' on the toxicity of chemical 'B' and one for the effect of chemical 'B' on the toxicity of chemical 'A'. These determinations will be classified according to whether the interaction is additive, greater than additive, less than additive, or indeterminate. The quality of the data is also taken into account. The data are graded according to how well the mechanism of interaction has been characterised, what the toxicological significance of the interaction is, whether the data apply to the anticipated route, duration and sequence of exposure, and whether the interactions data have been derived from in vivo or in vitro studies. All of this information is expressed as an alphanumeric classification that can then be converted to a single numeric BINWOE score with the use of weighting factors (see the ATSDR guidance document for further details; ATSDR (2004)). A positive score indicates a synergistic pairwise interaction and a negative score indicates an antagonistic pairwise interaction. For most mixtures it is likely that some interactions will indicate that toxicity is greater than additive, some will indicate that toxicity is less than additive, and some pair wise combinations will not show any interactive behaviour. Individual BINWOE scores are therefore summed to give an overall score. If the resulting overall score is positive and significantly different from zero, it is concluded that the toxicity of the mixture is likely to be greater than would be predicted based on the assumption of simple similar action. Conversely, if the overall score is negative and significantly different from zero, this suggests that the hazards of the mixture are unlikely to be greater than would be predicted assuming dose addition. Details of the procedure and the values of the numeric scores that are assigned in various circumstances are provided in the ATSDR document and in Mumtaz and Durkin (1992). Papers by De Rosa *et al* (1996) and Pohl *et al* (1999) and also the interaction profiles published on the ATSDR website at http://www.atsdr.cdc.gov/interactionprofiles/ illustrate the use of this approach to assess the hazards of chemicals released from hazardous waste sites.

The main advantage of this approach is that it provides a systematic framework for evaluating the effects of interactions in a component based assessment, and will help to identify sources of uncertainty in our understanding of the interactions that may be occurring.

Refinements to the weight of evidence (WOE) approach have been proposed by the US EPA (US EPA, 2000). Instead of the additive HI being modified by a single composite interactions factor, each term is modified according to the interactions of the other components and these modified terms are summed. This enables asymmetric interactions to be taken into consideration where chemical 'A' may have a synergistic effect on the toxicity of chemical 'B' but chemical 'B' is only additive for chemical 'A'. The approach also enables changes in interactions at different dose levels to be taken into account.

5.3.4 Additional component-based approaches for complex mixtures

Additional approaches have been considered for complex mixtures. Groten *et al*, (2001) outlined a decision tree approach that included suggestions for dealing with complex mixtures that cannot be tested in their entirety. The main solution offered was to reduce the number of components that have to be considered to a more manageable number by identifying those chemicals or classes of chemical that are of greatest concern and treating this subset as a simple mixture to which component-based approaches can be applied. This approach, called the top 'n' approach, or two-step approach, was previously outlined by Feron *et al* (1995). The initial step in the top 'n' approach is to derive a risk quotient for each chemical in the mixture.

The risk quotient represents the ratio between the estimated or measured level of exposure and the level of toxicity as expressed by a health-based occupational exposure limit or equivalent value. Where the assessment is being based on classes of chemicals, lumping analysis (see Box 6) might be used to group chemicals with relevant similarity, e.g. with the same target organ or similar modes of action (Verhaar *et al,* 1997). Only the chemicals or classes of chemical with the highest risk quotients (i.e. the smallest margin between exposure levels and doses causing toxicity) are carried forwards to the second step; a detailed assessment of the risks posed by simultaneous exposure. Feron *et al* (1995) recommended that no more than 10 substances should be included in the detailed evaluation.

Box 6. *Lumping Analysis:*

Lumping analysis was initially developed for the petrochemical industry to help ensure consistency in the composition of refinery streams. It enables similar components in complex mixtures to be grouped together into pseudocomponents. One actual component or a fictional average for the entire pseudocomponent may represent these pseudocomponents. How the chemicals are "lumped" depends on the behaviour of the chemical being studied but, for example, it could be done on the basis of partition coefficients or on the basis of a similarity of effect in a given target organ or cell or enzyme system. The advantage of this approach is that it allows modelling techniques developed for simple mixtures to be applied to complex mixtures. However, it assumes that the chosen pseudocomponents adequately represent all of the chemicals in the mixture. Verhaar *et al* (1997) discuss some of the issues raised by these assumptions and the mathematical techniques that can be used to estimate margins of error. They also discuss how the technique can be combined with other predictive tools such as QSAR and PBPK/PD modelling to study the toxicology of a complex fuel mixture (JP-5). More recently, PBPK modelling and lumping analyses have been used to characterise the pharmacokinetics of gasoline in rats (Dennison *et al*, 2003).

The top 'n' approach provides a pragmatic way of selecting the components that are likely to dominate the toxicological profile of the complex mixture, but it relies on the assumption that components not taken forward for a detailed analysis will have a negligible effect on the overall toxicity of the mixture.

Where the identity of a substantial proportion of a complex mixture is unknown, this assumption becomes increasingly unreliable. Also, it is not clear how data-poor components that are present in high concentrations would be handled using this approach.

An alternative approach that has also been used for mixtures of PAHs is the benchmark approach in which a marker compound is selected to act as a surrogate for the whole mixture (EPAQS, 1999; Pufulete *et al*, 2004; WHO, 2006). The philosophy behind this approach is that if the toxicity profile for a mixture is always dominated by one compound, then an assessment of the effects of exposure to the mixture could be based on the dominant compound. Such an approach may not be appropriate if the chosen marker compound is a relatively minor constituent or if the mixture includes dissimilarly acting components. In the case of PAH mixtures, BaP was chosen as the marker compound to estimate carcinogenic risks from PAHs in ambient air (Pufulete *et al*, 2004) and from PAH mixtures in food (WHO, 2006). This approach assumes that the carcinogenic potency of a PAH mixture will always be proportional to the BaP content and that the proportions of individual PAHs relative to BaP will be relatively stable. If more potent PAHs are present or if the BaP content is very small, BaP may not be the dominant carcinogen in the mixture. While this pragmatic approach may be appropriate for mixtures of PAHs in situations where the composition is fairly stable, wider application of the method to mixtures of other classes of chemicals needs to be underpinned by robust data to support the choice of surrogate compound.

The concept of marker compounds is also used in the context of classification and labelling for certain complex coal and oil derived substances, where the decision to classify a mixture as a carcinogen depends on the concentration of a particular marker compound. So, for example, if it can be shown that certain coal tar products contain less then 0.1% w/w benzene then there is no requirement to classify the product as a carcinogen. Equally, if it can be shown that certain petroleum-derived gases contain less than 0.1% butadiene, there is no requirement to classify the gas mixture as a carcinogen. This is based on the assumption that if the concentrations of the chosen marker compounds are below their respective thresholds, the concentration of any other potentially carcinogenic components will also be low. It should be noted that the basis for the numerical values of these cut-offs is not explained in the relevant European Directives and is likely to have been for convenience.

One other approach that has been considered for determining acceptable levels of chemicals in the environment in terms of their potential to cause adverse effects to human health is the application of an additional uncertainty factor to single substance Maximum Permissible Risk (MPR) levels to account for the potential for interactions to occur with other environmental pollutants. Although the extra margin will limit the potential for adverse effects to occur as a result of mixed exposures, this crude approach may produce standards that cannot practically be achieved (van Zorge, 1996; Henschler *et al*, 1996). Where uncertainty factors are applied to reference levels for single substances there needs to be a clear scientific justification, for example evidence for toxicokinetic interactions.

5.3.5 Which components need to be taken into account?

In any component-based risk assessment there is the question of which components should be taken into consideration. At what point can the concentration of a substance be considered too low for the substance to make any meaningful contribution to the overall toxicity of the mixture?

Within the Preparations Directive (1999/45/EC), a Directive of the European Union that lays down rules to be followed for the hazard classification of formulated products marketed within the EU, concentration limits are used to determine which components should be considered to contribute towards the classification of a formulated simple mixture and which can be excluded on the grounds that they are present at too low a concentration (see Table 4). If a chemical is present below these concentration limits then it does not need to be taken into account in deciding which classification to assign to the formulated mixture.

It should be noted that the rationale for selection of these generic concentration limits has not been published, and may well have been selected on pragmatic rather than scientific grounds. If there is evidence to indicate that these generic limits are not appropriate for particular substances, substance-specific concentration limits may be assigned. These could be higher or lower than the generic limits and is determined by effect levels for the specific substance to which they apply.

The Preparations Directive has been in force for a number of years. Over the next few years, it will be replaced by the Globally Harmonised Scheme (GHS) for hazard classification.

This has been adopted in the EU through Regulation (EC) no. 1272/2008 on classification, labelling and packaging of substances and mixtures (known as the CLP Regulation) (see http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:EN:PDF).

Table 4. Some of the generic concentration limits that are used in the Preparations Directive to determine which components should and should not be taken into account when determining the hazard classification for a formulated simple mixture.

Preparations Directive 99/45/EC		
Category of danger	**Concentration to take into consideration for**	
	Gaseous preparations % vol/vol	**Other preparations % w/w**
Substances classified as very toxic, toxic and category 1[1] or 2[1] carcinogens, mutagens and reproductive toxicants	≥ 0.02	≥ 0.1
Corrosive	≥ 0.02	≥ 1
Substances classified as harmful, irritant, sensitising and category 3[1] carcinogens, mutagens and reproductive toxicants	≥ 0.2	≥ 1
Dangerous for the environment, N		≥ 0.1
Dangerous for the environment, ozone	≥ 0.1	≥ 0.1
Dangerous for the environment		≥ 1

[1] Category 1 carcinogens and reproductive toxicants are those for which there is clear evidence of a causal relationship between exposure and adverse effects in humans. Category 1 mutagens are those for which there is clear evidence of a causal relationship between exposure and the occurrence of heritable genetic damage in humans.

[1] Category 2 carcinogens and reproductive toxicants are those for which there is sufficient evidence to provide a strong presumption that they present a hazard to humans. Category 2 mutagens are those for which there is sufficient evidence to provide a strong presumption that exposure may result in the development of heritable genetic damage in humans.

[1] Category 3 carcinogens and reproductive toxicants are those for which there is evidence for possible carcinogenic or adverse reproductive effects, usually based on evidence for an effect in animal models where there is uncertainty about the relevance of the findings to humans. Category 3 mutagens are those which cause concern for humans owing to possible mutagenic effects usually based on evidence from studies showing evidence that the substance can cause genetic damage in somatic cells *in vivo*.

