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Cumulative risk assessment of pesticide residues in food

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This manuscript is dedicated to the memory of Prof. Robert (Bobby) Kroes, a scientist, colleague and human being of a nature that is rare indeed.

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ABSTRACT

There is increasing need to address the potential risks of combined exposures to multiple residues from pesticides in the diet. The available evidence suggests that the main concern is from dose addition of those compounds that act by the same mode of action. The possibility of synergy needs to be addressed on a case-by-case basis, where there is a biologically plausible hypothesis that it may occur at the levels of residues occurring in the diet.

Cumulative risk assessment is a resource-intense activity and hence a tiered approach to both toxicological evaluation and intake estimation is recommended, and the European Food Safety Authority (EFSA) has recently published such a proposal. Where assessments have already been undertaken by some other authority, full advantage should be taken of these, subject of course to considerations of quality and relevance.

Inclusion of compounds in a cumulative assessment group (CAG) should be based on defined criteria, which allow for refinement in a tiered approach. These criteria should include chemical structure, mechanism of pesticidal action, target organ and toxic mode of action.

A number of methods are available for cumulating toxicity. These are all inter-related, but some are mathematically more complex than others. The most useful methods, in increasing levels of complexity and refinement, are the hazard index, the reference point index, the Relative Potency Factor method and physiologically based toxicokinetic modelling, although this last method would only be considered should a highly refined assessment be necessary.

Four possible exposure scenarios are of relevance for cumulative risk assessment, acute and chronic exposure in the context of maximum residue level (MRL)-setting, and in relation to exposures from the actual use patterns, respectively. Each can be addressed either deterministically or probabilistically. Strategies for dealing with residues below the limit of detection, limit of quantification or limit of reporting need to be agreed.

A number of probabilistic models are available, but some of there are geographically constrained due to the underlying datasets used in their construction. Guidance on probabilistic modelling needs to be finalised.

Cumulative risk assessments have been performed in a number of countries, on organophosphate insecticides alone (USA) or together with carbamates (UK, DK, NL), triazines, chloroacetanilides, carbamates alone (USA), and all pesticides (DE).

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; BMD, benchmark dose; CAG, cumulative assessment group; CMG, common mechanism group; CRI, cumulative risk index; CSAF, chemical-specific adjustment factor; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; FQPA, Food Quality Protection Act; GAP, good agricultural practices; HI, hazard index; HQ, hazard quotient; IESTI, International Estimate of Short Term Intake; LOD, limit of detection; LOQ, limit of quantification; LOR, limit of reporting; LP, large portion; MOA, mode of action; MOE, margin of exposure; MRL, maximum residue level; NOAEL, no observed adverse effect level; OP, organophosphate pesticide; PBTD, physiologically based toxicokinetics; PBTK, physiologically based toxicodynamics; PEF, potency equivalency factor; PPR Panel, EFSA Scientific Panel on Plant protection products and their Residues; RP, reference point (e.g. NOAEL); RPF, relative potency factor; RPI, relative potency index; RV, reference value (e.g. ADI); STMR, supervised trials median residue; TEF, toxic equivalency factor; UF, uncertainty factor.

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All identifiable assumptions and uncertainties should be tabulated and evaluated, at least qualitatively. Those likely to have a major impact on the outcome of the assessment should be examined quantitatively. In cumulative risk assessment, it is necessary, as in other risk assessments, for risk managers to consider what level of risk would be considered "acceptable", for example what percentile of the population should be below the reference value.
Criteria for prioritising CAGs for cumulative risk assessment include frequency of detection in monitoring programmes, high usage, high exposure relative to the reference value, large number of compounds (e.g. five or more) in a group.

1. Introduction

The risk assessment of pesticide residues in food is currently performed on a compound-by-compound basis. If potential exposure of consumers is below the relevant health-based guidance value (ARfD and ADI, for acute and chronic exposure, respectively), the use of that pesticide in crop protection is considered acceptable. However, frequently, consumers are exposed to more than one pesticide residue at the same time, or within a short space of time. In the European Union, in 53–64% of food samples, pesticide residues were not detectable, 32–42% contained detectable residues, which were below the maximum residue levels (MRL) and 3.0–5.5% contained levels above the MRL, respectively. Of particular note, 14–23% of the samples with detectable residues contained more than one active ingredient (CEC, 2007). As the analytical samples in monitoring programmes are composite samples, containing multiple units, it cannot be excluded that all of the detectable residues in a sample were present in the same unit. Combined exposures to different pesticide residues could therefore occur as a consequence of intake from a single food item containing multiple residues or from several food items each containing one or more residues. The consequence of such combined exposure has raised concerns amongst both consumers and regulators. This was recognised in the US Food Quality Protection Act (FQPA) of 1996 and more recently in Europe in Regulation (EC) No. 396/2005 on MRLs. This emphasises the importance "to carry out further work to develop a methodology to take into account cumulative and synergistic effects of pesticides". Indeed, at the time of the adoption of the Regulation, the European Parliament emphasised the need to develop and utilise such methodology as soon as possible in estimating the acceptability of MRLs.

There is currently no internationally agreed methodology to assess risks from combined exposures to pesticide residues. However, a number of international activities are ongoing in this or related areas. These include the development of a framework for cumulative risk assessment by the International Programme on Chemical Safety (WHO, 2008), the adoption of an Opinion by EFSA's Scientific Panel on Plant Protection products and their Residues (PPR) on the evaluation of existing methodologies and the identification of new approaches for cumulative risks assessment of pesticide residues (EFSA, 2007a, 2008), the development of approaches to assess the combined toxic effects of multiple chemical exposures by the Norwegian Scientific Committee for Food Safety (VKM, 2008), the development of a framework approach to the risk assessment of chemical mixtures by ILSI Health and Environmental Sciences Institute and the human health risk assessment of mixtures by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). This manuscript takes into account these and other developments, such as those within the US EPA in the areas of cumulative risk assessment.

2. Sources of exposure to pesticide residues

Exposure to pesticides can occur via a number of pathways (e.g. food, drinking water, residential, occupational) and routes (oral, inhalation, dermal). Generally, each exposure pathway is the responsibility of a different department or agency within national governments or international bodies. Hence, assessment by each route is generally undertaken independently. However, it is the totality of exposure that determines risk, and this was recognised in the USA by the Food Quality Protection Act (FQPA) of 1996. This resulted in the requirement to include so-called aggregate exposure in the risk assessment of pesticides, i.e. exposure through multiple routes, and multiple pathways (WHO, 2007). In Europe, Regulation (EC) No. 396/2005 requires similar considerations in the future.

The contribution of a given route or pathway to overall exposure will depend on the pesticide. For example, in a US evaluation of *N*-methylcarbamates (EPA, 2007), exposures via food predominated, followed by residential exposure and then drinking water. In contrast, with organophosphates (EPA, 2006a) residential exposure predominated, whilst for triazines (EPA, 2006c), drinking water predominated.

Under Council Directive 98/83/EC in Europe, the legally permitted limit for an individual pesticide in drinking water is 0.1 μg/L, whilst the total of all pesticides must not exceed 0.5 μg/L. These limits are based on analytical, not toxicological, considerations. Hence, even if several pesticides sharing a mode of action were present in drinking water, this should not be of toxicological concern.

Other uses of a pesticide, for example as a human or veterinary medicine could contribute to overall exposure. Ideally, all such sources would be taken into account in the assessment. However, in practice, relevant information on exposure by a number of these pathways is not available. Further work is required to develop methods to aggregate exposure via multiple pathways and sources. The US EPA have developed guidance that could the form the basis of this (EPA, 2001).

3. Types of combined action

Pragmatically, the combined toxicological effects of two or more compounds can take one of three forms: independent, dose addition or interaction (Wilkinson et al., 2000; Feron and Groten, 2002).

Independent action, also known as response-addition, simple dissimilar action, simple independent action or independent joint action, occurs when the toxicological effects of the individual compounds in a mixture are a consequence of separate mechanisms/modes of action. In such circumstances, the nature and sites of toxicity may differ amongst the chemicals. The effects of the chemicals are the same as if they were in the absence of the others, i.e. they do not influence each other's action. Hence, the effects of such a combination will be the sum of the effects of the component compounds when given alone, i.e. response or effect addition.

