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Deliverable 9.3

Report of EuroMix Workshops on International Harmonisation on the Risk Assessment of Combined Exposure to Multiple Chemicals

May 2019

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Task 9.3: Harmonisation and acceptance of mechanism-based testing

Report of EuroMix Workshops on International Harmonisation on the Risk Assessment of Combined Exposure to Multiple Chemicals

Under Task 9.3, EuroMix organised a series of four workshops on the international harmonisation of the risk assessment of combined exposure to multiple chemicals, with the aim of exploring options and potential limitations in the international acceptance of the approaches being developed by EuroMix. International acceptance has obvious implications for those commodities where Maximum Residue Limits (MRLs) have to be established for residues, in that if very different approaches were to be used for combined risk assessment, the acceptability of MRLs could vary markedly.

Workshops were organised as follows:

- 1st Workshop – Imperial College London, UK, 20-21 October 2016. Attended by 14 participants
- 2nd Workshop – Thon Hotel EU, Brussels, Belgium, 17 May 2017. Attended by 19 participants
- 3rd Workshop – Imperial College London, UK, 25 October 2018. Attended by 15 participants
- 4th Workshop – WHO HQ, Geneva, Switzerland, 15 April 2019. Attended by 18 participants

Participants at the workshops involved experts from North and South America, Europe, Australasia, Asia, and North Africa as well as national and international organisations, including the European Commission (DG SANTE, DG Environment), EFSA, The Joint Research Centre of the European Commission (JRC), OECD, Codex Alimentarius, WHO, FAO, US FDA and US EPA. Most of the participants were experts in risk assessment, but several were experienced risk managers, whose input was invaluable.

The first workshop focused on the scientific issues involved in the risk assessment of combined exposure to multiple chemicals and identified those topics of greatest priority for consideration at future workshops in the series. It was agreed that harmonisation of the approach used was highly desirable and in some areas such as pesticides it was essential, to ensure the safe and effective continuation of international trade in food commodities. A number of key issues were identified where harmonisation has yet to be achieved, such as the scope of cumulative risk assessments (which “chemical silos”), the basis for grouping chemicals into assessment groups, and how information on modes of action/adverse outcome pathways (AOPs) would be taken into account in such assessments. These topics should be discussed in more detail at later workshops in the series.

The aims of the second workshop were to understand current and upcoming legislative needs for cumulative risk assessment of chemicals (with a focus on the diet); how this varies across chemical sectors (e.g. pesticides, additives, contaminants) and the extent to which this might be harmonised; how this varies across geographical regions and the opportunities for harmonisation; the role that scientific research, and particularly that of EuroMix, might contribute to achieving these goals. The meeting concluded that, currently, there is no overarching approach to the risk assessment of combined exposure to multiple chemicals, either within the EU (across regulatory sectors) or internationally. Approaches to such risk assessment vary across regulatory sectors and geographies, sometimes markedly. In some areas, risk assessment of combined exposure to multiple chemicals is currently not a significant consideration, whereas in others there is appreciable concern. However, even in the latter case, approaches utilised in different regions show appreciable differences. The most common approach to date for developing cumulative assessment groups is use of common structure and/or co-occurrence and/or designed function (e.g. pesticidal mode of action). EuroMix is

exploring implications of different exposure and toxicology cut-offs for human health protection, both experimentally and by simulation. Work is underway both within and beyond the EU to explore harmonisation of approaches to risk assessment of combined exposure to multiple chemicals within and across chemical sectors. Case studies would be invaluable to explore these issues.

The objectives of the third workshop were to review the outcome of the first two workshops; to review ongoing work on harmonisation elsewhere, particularly at OECD and EFSA; to explore ways in which the EuroMix toolbox can contribute to the risk assessment of combined exposure to multiple chemicals; to compare and contrast different approaches to the risk assessment of combined exposure to multiple chemicals in the diet, in relevant legislation by means of illustrative case studies; to consider how the EuroMix toolobox might contribute to the different needs to risk assessors and promote greater harmonisation in the approaches used. It was concluded that there is considerable alignment of the principles for assessment of combined exposure to multiple chemicals in the guidance of IPCS, OECD, EFSA and other organisations. These all emphasise the importance of problem formulation, including specification of the objectives and acceptable degree of uncertainty for assessment, the basis for grouping and the selection of the assessment approach. There was general agreement on the need for tiered approaches for both hazard and exposure assessment, to avoid overly conservative assumptions. The use of mode of action/AOP information in refining assessment groups has also been broadly incorporated, as has been transparent delineation of uncertainties at each tier. In a number of chemical sectors, there is common application of these principles. However, in the area of pesticides, there are significant differences between the proposed approach in Europe and that which is in use in the USA. The EuroMix Toolbox has potential application, regardless of the approach used in different sectors or geographical regions. Whilst harmonisation of the specific risk assessment methodology might not be possible, at least in the short term, it should be possible to harmonise the principles used, the standard of reporting and data templates. The EuroMix Handbook will seek to provide best practice for the range of problem formulations that might concern risk managers and will encourage further harmonisation, to the extent possible.