The Regulation will apply to the classification of substances from 1st December 2010 and to the classification of mixtures from 1st June 2015. The Regulation outlines classification approaches for mixtures for the situation where whole mixture or component data are available. The CLP Regulation defines a mixture as "a mixture or solution composed of two or more substances". Like the Preparations Directive, it uses concentration limits to identify which components should be taken into consideration for the purposes of hazard classification. The generic concentration limits that have been adopted within the CLP Regulation are broadly similar, although not identical, to those used within the Preparations Directive.

As with the generic concentration limits in the Preparations Directive, there is no clear scientific rationale for the choice of value and pragmatism may well be the driver.

Generic concentration limits offer a pragmatic solution to the question of whether or not to include components in a risk assessment, and may be appropriate in certain contexts. However, for transparency in a risk assessment it is preferable to use science-based values. One generic science-based approach that has been developed to help prioritize chemicals in food for risk assessment is the Threshold of Toxicological Concern (TTC) approach (Box 7).

Box 7. *Threshold of Toxicological Concern approach:*

The TTC approach is similar in concept to the "Threshold of Regulation" first introduced by the US Food and Drug Administration (FDA) and used to assist regulatory assessments for food contact materials. The philosophy of the TTC approach is based on the de minimus concept; this acknowledges that there will be a practical human threshold value for chemicals below which there will be no significant risk to health. This threshold is referred to as the "threshold of toxicological concern". The TTC approach was developed as a means of reducing the need for extensive toxicity testing for novel chemicals present in food contact materials. As long as there are reliable exposure data to indicate that the substance is present below the appropriate TTC, then no further toxicity testing or safety evaluation is needed.

A detailed description of the derivation of TTC values is provided in papers by Kroes *et al* (2000) and (2004). Briefly, for non-cancer endpoints, TTC values were derived from the 5th centile of the distribution of NOELs for around 900 chemicals, obtained from mainly chronic oral administration studies, divided by an assessment factor of 100. TTC values have also been derived for carcinogens by a process of linear extrapolation from experimentally derived TD_{50} values for around 700 carcinogens in the Gold database (Gold *et al*, 1984, 1995) to determine doses estimated to present a one in one million lifetime risk or less for the development of cancer. Certain classes of substance were poorly represented in the reference database. The TTC approach is not recommended for these substances. These are non-essential metals, proteins, substances with polyhalogenated ring structures, substances with the ability to cause endocrine disruption at low doses, and potential allergens. Aflatoxin-like compounds, azoxy-compounds and nitroso-compounds are also excluded because of concerns for high carcinogenic potency.

The use of an approach such as the TTC that is based on dose rather than percentage concentration will provide a transparent method for screening out components that are present at trace amounts and therefore not likely to contribute to the overall toxicity of a mixture. One concern over the use of this type of approach for mixtures is the potential for toxicokinetic interactions that raise the levels of a toxicant at its target site. It is noted that, for non-cancer endpoints, the TTC thresholds are conservative, being based on the 5th centile of the distribution of NOELs and incorporating a 100 fold assessment factor. In most cases, this margin is likely to be sufficient to accommodate the effects of toxicokinetic interactions at dose levels in the range of the TTC values. It should be noted that the NOELs from which the TTC values have been derived are from oral data; hence, at present, it is only appropriate to consider the TTC approach in assessments of oral exposures.

Debate has started in the scientific literature on the establishment of a "Concentration of No Toxicological Concern" (CoNTC) for airborne exposures (Drew and Frangos, 2007). The debate is at an early stage. There needs to be discussion on the most appropriate data to use to identify CoNTC values; for example, the value suggested by Drew and Frangos for airborne organic material was extrapolated from an oral "threshold value" used by the US FDA for carcinogens. If the CoNTC concept gains regulatory acceptance in the future, this may provide a transparent means to screen out airborne components present at low concentrations from a mixture risk assessment.

Another concept that is receiving attention is that of hormesis or the "U-shaped" or "J-shaped" dose response curve. The hormesis theory suggests that exposure to very low doses of certain stressors may be beneficial to health because the exposure stimulates adaptive mechanisms in the body. As yet there is no clear consensus in the scientific literature about the types of chemicals to which this may apply or the dose ranges that may confer potential benefits to health. For mixture risk assessments, an adaptive response to one substance may potentially exacerbate an adverse response to another substance. This emphasises the need to have a good understanding of dose-response relationships and mechanisms of action when determining an acceptable level of exposure to substances in mixtures.

6. Decision Tree for Risk Assessment

The decision tree presented in this chapter provides an overview of the framework, which the IGHRC suggests should be followed when conducting a risk assessment for a chemical mixture. It is not an all inclusive step-by-step guide to chemical mixture risk assessment but highlights key issues that have to be considered depending on the type of mixture that is being assessed and the type of data that are available. The decision tree is presented in Figure 1. The following text provides additional clarification to help the assessor use the decision tree.

Step 1

The first step is to define the mixture for which a risk assessment is required and the context in which it is being assessed. For example, the assessment may be seeking to identify the risks posed by contaminants at a former industrial site with respect to potential future use of the site for housing. Alternatively, the assessment may be conducted to identify an acceptable level of occupational exposure to a particular mixture. The assessor must consider which populations may potentially be exposed, which pathways of exposure may be relevant, if there is variability in mixture composition, and whether there may be any relevant sequential exposures. Thinking about these issues as a first step will help the assessor to frame appropriate questions at later stages in the assessment and help to determine the adequacy of the available data or identify data gaps. Issues to consider when defining a mixture are discussed in Section 5.1.

Step 2

The next step is to determine whether the mixture and the circumstances in which it is being assessed fall within the scope of any regulations and, if so, whether there is a regulatory framework that dictates the way in which the risk assessment should be conducted. In the EU, this is applicable to mixtures and preparations falling within the Dangerous Preparations Directive 1999/45/EC and the REACH regulations (in the case of REACH mixtures are referred to as complex substances or substances of variable composition), as well as regulations relating to the approval of pesticide/biocide products. Approaches for assessing the hazards of mixtures under these regulations have been agreed between EU Member States and include guidance on how a risk assessment should be conducted, whether it is necessary to use whole mixture data or component data, and whether there is scope for additional information to be obtained. For mixtures that fall outside the scope of these regulatory schemes, and where there is no formally agreed risk assessment framework, this flow chart may be followed as an aid to the risk assessment process.

Step 3

If the mixture is not covered by a regulatory risk assessment framework, the next step is to consider the extent to which various target populations may be exposed. If it can be established that there is no significant exposure to particular target populations, there is no need to conduct a risk assessment for those groups. The term ‘significant’ is used here to indicate any exposure that may potentially give cause for concern. It is not intended to signify a predetermined exposure level below which all exposures will be deemed to be of no concern. At an early stage, it is therefore important to establish exposure criteria, including the key types of exposure of concern and susceptible groups. These criteria should be clearly defined when describing the scope of the risk assessment. It is likely that the definition of the mixture for which a risk assessment is required and the context in which it is being assessed will provide a starting point for identification of exposure type and the population groups exposed.

Step 3a

When considering the likely extent to which a particular population may be exposed, it is also important to consider the possible sources of exposure, the pathways of exposure, and whether exposure will be to the whole mixture or to a sub-fraction; for instance, if a mixture contains both volatile

and non-volatile components, it may be the case that only the volatile components will become airborne. Other issues to be taken into account include the extent to which the mixture will degrade and the potential for commonly occurring sequential exposures. The fate and behaviour of chemicals in the environment are important considerations and data gaps in this area will be a key source of uncertainty in a risk assessment.

Where exposure is used as a criterion for excluding groups from the scope of the risk assessment, there needs to be a good understanding of exposure pathways and a high degree of confidence in the exposure data for the mixture.

Step 4

Once the relevant target populations have been identified and the fraction of the mixture to which they may be exposed, including potential exposure to degradation products, has been established, the assessor needs to consider what hazard data are available. For example, is there information on the health effects of the whole mixture or the relevant sub-fraction and if so how extensive is this data? Section 5.1.3 provides further guidance in this aspect. It is also necessary to consider how variable the composition of the whole mixture or sub-fraction may be (see Section 5.1.4 for additional guidance). In order for hazard data to contribute to a whole mixture risk assessment, the composition of the mixture that was tested must adequately reflect the composition of the mixture that is being assessed (i.e. the mixture that has been defined in Step 1). If comprehensive data covering all relevant endpoints are available for the whole mixture or whole sub-fraction, it may then be possible to assess the mixture as if it were a single substance. Whole mixture or whole sub-fraction data could be supplemented with data for a similar mixture. Where data for a similar mixture are used, there is a need to consider any differences that exist between the similar mixture and the mixture or sub-fraction under assessment, and how these differences might impact on the assessment.

If insufficient whole mixture data are available, it is then necessary to consider whether component data can be used.

Step 5

In order to carry out a component based risk assessment, the components that contribute most to the hazardous properties of the mixture must, as a minimum, be known. Such components may not always be the most abundant in the mixture. In particular, components that have genotoxic potential, are persistent or bioaccumulative (i.e. have long biological half-lives), or are allergenic may pose risk at relatively low levels of exposure.

If the mixture has few components, all will probably be known as well as key information on hazardous impurities. The more components a mixture has, the greater the likelihood that not all will have been identified and the variability in composition not well understood. Incomplete understanding of the fate and behaviour of mixtures and their components in the environment will be a particular problem when assessing the effects of exposure to mixtures. A decision must be made to proceed with a risk assessment within the parameters of existing knowledge or, if the uncertainties surrounding composition and variability in composition are too great, whether a meaningful risk assessment can be achieved (further guidance is given in Section 5.1.4).

Step 5a

If it is not possible to carry out a meaningful risk assessment based on current knowledge it is necessary to consider whether it is possible to gather additional information about the composition of the mixture, for example by chemical analysis, and the factors that govern variability in composition.

When there is confidence that all components required for the risk assessment have been identified, a decision needs to be made about the most suitable component-based approach to use.

Step 6

Different approaches may be used for component-based assessments depending on whether the mixture is simple or complex. Definitions of what constitutes a simple and a complex mixture are given in Section 2. If the mixture is complex, i.e. many components and/or a highly variable composition, then the first step should be a comparison of approaches developed for other complex mixtures to examine the relevance of application to the current complex mixture.