Dose-addition, also referred to as simple similar action, similar joint action or relative dose-addition, occurs when the individual compounds in a mixture share the same mechanism/mode of action for their the toxicological effects, and they differ only in their potencies. The molecular basis for this is receptor occupancy/ligand binding site theory. This states that an allosteric binding site, such as a receptor, cannot distinguish between occupancy by different agonists (if they are full agonists). Occupancy is determined by affinity, so that for any given agonist, 50% occupancy is determined by the respective K_D, the dissociation constant (=$1/K_a$, the affinity constant). Fractional occupancy is determined by the ratio $[D]/([D]+K_D)$, where [D] is the concentration of ligand. Hence, the extent to which agonists of different potencies will occupy the receptor, will be determined only by their respective concentration and K_D. In general, the magnitude of the biological response is proportional to receptor occupancy. Fractional biological response $E/E_{max} = [D]/([D]+K_D)$. Hence, the factional response of a mixture will be determined from the concentrations of each agonist, once adjusted for differences in potency. Whilst a simplification, this theory is consistent with the cumulative risk assessments performed to date.

Such compounds are said to belong to a "common mechanism group" (CMG), and dose-addition implies that the effects of exposure to a mixture of these compounds are equivalent to the effects of the sum of the potency-corrected doses of each individual compound. Although US EPA have used the term CMG, more properly this should be common mode of action (MOA) group, on the basis of the definition used (EPA, 1999). "Mode of action" refers to the major steps ("key events") leading to a toxicological effect produced by a compound whereas "mechanism of action" is a detailed explanation of the individual molecular events leading to the toxic effect (Boobis et al., 2006). Membership of a CMG by a compound implies that a common mode of action has been established for this compound. However, as part of the tiered assessment proposed below, compounds may be considered for cumulative assessment on the basis of less stringent considerations. Hence, it is proposed that such be identified as cumulative assessment groups (CAGs).

The term interaction includes all forms of joint action that depart from either dose or response addition. Hence, the combined effects of two or more chemicals is either greater (synergistic, potentiating, supra-additive) or less (antagonistic, inhibitive, sub-additive, infra-additive) than that predicted on the basis of dose-addition (if the chemicals belong to a CMG) or response-addition (if they do not belong to a CMG).

4. Types of combined action relevant to the risk assessment of pesticide residues in food

In theory, exposure to a combination of pesticide residues could result in any of the types of combined effect described above. However, in considering the joint actions most likely, it is important to consider possible exposure levels. Pesticides require authorisation prior to use, and this includes consideration of appropriate risk management options to ensure that the levels of pesticide residues in food to which consumers are exposed are within acceptable limits. These limits are designed to ensure that maximum exposure is well below the no observed adverse effect level (NOAEL) for the critical toxicological effect upon which the risk assessment is based. Extensive monitoring studies throughout Europe and in many other countries have shown that this is being achieved, subject to use as approved. The annual EU-wide monitoring reports, which are currently available up to 2005, show that 5% or less of samples contained residues above the maximum residue limit (MRL), and even in those instances, estimated intakes were normally below the relevant reference value (RV), i.e. ADI or ARfD as appropriate. Hence, maximum exposure of a consumer to residues of an individual pesticide, providing this was from an approved use, would be at least one order of magnitude below the NOAEL. When exposure is to multiple pesticides, each would be at a level below its respective NOAEL. When compounds all act by different modes of action, in the absence of any interaction, their joint effect would be response or effect addition. Hence, in the absence of any response or effect of the individual compounds, there would be no toxicologically/biologically significant effect of the combined exposure. This is because response addition of a mixture is the sum of the responses of the individual compounds present. If each produces no response, the mixture will produce no response. Thus response addition is not a priority for consideration in the cumulative risk assessment of resides in food.

When individual compounds share a mode of action, combined exposures will act jointly by dose addition. Hence, in theory, even when individual compounds are present at levels below their respective NOAELs, combined exposures could result in a measurable effect, due to the summed doses of the individual pesticides. Where compounds obey dose addition, the magnitude of the response can be predicted by expressing the dose of each compound normalised on the basis of potency to a single reference compound. Where the molecular target is identifiable, for example the estrogen receptor alpha or the aryl hydrocarbon receptor, experimental studies have borne this out. However, there are persistent concerns that some pesticide combinations will act synergistically, that is their join action will be greater than that predicted on the basis of response addition (when they have different modes of action) or dose addition (when they share a mode of action). This topic has been reviewed extensively by a number of groups over the last few years (e.g. EFSA PPR (EFSA, 2008), IPCS (WHO, 2008), UK WIGRAMP (COT, 2002)). In all cases, it was concluded that the available evidence did not provide support for significant deviation from dose or response additivity at low levels of exposure, i.e. when all compounds were present at or below their respective NOAELs. However, on rare occasions there was some evidence for synergy, usually at doses above the NOAELs of one or more of the respective compounds, which was, in most cases, explicable on the basis of the established toxicokinetics or toxicodynamics of the compounds. Hence, the available data, whilst not excluding the possibility of synergy in some cases when compounds are present at doses below their respective NOAELs, do support the conclusion that such interactions are rare and much less likely to occur at these doses than at levels of exposure above their respective NOAELs.

As a consequence, for pesticide residues that have different modes of action (see below) the default assumption for cumulative risk assessment is that the combined exposure will not have any toxicological effect in consumers, given that the individual exposures do not exceed the respective RVs. For compounds that share a mode of action, where there is the possibility of combined exposure the default assumption is that they will exhibit dose addition. Where there is a plausible biological hypothesis, the possibility of synergy should be considered, on a case-by-case basis. An example would be where a compound was a potent inhibitor (NOEL for inhibition below toxicological NOAEL) of the metabolism of another, e.g. between isomalathion and malathion (Aldridge et al., 1979; Baker et al., 1978).

5. Exposure scenarios for consideration in cumulative risk assessment

There are four main exposure scenarios that could be considered within the context of a cumulative risk assessment:

MRL-setting (i.e. a theoretical exposure estimated on the basis that the residue of a compound under evaluation is present at its MRL (maximum residue limit)). Exposure could be either acute (1) or chronic (2).

Assessment of actual exposure (i.e. that which occurs from usage of the pesticide in practice). Again, exposure could be either acute (3) or chronic (4).

Whilst other exposure scenarios could be considered, for example within the context of use authorization, these would result in lower predicted exposures than those on the basis of MRL-setting. Hence, the four scenarios proposed would include an estimate of the highest (MRL-setting) and lowest (actual usage) exposure.

The acute MRL-setting scenario (1) addresses the situation where one commodity is consumed with a residue level at its MRL, and there is cumulative exposure to this pesticide in other foods and to others with the same mode of action. This allows a conclusion on whether the proposed MRL is acceptable against the background of existing uses of approved pesticides with the same mode of action (imported produce also has to be considered, see below). The chronic MRL-setting scenario (2) enables consideration of the risk from exposure to a pesticide present in one commodity with a residue at its MRL, under different assumptions, for example daily, once every year, against a background of cumulative exposure to this pesticide in other foods and to others with the same mode of action. Hence, the MRL-setting scenarios are relevant in determining whether cumulative exposure of consumers to residues from all of the pesticides in a CAG is at an acceptable level when a proposed legal limit (MRL) is set for a specific pesticide/commodity combination.

Actual exposure scenarios, either acute (3) or chronic (4), require probabilistic assessment of exposure on the basis of the distributions of the residue levels of each pesticide in the CAG, present in food as eaten. Such scenarios are of value in determining whether cumulative exposure of consumers to residues from pesticides as actually used is at an acceptable level.

6. Identification of a cumulative assessment group (CAG)

Compounds acting at the same molecular target, e.g. organophosphates on acetylcholinesterase, dioxins on the aryl hydrocarbon receptor, belong to the same CMG and can be readily identified as such. However, the information required to establish membership of a common mechanism group is substantial, and only a relatively few CMGs have been determined to date. Nevertheless, failure to consider compounds that exhibit dose addition together might not be adequately protective of consumers. Hence, one approach would be to use a tiered strategy in deciding which compounds should be considered members of a cumulative assessment group. In the lower tiers, relatively broad assumptions would be used to identify members of a CAG, for example common target organ, whilst in the higher tiers more refined criteria, such as consideration of chemical specific data on mode of action, would be used. As discussed below, a tiered approach would also be appropriate for exposure assessment.