At the fourth, and final, workshop, the specific objective was to explore the potential of the EuroMix Handbook to contribute to harmonised scientific approaches to the risk assessment of combined exposure to multiple chemicals in the diet and more generally, in relevant legislation. Perspectives on the Handbook (and the Toolbox) were invited from representatives of international organisations. Issues that might arise in utilising the Handbook and the Toolbox at international level were identified, for consideration at the FAO/WHO Expert Consultation on Dietary Risk Assessment of Chemical Mixtures (Risk Assessment of Combined Exposure to Multiple Chemicals), 16-18 April 2019, Geneva. The EuroMix Handbook was generally well received. Participants felt that it was clearly laid out and that, together with the Toolbox, it should make a significant contribution towards international harmonisation of approaches and methods used in the risk assessment of combined exposure to multiple chemicals. Participants made a number of suggestions on how the Handbook might be clarified or extended. These centred primarily around ensuring maximum flexibility of the Handbook and the recommended approaches, to ensure fitness-for-purpose for the range of needs of risk managers, in different chemical sectors and geographical regions; greater transparency and guidance in some areas, such as when it is not possible to establish an AOP, and alternatives to a full probabilistic exposure assessment; ensuring that data, tools and models comply with agreed standards, to facilitate information exchange and sharing. The participants concluded that there were several sections of the Handbook and modules in the Toolbox that could potentially contribute to risk assessment of combined exposure to multiple chemicals by JECFA/JMPR and that should be considered at the FAO/WHO Expert Consultation. However, potential limitations were also identified, including availability of suitable data on exposure, formats for consumption data, transparency of the models used in the Toolbox, and the need for verification of methods, algorithms and software if they were to be used within the JECFA/JMPR process.

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Report of EuroMix First Workshop on International Harmonisation on the Risk Assessment of Combined Exposures to Chemicals

20 -21 October 2016, Celia Hensman Suite, W12 Conferences, Imperial College London, Hammersmith Campus, London W12 0HS

Background

EUROMIX organised the first of a series of workshops on the international harmonisation of the risk assessment of combined exposures to chemicals from 20-21 October, 2016 at Imperial College London, UK. The aim of these workshops is to explore options and potential limitations in the international acceptance of approaches to the assessment of combined exposures to chemicals. This has obvious implications for those commodities where Maximum Residue Limits (MRLs) have to be established for residues, in that if very different approaches were to be used for combined risk assessment, the acceptability of MRLs could vary markedly. This first workshop focused on the scientific issues involved and identified those topics of greatest priority for consideration at future workshops in this series.

The workshop took place over 1.5 days, from 14:00 on day 1 until around 16:00 on day 2. The programme of the workshop is provided in the Annex. Participants were selected from representative geographical regions and organisations, with participation from Europe, North and South America, Australasia and North Africa. The following individuals attended the workshop:

Name	Country/Region	Organisation
Luc Mohimont	Europe	EFSA
Eeva Leinala (Day 2 only)	International	OECD
Vittorio Fattori	International	FAO
Cecilia Tan	USA	EPA
Yasunobu Aoki	Japan	National Institute for Environmental Studies
Matthew O'Mullane	Australia	APVMA
Mohammed El Azzouzi	North Africa	University of Rabat
Andrew Worth	Europe	JRC
Bette Meek	Canada	University of Ottawa
Angelo Moretto	Italy	University if Milan
Jacob van Klaveren	The Netherlands	RIVM
Eloisa Dutra Caldas	Brazil	University of Brasilia
Roland Solecki	Germany	BfR
Alan Boobis	UK	Imperial College London

The meeting room was arranged in board room style. The meeting was chaired overall by Alan Boobis. Each topic on the agenda started with a short introduction by a designated participant, followed by a round table discussion during which existing areas of harmonisation were identified

and those where further work would be needed before harmonisation would be possible were agreed. The intention at the first meeting was not necessarily to resolve outstanding issues but list and prioritise these for further discussion at a later date.