Step 6a

The component-based approaches developed for complex mixtures have usually been developed for specific mixtures and cannot readily be adopted for other complex mixtures without careful evaluation. One approach that has proved successful for complex mixtures of chemicals from the same chemical class which can be shown to cause the same effects by the same mechanisms, is the use of TEFs. This has been used for mixtures of dioxins and dioxin-like PCBs. A separate TEF scheme has also been developed for acetylcholinesterase inhibiting organophosphates. Attempts to develop a TEF scheme for mixtures of PAHs were less successful because of limited data (see Section 5.3.1 for details). Other approaches that have been considered are outlined in Section 5.3.4 and include approaches that try to reduce the number of components that need to be considered (top 'n' approach) and the use of marker compounds to act as surrogates for the whole mixture; for example, BaP has been used as a marker compound to estimate carcinogenic risks from mixtures of PAHs. In order for such approaches to be used successfully, it is important to understand which components contribute most to the hazardous properties of the mixture, the factors that determine variations in the relative proportions of components, and the way in which interactions between components could affect the hazards of the mixture.

Steps 7 and 7a

The next step for evaluating simple mixtures and simplified complex mixtures is to consider whether there is any information to suggest that interactions between components could influence the overall toxicity of the mixture (see Section 3.2 for information on different types of interaction). If sufficient data are available, it may be possible to make a quantitative assessment of the effect of interactions on the toxicity of the mixture. Where this is not possible, but there is a plausible biological hypothesis suggesting the potential for interactions between components, a qualitative assessment of the impact of any potential interactions can be attempted. The BINWOE framework has been developed to help risk assessors make such qualitative assessments, but this is a resource intensive approach and requires the existence of data to inform the assessment process (see Section 5.3.3). The outcome of a BINWOE assessment will provide qualitative information to enable an assessor to judge the potential toxicological consequences of any potential interactions. This will help to inform decisions about whether or not additional assessment factors may be required and, if so, what size/scale of assessment factor. Alternatively, the risk assessor may decide that the qualitative assessment is insufficient and there is a need to obtain additional information to enable a quantitative risk assessment to be performed.

In many cases there may be no clear information on whether or not there is a potential for interactions to occur that could influence the toxicity of the mixture. If this is the case, there is no scientific basis on which interactions can be taken into account even in a qualitative sense. It is therefore most appropriate to use a default approach that assumes there are no interactions. This will be suitable for a preliminary (Tier 1) risk assessment. The absence of data to judge the likelihood of an interaction should be regarded as a data gap which will contribute to the overall uncertainty of the risk assessment.

It is acknowledged that the "no-interaction" assumption may be seen as a less precautionary approach. The picture emerging from robust mixture studies suggests that interactions are not observed at dose levels below thresholds of effect (see Section 3.2.3.2 for details). This document deals with situations where exposures are likely to be low. It is noted that conservative assessment factors are generally used to estimate thresholds of effect for data sparse substances. On this basis, providing that each component is below its estimated threshold of effect (or, for groups of components that cause similar functional effects, exposure to all components in the group is below the threshold of effect for the most hazardous chemical in the group), a no-interaction model is likely to be adequate. When considering the potential for interactions to occur in relation to thresholds of effect, it is important that the influence of any potential toxicokinetic interaction in the relationship between external dose and the level of the toxicant at its target site has been taken into account. The uncertainties associated with the choice if a default no-interaction model should be described in the risk assessment. Guidance on the choice of no-interaction model is given in Step 8.

Step 8

The subsequent steps in the flow chart guide the user in the selection of the most appropriate no-interaction default, i.e. dose addition or independent action (the models for component based risk assessments are described in Section 5.3). The flow chart suggests options to deal with data-rich as well as data-poor components. For a no-interaction component-based assessment, each component will either be considered to show dose addition with other components or will act independently. The type of action is informed by the target organs affected by each component and the mechanism of action. In some cases, with data sparse chemicals, understanding of the toxicological properties may be limited and hence all potential target organs for the chemical may not be identified. The most precautionary approach for data sparse components in a risk assessment is to assume that they show dose addition. The Hazard Index approach can be applied in this situation (see Section 5.3.1). In most cases this will overestimate the overall toxicity of the mixture. The lack of toxicological data on specific components will be a key source of uncertainty. If a good understanding of potential target organs for a particular component is lacking, the threshold of effect for that component will not be known. It is therefore most likely that the preliminary (Tier 1) risk assessment will conclude that there is a concern with the mixture. Rather than concluding that there is a real risk in this situation, the most appropriate course of action will seek to obtain additional information before risk management options are considered (see Step 11 below).

Step 9

Where there is information on target organs, the next step is to consider what information is available concerning the mode/mechanism of action of each component. If the mode/mechanism of action is known, and groups of components that share the same mode/mechanism of action can be identified, dose addition should be assumed. The Hazard Index approach could be applied. For data-rich groups, it may be possible to consider deriving relative potency factors to aid the assessment process. Dose addition should also be considered for components that produce functionally similar effects in the same target organ even if the molecular mechanism is different. It has been demonstrated that the effects of exposure to mixtures of endocrine modulating chemicals that produce similar effects by different mechanisms are best modelled by dose addition (Kortenkamp, 2007).

Independent action should be assumed for components that affect different target organs, and may also be considered for components that have an effect on a common target organ but by differing mechanisms. A decision will need to be taken as to whether the simple approach to assessing independent action or the more statistical approach used for response addition calculations is appropriate (see

Section 5.3.2). If the mode/mechanism of action cannot be determined from the available data, the decision on whether chemicals show dose addition or independent action must be made on the basis of target organs. In each case, the rationale for choosing a particular no-interaction model should be explained, especially where independent action is assumed for components that affect a common target organ but by different toxicological mechanisms.

Step 10

In the absence of information on the mode/mechanism of action, the most precautionary approach assumes that chemicals sharing target organs will show dose addition. There is no scientific basis to assume dose addition for chemicals affecting dissimilar target organs and hence independent action should be assumed in this case. Even though the mode/mechanism of action for each component may not be fully understood, it may be possible to identify a threshold of effect. This will provide a benchmark against which to assess exposure. If the preliminary (Tier 1) risk assessment concludes that there are no concerns, then the mixture would be of low priority for further work. If concerns are identified, this may be a result of the use of precautionary default assumptions rather that an indication of real risk. Therefore, the first action should be to consider whether it is possible to obtain additional information to refine the risk assessment (see Step 11 below).

Step 11

If a preliminary (Tier 1) risk assessment has identified concerns with a particular mixture and understanding of the toxicological properties of various components of the mixture is inadequate, then the possibility to obtain additional data to refine the risk assessment should be considered as the first action. If the hazards of the materials are not well understood and the appropriate exposure not identified, there can be no certainty that appropriate decisions have been made. This problem will be most acute where mixtures include data sparse components with poorly understood effects. In this situation, gaps in our understanding may be so great that we cannot identify a level of exposure below which there will be an assurance of health protection. In the absence of additional data, we may need to resort to stringent and potentially costly regulatory positions. The use of a tiered approach to help target further work is outlined in Section 6.1.

6.1 Use of a tiered approach for risk assessment of chemical mixtures

Increasingly, where data are sparse, risk assessors are adopting a tiered approach to risk assessment. Typically, the tiered approach will entail the use of precautionary default assumptions to compile a preliminary (Tier 1) assessment of risk. The Tier 1 risk assessment may then be refined by replacing precautionary defaults with measured data or the use of a more resource intensive modelling approach, e.g. PBPK modelling, to predict systemic doses. Where a mixture risk assessment is being performed, it is recommended that use should be made of all available data at each stage of the risk assessment process. Defaults should only be considered where actual data are lacking or are of questionable validity. In the absence of reliable data, a tiered approach may be useful to aid prioritisation of mixtures for further work.

There are several stages in the flowchart where precautionary assumptions may need to be made to compensate for poor information. It is recommended that each time it becomes necessary to use a default position, there should be some analysis to determine the extent to which this default contributes to the overall uncertainty in the risk assessment. The use of qualitative and quantitative uncertainty analysis to inform risk assessment has been described in the Reference Technical Guidance Document for registrants preparing a chemical safety report under REACH (EHCA, 2008). The general principles given in this document can be applied to mixtures of chemicals. A qualitative uncertainty analysis consists of

the characterisation of sources of uncertainty and judgement about whether this uncertainty could lead to under or over prediction of risk, allowing the most relevant sources of uncertainty to be identified. Quantitative uncertainty analysis may take a deterministic approach to examining the effects of changing certain critical assumptions and input parameters on the outcome of the assessment. Probabilistic methods are also available, but can generally only be used where there is good data. Probabilistic uncertainty analyses may be accompanied by a sensitivity analysis, which will identify the contribution of each source of variability or uncertainty to the overall outcome. Uncertainty analysis will help to refine the risk assessment by pinpointing the areas where there is the greatest need for refinement. A tiered approach that is informed by the results of these uncertainty analyses can then be developed to structure future work.

It will be most beneficial to fill data gaps in a particular sequence, with any necessary data on composition and exposure obtained before additional hazard characterisation is considered. The following outline may help risk assessors construct a tiered approach:

Tier 1

A Tier 1 risk assessment will incorporate several sources of uncertainty. These may relate to the composition and factors that affect variability in composition, pathways of exposure, the extent to which different groups/populations are exposed, and the toxicological hazards of the mixture. If a component-based assessment has been performed, there may also be uncertainty about the types of joint actions between components. Within the hazard assessment, it may have been necessary to use default assumptions about systemic doses and about the potential for interactions between components. Based on an analysis to identify the key sources of uncertainty, the risk assessor should determine whether or not the Tier 1 risk assessment is sufficiently robust to support any proposed regulatory action. If it is considered robust then no further work is necessary. If the uncertainty is deemed too great to support regulatory decision making, it will be necessary to consider refining the risk assessment. The uncertainty analysis may pinpoint the sources of uncertainty in most need of attention.

