TOX/2021/39

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Draft EFSA Scientific Committee Opinion on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals.

Introduction

1. EFSA has released draft guidance, prepared by its Scientific Committee, on the grouping of chemicals for risk assessments of combined exposure, to which an assumption of dose addition would apply. A link to the draft opinion is given in Annex A.

2. The consultation closes on the 10th of July 2021. Members' comments are invited so that a COT response can be submitted. Members were asked to submit comments prior to the meeting and the comments received so far are attached at Annex B

Previous COT evaluations of relevance to mixtures

3. In 2002, the COT published a report on the risk assessment of mixtures of pesticides and similar substances (COT, 2002). A key conclusion was that "the default assumptions should be that chemicals with different toxic actions will act independently, and those with the same toxic action will act additively (simple similar action). In specific instances the possibility of interaction, particularly potentiation, may have to be considered."

4. In 2004, the COT made general conclusions regarding mixtures of chemicals in food, extending the conclusions of its 2002 report to take account of the possibility that exposure to some food additives and ingredients of very low toxicity may be much higher than exposure to pesticides and veterinary medicines (COT, 2004). These conclusions included the following:

- "Several studies claim to have identified synergistic interactions of some mixtures. However, for the most part, these studies have been inadequately designed and based on an incomplete understanding of the concepts involved, but a few well-designed studies have demonstrated the occurrence of both synergistic and antagonistic interactions, as well as additive effects in mixtures. These effects have usually been demonstrated at high concentrations or high experimental exposure levels, which are probably unrepresentative of exposure doses to chemicals present at very low levels in food".

- "Studies in vivo with chemicals that exhibit the same mode of action in the same target organ have shown that the effects of mixtures of similarly acting toxicants show additivity (dose addition), which results from simple similar action. This is the case, over the whole dose range".
- "Generally, when exposure levels of the chemicals within a mixture are in the range of the NOAELs, and the components of the mixture have different modes of toxic action, no additivity and no potentiating interactions are found, indicating the applicability of the basic concept of "simple dissimilar action", which suggests that adverse reactions would be unlikely".

5. In 2011, the COT considered the results of a programme of research that was conducted to address research recommendations made in its 2002 report. The COT concluded that the results provided reassurance that combined risk assessment based on dose/concentration addition was adequately protective for compounds with similar modes of action (COT, 2011).

6. In 2018, the COT responded to a previous EFSA consultation on mixtures entitled "guidance on harmonised methodologies for human, health, animal health and ecological risk assessment of combined exposure to multiple chemicals". EFSA published this guidance in 2019 (EFSA, 2019). In the minutes of the COT meeting when this draft guidance was discussed, the Committee noted that "there is little specific guidance on the use of MOA/AOP in deciding which chemicals should be included in assessment groups" (COT, 2018).

Background to the draft EFSA guidance

7. EFSA has requested the Scientific Committee to develop a guidance document addressing scientific criteria for the grouping of chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals, taking into account various aspects, namely:

- the general principles described in the guidance on "harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals" (EFSA, 2019);
- the need for prioritisation methodologies for the grouping of chemicals when a vast number of chemicals will need to be assessed and resources are limited; and,
- available hazard information (e.g. reference points, specific toxicological effects in target organs, mode of action) and exposure information.

8. A hyperlink to the guidance document is provided in Annex A. The finalised EFSA Opinion will be published after the public consultation, together with the technical report of the public consultation, by the autumn of 2021.

The present guidance

Problem formulation

9. In the problem formulation, it is decided whether a risk assessment of combined exposure to multiple chemicals is required - this is known as the "gatekeeper step". If so, then an initial group of chemicals is defined, based on legal requirements, specific concerns or pragmatic considerations, and terms of reference (ToR).

Sorting into assessment groups

10. The draft guidance document presents a framework where the chemicals (that were pre-defined in the problem formulation step) are sorted into "assessment groups". The sorting is based on similarity of toxicological properties, and common mode of action (MoA) or adverse outcome pathway (AOP) information involving key events (KEs). More details on AOPs are available in OECD documents (e.g. OECD, 2018).