The meeting started with participants introducing themselves, which was followed by a brief description of the EU funded EuroMix project (grant agreement number 633172) by Jacob van Klaveren. The key focus of EuroMix is developing methods and approaches for mixture toxicology that will help inform risk management in Europe, and elsewhere. This will be achieved by proof-of-principle studies of a tiered approach to assessing the risk from combined exposures and a test strategy to confirm or to refine the assumptions made in current cumulative risk assessment proposals or practices in Europe and elsewhere. An important aspect of this is international harmonisation, to the extent possible, of the approaches proposed.

One of EuroMix's deliverables is a survey of the legal requirements for cumulative risk assessment in different regions and countries. An advanced draft of the report is now available. It was agreed that it would be very helpful for this to be circulated to participants and ask for feedback, particularly from those countries not well described at present.

Problem formulation

Key messages

- Problem formulation by risk managers, in dialogues with risk assessors, is critical to success
- A tiered approach using existing tools enables pragmatic decisions
- Terminology for cumulative risk assessment should be harmonised to achieve a shared global understanding

The first topic addressed at the workshop was **problem formulation**, introduced by Bette Meek. From an international perspective, the key question is what is the purpose of assessing the risks from combined exposure to chemicals? Is the objective to harmonise methodology, the approach to setting MRLs, or some form of global risk assessment of real world exposures?

In the context of international harmonisation, it was agreed that in the short term, harmonisation might be possible for pesticides, due to the relatively limited number of chemicals in this sector, but that for other chemicals such as contaminants, more work would be needed before harmonisation is likely to be achievable.

In general, problem formulation is not well developed for the assessment of combined exposures to chemicals. It is often not well articulated, leading to lack of transparency. Elements in problem formulation should include the nature of the chemical sector, the regulatory context (legislative and policy considerations), the objective of the assessment, the timescale within which the assessment was required and the resources available, and the level of uncertainty that would be acceptable. It was agreed that clarity of problem formulation is critical.

Of the two major exposure scenarios (for authorised compounds such as pesticides), actual (real world) exposure (based on specific measurements of the compounds in question) and that for MRL setting ('worst case', based on conservative assumptions), harmonisation would be easier to achieve for the approach to the latter, though it should be possible to harmonise at least the methodology used for the former as well.

A key component in problem formulation is agreement on how chemicals should be grouped for assessment (cumulative assessment groups, CAGs). Should this be based on a common phenotypic

effect or MOA (this was discussed in more detail later in the workshop – see below). Information on both hazard and on exposure would be necessary, although the weight given to them will vary with the chemical sector. For new pesticides, particularly when considered authorisation, most information available is on hazard and a typical average or high end consumer is used to estimate potential exposure. However, when considering combined exposure, for example when a new pesticide shares a similar adverse outcome with several pesticides already on the market, sufficient exposure data are available in Europe on the other pesticides in the group, but might be lacking in other parts of the world. In contrast, when assessing possible risks from exposure to commodity chemicals, particularly in the form of contaminants in food, often more information is available on exposure although this varies considerable among chemical classes and monitoring practices. For chemicals migrating from food packaging materials very little data exist, whereas levels of dioxins and PCBs are well monitored because of EU regulation.

The importance of tiered approaches was emphasised, only doing what is necessary to address the problem, but this varies with the chemical sector (problem formulation). The WHO Framework for assessing combined exposures to multiple chemicals was a good starting point for this purpose[1]. However, this will require that the level of uncertainty is specified and that the uncertainty associated with the various tiers can be determined. This should then be linked to regulatory consideration of what is an acceptable margin of exposure and generally this is lacking in the problem formulation. Lower tiers are associated with higher uncertainties and hence require larger margins of exposure compared to higher tier assessments, where more data are available or refined modelling approaches can be utilised. It is necessary to determine where the best options are for refinement of the groupings, and should this be based on hazard or on exposure. In practice, this will be determined by both scientific and by policy considerations.