Tier 2

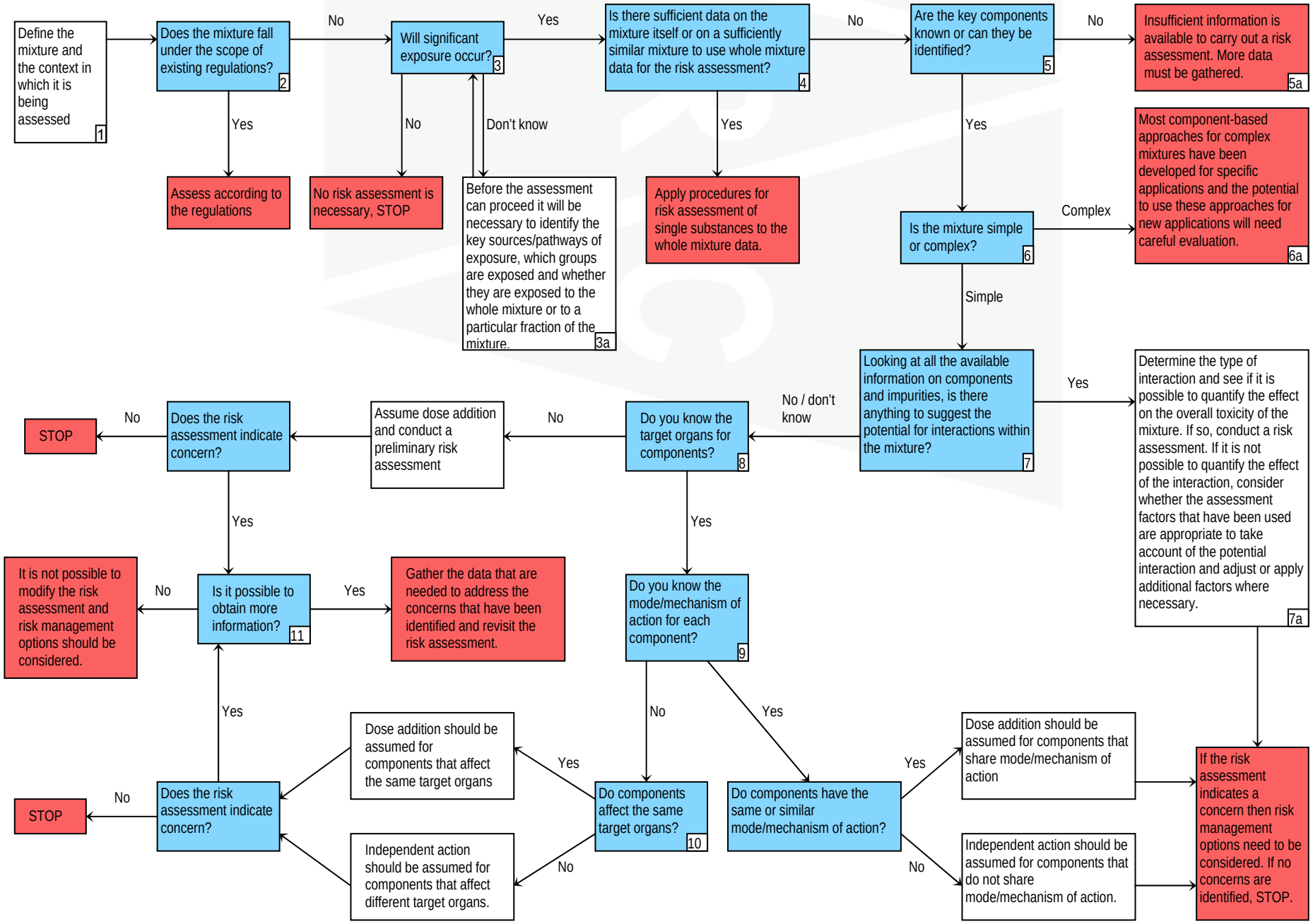
The risk assessor should first seek to clarify uncertainties relating to the composition of the mixture and the exposure assessment. Gathering additional information on composition will allow the risk assessor to identify more clearly the components that are of greatest importance in a component-based assessment. Refinements to the exposure assessment may be made by gathering additional information on exposure pathways and using probabilistic rather than deterministic modelling approaches, or obtaining measured exposure data if available.

Tier 3

If concerns remain after the additional information on composition and exposure has been obtained, the next step is to consider refinements to the hazard characterisation. The nature of the information required will depend on the specific issues of concern for the particular mixed exposure situation. The risk assessor will have to decide on a case-by-case basis how best to address these issues. Modelling approaches may be considered as a mechanism of refining hazard characterisation. Increasingly sophisticated PBPK/PD models are being developed that more accurately reflect the complexity of biological systems. For example, reaction network modelling, which has been used successfully in the area of petroleum and chemical engineering to understand complex chemical processes, is now being applied to biological systems (Liao *et al*, 2002). Reaction network modelling predicts the amounts of reactants, intermediates and products as a function of time for a very large series of coupled chemical reactions and has the potential to allow interactions to be modelled at the molecular mechanism level. Mayeno *et al*, (2005) demonstrated the

use of reaction network modelling to predict the metabolites formed with a four-component mixture of volatile organic solvents (trichloroethylene, perchloroethylene, methylchloroform and chloroform) that share aspects of their metabolic pathways. The points at which the metabolic pathways for the four substances intersected was visualised and depletion points for endogenous compounds such as glutathione that are involved in detoxification processes were revealed. Another development is the use of PBPK/PD models to investigate interaction thresholds. El-Masri (2007) gives three examples of the use of PBPK/PD models to examine interaction thresholds between two-component mixtures. By modelling the dose combinations required to produce a specific effect, it is possible to identify the range of dose combinations that conform to non-interactive behaviour. The boundary at which dose combinations start to deviate from non-interactive behaviour is referred to as the interaction threshold boundary. By modelling dose combinations beyond the interaction threshold boundary, it may be possible to gain an understanding of the direction (i.e. synergistic or antagonistic) that an interaction may take. PBPK/PD tools are best suited to answering specific questions relating to toxicological mechanisms, and to help understand the types of interactions that occur and the dose combinations that result in synergistic effects. They should be used as a complementary technique alongside focused experimental work where there is a need to refine hazard assessments for chemical mixtures.

Figure 1. Flow chart outlining the risk assessment process for chemical mixtures



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Glossary

The following glossary is included to provide the reader with a convenient set of definitions of toxicological and risk assessment terms used in government departments and agencies. The list is not intended to be exhaustive but to provide a level of inclusion sufficient to allow a clear understanding of terms and expressions used in this document.

The definitions are based on those provided in government publications, in particular the COT (2002) report on Risk assessment for Mixtures of Pesticides and Similar Substances and the IGHRC publications on route-to-route extrapolation and on uncertainty factors available at http://ieh.cranfield.ac.uk/ighrc/igpublications.html.

Absorption: The process by which a chemical is transferred into the body from the gastrointestinal tract, the lungs or across the skin.

Acceptable daily intake (ADI): Dose of a compound, which, on the basis of present knowledge, can be ingested every day over a lifetime.

Acute reference dose (ARfD): Dose of a compound, which, on the basis of present knowledge, can be ingested over a day or at a single meal.

Additivity: In dose additivity, each of the chemicals in a mixture contributes to the toxicity of the mixture in proportion to its dose, expressed as a percentage of the dose of that chemical alone which would elicit the given effect of the chemical. Response additivity is a situation which exists where each individual in a population has a certain tolerance to the individual components of a mixture and will only exhibit a response where the dose exceeds the tolerable dose. Response additivity can be determined by summing the quantal response of the animals to each toxicant in a mixture.

Adverse effect: Change in morphology, physiology, growth, development or lifespan of an organism, which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Aggregate risk assessment: A term introduced by the Food Quality Protection Act (1996) in the USA which refers to an assessment of exposure to a single chemical from multiple sources, specifically food, drinking water and residential-use sources.

ALARP – As Low As is Reasonably Practicable: A risk management approach under which exposure to a substances or mixture is reduced to the lowest level that it is deemed to be reasonably practicable to achieve in particular circumstances.

Antagonist: A substance that interacts with one or more other substances to reduce toxicity.

Antagonistic interaction: An interaction in which two or more chemicals interact resulting in a reduction of the toxicity of each chemical.

AOEL – Acceptable Operator Exposure Level: The maximum amount of active substance to which an operator may be exposed without any adverse health effects.

Assessment factor: Numerical adjustment factor used to extrapolate from experimentally determined (dose-response) relationships to estimate the exposure below which an adverse effect is not likely to occur.

ATSDR – Agency for Toxic Substances and Disease Registry: A government agency of the United States of America (USA).

Benchmark dose (BMD): A mathematically derived alternative to the NOAEL/LOAEL, using the data from a dose-response relationship as a toxicological reference point for use in risk assessment.

BINWOE – Binary Weight of Evidence approach: A risk assessment approach that allows qualitative judgements to be made about the potential effect of interactions between components on the overall toxicity of the mixture.

Bioavailability: The proportion of a substance that reaches the systemic circulation after a particular route of administration.

Combined action: The joint effects of two or more chemicals.

Common mechanism group (CMG): Group of compounds sharing a common mechanism of action.

Complex mixture: For the purpose of this report, a complex mixture is one that consists of many chemicals for which the composition is not fully characterised qualitatively or quantitatively.

Concurrent exposure: Exposure to two or more chemicals at the same time usually because the chemicals are all present in a single mixture.

Contaminant: A substance that is unintentionally present in food, in drinking water or in the environment.

Congener: One chemical from a family of chemicals of the same chemical class.

COC – Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment: The COC is an independent advisory committee that provides advice to government departments and agencies on the potential carcinogenicity of chemicals, from natural products to new synthetic chemicals used in pesticides or pharmaceuticals.

COM - Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment: The COM is an independent advisory committee that provides advice to Government departments and agencies on matters concerning the potential mutagenicity of chemicals, from natural products to new synthetic chemicals used in pesticides or pharmaceuticals.

COT – Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: An independent scientific committee that provides advice to the United Kingdom (UK) Food Standards Agency, the UK Department of Health and other UK government departments and agencies on matters concerning the toxicity of chemicals.

Critical health effect: The critical effect(s) is/are the effect(s) of a substance that drive the risk assessment. The critical effect that is chosen will depend on the anticipated route and duration of exposure.

Cumulative risk assessment: A term introduced by the Food Quality Protection Act (1996) in the USA which refers to an assessment of exposure to multiple chemicals with the same mechanism of toxicity.

CVMP – Committee on Veterinary Medicinal Products: The Committee which prepares the opinion of the European Medicines Evaluation Agency on any matter to do with the evaluation of veterinary medicinal products.

DEFRA - Department for the Environment, Food and Rural Affairs: UK government department.

DH – Department of Health: UK government department.

Distribution: The process of transport of a chemical around the body and its transfer into tissues and cells.

Dose: Total amount of a substance administered to, taken or absorbed by an organism.

Dose additivity: See “Additivity”

Dose-response: The relationship between the dose or level of exposure to a chemical and the incidence or severity of the associated adverse effect.

ED_{10}: A dose level causing an effect in 10% of the exposed population.

Effect addition: See “Response addition”.