Several criteria have been proposed for identification of membership of a CMG (EPA, 1999, 2002a,b; ILSI, 1999). This is based on a step-wise approach to constructing a CMG, as opposed to performing a combined risk assessment, as discussed later, on a CAG. According to the approach adopted by EPA, preliminary identification should be based on one or more of the following criteria:

(a) Chemical structure (known or potential toxicophores, based on core molecular structure, specific functional groups, or their metabolic precursors). A number of software approaches, based for example on substructure databases for toxicophores, quantitative structure activity relationships, knowledge-based systems, are available for this purpose.
(b) Mechanism of pesticidal action. The mechanism of mammalian toxicity of a number pesticides is similar to that responsible for their activity against target pests, e.g. organophosphates.
(c) General mode/mechanism of mammalian toxicity. This is based on a relatively broad consideration of mode of action, and not a detailed evaluation of key events, as discussed below.
(d) A specific toxic effect. It is possible that similar toxic effects are caused by structurally unrelated compounds via the same MOA. It would be important to consider such compounds in the same CMG, as they would exhibit dose addition. However, non-specific effects such as changes in body weight, unless they are due to a specific mechanism, should not be used as a basis for membership of a CMG.

In this approach, a common group identified as above, is further refined by a number of steps. These start with definitive identification of those compounds that cause the same toxic effect, on the basis of both site and nature of toxicity. Compounds causing some other effect are excluded from further consideration in the combined assessment. The MOA for the toxic effect caused by each substance is then determined, by consideration of the key events involved. It is acknowledged that it is often not necessary or even possible to determine all of the specific biochemical events leading to toxicity, but sufficient information on common key events should be available to enable a conclusion to be reached as to whether compounds share a MOA. Those that do not are excluded from further consideration in the combined assessment.

Whilst full consideration of these criteria will result in the most robust grouping of compounds into a CMG for a combined risk assessment, this is a resource intense exercise and it may not be necessary or even possible for all possible CAGs. It may still be possible, and indeed sufficient, to group compounds for a combined assessment on the basis of less refined criteria, (e.g. target organ toxicity), even in the absence of detailed information. In the EFSA PPR Opinion (2008), such a group of compounds has been defined as a cumulative assessment group (CAG), to distinguish it from a group of compounds for which a common mode/mechanism of action has been rigorously established, as above, i.e. a common mechanism group (CMG). As a consequence, a CMG represents a CAG, but the reverse is not necessarily true.

In defining a CMG, members of which would be assumed to exhibit dose additivity, it is important to consider the fact that for some endpoints, such as those caused by certain endocrine disruptors, dose-additivity may occur even if they do not share a common molecular target (see Kortenkamp, 2007). Hence, the definition of a "common MOA" for such compounds needs to be modified. Thus, compounds impairing male sexual development via effects on steroid biosynthesis would be considered a separate CMG from those acting by antagonism of the androgen receptor, if a narrow definition of MOA were adopted. However, combinations of such compounds exhibit dose additivity (Gray et al., 2001; Hotchkiss et al., 2004). This is also true of estrogenic and estrogen-like compounds (Picard, 2003). Hence, in these instances, the criteria for identifying membership of a CMG should be based on induction of a common endocrine-related effect (e.g. altered ano-genital distance) (Kortenkamp, 2007).

7. Methods for cumulating the toxicity of compounds in the same cumulative assessment group

A number of methods have been developed for assessing the combined hazard of compounds that share a common mode of action (ILSI, 1999; US EPA, 1999, 2000a,b; Wilkinson et al., 2000; Groten et al., 2000; Feron and Groten, 2002; DVFA, 2003; Jonker et al., 2004; Van den Berg et al., 2006). The combined hazard of the individual compounds in a CAG can be determined using either the respective reference points (RPs, also known as points of departure or PODs) or the reference values (RVs, health based guidance values, e.g. ADI, ARfD). When RPs are used, uncertainty factors are applied to the estimate of combined hazard, whereas when RVs are used, uncertainty factors are applied for the individual compounds, before obtaining the estimate of combined hazard.

The method of dose addition after normalising the potency of each compound to that of an index compound was first developed for dioxins, when it was termed the toxic equivalency factor (TEF) method (see Haws et al., 2006; Van den Berg et al., 2006). It was later generalised to other groups of chemicals sharing a MOA, including certain classes of pesticide. This method is known as the relative potency factor (RPF) method (EPA, 2000a) or the potency equivalency factor (PEF) method. In this method, risk characterisation occurs after the toxicological evaluation has taken place.

Each of the methods has its own advantages and disadvantages. None is ideal.

7.1. Hazard index and adjusted hazard index

The hazard index (HI) is the sum of the hazard quotients (HQ), i.e. the ratio between exposure and the respective RV for each compound in the CAG.

$$\mathrm{HI} = \frac{\mathrm{Exp}_1}{\mathrm{RV}_1} + \frac{\mathrm{Exp}_2}{\mathrm{RV}_2} + \frac{\mathrm{Exp}_3}{\mathrm{RV}_3} + \cdots$$

When the basis of the RV of a compound is an effect other than the common toxic effect, the uncertainty factor used in the derivation of the RV may include adjustments unrelated to the common toxic effect. In this case, the HQ can be refined in a higher tier assessment, by calculating a putative RV for the common toxic effect and adjusting the HQ, accordingly. An adjusted HI (aHI) is then calculated.

When the HI is less than 1, the combined risk from exposure to the compounds in the CAG is considered acceptable. Extrapolation of the no-effect level for the toxic effect in animals (when the RV is based on animal data) to an exposure considered to be without appreciable risk to humans is taken into account in determining the individual RVs.

7.2. Cumulative risk index (CRI)

The risk index (RI) of a compound is the MOE (RP/exposure) divided by the UF for that compound, which is equivalent to the respective RV (when this is based on the same endpoint as the MOE) divided by the estimated or measured exposure to the compound. Hence, it is the reciprocal of the HQ. Thus,

$$\mathrm{RI} = \frac{\mathrm{RP}}{\mathrm{Exposure} \times \mathrm{UF}} = \frac{\mathrm{RV}}{\mathrm{Exposure}} = \frac{1}{\mathrm{HQ}}$$

The CRI is the reciprocal of the sum of the reciprocals of the RIs of the individual compounds in the CAG. As this is equivalent to the reciprocal of the sum of the HQs, the CRI is the reciprocal of the HI.

$$\mathrm{CRI} = \frac{1}{\mathrm{Exp}_1/\mathrm{RV}_1 + \mathrm{Exp}_2/\mathrm{RV}_2 + \mathrm{Exp}_3/\mathrm{RV}_3 + \cdots} = \frac{1}{\mathrm{HQ}_1 + \mathrm{HQ}_2 + \mathrm{HQ}_3 + \cdots} = \frac{1}{\mathrm{HI}}$$

When the CRI is greater than 1, the combined risk from exposure to the compounds in the CAG is considered acceptable. If the RV is used in calculating the CRI, similar considerations with respect to the toxicological basis of deriving the HQ apply to the CRI. Extrapolation of the no-effect level for the toxic effect in animals (when the RV is used and it is based on animal data) to an exposure considered to be without appreciable risk to humans is taken into account in determining the individual RVs. If the RP is used, such extrapolation is taken into account by the choice of the respective UFs for the individual compounds.

7.3. Reference point index (RPI)

The reference point index (RPI) is determined by summing the exposure to each compound in the CAG expressed as a fraction of its respective RP for the common toxic effect (e.g. as RP, the dose that causes a 10% effect, BMD10; or the NOAEL, could be used).

$$\mathrm{RPI} = \frac{\mathrm{Exp}_1}{\mathrm{RP}_1} + \frac{\mathrm{Exp}_2}{\mathrm{RP}_2} + \frac{\mathrm{Exp}_3}{\mathrm{RP}_3} + \cdots$$

The RPI is then multiplied by a group uncertainty factor, usually 100. If the RPI is less than 1, the combined risk from exposure to the compounds in the CAG is considered acceptable.

In this method, extrapolation of the no-effect level for the toxic effect in animals (when the RP is obtained from animal data) to an exposure considered to be without appreciable risk to humans is taken into account using a common uncertainty factor applied to the combined exposure.