11. It is proposed that common KEs (i.e. sub-cellular or molecular targets) are used a basis for defining assessment groups. Thus, different chemicals should be in the same assessment group if they converge on any common KE. The rationale for this approach is that combined toxicity (for different chemicals with common KEs) has been best described using dose addition (EFSA, 2019).

12. This requires a weight of evidence (WoE) approach to assemble, weigh, and integrate the available lines of evidence (LoEs) on toxicity at different levels of biological organisation (molecular, cellular, organ level, whole organism) for each chemical. Methods for weighing and integrating the evidence can include qualitative approaches (simple description), semi-quantitative methods (low, moderate, high) or quantitative methods (probabilistic scale) (EFSA, 2017). Some case studies using the WoE approach are provided (appendices B-E).

13. Toxicokinetic (TK) data can be useful for grouping (though it should not be used in isolation for defining assessment groups), for example when different chemicals are substrates of the same toxicologically relevant enzymes or transporters. Additionally, TK data or TK models in test species or humans can be used to refine grouping, or to compare risk metrics based on internal dose (EFSA, 2019).

Poorly characterised chemicals

14. If a chemical's MoA or AOP is unknown, grouping can still be done using other hazard criteria such as a common adverse outcome. The rationale for this approach is that different AOPs can converge on the same adverse outcome even if they do not have any KE in common. However, this approach is associated with greater uncertainty in the assessment, and may imply the inclusion of many chemicals in an assessment group, leading to an overestimation of the risk of combined toxicity.

15. Chemicals with little or no toxicological information may be included in an assessment group if *in vitro* or *in silico* approaches are used to provide additional

information on their toxicological properties, showing similarity to data-rich members of an assessment group. For example, molecular docking and machine learning tools can be used to identify specific chemical moieties or structural features which may lead to a toxic effect (e.g. Allen *at al*., 2020). However, it is necessary to assess the applicability domain of each model and integrate the prediction results of multiple models for the prediction of the same property, using WoE methods.

Approaches to prioritisation

16. Risk- and exposure-based approaches for prioritisation (described in further detail below) are proposed to identify 'low priority chemicals' which can be excluded from an assessment group when they fall below a certain threshold. The threshold value represents a protection goal and is thus defined by risk managers. The threshold value may also depend on data availability and the number of chemicals under consideration. For each approach, exposure estimates can be based on external or internal dose.

Combined risk-based approach

17. In this approach, a combined risk is calculated using hazard information for a common effect or target organ/system. This approach uses dose addition as the default assumption (e.g. combined, or total margin of exposure (MOE_T).

18. Subsequently, the relative contribution of each individual chemical to the combined risk (including the uncertainty in estimates) can then be used to identify low priority chemicals.

19. As a starting point, a default cut-off value of < 10 % contribution of a single chemical to the combined risk is proposed, so that any chemical contributing < 10 % to the combined risk (threshold value) is excluded from the assessment group. However, this threshold value (which should be documented) depends on the context of the assessment and the statistical methods used. For example:

- when a high number of chemicals have a contribution slightly below the threshold value, it is recommended to reduce the threshold value to ensure that the total contribution of retained chemicals accounts for at least 90 % of the combined risk
- when individual chemicals contribute to the combined risk below the threshold value, these contributions may be strongly correlated (i.e., when contribution of chemical A is at its highest, the contribution of chemical B is also at its highest). When such correlations are identified between chemicals, it is recommended to retain those chemicals for refinement of the grouping, regardless of their individual contributions.

20. Appendix D provides an example where pesticides with acute neurotoxic effects are sorted into assessment groups, using a combined MOE approach exclude low priority compounds.

Individual risk-based approach

21. When hazard metrics are available only for a chemical's critical effect (i.e. the reference point for HBGV or MoE calculation) and the approach above cannot be applied, then the individual risk for each chemical under consideration is calculated. When the individual risk metric of a chemical falls below a pre-defined threshold, it is excluded from the assessment group.

22. A pre-defined threshold value < 10 % of the relevant health-based guidance value (HBGV) or a MOE that is > 10-fold of the adequate MOE for each individual chemical have been proposed (FAO/WHO, 2020). EFSA proposes that these threshold values can be lowered, depending on the context of the assessment. The rationale for deviating from the proposed threshold value should be documented.