Problem formulation should stipulate the degree of discrimination required, i.e. what level of uncertainty is acceptable and hence what margin of exposure is acceptable as a threshold for regulatory consideration at each tier. This is a risk management issue, but is often not stated explicitly. With the move to probabilistic approaches, particularly for exposure (see discussion below), agreement will be needed on which percentile (or percentiles) should be assessed within each tier, for the distributions used (e.g. population exposure level, commodity consumption, incidence of toxicological effect).

The value of mapping the risk assessment tools developed by IPCS against the various tiers for assessment of combined exposures was emphasised.

There are issues with the terminology used in cumulative risk assessment, which is still not harmonised.

Exposure considerations

Key messages

- Problem formulation and available risk management options shape exposure considerations
- Both toxicokinetics and toxicodynamics need to be taken into account
- Chemicals should be grouped based on relevant use patterns and biological characteristics

The second and third sessions, introduced by Alan Boobis were discussed together. These were on: what is the **definition of an exposure combination of concern**, i.e. what is the chemical domain of

[1] Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV and Vickers C (2011). Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul Toxicol Pharmacol 60: S1-S14.

concern taking account of “legislative/regulatory silos” and **what is meant by co-exposure** (i.e. how should toxicokinetic and toxicodynamic considerations be taken into account).

There was general recognition that humans are exposed to a wide variety of chemicals from many categories of product, by many routes. Exposure levels vary markedly. In general, different categories of chemical are regulated under different legislation and often by different departments/agencies with little or no interaction.

The scenario determining the exposure combination of concern should be identified in problem formulation. Is the objective of the assessment limit setting – where issues of product approval and permitted conditions of use are factors, or is it determination of the risk of the population to actual exposures – where consideration needs to be given to existing scenarios and to the change that would result from the introduction of a new product. An important issues is what risk management options are available and feasible.

In considering co-exposure, exposure to different chemicals may occur simultaneously in time and space (e.g. pre-formed mixtures), separated by time, separated by space or separated by both. One possible definition of co-exposure would be chemical exposure in space and time such that there is simultaneous systemic exposure to, or simultaneous effects of, more than one chemical. This would require consideration not only of toxicokinetics but also of the persistence and reversibility of the toxicodynamic response.

Examples are known of chemical combinations where, for any combined effect, exposure has to be at the same time and space due to rapid elimination and reversibility; where there can be separation of some time or space between exposure to the different chemicals due to slow elimination and/or slow reversibility; or where there can be a considerable separation between exposures (months or years), for example cancer initiation and promotion. This has implications for the scope of a combined assessment and is therefore critically dependent on problem formulation – the objective of the assessment and the options that would be available. It will also impact on how assessment groups are constructed. For example, the chemical grouping that would need to be considered for possible initiation/promotion interactions would be very different from that needed to consider acute additive effects from simultaneous exposure.

How should the toxicokinetic and toxicodynamic characteristics of chemicals in an assessment group be assessed and taken into account when exposure is separated in time and/or space? In the case of toxicokinetics, information will often be available (or can be predicted) on persistence, e.g. half-life. For toxicodynamics, it will be important to consider the nature of the effect, for example the MOA, reversibility, role of adaptation and repair, indirect effects (e.g. cardiac toxicity influencing renal function). It will also be necessary to consider potential windows of susceptibility, for example during early development.

Information on the use profile of chemicals will be of value in assessing the likelihood of co-exposure. Depending on the scope of the assessment, if this becomes very broad, agreement will be needed on default assumptions regarding co-exposure. Methods are being developed to determine which real world combinations of chemicals co-occur in food, using probabilistic approaches (see below).

For pesticide residues and residues of veterinary drugs, levels in food are generally very low however for other chemicals this is not always the case. For these, regional use profiles would be of value, for example for food additives, although this information is often not available in some parts of the world. Use of common methodology to obtain and evaluate such data would be beneficial.

Biomonitoring data is invaluable in determining real-world co-exposures, as it provides direct information on the nature and levels of systemic co-exposure occurring in individuals and it takes into account multiple routes of exposure (food intake, inhalation, dermal contact).

Advances in computational biology will result in the increasing use of modelling to predict the effects of combined exposures. This brings with it a number of additional issues with respect to any international harmonisation, but this aspect was not discussed further at the first workshop.

Formation of cumulative assessment groups requires some biological basis for grouping. However, prior to considering the biological effects of chemicals, an alternative would be to consider likelihood of co-exposure and the levels of exposure occurring. Most authorities group on hazard first and then consider exposure, but this is for specific chemical sectors, where there is a limited number of chemicals in scope (e.g. pesticides). Chemical groups based on biology could be assessed using a tiered approach, taking account of potency, MOA and exposure.