EFSA – European Food Safety Authority: EU agency responsible for risk assessments regarding food and feed safety.

EFTA – European Free Trade Association: An intergovernmental organisation set up for the promotion of free trade and economic integration to the benefit of its four Member States: Iceland, Liechtenstein, Norway and Switzerland.

EINECS – The European Inventory of Existing Chemical Substances: A list of chemical substances being marketed within the EEC between 1 January 1971 and 18 September 1981.

Elimination: The process by which chemicals are removed from the body.

Enterohepatic circulation: A cycle in which a substance absorbed from the intestine is transferred by the liver into the bile and re-enters the intestine, from where it is reabsorbed. The overall effect is to prolong the time it takes to clear a chemical from the body.

Enzyme induction: The process by which the body increases the amount of certain enzymes involved in metabolism in response to exposure to chemicals that are metabolised by those enzymes.

Enzyme inhibition: See “Inhibition”.

EPAQS – Expert Panel on Air Quality Standards: A committee that provides UK government departments with advice on non-occupational air quality standards and other aspects of air pollution.

ESR - **The Existing Substances Regulation**: A chemical hazard and risk assessment programme initiated by the EU in 1993 that will be superseded by REACH.

FSA – Food Standards Agency: UK Government agency concerned with food safety.

Genotoxicant: A substance that has the ability to damage DNA, either directly or after metabolic activation.

GHS – Globally Harmonised System: The globally harmonised system for hazard classification and labelling of chemicals.

Group ADI: ADI assigned to a group of similarly acting chemicals from the same chemical class.

Half-life: The time taken for the level of a chemical substance in the body to fall to one half of the starting concentration. This can be used to determine the likely persistence of a chemical in the body.

Hazard: The inherent properties of a substance, or mixture of substances, that make it capable of causing adverse effects in organisms.

Hazard characterisation: The quantitative (potency) evaluation of any adverse effects observed, usually by dose-response assessment, and the identification of mechanisms of action and species differences in response.

Hazard identification: The identification from animal studies, *in vitro* studies and structure-activity relationships, of adverse health effects associated with exposure to a chemical.

Hazard Index (HI): A risk assessment approach based on the assumption of dose additivity.

HPV – High production volume: A term used within certain chemical hazard assessment programmes to denote chemicals that are produced in quantities of 1000 tonnes per annum or more.

HQ – Hazard Quotient: The contribution that an individual chemical makes to the overall toxicity of a mixture. It is determined by dividing the level of exposure to the chemical in the mixture of interest with some reference level e.g. a tolerable daily intake or soil guideline value.

HSE – Health and Safety Executive: A UK government body with responsibility for regulating workplace health and safety. HSE is the UK competent authority for REACH.

IGHRC – Interdepartmental Group on Health Risks from Chemicals: The IGHRC comprises representatives of UK government departments, research councils and agencies, and aims to stimulate the development of improved approaches to the assessment of risks to human health from chemicals.

ILSI – International Life Sciences Institute: A non-governmental organisation affiliated to the WHO.

Impurity: A substance that is present unintentionally as a result of a manufacturing process. It may derive from the starting materials that are being used or from the production process itself.

Incidental exposure: The term 'incidental' is used in this document to distinguish between exposures that arise as a consequence of normal daily activity e.g. through the diet, via the environment or the workplace, and exposures that are deliberate e.g. the use of medicines.

Independent action: The situation in which individual chemicals in a mixture act by different mechanisms and do not affect the physiological response to other chemicals in a mixture, hence the effect of exposure to a mixture of independently acting chemicals is expected to be qualitatively and quantitatively similar to the effects of the individual components when given alone.

Inhibition: **1) Enzyme kinetics**. The situation where one chemical blocks enzymatic breakdown of another chemical.

2) Mixture toxicology. Describes a toxicological interaction between two or more compounds resulting in reduced toxicity. The term inhibition has been used interchangeably with the term antagonism. In this report, inhibition is used to describe the situation where one chemical will reduce the toxicity of another chemical but is itself unaffected.

Interaction: The situation in which individual chemicals in a mixture influence the way the body responds to other chemicals in a mixture. This is of concern when it leads to a harmful outcome.

ISO – International Organization for Standardization: Develops and publishes International Standards

JECFA – Joint Expert Committee on Food Additives: Committee convened jointly by the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organisation (WHO) to consider reference doses for food additives and veterinary residues.

JMPR – Joint Meeting on Pesticide Residues: Meeting convened jointly by the Food and Agricultural Organization of the United Nations (FAO) and the World health Organization (WHO). It advises the Codex Alimertarius Commission on pesticide residues in food.

LOAEC - Lowest observed adverse effect concentration: The lowest exposure concentration at which an adverse effect is seen.

LOAEL - Lowest observed adverse effect level: The lowest administered dose in a study at which an adverse effect is seen.

Masking: A situation in which the effects of one chemical in a mixture conceal the effects of another chemical.

Mechanism of action: A detailed molecular description of the events involved in the physiological response of an organism to a chemical agent.

Metabolic activation: The process by which relatively stable substrates are converted to highly reactive, generally electrophilic products with the capability of producing damage to critical cellular macromolecules. The term is occasionally used to refer to the metabolism of therapeutically inactive pro-drugs to the active form of the drug.

Metabolism: The process by which chemicals are broken down and changed in the body.

Metabolite: Product formed from the original compound by enzymic/hydrolytic reactions in the body or cell.

Mode of action: A general description of the key events and processes involved in the physiological response of an organism to a chemical agent.

MRL: **1) Maximum Residue Level** (for pesticides). Legally enforceable limit on the maximum concentration of a pesticide or allowed in food. For pesticides, it is calculated from trials data and is not a safety limit per se.

2) Maximum Residue Limit (for veterinary products). Legally enforceable limit on the maximum concentration of a veterinary drug allowed in food. The MRL for veterinary drugs is a safety limit.

NOAEC - No observed adverse effect concentration: The highest concentration in a study at which no adverse effects are seen.

NOAEL - No observed adverse effect level: The highest administered dose in a study at which no adverse effects are seen.

NOEL – No observed effect level: The highest administered dose in a study at which no effects are seen,

Non-genotoxic carcinogen: A substance which induces tumours via a mechanism which does not involve direct damage to DNA.

NONS - Notification of New Substances Regulation: A chemicals hazard and risk assessment programme introduced within the EU in 1981. This will be superseded by REACH.

OECD – Organisation for Economic Co-operation and Development.An organisation that brings together the governments of countries committed to democracy and the market economy from around the world.

OEL – Occupational Exposure Limit: Generic term used to describe a limit set to restrict the amount of a substance that a worker can be exposed to for short or long durations in their working day. OELs may be set at a level at which no adverse effects are expected, based on present knowledge, for someone exposed for a working lifetime. OELs may also be set at a level which reflects a certain standard of control and may not always carry a guarantee of health protection.

Organelle: Structure within a cell that supports the cell's function.

Organophosphate (OP): A chemical class most usually associated with pesticides.

PAH – Polycyclic aromatic hydrocarbon(s): Chemical compounds that consist of fused aromatic rings.

PBPD modelling – Physiologically-based pharmacodynamic modelling: See "PBPK modelling".

PBPK modelling – Physiologically-based pharmacokinetic modelling: A mathematical approach to aid understanding of the kinetics of a substance. When applied to pharmaceutical compounds the term pharmacokinetic is commonly used. When applied to other types of chemical the term toxicokinetic may be used, however, the two terms are interchangeable.

PBTK modelling – Physiologically-based toxicokinetic modelling: See "PBPK modelling".

PCB - **Polychlorinated biphenyl**: A family of chemicals, some of which have toxicological properties similar to dioxins.

Pesticide: Substance intended to kill unwanted living organisms.

Potentiation: A toxicological interaction between two or more compounds resulting in enhanced toxicity. The term potentiation has sometimes incorrectly been used interchangeably with the term synergism. In this report, potentiation is being used to describe the situation where one chemical will enhance the toxicity of another chemical but is itself unaffected.

Potency Equivalency Factor (PEF): See "Relative Potency Factor".

PSD – Pesticides Safety Directorate

QSAR – Quantitative Structure Activity Relationships: Computational tools that enable the toxic effects of chemicals to be predicted based on an analysis of the chemical structure.

REACH – European legislation governing the Registration, Evaluation, Authorisation and Restriction of Chemical substances. It came into force in the EU on 1st June 2007.

Relative Potency Factor (RPF): A numerical indicator of the toxicological potency of a chemical in relation to that of an index chemical from the same chemical class. May also be referred to as a Potency Equivalency Factor (PEF) or Toxic Equivalency Factor (TEF).

Response addition: A component-based assessment approach used to determine the effects of exposure to a mixture of independently acting chemicals. It takes account of the fact that the sensitivity of individuals within a population will be different to different components of the mixture.

Risk: Probability that a harmful event (e.g. death, injury or loss) arising from exposure to a hazard may occur under specific conditions.

Risk assessment: The evaluation of the potential for adverse effects to occur from exposure to a hazard.

Risk characterisation: The integration of hazard identification, hazard characterisation and human intake with exposure assessment in order to assess the probability or occurrence of a risk to human health.

Saturation: When the body reaches its maximal capacity to metabolise a chemical by a particular enzymatic pathway. This may refer to binding, transport or a particular step in metabolism. Saturation of detoxification pathways can result in a chemical being metabolised along a different pathway, potentially leading to a more toxicologically active metabolite and a step change in the dose-response relationship.

Sequential exposure: Exposure to two or more chemicals at different times. This is of concern where a chemical has an influence on the toxicological effects of subsequent chemicals to which an individual is exposed.

SGV – Soil guideline value: Scientifically based generic assessment criteria to help evaluate long-term risks to human health from chemical contamination in soil. Can be considered to be "trigger values". Where soil concentrations exceed SGV, there may be a cause for concern to human health and hence a need for further investigation.

Simple mixture: For the purposes of this report, a simple mixture is one that consists of a small number of chemicals, which is characterised both qualitatively and quantitatively.

STEL – Short Term Exposure Limit: A STEL is an occupational exposure limit that is set where there are concerns that adverse health effects may arise from brief exposures. The reference period is typically 15 minutes, though other reference periods may be used.

Synergist: A substance that interacts with one or more other compounds to enhance toxicity. A pesticidal synergist is defined as "any substance other than water, without significant pesticidal properties, which enhances or is intended to enhance the effectiveness of a pesticide when added to that pesticide".