Exposure-based approach

23. When the risk assessment question deals with a large number of chemicals (e.g., all contaminants in human blood or breast milk), but hazard information is unavailable, then an exposure-driven approach can be used.

24. Chemicals that have a probability of co-exposure in humans above a pre-defined threshold would remain under consideration for grouping. It is important to assess the timeframe(s) of exposure and the chemicals' biological half-lives.

25. The exposure-based approach has a drawback since potent compounds with a low probability of co-exposure might be excluded from grouping. Indeed, EFSA notes that this approach currently has limited applications in risk assessments conducted by EFSA panels.

26. Appendix E provides an example which uses the exposure-driven approach as a prioritisation method for multiple contaminants in human breast milk.

Recommendations for future work

27. The Scientific Committee made several recommendations for future work:-

- test the guidance in relevant EFSA panels using case studies, including threshold values for defining low priority chemicals;
- update the OpenFoodTox database with chemical hazard information;
- further integrate data from *in silico* tools (e.g. QSARs, TK models) or new approach methodologies (NAMs) to help sort chemicals into assessment groups; and,
- develop open-source software tools where the prioritisation methods can be applied to chemical mixtures.

Appendix B - a generic example to illustrate application of the WoE approach for sorting chemicals into assessment groups:-

28. There is combined exposure to five contaminants (A, B, C, D, and E), and hazard information is available for each contaminant. In order to group these contaminants into an assessment group, a WoE approach is taken which involves the following steps:

1. Assemble hazard evidence

Hazard information (e.g. reference points, adverse outcomes, *in silico* predictions) is evaluated to generate LoEs on:

- dose-response relationships for specific effects;
- clinical evidence for the effect;
- biochemical evidence for the effect; and
- MoA supporting the effect.

2. Weigh and integrate the hazard evidence

The reliability, relevance and consistency of the evidence is assessed, using qualitative (e.g. expert judgement) or quantitative (e.g. scoring) methods described elsewhere (EFSA, 2017). In this example, the four LOEs for each contaminant were weighted as low, moderate, and high. Then expert judgement was applied to conclude on the probability of inclusion to the assessment group (extremely likely, 99-100 %; very likely, 90-99 %; likely, 66-90 % etc.)

3. Summarise results

The outcome of the WoE assessment (from steps 1 & 2) is shown - in this case, the grouping of chemicals A, B, and C (associated with adverse outcome 1) into common assessment group 1 (MoA1), and chemicals D and E (associated with adverse outcome 2) into common assessment group 2 (MoA2). MoA1 and MoA2 are different MoAs which produce different adverse outcomes.

Appendix C - statistical methods to assess the probability of co-exposure

29. As previously described, chemicals may be sorted into assessment groups using the exposure-based approach, where chemicals with a probability of co-exposure in humans below a pre-defined threshold are removed from the group. This appendix describes some statistical methods for quantifying the probability of co-exposure.

30. Spearman and Pearson correlation coefficients are commonly used to assess the strength and direction of association between two variables. A positive correlation coefficient indicates that when the first variable increases, the second variable increases too. Likewise, a negative correlation coefficient indicates that when the first variable decreases, the second variable decreases too. The closer the correlation coefficient is to 1 (or to -1), the stronger the dependencies between the variables.

31. It is proposed that a chemical with no or low magnitude of correlation with other chemicals ($r < 0.4$) can be excluded from the assessment group, whereas if $r \geq 0.4$ the probability of co-exposure is considered relevant, and an r value > 0.6 or 0.7 is considered strongly relevant.

32. As previously described, chemicals may be sorted into assessment groups using the combined risk-based approach. Here, use of the Maximum Cumulative Ratio (MCR) is also proposed. The MCR is the ratio of the combined risk estimate to the highest risk calculated for a single chemical within the assessment group. The MCR thus provides a measure of whether combined risks are dominated by a single chemical or from the contribution of multiple chemicals. An MCR of 1 for a chemical in an assessment group indicates that the combined risk metric is dominated by a single chemical and that a combined risk assessment is not needed. At its maximum value, the MRC equals to the number of chemicals assessed, where all chemicals have an equal contribution to the combined risk and thus all chemicals should be prioritised for further/refined assessment (EFSA, 2019).