7.4. Combined margin of exposure (MOE_T)

The margin of exposure (MOE) is the ratio of the RP for a compound to the estimated or measured level of exposure to that compound in humans. Hence, it is the reciprocal of the RPI. For example, if the BMD10 is used as RP, the MOE = BMD10/exposure. The combined MOE is designated the $\mathrm{MOE_T}$, and is determined by taking the reciprocal of the sum of the reciprocals of the individual MOEs.

$$\mathrm{MOE_T} = \frac{1}{(1/\mathrm{MOE}_1) + (1/\mathrm{MOE}_2) + (1/\mathrm{MOE}_3) + \cdots}$$

When the $\mathrm{MOE_T}$ is greater than 100, or whatever other value is considered appropriate by the risk manager, the combined risk from exposure to the compounds in the CAG is considered acceptable.

In this method, extrapolation of the no-effect level for the toxic effect in animals (when the RP is obtained from animal data) to an exposure considered to be without appreciable risk to humans is taken into account by agreeing an acceptable combined margin of exposure (e.g. 100).

7.5. Toxic equivalency factor (TEF)/potency equivalency factor (PEF)/relative potency factor (RPF) methods

The TEF method was developed initially for dioxins and other Ah receptor agonists (see Haws et al., 2006; Van den Berg et al., 2006), whereas the PEF or RPF is a more generalised method that has been used for compounds such as polycyclic aromatic hydrocarbons

(Pufulete et al., 2004) and certain pesticides (e.g. organophosphorus compounds) (EPA, 2000a). Both these methods require the identification of a so-called "index compound" (IC), such as 2,3,7,8-TCDD for dioxins and dioxin-like PCBs, and the potencies of all chemicals of the group are normalized to a single potency scale, that of the IC. Potencies are normally derived from the respective dose response curves, using the same benchmark response (e.g. 10%) for each compound. However, is also possible to use the NOAELs, and this could serve in a lower tier assessment. As an example, if the BMD10 for a compound is 10-fold greater than that of the index compound, the relative potency factor for the compound would be 0.1 and exposures to the compound would be corrected by a factor of 0.1 to convert them to IC equivalents. This is because it would require 10 times more of the compound to produce the same effect as the index compound.

The activity of the CAG is then determined as the sum of the IC equivalent (i.e. potency-normalised) doses to provide a total exposure expressed as IC equivalents. This total equivalent exposure is compared to the RV of the IC. If lower than the RV of the IC, the combined risk from exposure to the compounds in the CAG is considered acceptable. An alternatively approach would be to determine the MOE of the total equivalent exposure from the CAG relative to the RP of the IC. Any UFs required, other than the "standard" default values, would be applied to the individual RPFs before calculating the MOE. Extrapolation of the no-effect level for the toxic effect in animals (when the RV is based on animal data) to an exposure considered to be without appreciable risk to humans is taken into account in determining the individual RVs.

8. Advantages and disadvantages of methods for cumulating the toxicity for compounds in a CAG

It is envisaged that most cumulative risk assessments of pesticide residues will be based on existing data, for example that submitted as part of the normal dossier, and that no new data will be generated specifically for this purpose. Hence, the discussion that follows is based on this assumption.

All of the methods utilise the same dataset and similar concepts in the calculations. They differ only in the way in which the outcome is expressed. Indeed, some are related arithmetically to others, according to a simple transformation. Hence, the CRI is the reciprocal of the HI and the MOE_T is reciprocally related to the RPI, respectively. Whereas the HI and CRI are based on the existing RVs, the MOE_T and the RPI are based on the RPs. RVs are readily easily obtained, whereas RPs require access to the original data. In addition, the choice of UF has already been made in deriving an RV whereas when starting with the RP, it will be necessary to decide upon an appropriate UF. The RPF method is somewhat different, in that all of the compounds in a CAG are normalised to an IC, which requires consideration of the individual dose–response curves. Extrapolation to acceptable human exposure is on the basis of the IC. Unlike in the other methods, exposure considerations take place towards the end of the process.

All of the methods are sensitive to the choice of toxicological endpoint used to cumulate toxicity. Ideally, these would be derived in the same species using the same study design. In practice, however, they may be derived in different species and/or with different study designs, e.g. duration of treatment, target tissue for determining common biological response. Different uncertainty factors may have been used to derive RVs, or the RP may have been obtained using different methods (e.g. BMD10, BMDL10, NOAEL). These factors all contribute to the uncertainty in the overall evaluation and should be clearly stated in the report of the assessment (see below).

8.1. Hazard index (HI) and cumulative risk index (CRI)

The advantage of the HI is that it is based directly on the RV, which is a well-understood estimate of the acceptable hazard to consumers. The RV (particularly the ADI) has been used in risk assessment for some time. It provides a relatively transparent, understandable means of assessing risk, and has the advantage that individual compounds will already have been approved on the basis of all necessary assumptions, if the RV is used unmodified. This allows ready translation of the relative risk of the CAG compared to that of the individual compounds comprising the group. These indices can be (relatively) rapid and simple to calculate, as the individual RVs should be readily available. It is also possible to accommodate chemical-specific adjustment factors (CSAFs), as these would have been used as appropriate in establishing the RVs.

Whilst these indices provide a rapid and relatively simple approach in lower tier assessments, among possible disadvantages is that RVs already incorporate uncertainty factors (UFs). These may include factors in addition to the conventional inter-species and intra-species factors, based on policy (e.g. additional UF for children as required in US or for severity of effect) or scientific (e.g. for incompleteness of the database unrelated to the common toxic effect) considerations. In such cases, the RV may no longer provide a true measure of relative toxicological potency of the respective compounds. A further potential disadvantage is that the toxicological basis for the RV may not be the common toxic effect upon which the CAG is based. Hence, it would be necessary to determine the toxicological basis for the RV for each compound and to calculate a putative RV based on the common effect as necessary. This would clearly require appreciably more effort than simply accepting the RVs as reported. However this might be required only if refinement for a higher tier assessment is considered necessary.

The CRI is not as transparent and is more difficult to understand than the HI. It also involves a more complex calculation than the HI. Hence, as similar considerations apply to this method as to the HI, it has no inherent advantages over the HI as an initial screening method.

As discussed above, when the endpoint for a compound upon which its RV is based does not serve as the common endpoint for a CMG, refinement in higher tier assessments may include deriving a putative RV based on the common endpoint. Even though it is likely that this RV will be greater than the "true" RV, the compound should be included in the CAG. This is because cumulative exposure to this compound with the others in the CAG may result in a combined exposure above that which is considered acceptable.

8.2. Reference point index (RPI) and combined margin of exposure (MOE_T)

These methods have the advantage that they sum exposures to the different compounds in a CAG in relation to their relative potencies, expressed as the RP. Mathematically, they are closely related to the RPF approach, although implementation is somewhat different. Use of the RP means that calculations are based directly on potency with no assumptions necessary about the appropriateness of the UF. In addition, as the RP will be specific to a toxicological endpoint, the appropriateness of the endpoint for the CAG is readily apparent. The RPI is probably more intuitive and is mathematically simpler than the MOE_T, and indeed than most of the other methods. Normally, a single UF is applied as the last step in the process, to the entire group. This is a potential limitation of these indices, as it is not possible to apply CSAFs, or other specific adjustments such as for the use of human data (default UF of 10 rather than 100), unless this is done for each compound, prior to calculating the cumulative index. In this case, the individual RPs are adjusted

as appropriate prior to entry into the calculation of the cumulative index. The group UF is then based on residual uncertainty, not addressed by the use of compound specific factors. Thus, if CSAFs are used to adjust the RP for one or more of the compounds, the default for this component of uncertainty would need to be applied to the remaining compounds, prior to calculating the cumulative index. Ideally, study design should be the same for all compounds.

8.3. Relative potency factor (RPF/PEF)

The RPF approach is transparent, and relatively easy to understand because potency correction is separated from considerations of exposure. As a consequence, it provides an effective means for standardising the dose metrics for the toxicity of the different compounds in a CAG. As RPFs are potency factors, when they are based on a common benchmark response, e.g. 10% when the BMD10 or BMDL10 is used, in theory they should be applicable over the entire dose range and hence could be used potentially to estimate risks posed by exposure to the members of a CAG at or above the RV of the index compound. This would require the assumption that the dose–response curves were parallel.

A potential limitation of the RPF is the considerable reliance it places on the quality of the toxicology database of the index compound. Choice of IC would normally be from amongst the compounds in a CAG with a toxicological database such that it provides the least uncertainty. Uncertainty in the data for the IC would translate into uncertainty about the adequacy of the margin of combined exposure to the compounds in a CAG. Clear criteria need to be established for selection of index compounds.