Synergistic interaction: An interaction resulting in an increase in the toxicity of a chemical or chemicals. This term has been used interchangeably with the term "potentiation". In this report, the term "synergistic interaction" is used to describe the situation where two or more chemicals interact resulting in an increase in the toxicity of each chemical.

Systemic dose: The total amount of an agent that is administered and subsequently absorbed into the body.

Systemic toxicity: Toxicity expressed in tissues/organs distant from the site of administration.

TDI - Tolerable daily intake: An estimate of the amount of contaminant, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.

TEF – Toxic equivalency factor: This is a factor that expresses the toxicity of one congener from a chemical class relative to an index compound. In the case of dioxins and dioxin-like substances, TEFs express the toxicity of individual congeners relative to the index compound 2,3,7,8 tetrachloro-*para*-dibenzodioxin (2,3,7,8-TCDD).

TEQ – Toxic equivalence quotient: The TEQ for a mixture of chemically related substances expresses the toxicity of the mixture in terms of an equivalent dose of a key indicator chemical from that category of substances.

Threshold of effect: Dose or exposure concentration below which a chemical does not exert an effect.

Top 'n' approach: A risk assessment approach for complex mixtures that involves the identification of those components that contribute the most to the toxic effects of the mixture.

Toxicity: An inherent property of an agent that causes an adverse biological effect.

Toxicodynamics: A description of the adverse effects that toxic chemicals exert on the body at their target site. This may include interaction of the substance or its metabolite with the target tissues (including cells, organelles and biological macromolecules) and any resulting pathophysiological consequences that lead to expression of toxicity.

Toxicokinetics: A description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and elimination.

TTC – Threshold of Toxicological Concern: The concept that there will be a single dose/exposure concentration for every chemical below which it is of no toxicological concern.

TTD – Target organ Toxicity Dose: A modification of the hazard index approach in which all target tissues for each chemical are taken into account.

TWA – Time-weighted average: Term used to indicate that exposure measurements have been averaged over a period of time.

Uncertainties: Those elements in the risk assessment process about which knowledge is absent or imprecise.

Uncertainty factor: A numerical factor applied to a toxicological reference point to allow for uncertainties in risk assessment. These factors may be default values used in the absence of specific information on a chemical and may be modified in the light of specific information.

US EPA – United States Environmental Protection Agency: Leads environmental science, research, education and assessment efforts across the United States, with the mission of protecting human health and the environment. .

US FDA – United States Food and Drug Administration: The federal United States agency responsible for ensuring the safety of food, human and veterinary drugs, biological products, medical devices, cosmetics, and electronic products that emit radiation.

VMD – Veterinary Medicines Directorate (agency of DEFRA): A UK government agency.

VMP – Veterinary Medicinal Product: medicines and associated products associated with veterinary use.

VPC – Veterinary Products Committee: An independent scientific committee that provides advice to the United Kingdom (UK) ministers on any aspect of veterinary medicinal products and animal feed additives.

WATCH – Working Group on Action to Control Chemicals: A UK government advisory committee that considers issues relating to the control of chemicals in the workplace.

WEL – Workplace Exposure Limit: Occupational exposure limits set under the UK Control of Substances Hazardous to Health Regulations 2002 (as amended). WELs represent a level of exposure that can be achieved where good occupational hygiene practices are being followed.

WHO – World Health Organization: WHO is the directing and co-ordinating authority for health within the United Nations system. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends.

IHRC

Current Policy of UK Departments and Agencies on Chemical Mixtures

IHRC

Annex A: Environmental Agency – current approach to mixtures

Introduction

Most toxicological and epidemiological data come from studies that have investigated the impacts of individual chemicals. However, environmental contaminants are rarely present in isolation. Often, the environment is contaminated by complex mixtures, such as petroleum products, whose content is variable and poorly defined. The health effects of some components of mixtures of environmental chemicals could be additive. Synergistic or antagonistic interactions are also potentially possible, but are less likely to be relevant to the health impacts of contaminants at environmental concentrations.

Data on the toxicity of mixtures are usually lacking and, because there are so many possible combinations of environmental contaminants, mixture testing is rarely practicable. In addition, the composition of a mixture released to the environment will change with time, due to the different environmental fates of the various components (e.g. during the 'weathering' of petroleum products).

Risk assessment of mixtures

The practical difficulties in testing the toxicity of mixtures are well recognised. Consequently, toxicological criteria (tolerable daily intakes etc) are generally derived for individual substances. However, in some cases, these may be set for a class of substances with similar effects, especially if they are likely to be present in combination.

Hazard quotient / hazard index approach

As outlined in its guidance for assessing the potential health effects of land contamination (Environment Agency, 2008), the Environment Agency considers the possible additivity of more than one contaminant where there is evidence that the chemicals share a common toxicological pathway leading to the same adverse effects on the same target organ, i.e. simple similar action giving rise to dose additivity.

For substances exerting effects for which there is a threshold, the Environment Agency favours use of the hazard index (HI) / hazard quotient (HQ) approach. HQs for each of the components of the 'additive' mixture are calculated by dividing the estimated exposure by the tolerable daily intake (TDI). The HQs are then summed to produce the HI. If the HI is lower than unity (i.e. 1), there is no cause for concern (see equation below). If the HI exceeds unity, this is considered to be equivalent to a TDI being exceeded by a single contaminant, and further expert consideration is needed.

$$\frac{Exposure_1}{TDI_1} + \frac{Exposure_2}{TDI_2} + \Lambda + \frac{Exposure_n}{TDI_n} \leq 1$$

Toxic equivalence (dioxin-like compounds)

Polychlorinated-dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain polychlorinated biphenyls (PCBs) exert their biological effects by the same mechanism of action (via interaction with the aryl hydrocarbon receptor) and are almost always found as complex mixtures in the environment. Toxic equivalency factors (TEFs) for the active congeners have been derived, taking into account factors such as relative potency at the receptor and half-life in the body (see van den Berg *et al*, 2006; COT, 2006). This approach allows the calculation of the toxic equivalent (TEQ) for each component of the mixture by multiplication of the concentration of the congener by its TEF. The overall TEQ of the mixture is the sum of the TEQs for the constituent congeners. The TEQ of the mixture can be compared with the TDI for the group to allow an assessment of the risk posed by the mixture. The Environment Agency uses this approach in its evaluations of risks from these compounds (e.g. Defra and the Environment Agency, 2003).

Indicators and/or fractions (petroleum hydrocarbons)

Petroleum hydrocarbons are common contaminants at sites investigated for land contamination. Petroleum products are complex mixtures of variable composition. Assessing the risks they pose at contaminated sites is further complicated because the profile of components present in the weathered product can be very different from the composition of the fresh product. A wide range of hydrocarbons might be present at a site and, for many, toxicity data will be lacking. However, many would be sufficiently similar in structure to expect that they might have similar toxicities. In view of these factors, the risks to health from petroleum hydrocarbons should be assessed as a mixture rather than as individual substances.

One methodological approach to assessing health risks from petroleum hydrocarbons in soil involves both indicator compounds and hydrocarbon fractions (Environment Agency, 2003). This approach is similar to that developed by the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG. 1999). Although the toxicological reviews and derivation of soil guideline values (SGVs) for total petroleum hydrocarbons (TPH) are still under development, the principles that will be adopted have been published (Environment Agency, 2005).

Indicator compounds represent the most toxic contaminants – including those known to be non-threshold carcinogens – and those found most frequently at petroleum hydrocarbon contaminated sites. These include compounds such as benzene, toluene, ethylbenzene and xylene (BTEX) and some polycyclic aromatic hydrocarbons (PAHs). Risks from these compounds are assessed individually. It is anticipated that the risks at many contaminated sites will be driven by the presence of indicator compounds.

Nonetheless, only a few of the many components of TPH can be assessed in this way. In order to ensure that potential risks from other components of the hydrocarbon mixture are not overlooked, the threshold effects of the whole TPH mixture will be assessed using an approach based on fractions. Fractions represent groups of hydrocarbons with similar fate and transport and toxicity characteristics. They are defined based on effective carbon (EC) number and whether they are aromatic or aliphatic in structure (see Table A below). TDIs for each fraction will be derived using the most appropriate available toxicity data for observed threshold effects. These data might be for a representative mixture of compounds falling within the fraction or, more often, selected from studies on single ‘surrogate’ compounds within the fraction (see TPHCWG, 1997 for examples of the approaches used).

Table A: Petroleum hydrocarbon fractions (based on equivalent carbon number) for use in risk assessment of UK land contamination (Environment Agency, 2005)

Aliphatic fractions	Aromatic fractions
>5–6	>5–7
>6–8	>7–8
>8–10	>8–10
>10–12	>10–12
>12–16	>12–16
>16–35	>16–21
>35–44	>21–35
	>35–44
>44–70	

Fraction-specific SGVs will be generated using representative fate and transport data for the fraction and the fraction-specific TDIs in the Contaminated Land Exposure Assessment (CLEA) model. These SGVs represent a concentration in soil at or below which resulting human exposure can be considered to represent a tolerable level of risk. Soil concentrations of TPH fractions are compared with the SGV for the fraction to assess whether health risks are likely. However, even where SGVs for individual fractions are not exceeded, potential additivity of toxicological effects between the fractions means that a risk could exist. To address this issue a hazard index/hazard quotient approach is adopted.

The HI should be calculated in accordance with the approach described previously.

The HQ for each fraction is calculated by dividing the average daily exposure (ADE) from soil for each fraction by its TDI, after allowance for exposure from other (non-soil) sources. The HQs are then summed to give the HI. This process is repeated for each relevant route of exposure. In practice, however, an approximation of the HQs and HI can be more practicably achieved by dividing the measured concentration of each fraction in soil by its SGV, and summing these (see Figure A below).

Where the HI exceeds unity, this indicates a potential risk to human health and the investigation proceeds to the next stage (detailed quantitative risk assessment or options appraisal).

$$HI = \sum_{F_i=1}^{16} HQ\,F_i = \frac{Concentration\ in\ soil\ F_i\ (mg\,kg^{-1})}{SGV\ F_i\ (mg\,kg^{-1})}$$

where HI = Hazard Index
HQ = Hazard Quotient
F_i = Fraction $_i$
SGV = Soil Guideline Value

Figure A: Hazard index determination using soil guideline values.

Fractions exhibiting different toxicological properties might be excluded from the HI. Guidance on this issue will be developed along with the derivation of the TDIs for the various petroleum hydrocarbon fractions.