Appendix D - combined risk-based approach as a prioritisation method for grouping pesticides into assessment groups

33. This example starts with 100 pesticides from the study of van Klaveren *at al*. (2019). Hazard metrics (acute reference doses, ARfDs) and pesticide concentrations are available for 96 of these pesticides.

34. Two exposure scenarios were used in the risk assessment: 95th and 99.9th percentiles of the exposure distribution.

35. Hazard quotients (HQ) were calculated for each of the 96 pesticides, as individual ratios between each exposure percentile (95th and 99.9th) and the relevant acute reference dose. The pre-defined threshold values for identifying low priority pesticides were 1 % and 10 % of the ARfD, corresponding to HQ values of 0.01 and 0.1 respectively.

36. Subsequently, at the 95th exposure percentile, 53 pesticides had HQ > 0.01, of these 11 had HQ > 0.1, whilst at the 99.9th exposure percentile, 78 pesticides had HQ > 0.01, of these 46 had HQ > 0.1. These remaining pesticides were subsequently sorted into assessment groups based on the collection of further hazard information.

37. Indeed, two chemical assessment groups (CAGs) were formed based on common effects: 1) CAG-NAN (brain and/or erythrocyte acetylcholinesterase inhibition), and 2) CAG-NAM (alterations of the motor division).

38. Combined MOEs (MOE_Ts) were calculated for each of the two CAGs, using specific NOAELs for each assessment group. MOE_Ts of > 100-fold were interpreted as of low concern (EFSA, 2019).

Appendix E - Exposure-driven approach as a prioritisation method for grouping multiple contaminants from breast milk and comparison with a risk-based approach for single chemicals

39. The example presented here illustrates the use of an exposure-driven approach to identify low-priority contaminants in human breast milk for grouping.

Hazard metrics for critical effects were not available for all chemicals defined in the terms of reference. Therefore, an exposure-based approach for prioritisation was initially used.

40. The assessment includes 32 chemicals with positive concentrations in 180 breast-milk samples (Crépet *at al*., 2021). Combined exposure for infants was calculated by multiplying each chemical concentration with a mean consumption of breast milk of 763 ml/day and a mean body weight of 6.1 kg. The Sparse non-negative matrix under-approximation (SNMU) method was applied to the exposure matrix (180 by 32). Chemicals with a low probability of combined exposure were considered as low priority whereas chemicals with high probability of combined exposure were prioritised.

41. In order to compare the results with the risk-based approach for single chemicals, HQs were calculated as the individual ratio between exposure and the HBGVs for 26 of the 32 chemicals for which HBGVs were available. Similar to the example presented in Appendix D, pre-defined trigger values for identifying low priority chemicals were set at 1 % and 10 % of the HBGVs corresponding to HQ values of 0.01 and 0.1, respectively (EFSA, 2019).

42. The prioritisation methods led to the selection of 19, 20, and 17 chemicals using the combined exposure metrics, risk metrics for single chemicals (using a threshold of 1 %) and risk metrics for single chemicals (using a threshold of 10 %), respectively. The Hazard Index (HI, the sum of each chemical's Hazard Quotient) was calculated for the prioritised chemicals in each of these scenarios. Its value was close to the HI obtained from the initial 26 chemicals under consideration, additionally HI values across the two prioritisation approaches used were similarly high.

Summary

43. The present guidance document presents a framework where chemicals present in a mixture can be sorted assessment groups, based on similarity of common mode of action (MoA) or adverse outcome pathway (AOP) information, namely KEs. Three approaches for the prioritisation of chemicals within these groups have been proposed, where 'low priority' chemicals can be excluded from the assessment group: 1) combined risk-based approach, 2) individual risk-based approach, and 3) exposure-based approach. These approaches require successively fewer hazard information, and are thus associated with increasing uncertainty.