Between the two options, group by biology, followed by consideration of exposure and group by co-exposure, followed by consideration of biology, it is likely that the choice will depend on problem formulation. Harmonisation on this should be possible. For example, in assessing pesticides it could be agreed that the first approach should be adopted.

Cumulative assessment groups

Key messages

- There is a need to harmonise how chemicals are combined into assessment groups
- The rationale for an assessment group needs to be clearly defined, whatever its basis
- While synergy is highly unlikely, guidance should be developed to help consider it as needed
- The use of data generated using non-animal methods will need careful integration into the entire weight-of-evidence

The second day started with a session on **how should chemicals be combined into assessment groups**, introduced by Angelo Moretto. This is an area where there is currently little international harmonisation. Amongst the key issues that need resolution are whether an inclusion approach (as used by US OPP) or an exclusion approach (as proposed by EFSA's Pesticides Unit) should be employed and how information on MOA/AOP should be used to inform the assessment of combined exposure to chemicals. Additional areas where there could be an improvement in consistency across authorities are: the information used as the basis of grouping chemicals (e.g. chemistry, function/target, common phenotypic effect, common MOA/AOP, some combination of these), common understanding on what is meant by a shared mode of action, the minimum information required to include or exclude a shared mode of action and related uncertainty, and whether the relative potency between the common and the critical effect should be taken into account in some way. In addition, there is the question of how rare but possible synergy (or inhibitory interactions) should be addressed. Finally, agreement is needed on what the default assumptions (e.g. dose-addition or response addition, when the possibility of synergy needs to be considered) should be regarding combined action.

It was noted that assessment groups based on common target organ (e.g. liver) or even phenotypic effect (e.g. hepatic steatosis) can lead to large groupings, even for chemical sectors with less than 1000 members in total, such as pesticides. An appreciable number of compounds belong to more than one CAG, based on phenotypic effect, but some of these effects form part of a toxicological continuum so should not be treated independently. Given that the focus of EuroMix and many other initiatives is the use of non-animal methods for regulatory toxicology, there will need to be agreement on how these methods can be used to help in grouping of chemicals based on AOPs.

What type and how much information would be needed? Perhaps of equal importance to demonstrating that compounds share the same AOP, it will be important than non-animal methods can be used to exclude involvement in a shared AOP. It will be necessary to determine the confidence in such a conclusion.

EFSA will conduct a cumulative risk assessment for two of its assessment groups (thyroid and neurotoxicity) in Q3-Q4, 2017, using monitoring data to inform the exposure assessment. In the meantime, the assessment groups based on the other target organs will be developed one by one. When all assessments groups have been established and an impact assessment completed application in MRL setting will commence. EFSA have indicated that when relevant information on MOA is available this will be taken into account in cumulative risk assessment. It is likely that EFSA will identify options or make recommendations for research to refine its CAGs before their use in a regulatory context. In this respect, EFSA and DG SANTE are working in close cooperation to determine the fitness-for-purpose of the methodology developed for the regulation of pesticides.

Some authorities such as US EPA OPP have used chemical structure as one of the criteria for grouping chemicals for cumulative risk assessment. However, use of such information is nuanced and not as transparent as it might be. Compounds with the same structure may be excluded from a group but the reasons for this (e.g. because exposure is negligible) are not always obvious from the assessment report

Adoption of non-animal methods will necessitate consideration of the possible role of metabolism in the cumulative effects of chemicals. The parent compound may be converted to a metabolite in vivo, e.g. in the rat or human, which is not produced in the non-animal models used. As this metabolite might share an MOA/AOP with an assessment group, separate evaluation of such a possibility will be needed. Metabolic prediction software can be used to assess the potential formation of reactive metabolites and though perhaps not as reliably, the potential formation of stable metabolites. An alternative in the latter case is to test metabolites identified in plant or target species using non-animal methods, to assess whether they share AOPs with other chemicals.

EuroMix is developing novel approaches and methodology for combined exposure assessment (see below). With increasing reliance on non-animal methods, quantitative exposure assessment will assume critical importance. There is a need to extrapolate from in vitro findings to the in vivo situation. Chemicals may activate key events in vitro but produce no effect in vivo, because the necessary concentration for the effect is not achieved at the active site. Hence physiologically-based pharmacokinetic modelling will play a key role as will consideration of the active site concentrations attained in individuals on exposure to the chemicals in an assessment group.