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Annex B: Food Standards Agency – current approach to mixtures

Introduction

Food is a complex mixture of chemicals and the Food Standards Agency (FSA) recognises that it is not feasible to systematically assess all possible combinations. Risk assessment of chemical mixtures is based on advice from the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), an independent scientific committee that provides advice to the FSA, the Department of Health and other Government Departments and Agencies on matters concerning the toxicity of chemicals. The view of COT is that mixtures of similarly acting toxicants show additivity (dose addition), which results from simple similar action, and that this occurs over the whole dose range. When the components of a mixture have different modes of action, no additivity and no potentiating interactions are expected when exposure levels to the chemicals within the mixture are in the range of their no-observed-adverse-effect-levels (NOAELs). The FSA therefore evaluates mixtures of single substances with different mode of actions by assuming that the Acceptable Daily Intake/Tolerable Daily Intake (ADI/TDI) approach is protective against interactions.

Selection of the approach to be adopted for mixtures is determined by the COT, or by the scientific advisory committees of the European Food Safety Authority (EFSA) or World Health Organization (WHO) on a case-by-case basis, depending on the nature of the toxicity and the available database. Examples of the main approaches taken are given below.

Toxic Equivalency Factors (TEFs)

TEFs have been proposed for a number of chemical classes with similar mode of action, and can be described as a measure of the relative toxicological potency of a chemical compared to a well-characterised reference compound usually the most toxic. The summation of TEFs for all compounds in the mixture is used to give a measure of the total toxic equivalence (TEQ). The most widely accepted TEF system for chemicals in food is that used for the chlorinated dibenzodioxins, dibenzofurans and dioxin-like polychlorinated biphenyls, which have similar structures, and act via a common receptor. The TEFs have been set relative to the toxicity of 2,3,7,8-tetrachlorodibenzo[*p*]dioxin (TCDD), and the total dietary TEQ is compared with the group Tolerable Daily Intake set on the basis of the toxicity of TCDD.

Surrogate markers

If the toxicokinetic and toxicological data are inadequate to establish TEFs, the risk assessment for the mixture may be based on a single component, or surrogate. For example, there are insufficient data to derive TEFs for oral exposure to polycyclic aromatic hydrocarbons (PAHs). However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that benzo[a]pyrene could be used as a marker of exposure to, and the carcinogenicity of, the PAH present in food. The risk assessment compares dietary exposure to benzo[a]pyrene with the benzo[a]pyrene content of a relevant PAH mixture that has been tested for carcinogenicity (WHO, 2005).

Another type of surrogate approach, used when fewer data are available, is the precautionary assumption that the toxicity of the mixture is equivalent to the toxicity of its most potent component. Thus for the polybrominated diphenyl ethers (PBDEs), the COT compared the total dietary exposure to PBDEs with the NOAEL for the most potent congener (FSA, 2004).

Group Acceptable Daily Intake (ADI)

For groups of food additives or pesticides with similar structures acting through a common metabolite or with similar toxicity, a group ADI may be used i.e. one compound could be consumed at the ADI level, or four compounds could be consumed at 25% of the ADI.

Research

In order to reduce uncertainties in the risk assessment of mixtures of pesticides and similar substances in food, the FSA has commenced a research programme to develop and validate the necessary tools to investigate whether interactions might occur at the low levels of residues to which consumers are exposed. Research projects are addressing areas of relevance to the COT's recommendations (FSA, 2002). These include the development of biomarkers of exposure and effect, the characterisation of possible variability in human responses to mixtures of residues, the nature and dose-response relationships for combined actions of chemical mixtures, and the effect of food processing and preparation on the bioavailability and chemical nature of residues.

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Annex C: Health and Safety Executive – current approach to mixtures

Introduction

The approach of the Health and Safety Executive (HSE) to mixtures of industrial chemicals can be divided into the approaches taken to assess workplace exposures to mixtures (these are described in EH40; HSE, 2005) and the approaches taken to determine the appropriate classification and labelling of mixtures formulated for sale (as described in the EU Dangerous Preparations Directive). HSE also has a role in approving pesticides/biocides for non-agricultural uses. In addition to extensive data requirements for active ingredients, companies seeking approval must submit short-term toxicity data for the formulations they intend to market. HSE will use formulation data in the approvals process in essentially the same way that PSD use such data in the approvals process for agricultural pesticides, therefore this will not be discussed further here.

Guidance on assessing mixed exposures in the workplace

There are very few workplace situations where workers will be exposed to one single substance. Most workplace exposures will be to mixtures because of the use of formulated products and/or because of process emissions. For many process emissions, the composition may not be well defined and may change during the working day and from day-to-day. Some guidance on how to decide whether control of these mixture exposures is adequate is published in EH40 (HSE, 2005).

Occupational Exposure Limits (OELs) are available for a few specific mixtures e.g. wool process dust or ferrous foundry particulate. These limits have been derived using whole mixture data and by treating the data as if for a single substance. Compliance with these whole mixture limits is assessed by measuring total particulates.

A procedure called the Reciprocal Calculation Procedure (RCP) has been developed to enable OELs to be calculated for mixtures of certain hydrocarbon solvents based on OELs for individual components. The RCP is a pragmatic approach for deriving OELs for mixtures of certain hydrocarbon solvents. These are mixtures containing aliphatic hydrocarbons in the range C_5 to C_{15}, cycloalkanes in the range C_5 to C_{16} and aromatics. Halogenated and oxygenated hydrocarbons are specifically excluded. The procedure only applies to vapours (mists are excluded) and concentrations must be expressed as mgm^{-3}. The RCP was adopted by the UK on the recommendation of an Advisory Committee on Toxic Substances (ACTS) working group and following endorsement by WATCH (WATCH/30/93) and ACTS (ACTS/43/93). The formula as provided in EH40 is:

$$\frac{1}{OEL_{sol}} = \frac{FR_a}{OEL_a} + \frac{FR_b}{OEL_b} + \frac{FR_n}{OEL_n}$$

where:

OEL_{sol} = OEL for the hydrocarbon solvent mixture (in mgm^{-3})

OEL_a = OEL or guidance value for component 'a' (in mgm^{-3})

FR_a = Fraction (w/w) of component 'a' in the solvent mixture.

The RCP is based on two key assumptions. The first assumption is that the toxicological effects of the mixture components will be additive (the components will show simple similar action) and hence the OEL for the mixture can be derived by scaling OELs for the individual components of the mixture. The second assumption is that the airborne fraction will have the same relative composition as the source material.

For the majority of mixtures, no mixture-specific OELs are available and the adequacy of control has to be judged using substance specific OELs. With the exception of the RCP, the approaches recommended by HSE do not enable an OEL to be derived for a mixture based on its components, nor do they enable an employer to determine what the toxicological hazards of the mixture will be.

Instead they help to provide an answer to the question “Am I controlling exposures to the combination of substances present in this workplace adequately or are the exposure levels too high?”

EH40 indicates that assessments of exposure to mixture components should be based on the concentrations of each component in the workroom air since the relative concentrations in the air may be very different to the concentrations in the source material. The first step for an employer to take is to ensure that each substance present in the workroom air is adequately controlled in relation to its own specific limit. Employers should then look to see if there is a need to further reduce exposures to counteract a possible risk of adverse effects arising from mixed exposures. EH40 provides guidance on approaches to be taken where particular types of joint action are anticipated. Employers are advised to consider the potential for synergistic behaviour first, then consider the potential for additive behaviour and finally consider substances thought to act independently. The recommended approaches for each type of joint action are discussed in more detail below. Since the mechanism of joint action between components in a mixture may not always be clear EH40 recommends that it may be prudent to treat all non-synergistic systems as though they were additive.

Synergistic substances

If there is reason to suspect that components in a mixture may act synergistically, EH40 recommends that employers should seek specialist advice. Such mixtures would need to be assessed on a case-by-case basis.

Additive substances

Where additive behaviour is expected e.g. where there is reason to believe that the effects of the constituents are additive, and where the exposure limits are based on the same health effects, EH40 recommends the use of the hazard index method to determine whether the measured airborne concentrations should be regarded as having exceeded the occupational exposure limit. Hazard quotients for each substance are derived and summed together using the following formula:

$$\frac{C_1}{L_1} + \frac{C_2}{L_2} + \frac{C_3}{L_3} \ldots < 1$$

where:

C_1, C_2, etc = the time weighted average (TWA) concentrations of constituents in the air

L_1, L_2, etc = the corresponding occupational exposure limits.

EH40 stresses that L_1, L_2, etc should relate to the same reference period i.e. be either 8-hour TWAs or short-term exposure limits (STELs).

Independent substances

Where no synergistic or additive effects are anticipated, for example if the chemicals in the mixture act at separate sites and produce different effects then EH40 indicates that control of exposure is adequate providing the individual OELs have been adhered to. No specific formula is provided in EH40 but the concept can be expressed thus:

$$\frac{C_1}{L_1} \leq 1; \quad \frac{C_2}{L_2} \leq 1; \quad \frac{C_3}{L_3} \leq 1; \text{ etc};$$

where:

C_1, C_2, etc = the measured airborne concentration

L_1, L_2, etc = the OEL for the substance.

Classification and labelling of preparations (EU preparations directive)

HSE uses the procedures laid down in the Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 to determine the appropriate classification and labelling for preparations that are supplied onto the EU market (Statutory Instrument, 2002). These procedures are based on the rules for hazard classification and labelling of substances and preparations laid down in the Dangerous

Substances Directive (67/548/EEC) and the Dangerous Preparations Directive (99/45/EC) and adaptations to technical progress of these directives. The approaches recommended in these directives are endpoint specific and are based on the assumption that for each endpoint, chemicals will either act in an additive manner or an independent manner. No account is taken of the potential for substances to show synergistic behaviour. For each endpoint, concentration limits are used to determine whether individual components will contribute to the overall classification of the preparation. In some cases, chemical specific concentration limits will be available. For most substances generic concentration limits will apply. For example, if a liquid preparation contained a substance classified as a skin sensitiser at a concentration of 1% or more, the preparation would also be classified as a skin sensitiser.

Endpoints for which additive behaviour is assumed are acute toxicity, narcotic effects and corrosivity/irritation. The calculation to determine classification is based on the hazard index approach. As an illustrative example, to determine whether a preparation containing very toxic, toxic and harmful substances should be classified as harmful, the following calculation would be performed:

$$\sum \left(\frac{P_{T+}}{L_{Xn}} + \frac{P_T}{L_{Xn}} + \frac{P_{Xn}}{L_{Xn}} \right) \geq 1$$

where:

P_{T+} = the percentage by weight or by volume of each very toxic substance in the preparation

P_T = the percentage by weight or by volume of each toxic substance in the preparation

P_{Xn} = the percentage by weight or by volume of each harmful substance in the preparation

L_{Xn} = the respective harmful concentration limit specified for each very toxic, toxic or harmful substance, expressed as percentage by weight or by volume.