9. The derivation of RPFs and the application of the method is quite demanding of resources

Although each of the above approaches utilises the same dataset, the mathematical approaches differ, However, as discussed above, in general these are all interconvertible, and Wilkinson et al. (2000) have shown that, when the evaluation of all of the compounds in a CAG is based on the same toxicological endpoint and study design (duration of dosing, test species, type of RP, e.g. BMD10), and the value of the UF is the same for all of the compounds, the outcome of the assessment is the same regardless of the method used. The corollary is that, as the type and quality of toxicity data available are likely to vary from one chemical to another in a CAG, the choice of method may have some impact on the outcome of the assessment.

In choosing datasets for calculating a cumulative index, it is important to consider possible differences in the time-course of the common effect when choosing the RV or the RP. For example, when performing a cumulative acute assessment, the use of estimated short term intake to compare with the relevant RV, the ARfD, may underestimate the risk from compounds with a long half-life (kinetics) or that produce a persistent effect (dynamics). In the first instance, there will be significant carry over of compound (or relevant metabolites) to the next day, whereas in the second instance the effect may persist until the following day (e.g. inhibition of acetylcholinesterase by an OP). In either case, exposure to another member of the CAG on the next day could result in cumulative exposure above that initially estimated. A case-by-case approach is necessary in such instances.

As indicated above, all of the methods are based on the assumption that the dose–response curves for the compounds in a CAG are parallel, which is not necessarily true (e.g. see Moser, 1995) and hence this is a source of uncertainty in the assessment. However, in the absence of specific information is not possible to determine whether this is likely, nor whether the consequence would be greater or less conservatism in the assessment, which will vary case by case. In addition, this will be the situation regardless of the RV used, i.e. NOAEL, BMD or some other RV. Whilst the dose–response curves may be not be parallel in the range of observable responses, the curves may relate to each other differently at levels of consumer exposure.

In contrast to the BMD(L), the NOAELs for the compounds in a CAG are likely to represent different response levels, as this will depend on dose-spacing. For one compound the NOAEL may be lower than the no adverse effect level (NAEL), i.e. biological threshold for the effect, because of appropriate choice of dose spacing, whereas for another compound, the NOAEL may be above the NAEL, because the response is not statistically significant at this dose, despite poor choice of dose spacing, because of the number of animals in each dose group. Clearly, the dose margin before producing a significant effect will vary for the two compounds. This is not such a problem when the BMD(L) is used as RV, as this represents a uniform response level for all of the compounds. The method for determining the BMD(L) takes into account the shape of the dose–response curve and, in the case of the BMDL, variation in the data (Filipsson et al., 2003).

10. Physiologically based toxicokinetics (PBTK) and toxiccodynamics (PBTD) and other higher tier approaches

Physiologically based approaches to modelling toxicokinetics and/or toxicodynamics may be of value in higher tier assessments. Whilst to date, most emphasis has been on toxicokinetics (PBTK), approaches are also available to model toxicodynamics (PBTD) and indeed to produce linked TK/TD models (El-Masri, 2007). PBTK enables differences in absorption and disposition to be taken into account (Jonker et al., 2004) and also allows the concentration of a compound to be estimated at its toxicological target site. In cumulative risk assessment, the concentrations of each compound in the CAG would need to be corrected for potency, for example by using RPFs. Such modelling is not restricted to dose/concentration addition, but can be used to explore other possible types of combined action.

The use of physiologically based modelling in risk assessment has been described in Teuschler et al. (2004), where combinations of disinfection by-products were assessed. Such approaches have also been described for the cumulative risk assessment of pesticides (Conolly et al., 2005; Lowit et al., 2004), but to date they have not been utilised in the regulatory assessments. Physiological modelling provides a highly refined methodology, in which it should be possible to reduce uncertainty, but it is resource intensive and requires specialised expertise. More advanced modelling approaches are also possible, but it is unlikely that they will see application in cumulative risk assessment of pesticide residues in food in the near future. In part, this is because the margins of exposure to pesticide residues, even as CAGs, are such that it is likely that consumer protection can be assured before such higher tier assessments are necessary.

Probabilistic approaches provide a more realistic, and less uncertain estimate of variability within the population to be determined. Unlike deterministic approaches, in which the population is represented by a single value (possibly with an estimate of uncertainty such as the 95% confidence interval), probabilistic approaches produce a distribution of values for the population or sub-populations. To date, such approaches have been applied mainly to exposure assessment (see below), but there is increasing interest in their use in hazard characterisation as well (Bosgra et al., 2005). Probabilistic approaches could provide refined, higher tier methods in cumulative risk assessment, but it is likely that considerable development work would be necessary before this were routinely possible, particularly in the area of hazard characterisation.

11. General consideration of cumulative exposure assessment

As discussed above, cumulative exposure assessments can be used to determine the risk both from patterns of actual use of pesticides and for MRL-setting. Based on the toxicological properties of the CAG, relevant exposures will either be acute, chronic or both. Whilst these are the timeframes normally considered in the risk assessments of individual pesticides, intermediate timeframes may also be relevant in a cumulative risk assessment, in part to avoid undue conservatism in the exposure estimates. The toxicological profile of the compounds in a CAG should be used as a guide to the choice of type of exposure assessment required.

In the actual exposure scenarios, residue levels from monitoring programmes should be used for all commodity/pesticide combinations in the assessment and the total population of interest should be considered, i.e. both consumers and non-consumers.

In the acute cumulative MRL-setting scenario, residues at the level of the MRL should be considered only for the commodity/pesticide combination for which the MRL is to be set. For all other commodity/pesticide combinations in the assessment, background residue levels (e.g. from monitoring programmes) should be used. In this scenario, only those who consume the commodity of interest should be considered.

In the chronic cumulative MRL-setting scenario, it is very likely that patterns of pesticide usage, and hence exposure, will change over a lifetime. Hence, as in the assessment of individual pesticides, assessment of chronic cumulative exposure represents only a snapshot in time. The uncertainty that potential changes in usage pattern over a lifetime will introduce into the assessment will be much greater for a CAG than for individual compounds. The starting assumption for the combined assessment needs to be defined, i.e. how often is exposure to a pesticide at the level of its MRL assumed to occur. In the assessment of individual pesticides, it is assumed that worst case exposure would occur when consumption is at the MRL (or supervised trials median residue (STMR)) for a lifetime. However, in a cumulative assessment where there are residues from multiple pesticides and multiple commodities, this is highly unrealistic, even if one were to assume exposure at the STMR for a single commodity/pesticide and combine this with background exposure for all other commodities/pesticides. However, it could serve as a lower tier assessment, although it many cases some refinement in higher tiers would be necessary.

Actual chronic cumulative exposure assessments should provide information on the risk over a lifetime from being exposed to multiple pesticides in a CAG and hence the acceptability of the individual members of the CAG. However, this would be applicable only for those pesticides for which there were monitoring data.

12. General requirements for residues data

Pesticide residue means any specified substance in food, agricultural commodities or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance (Codex, 2007). Ideally, a pesticide residue definition should include substances of toxicological concern for dietary intake estimations (exposure assessment) and should be suitable for monitoring compliance with good agricultural practices (GAP). However, it is not always possible to achieve both with the same residue definition. When different definitions are required, that for dietary risk assessment should include those metabolites and degradation products of toxicological concern whereas that for monitoring for MRL compliance should be chemically simple (i.e. indicator molecule) so that routine monitoring can be performed at reasonable cost (FAO, 2002). Residue definitions (particularly for risk assessment) can change over time, as new information on the toxicological relevance of metabolites and degradation products becomes available.

For cumulative risk assessment, only those compounds (parent, metabolites degradation products) producing the common effect serving as the basis of a CAG are of concern.

Ideally, in a cumulative risk assessment, the distribution of residues should be characterised, i.e. occurrence and representative concentrations in food commodities, of residues of each compound in the CAG, in foods as consumed. However, in reality such data are rarely available. More usually the information available is from supervised residue trials and from targeted monitoring. Although less suitable, such data can still be used in a cumulative risk assessment. In an acute assessment it is important to characterise the upper regions of the residue distributions, whilst for chronic assessments information on average residue levels is required. These distributions also provide the background for assessments in the MRL-setting context.

12.1. Supervised trials and other residues data

In Europe, marketing authorizations (Directive 91/414/EEC) and the establishment of MRLs (Regulation 396/2005) for plant protection products require data from supervised trials, which reflect the critical GAP (EC, 1997). Residues data may not be required for every edible crop on which use is permitted because “extrapolation” of residues data is permissible from certain plants or plant products to others (EC, 1997). Where any such uses are permitted, available residues data for both the tested and extrapolated commodities should be used in a cumulative assessment.