Questions for the Committee

44. Members are invited to provide general and specific comments on the draft guidance.

i) Does the Committee agree with the sorting of different chemicals into assessment groups on the basis of common KEs?

ii) For each prioritisation approach (to identify 'low priority chemicals' in an assessment group), does the Committee agree with the default threshold values for a chemical's inclusion/ exclusion (as described above in text):

- Combined risk-based approach,
- Individual risk-based approach, and
- Exposure-based approach?

iii) EFSA notes that the exposure-based approach has a drawback since potent compounds with a low probability of co-exposure might be excluded from grouping (see paragraph 24). In the absence of hazard information of the chemicals involved, is there any way to gauge the potency of such chemicals, such as the read-across approach?

iv) Any other comments?

Secretariat
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List of abbreviations

AOP – adverse outcome pathway

ARfD – acute reference dose

CAG – chemical assessment group

HBGV – health-based guidance value

HI – hazard index

HQ – hazard quotient

KE – key event

LoE - line of evidence

MCR - maximum cumulative ratio

MoA – mode of action

MoE – margin of exposure

MoE_T – combined margin of exposure

NAM – new approach methodology

NOAEL – no observed adverse effect level

QSAR – quantitative structure-activity relationship

SNMU - Sparse non-negative matrix under-approximation

TK – toxicokinetic

ToR – terms of reference

WoE – weight of evidence

Annex A to TOX/2021/39

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of the EFSA Scientific Committee Opinion on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals.

The link to the draft Scientific Committee opinion is given below:

https://connect.efsa.europa.eu/RM/s/publicconsultation/a0c1v00000HnXIB/pc0014

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Annex B to TOX/2021/39

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of the EFSA Scientific Committee Opinion on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals.

Comments received from COT Members and Assessors.

General comments

- The proposed guidance provides a pragmatic and scientifically sound approach for grouping chemicals for a combined risk assessment. The recognition that such assessments are predicated on problem formulation, which will often define the scope, is welcome. The focus is on mode of action/adverse outcome pathway, which is appropriate given much previous work and guidance, e.g. SCHER, EPA, WHO, OECD in this area. This focus provides a means of integrating information obtained from New Approach Methodologies. However, further guidance on this aspect is needed and would be welcome. EFSA are to be congratulated on producing this guidance. It is a complex area, and the proposals provide clear, pragmatic advice.

- Overall, a clear and well balanced document.
- The key concepts are covered.

- A lay summary would beneficial given the public interest in this topic

- How/when will emerging information be assessed & added?
- How well are different contaminant types accounted for when assessing mixtures ?- e.g. mycotoxin/organic contaminants
- The potential of different methodologies - e.g. immunoassay, cell toxicity

- The opinion discusses dose addition but not response addition.
- There is no consideration of antagonism or synergy although these terms are covered in the abbreviations so may have been considered at some stage and subsequently removed. It would be useful to consider these somewhere in the text as different types of interactions.

Section 3.1

P14, 448: There are different possible options following this exercise, and it is not clear which is recommended. For example, are some chemicals excluded from the group based on low probability of membership (it will never be zero) or will all be included, but weighted for probability of membership? Perhaps some text could be added, linking to section 4.

P14, 460: MOA includes only kinetic factors that are necessary for the outcome, for example metabolic activation or active uptake into the target cell. It does not include general systemic kinetics.

Section 4.1

P17, 547: Exposure-based approaches may also have a role to play earlier on in this assessment, depending on problem formulation. For example, if the problem is combined exposure to low calorie sweeteners, those for which exposure is likely to be very low or zero in the target population, for example because of use pattern, need not be considered further. i.e. Likelihood of co-exposure should be a gate-keeper step (and perhaps it is).

P20, Fig 4: Note that the uncertainty using the various approaches may differ markedly, depending on whether the Ref Val or the Ref Point is used, when the Ref Val is based on an effect that occurs at a much lower dose than the common effect. This is recognised in page 16, line 525, but is then not considered elsewhere.

Section 5 Recommendations

P21: There are still open questions on how data from NAMs will be used in mixture toxicology, and it would be helpful if EFSA could develop some guidance on this.