If the sum exceeds 1, the preparation is classified as harmful. Separate calculations would be performed to determine whether the preparation should be classified as toxic or very toxic using the respective toxic or very toxic concentration limits as the denominators.

For all other endpoints, i.e. non-lethal irreversible effects after single exposure, severe effects after repeated exposure, skin sensitisation and asthma, cancer, genotoxicity and reproductive toxicity, the assumption is made that the chemicals in the mixture will act in an independent manner. The preparation is only classified for these endpoints if any individual component exceeds the generic or substance specific concentration limit for a specific endpoint.

This approach is also used to determine whether impurities present in a chemical would require that the classification and labelling for the chemical reflect the properties of the impurity. For example, if a chemical contains a mutagenic impurity, is the concentration of the impurity such that the chemical itself should be classified as a mutagen? It is also used as an administrative solution to the problem of determining the correct classification for some complex substances, such as complex coal- and oil-derived substances that are classified on the basis of the content of specified marker substances.

References

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Statutory Instrument (2002) *The Chemicals (Hazard Information and Packaging for Supply) Regulations 2002* (ISBN: 0-1104-2419-0); Avail [April 2004] at: http://www.legislation.hmso.gov.uk/si/si2002/20021689.htm

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Annex D: Pesticides Safety Directorate – current approach to mixtures

Introduction

Current assessments of pesticides concentrate on the acceptability of an individual active substance in isolation. The potential for the toxicity of an active substance to be altered by other components of the formulation or other residues on a crop is not investigated in as much detail as the properties of the individual active substance.

The acute toxicity of the formulation as sold must be addressed, primarily for classification and labelling. If data show the toxicity of the formulation to be significantly different from that expected based on its individual constituents this will be investigated further. It is unusual for repeat dose oral studies to be performed on a formulation therefore any sub-acute interactions within a formulation are unlikely to be identified.

Formulations may contain more than one active substance. When assessing formulations containing two or more active substances with a common mechanism of action or similar toxicity profiles, the potential for interaction is considered.

There are no generic restrictions on a grower applying sequentially two or more compounds with a similar mechanism of action to the same crop. Similarly, there are few restrictions on applying two pesticides concurrently. If a pesticide formulation specifically indicates that it must be used in combination with another formulation, a limited assessment of the combination will be performed. The only specific restrictions concern the mixing of two or more anticholinesterase compounds in the same spray tank. Therefore it is likely that, at the time of consumption, a crop could contain residues of several pesticides. There is also the potential to have simultaneous exposure to more than one pesticide if a meal consists of a number of food items that have been treated with pesticides

Current UK and international assessments of pesticides do not routinely consider the risk to

consumers from the potential for interaction of residues of different pesticides or to operators, re-entry workers and bystanders from the potential effects of simultaneous or sequential exposure to different active substances. Cases where combined assessments are performed are described below.

Formulation acute toxicity / Classification and labelling

These can be addressed either by the pesticide company performing toxicity tests or by adopting the 'calculation method' described in the Dangerous Preparations Directive (99/45/EEC).

If the results of any studies show that the acute toxicity is clearly higher than would be predicted from information on the individual components, additional investigations will be performed. For example, penetration through the skin could be shown to be enhanced by the presence of solvents in the formulation and this will be taken into account in the operator exposure assessment.

Formulation assessments[1]

i. Formulations containing only one active substance

Many of the non-active chemicals in pesticide products (termed co-formulants) are basic industrial chemicals and have not been well studied toxicologically and little information is available on their mechanism of toxicity. They are not designed to be biologically active and it is assumed that they will not act with dose additivity with the active substance.

[1] Full details are available at http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/CombinedToxicity20050408.pdf

ii. Formulations containing more than one active substance

When a formulated product contains two or more active substances there is a need to consider the potential for interaction in an additive manner that might impact on the consumer and operator/ worker/ bystander risk assessments. This concept has been part of Advisory Committee on Pesticides (ACP) considerations for a number of years[2]. The default assumptions are that active substances with a common mechanism of action or common target tissue will act with dose additivity. Active substances with different mechanisms of action or target tissues are considered to act with response additivity. A hazard index approach is adopted, with exposures being assessed by summing the proportion of its reference dose that each active substance contributes. This approach is adopted for all relevant exposure scenarios, i.e. acute and chronic intakes from residues on food; operators using the pesticide; workers that could come into contact with the treated crop; bystanders present at the time of application or living close to the treated crop. If the reference dose summation exceeds unity, additional investigations will be required. Approval will not be granted unless exposures can be shown to be acceptable.

iii. Products containing synergists, agonists, herbicide safeners or other components designed to alter the properties of active substances

Synergists, agonists and herbicide safeners are designed to be biologically active and modify the action of the active substance to enhance the effectiveness against the pest or to protect the crop. The default assumption in performing human health risk assessments on formulated products containing such biologically active compounds is that the effects induced in the pest or crop will also apply to human exposures.

The applicant must present data or a reasoned case to address the impact on the human health risk assessment of co-exposure to the active substance and the synergist / agonist / safener. In some cases a repeat dose toxicity study has been performed on the combination of active substance and synergist, agonist or safener.

Surveillance of residues in foodstuffs

Surveillance work by the Working Party on Pesticide Residues (WPPR) and its successor the Pesticide Residues Committee (PRC) has shown the presence of multiple residues in a single sample. There is also the potential for exposure to more than one residue from different foods in the diet. This was one of the stimuli for the formation of the Working Group on the Risk Assessment of Mixtures of Pesticides (WIGRAMP). When such multiple residues are found, a risk assessment is performed using the approach described above, regarding additivity and summation using a hazard index approach.

Cholinesterase inhibiting pesticides

As a concluding part of the UK review of anti-cholinesterase pesticides (organophosphates and N-methyl carbamates), PSD is developing a methodology for the performance of a combined assessment of residues of anti-cholinesterase pesticides. The default assumptions are that the anti-cholinesterase compounds will act with dose additivity, as there is a common mechanism of action.

[2] Page 11 of the ACP report for 2000 assessment of triticonazole; Page 12 of the ACP report for 2001 assessment of flufenacet.

An initial exercise to test the probabilistic modelling program developed for this purpose was performed using a toxic equivalence factor approach. The reference compound was chlorpyrifos, chosen because it had an extensive, high quality database. A hazard index approach would have required much more data to work effectively with the modelling software.

The test was successful but as it used old residue data and food consumption data from the Netherlands that were already integrated into the model, the results will be of limited relevance to current UK exposures.

Work is ongoing to update the toxicity, consumption and residue databases used in the model to better reflect current UK data. When this is completed, a combined assessment of exposures to anti-cholinesterase compounds in the UK diet will be performed.

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Annex E: Veterinary Medicines Directorate – current approach to mixtures

Introduction

Veterinary Medicinal Products (VMPs) are manufactured to specific formulations, which normally contain a single "active substance", or in a few cases, two or three "active substances". These active substances are responsible for the "action" or "purpose" of the medicine, (i.e. what it is aiming to treat). In addition, there are several other "excipient substances" such as surfactants, stability agents, solubilising agents, flavourings and preservatives, which are required to formulate and manufacture the product.

Mixtures of veterinary medicinal products

Veterinarians, farmers, kennel workers, or pet owners, can be exposed to VMPs as a result of administration of the product and subsequent handling of the treated animal. In general a single product is administered and so mixtures of different VMPs in relation to user exposure is not considered to be a potential risk. In the more unusual event of one or more veterinary medicines being administered as the recommended treatment, then the potential user exposure and hazard of the VMPs would be part of the assessment procedure.

VMPs administered to food-producing animals may leave residues in meat, milk and dairy products, eggs, fish and honey. Some products, particularly ectoparasiticides (such as sheep dips) may contain active substances that are also used in pesticide formulations. All active substances used in food-producing species must have an EU maximum residue limit status[1].

During the MRL evaluation process, the use of the substance in other products, such as pesticides, is considered and the MRL is established to allow for this.

With respect to residues, an animal may, during the course of its life, be administered several VMPs, depending on conditions and indications that occur, resulting in a mixture of residues. However, it is not possible to make an assessment of this potential mixture because it is not possible to know what treatments an animal may require or how many different treatments it would receive or over what time period. Treatments that recommend two VMPs are rare, but potential consumer issues would be considered during assessment.

The evaluation of veterinary medicinal products

The evaluation of VMPs is in accordance with EU legislation[2]. Applications for VMP marketing authorisations (MA) are made in accordance with the Rules Governing Medicinal Products in the EU as given in the Notice to Applicants[3] and the benefit risk balance is evaluated before a MA can be granted. MRLs are established in accordance with EU legislation and guidance[4].

The assessment procedure is based on the formulation of each VMP and its active substance. A small number of marketing authorisations have been granted for VMPs that contain two active substances and these are considered in accordance with

EU guidance for "fixed combination products"[5] which addresses the potential interaction between the active substances.

[1] An entry into Annex I, II or III of Council Regulation 2377/90
[2] Directive 2001/82/EC as amended by Directive 2004/28/EC
[3] The Rules Governing Medicinal products in the EU. Notice To Applicants Vol 6 & Vol 8
[4] Council Regulation 2377/90
[5] CVMP Guideline on Pharmaceutical Fixed Combination Products [EMEA/CVMP/83804/2005]

Safety & residues data for fixed combination VMPs

For the evaluation of a fixed combination VMP, it is necessary to provide pharmacological data for the combination in order to demonstrate the mode of action and to investigate the possibility of interactions. It may also be necessary to provide toxicological data for the combination if there are interactions between the active substances and/or excipients or a possibility of masking toxicity. In all cases where there is a synergistic effect, more detailed toxicological data would be required.

User safety studies relating directly to effects on the person administering the product, or any other person exposed during or after treatment (e.g. children handling treated animals) such as skin and eye irritation, sensitisation and inhalation studies, should always be carried out with the final formulation, (i.e. the fixed combination formulation).

Environmental Impact Assessment is targeted at the effects of the combination product and if scientifically justified, data in accordance to VICH phase I and phase II guidelines may be provided for the individual substances only.

For food-producing animal VMPs, a withdrawal period must be established to ensure consumer safety. Residues depletion studies for foodstuffs (according to species) must be conducted with

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