In supervised trials, substances of toxicological concern should be analysed, enabling dietary intake estimations for cumulative risk assessment. However, the residue is determined in the raw agricultural commodity, and may not be present at the same levels in the portion of the commodity consumed. Information on processing (e.g. peeling, canning, cooking) may be available for some compounds. This would allow a more realistic estimate of intake. Where available and appropriate (EC, 1997; BfR, 2007), such information should be used in cumulative risk assessment.

Use of data from supervised trials will generally overestimate levels of intake and the frequency of residues in market samples because in trials, for example, all of the commodity sample will have been treated at the maximum dose when this is very unlikely to be the case in practice. Although there are often wide differences in the distributions of residues between trials and monitoring data, for acute intakes it is reasonable to assume that foods will occasionally contain residues at concentrations observed in supervised trials, or even at the MRLs.

Pesticide residues can occur not only in treated crops, but also in crops grown in areas previously treated, i.e. rotational crops. Residues may also be found in animal products (e.g. meat, milk and eggs), due to transfer of residues from animal feedstuffs. When there is reason to believe that such residues are not negligible, an estimate of possible residue levels may be required (EC, 1997). Hence, in a cumulative risk assessment, relevant evaluations should be reviewed for evidence of such possibilities and, where found, the impact on the cumulative assessment should be considered.

12.2. Monitoring data

In Europe, information on residues data in marketed commodities is most frequently available from national monitoring programmes. Most data are obtained from random sampling, with some targeted sampling, although the degree of targeted sampling

varies widely among countries. However, because random sampling as specified by Directive 2002/63/EC refers to the selected lot, it is also important to ensure that selected lots are such that their sampling probability is proportional to throughput and occurrence in the food supply. Such a procedure is followed in the US in the Pesticide Data Program (PDP).

Residues data may be available, in addition to those from official monitoring programmes, for example from retailers (e.g. Supermarkets), growers, and processors that may be useful for use in cumulative risk assessment.

When the residue definition used for monitoring/enforcement does not include all substances of toxicological concern for the cumulative assessment, a conversion coefficient may occasionally have been determined. It would be important to include this information in the assessment, though it will introduce additional uncertainty.

Where processing data are available, this should be used where possible to estimate residue levels in foods as eaten, particularly for commodities such as citrus fruits, banana, pineapple and melon, which are almost always usually peeled before consumption.

Monitoring programmes typically do not include information on all commodities that are consumed. Hence, the impact of such uncertainties on the cumulative risk assessment should be considered.

Where residues data are "extrapolated" between commodities for the purposes of MRL setting, it may be possible to extrapolate monitoring results from the commodity used as the basis of the MRL to commodities that have not been monitored. In such situations, an analysis of the potential impact of this on the assessment should be undertaken. Other extrapolations may also be possible. Details can be found in EFSA (2008)

An important issue is the censoring (truncation) of monitoring data, where the lowest values are unknown or unreported. This is not usually of consequence when evaluating residues for a single pesticide, but may be of concern when considering residues from multiple pesticides. This is because treatment of censored data can affect the distribution of estimates of high intakes.

12.3. Use of censored data

Data may be censored (truncated) as follows:

- In monitoring programmes and supervised trials, residues may not be above the limit of detection (LOD), i.e. true non-detects.
- In monitoring programmes and supervised trials, residues may be above the limit of detection, but below the limit of quantification (LOQ).
- In monitoring programmes, levels may be below a standard reporting level, even though they are quantifiable, being reported simply as "not found" or, more correctly, less than the reporting level.

Where residues are below the LOD in supervised trials, they can be regarded as zero if substantiated by the plant metabolism studies. If not, non-detects should be treated by one of the approaches discussed below.

The treatment of non-detects in monitoring data is more problematical, as these could either be a result of non-treatment or of the presence of low, non-detectable levels. In surveys where no residues have been detected above the LOD, it can be assumed that these are zero. However, the impact that this assumption has on the assessment should be investigated by sensitivity analysis. If this shows that the assumption affects the outcome, evidence to support the assumption is required, for example data from plant metabolism studies, information on proportion of the commodity treated, effect of processing.

Residues above the LOD, but below the LOQ, are often assigned a value of $0.5 \times$ LOQ. In such cases, sensitivity analysis should be undertaken to determine the impact of this assumption. If this shows that the assumption affects the outcome, a more refined analysis is necessary (e.g. maximum likelihood estimation). However, this approach is unlikely to be applicable to data from supervised trials as numbers of samples will be too small.

Reporting levels for monitoring programmes are often relatively high, which may lead to appreciable uncertainty in the intake estimates. The introduction of a default MRL of 0.01 mg/kg in Europe and the needs of cumulative risk assessment provide a reasonable basis for reducing the reporting levels.

It might be possible to improve the estimate of intake by taking into account the proportion of the commodity that has not been treated. The US EPA does this for domestic crops that are treated (EPA, 2000a), and the approach is described in (EPA, 2002a). In some European countries, data are available for this parameter for some crops. However, it is not possible to estimate this parameter for imported commodities.

13. Residue data in acute intake assessments

Ideally, for acute risk assessments, residues data for single items (units of food, e.g. an apple) rather than for composite samples should be used. In practice, however, such data are rarely available and hence it is necessary to consider inter-unit variability. This can be achieved by applying a variability factor to the value for composite samples. EFSA PPR (EFSA, 2007b) has reported that the way in which the variability factor is represented in probabilistic modelling (fixed factors of 1, 5 and 7 versus a range of values in a distribution) has little effect on the outcome. However, in models using parametric distributions, some will be sensitive to the choice of variability factor. Sensitivity analysis should be undertaken to determine the impact of the assumptions made.

In MRL-setting acute cumulative assessments, the MRL value should be used for the contribution of the active substance/commodity combination under consideration combined with background contribution from the active substance in other commodities and background contribution of other compounds in the CAG, using monitoring data if available. Where such data are not available, data from supervised trials could be used, combined with sensitivity analysis to determine the impact of the assumptions made.

14. Residue data in chronic intake assessments

Chronic assessments can be based on data from composite residue samples. However, it is important that the monitoring data are representative of the food supply, with respect to seasonality, geographical source and range of commodities sampled.

In MRL-setting cumulative assessments, the STMR value from supervised trials should be used for the contribution of the active substance/commodity combination under consideration combined with average concentrations for the background contribution from the other active substance/commodity combinations plus the background contribution of other compounds in the CAG, using monitoring data when available. Where such data are not available, data from supervised trials could be used, combined with sensitivity analysis to determine the impact of the assumptions made.

For actual chronic cumulative assessments, data from supervised trials could be used in a lower tier.

15. General requirements for consumption data

There are a number of methods for assessing dietary consumption, which include food consumption surveys at individual or household level or, less accurately, from food production statistics. Consumption can be recorded by one or more prospective diaries, retrospective dietary recall and food frequency questionnaires. Food production statistics provide data on consumption of foods in the raw form as produced, which can then be adjusted to account for losses.

Food consumption data should reflect differences between geographical regions. Information should be included on factors influencing consumption, such as the demographics of subjects surveyed, body weight, geographical region, day of the week and the season in which the survey was performed.

The data should reflect consumption over the whole year, as well as the demographics of the population, including sex, age, cultural background and socioeconomic status. Data should be available for subgroups of interest, such as infants, toddlers, women of child bearing age, as well as for individuals at the upper ends of the distribution. Detailed information on the collection of food consumption data is available in the report of the 2005 FAO/WHO Workshop on Exposure Assessment for Chemicals in Food (WHO, 2007) and in De Vriese et al. (2005).

The food items consumed should be clearly described, using a food coding system, e.g. LanguaL, to assist in unequivocally assigning ingredients to the foods as consumed. Consumption data are recorded on foods "as eaten" (e.g. hamburger, soup) but as the pesticide residues data from monitoring programmes are usually reported with respect to the raw agricultural commodities (e.g. potatoes, oranges, olive oil), it is necessary to be able to convert food consumption data from "as eaten" to food commodity, for which standard recipes are important. In the US, consumption data are obtained from the Department of Agriculture's Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96/1998. Standard recipes are available from the National Technical Information Service of the US Department of Commerce as the Food Commodity Intake Database (FCID).

No such food intake database is available for Europe as a whole, although they are available in some countries, such UK, the Netherlands and Germany (children only). It would be of value to perform a consumption survey in Europe, for example on the basis of the four European GEMS/Food cluster diets (WHO, 2006), rather than on the basis of national boundaries (Brussaard et al., 2002).

16. Food consumption data in acute intake assessments

Acute intake assessments require statistically robust data on one-day food intakes. However, not all consumption databases are such that probabilistic assessments of acute intake are possible. Dietary records should be available on a single day (24 h) basis. Although data may be collected over multiple days (consecutive or non-consecutive), the individual daily records should be available. Ideally, information on the times of consumption should be recorded. Data recorded should include the quantities of all raw, cooked or processed foods consumed, in g per day per person. Additional information should include body weight, sex and demographic characteristics (e.g. age, cultural background, geographical region) and any special dietary behaviour (e.g. vegetarian, diabetic).

The survey should be performed in such a way that it is adequately representative of day of the week and season. Some measure of both intra-individual and inter-individual variability should be obtained. This can be achieved by recording at least two non-consecutive time periods, each comprising more than one day (e.g. 2 periods × 3 days per period).

In an acute deterministic assessment, the large portion (LP), 97.5th percentile of "consumers only" in g food/day (for individual commodities only) is required (WHO, 1997). The LP should correspond to the raw agricultural commodity to which the residue data relates. The LP should be derived for the raw commodity for those that are eaten fresh, e.g. apples and from the processed commodity when this is primarily consumed in a processed form, e.g. bread, as long as residue data are also available for the processed food. Information will also be required on the edible portion in the raw versus the processed commodity.

Where the number of consumers is too small to derive a statistically valid 97.5 percentile some other approach may be used (Travis et al., 2004), although this may overestimate consumption of the commodity of interest.

In surveys consisting of more than one day of dietary recall, the 97.5th percentile can be determined either by considering all days as single days or by determining the maximum consumption for each person and obtaining the 97.5th percentile of these values. Using the 97.5 percentile obtained by the first approach will not necessarily ensure protection of 97.5% of the population, because intra-individual variability is not taken into account. The second approach is the more accurate.

In a probabilistic acute assessment, the distribution of exposure per person per day is required, for which it is necessary to construct a distribution of consumption. This would then enable exposure to be estimated at any desired percentile of the population and vice versa. Data collected on single days should be adequate for this purpose. When data are available for more than one day, only one day per person should be used in the analysis (see above).

17. Food consumption data in chronic intake assessments

Ideally, such data would be obtained form a statistically representative group of individuals, in whom daily records of food consumption would be collected over a sufficiently long interval to cover seasonality and days of the week. These data, when combined with information on pesticide residues would then enable the distribution of long-term average exposures to be determined. However, methodological limitations mean that it is difficult to obtain such data, for example eating patterns can change over long periods of time, and accuracy of recording decreases with time. Thus, longer-term, average intake estimates are normally based on shorter-term consumption data, which are then extrapolated to longer-term periods.

For long-term deterministic assessments, it is the average intake for an individual that is of concern. In such an assessment, it would be possible to use the average of individual mean intakes, or some percentile of the distribution of averages.

For long-term probabilistic assessments, the distribution of consumption is required, so that the mean individual daily exposure within a specific time period (e.g. one week, month, year) can be determined, based on a specific percentile of the distribution. Either the distribution of average individual consumption (variability) or the uncertainty distribution for the average of the averages could be used.

18. Methods to cumulate exposure

As described above, four relevant exposure scenarios can be identified within the context of cumulative risk assessment. In all of the scenarios, assessment of exposure can be either deterministic (providing 'point-estimates', obtained by multiplying a residue value with a consumption value) or probabilistic (multiplying a distribution of residue values with a distribution of consumption

values). If the same assumptions are used for both approaches in acute intake estimates, the deterministic approach should result in a point estimate at the high end of the intake distribution, determined probabilistically. The deterministic method is relatively easy to perform, and does not require specific software. The probabilistic method provides more realistic estimates and information on the probability of the outcome but requires more data.

In general, is not possible to refine a cumulative exposure assessment without using probabilistic approaches. However, in the situation where a single commodity contains multiple residues of pesticides within a CAG, a deterministic assessment can be undertaken using the IESTI (International Estimate of Short Term Intake) equations. Those assessments using this approach to date have used monitoring data for this purpose, i.e. assessments of actual exposure.

A number of models are available for probabilistic exposure assessment. Any model used should have sufficient power for the intended purpose. The data upon which the distributions are based should permit a suitable number of iterations to be performed, which will depend upon the percentile of the exposure distribution (e.g. 97.5, 99.0 or 99.99 percentile) requested by the risk manager

The US EPA have established criteria for the acceptability of probabilistic models for exposure assessment. The model should be transparent, peer-reviewed and freely available. At least eight models are currently available. These are the US models (1) DEEM/Calendex, (2) CARES, (3) Lifeline) and (4) SHEDS, and the European models (5) MCRA 5.1 (RIKILT, Netherlands), (6) CREMe 2 (CREMe, Ireland), (7) Uni HB (University of Bremen, Germany), and (8) CSL (CSL, United Kingdom) (EFSA, 2007b).

Although there are differences (EFSA, 2007c), all of the European models are the same in a number of respects. Intake is calculated per kg body weight, and body weights are available for each individual in the surveys. Food-as-eaten is converted to agricultural products using recipe data. Concentrations for individual items are sampled from a lognormal distribution around a sampled batch mean (the width of which is determined by a variability factor). Concentrations are corrected by fixed processing factors when information on this is available.

There is no *a priori* basis to choose one model over another. A key issue is that consumption data for the region of interest should be linked to the model chosen. Hence, currently some models can be used only in some geographical regions, because of the origins of the data already included in the model.

Most of the models in current use can provide an estimate only of the fraction of person-days, not of individuals for a given percentile, i.e. the probability that the specified consumption of an individual on a certain day will lead to exceedance of the (acute) RV. It is not possible to obtain an estimate of the fraction of the population that will exceed the RV on at least one day over a given interval, nor is it possible to obtain an estimate of the frequency with which such exceedances would occur in individuals. One of these outcomes may be more relevant then the other, depending upon the common toxicological effect. As an alternative, it is possible to obtain estimates of between and within individual variation for use in probabilistic models of intake (Slob, 2006). However, this is an area that needs further development.

Although increasing use is being made of probabilistic models in exposure assessment, there is currently no agreed guideline available in Europe. A draft guideline has been prepared on the basis of the Fifth Framework Monte Carlo project (Institute of European Food Studies, 2003). Given the importance of such modelling in cumulative risk assessment, it is would be of value if the draft guideline could be finalized.

In reporting the results of risk estimates based on probabilistic modelling, it is important that certain minimum information be provided (EPA, 2000b). This includes:

- Is the risk assessment dependent on a "high-end" consumption value (in many cases, this is not the case, and hence does not explain high estimated exposures at the tails of the distribution).
- How extreme are the upper tails of the consumption curve (e.g. is the 95th percentile value >4× mean consumption? Is the 99th percentile value >6× mean consumption?)
- How does the estimated high-end consumption value compare with that expected from the pattern of reported consumption values in the lower percentiles.
- What is the size of the affected subpopulation and would exposure estimates likely to be subject to undue effects of outliers (this would have more impact in small subpopulations than a larger subpopulations).
- How likely is the high-end value to be a valid reported consumption value, on the basis of dietary behaviour (for example, although probabilistically equally extreme, consumption of three gingko fruits per day might be considered more likely than consumption of 10 apples).
- The nature of the inputs both in the overall assessment, and particularly those driving the assessment (for example, do the input residues data include field trials or monitoring data; are default or actual processing factors used; have data on percent crop treated been included).

In conclusion, it is important that the assessment is adequately characterized, including any biases and uncertainties, and that sensitivity analysis is performed, where appropriate, to determine the plausibility of the upper-end percentile estimates.

It should be noted that a number of the issues highlighted as of concern in cumulative risk assessment are common to risk assessments of individual chemicals. However, the fact that the data are used cumulatively magnifies the issues identified, and therefore it was considered important to highlight them here.

19. Cumulative risk assessments of pesticide resides performed to date

To date, reports on cumulative risk assessments and guidance on the conduct of such assessments have been published by the US Environmental Protection Agency (EPA), the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (2002) and Advisory Committee on Pesticides (ACP), the Dutch RIKILT (Boon and van Klaveren, 2003), of the Danish Veterinary and Food Administration (Jensen et al., 2003), the German Chemisches und Veterinaeruntersuchungsamt Stuttgart (CVUA, 2007), the Norwegian Scientific Committee for Food Safety (VKM, 2008) and The Intentional Life Sciences Institute (ILSI, 1999). Details of most of these can be found in EFSA (2008).

The EPA have conducted cumulative assessments for four groups of compounds, organophosphates (EPA, 2006a), chloroacetanilides (EPA, 2006b), triazines (EPA, 2006c) and *N*-methylcarbamates (EPA, 2007). Although EPA has published detailed guidance on the identification of CMGs (EPA, 2002b), not all assessments used the higher tiers of this approach. For example, the RPF approach was not always used. In the case of the triazines it was assumed that all compounds were equally potent, and for the chloroacetanilides the NOAEL was used to obtain potency estimates rather than the BMD. This was because the lower tier assessments, which although considered to be more conservative, but were less resource intensive, indicated that there was no appreciable risk to consumers from combined exposure to the compounds in the respective CMG. It should be noted that when RPFs are calculated using a benchmark

dose approach, other than for the index compound, the BMD rather than the BMDL is often used.

Because the method used to cumulate toxicity utilised a single uncertainty factor, it was necessary in some cases to allow for the application of different UFs for different compounds. These were necessary for inter-species extrapolation (some human data were used) and for age-related differences (as mandated by FQPA (1996)), in the assessment of *N*-methylcarbamates. In this case, the additional UFs were applied to the RPFs and only the intra-species UF was considered in the combined assessment, i.e. the target MOE was 10.

EPA has reported that the cumulative risk assessment for OPs required appreciable resources, particularly the derivation of the RPFs.

In EPA assessments, some compounds initially included in a CMG on the basis of their toxicological effects were excluded from the cumulative risk assessment on the basis of exposure considerations. For example, propazine was excluded from the triazine CMG because estimated exposure was so low that it would not have contributed significantly to total exposure. Butachlor was excluded from the chloroacetanilide CMG because there were no registered uses in the USA. Hence, continuing dialogue between toxicologists, exposure assessors and risk managers is important to ensure the best use of available resources.

In the UK, it was reported that exchange with others who had experience of performing cumulative risk assessment, especially the US EPA, was of value in conducting their own assessments.

The UK found that BMD approaches are not well suited to data obtained using standard regulatory toxicity study designs (three dose groups and control) and hence NOAELs were used for determining potency values. However, as indicated above, it should be noted that use of the NOAEL rather than the BMD introduces greater uncertainty due to limitations of dose spacing and group size.

The plausibility of the modelling outcomes should be checked, with respect to predicted major and minor contributors.

In the UK analysis for OPs, there was less than three-fold difference in the predicted 99.9 percentile intake whether the RPFs were based on the RV or the NOAEL (RP). Hence, in this instance the choice of potency estimate for derivation of the RPF was not critical, but this may reflect the fact that the RVs for OPs are based on a consistent toxicological effect and the UFs were comparable for the different compounds.

There have been several cumulative assessments of anticholinesterase compounds to date, either OPs alone, *N*-methylcarbamates alone or the two groups combined. These have all indicated that there is no appreciable risk to consumers from combined exposures to residues of these compounds as used.

20. Dealing with uncertainty

Cumulative risk assessments involve consideration of exposure and toxicity for a number of different pesticides, and hence will be subject to a larger number of potential sources of uncertainty than assessments of the individual compounds. The degree of uncertainty associated with risk estimates should be adequately characterised, to assist in risk management decisions (Madelin, 2004; Codex, 2007).

EFSA (2006) has published guidance for dealing with uncertainty in exposure assessment. Although specific guidance is not available for toxicological assessment, similar considerations will apply and both should be applicable to cumulative risk assessment.

Each step of the assessment should be examined systematically for potential sources and types of uncertainty. It may be helpful to use a tabular approach to help highlight the important sources of uncertainty. An example of the tabular approach is provided in EFSA (2008).

It might be convenient to use a tiered approach to assessing uncertainties (EFSA, 2006). In this approach, each potential source of uncertainty can be analysed at one of three levels: qualitative, deterministic or probabilistic. Only the most substantial uncertainties need be quantified. In the first tier, all uncertainties are analysed qualitatively. If this provides sufficient information to enable risk managers to reach a decision, no further action would be necessary. Otherwise, any uncertainties critical to the outcome can be analysed deterministically or probabilistically. Key uncertainties and their impact on the risk assessment can be determined by sensitivity analyses. Use of all three levels of uncertainty analysis is illustrated in EFSA (2007b).

21. Selecting and prioritising cumulative assessment groups (CAG)

There is an increasing requirement, mandated by legislation in for example the US and in Europe, that the risk to consumers of combined exposures to pesticide residues is assessed. As discussed above and elsewhere, the priority is to ensure that the combined level of residues acting by the same mode of action does not give rise to a dose additive effect of a magnitude such that it poses an appreciable risk. Hence, there is a need to consider which pesticides should be included in a CAG, and if so, in which CAG should they be included. Possible criteria (both toxicological and exposure-based) upon which to base these decisions include the following:

- Pesticide groups that include compounds frequently detected in monitoring programmes or with high use on the basis of surveys or sales statistics.
- Pesticides for which there is evidence from biomonitoring for high intake in the general population or in some sub-populations.
- Groups of pesticides that include some with a high HQ (e.g. >0.25–0.5).
- Cumulative risk assessment performed elsewhere that has shown possible unacceptable exposure.
- CAG that includes a large number of compounds (e.g. five or more).
- Assumptions on future trends in use of pesticides.

The need to assess specific pesticide combinations should be considered when there is a strong biological hypothesis that the pesticides might interact below their respective NOAELs.

22. Conclusions

The available data suggest that the risk from combined exposures to residues of pesticides with different modes of action is not appreciably greater than the risk from residues of the individual pesticides, when exposure is below the respective ADIs or ARfDs. In this situation, the overall risk is determined by the compound that poses the greatest risk (e.g. the highest HQ). Hence, there is no need to assess combined exposure to those pesticides with different modes of action and different target tissues, occurring as residues in foods.

Several methods are available to cumulate risk. Some are relatively easily undertaken and are readily understood (e.g. the HI), whereas others require more detailed analysis of the toxicological data (e.g. RPI) or additional computation (e.g. RPF based on BMDs). In increasing complexity and refinement, the methods of choice would be the HI, the RPI, the RPF and PBTK modelling. The assessment can be refined when using any of these methods, for

example by better identifying the relevant end-point for the CAG, by applying CSAFs, by using the BMD rather than the NOEAL as a potency estimate. EFSA has proposed a tiered approach for this (EFSA, 2008).

Four exposure scenarios are of relevance in a cumulative risk assessment. These are assessment of acute and chronic exposure from (1) actual usage in practice, based on monitoring data and (2) MRL-setting, based on theoretical exposure based on data from supervised trials. It is possible to address each scenario either deterministically or probabilistically, in which exposure assessment can be increasingly refined.

Where there are different UFs for different pesticides, or it is possible to drive chemical-specific adjustment factors for some of the pesticides, this can be accommodated by adjusting the metrics for individual compounds for those UFs that vary between compounds and to use the "common" UF in any combined comparison necessary.

The common toxicological effect that is the basis of a CAG may require inclusion of compounds where this is not the endpoint that drives the RV. In higher tier assessments, this could be taken into account by calculating a putative RV on the basis of the common effect.

Sensitivity analysis should be performed to determine the consequences of excluding certain compounds from an assessment.

Prioritisation of CAGs for assessment should be based on the toxicological characteristics and likely exposure of the compounds in the CAG. Prioritisation should be based on potential risk rather than hazard, requiring dialogue between different members of the risk assessment community and with risk managers.

As proposed by EFSA (2008), a tiered approach would be the most efficient means of conducting a cumulative risk assessment. The assessment would commence with a combination of lower tier methods for both exposure and toxicity assessment. If this does not give adequate assurance of safety, then the assessment progresses to higher tier methods, for either or both the toxicity and exposure assessment.

Cumulative risk assessment for actual exposure is less resource intensive that for MRL-setting scenarios. This is because for actual exposure, all pesticide/commodity combinations are included in one model-run, whereas for MRL-setting scenarios, the model has to run for each pesticide/commodity combination for which an MRL is needed.

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