There is little international agreement on whether or how to take potency for the common effect into account in developing or refining assessment groups. One possibility is to compare the potency for the common effect amongst members of a CAG. Those compounds with a very low potency (e.g. as judged using the RISK21 methodology[2]) could then be considered for exclusion from the CAG in order to prioritise potential risk management focus on those compounds of higher concern. The need for such an approach will depend, in part, on the total number of chemicals to be addressed in the assessment. If any compound exceeds its respective health based guidance value (ARfD, ADI, etc), it would be logical to exclude it from consideration of the risk from the combined effects of this CAG, until risk management measures have been taken to address concerns about this compound. The potency for the common effect could also be compared with that for the critical effect (i.e. the effect that drives the establishment of health based guidance values) for the same chemical. Where

[2] Embry MR, Bachman AN, Bell DR, Boobis AR, Cohen SM, Dellarco M, Dewhurst IC, Doerrer NG, Hines RN, Moretto A, Pastoor TP, Phillips RD, Rowlands JC, Tanir JY, Wolf DC and Doe JE (2014). Risk assessment in the 21st century: roadmap and matrix. Crit Rev Toxicol 44, Suppl 3:6-16

the difference in potency for the two effects is very large, controlling exposure for the critical effect would ensure that the common effect from that chemical would be at a very low level. This is an option that might be considered in particular when dealing with combined exposure to a large number of environmental contaminants. Whilst the view was expressed by some that compounds should not be removed from a CAG on the basis of their relative risk, there may still be scope to explore conservative defaults (determined by database analysis). Decisions on whether or not to use any of these approaches would be the responsibility of risk managers, in discussion with risk assessors.

It was noted that determining the POD for a common effect, if is not the critical effect for that chemical, would take time and effort as intermediate effects are not subject to the same scrutiny and peer review as is the critical effect when conducting chemical risk assessment. It was noted that in the case of pesticides, EFSA was already preparing a list of common effects and their NOAELs for each member of its CAGs.

There was general agreement that whatever the basis used for grouping chemicals, this should be transparent and explicit, which has not always been the case. It should be clearly stated in the problem formulation.

There is appreciable variation in the choice of POD (e.g. BMDx, BMDLx, NOAEL) for cumulative risk assessment. There was agreement that as a minimum a consistent POD should be used for members of a CAG. However, there was no conclusion as to which POD should be used, though there is a scientific preference for the use of the BMD approach. Choice of POD will also impact on calculation of relative potency factors, as will the member of the CAG selected for this purpose (index compound). In addition, there is a lack of consistency in the criteria used for index compound selection, although it is generally preferred that this is a well-studied compound, in order to minimise the uncertainty in the hazard characterisation.

There is currently no consistent method for assessing the potential for chemicals in an assessment group to act synergistically. However, most authorities (e.g. EFSA, USA EPA) have concluded, based on scientific review of the available information, that this is not an issue of concern at human relevant exposures to dietary residues. The possibility of synergy should be considered on a case-by-case basis, but consistent guidance for how this might be done is lacking.

For chemicals with internationally accepted limit values, such a pesticides, there is a need for harmonisation of the approaches used to establish assessment groups.

The European Commission is currently discussing how to apply cumulative risk assessment methodology for pesticide MRL setting.

Exposure assessment

Key messages

- Refinements in exposure assessments are ongoing, with a shift in focus to probabilistic methods, and in particular to individual co-exposures
- Harmonisation of probabilistic exposure assessments will compliment efforts to harmonise how chemicals are combined into assessment groups.

The last topic addressed was **exposure assessment**, introduced by Jacob van Klaveren. What methodology should be used and what assumptions are made?

Currently, a deterministic approach is used for exposure assessment of individual pesticides in Europe, using the PRIMo model. This model is derived from 10 diets with uncertain consumption data. Information from 52 diets is now available within the EFSA data warehouse, which can be used fully probabilistically via web-based interfaces with calculation times of only a few hours, but it is not yet being used in pesticide risk assessment. Deterministic approaches such as PRIMo have a number of significant limitations, particularly for cumulative exposure assessment. However, until recently it was not possible to change the approach used in Europe within the regulatory context, for several reasons including reproducibility of the modelling approach.

The US EPA developed probabilistic approaches some time ago and has been applying them routinely for cumulative risk assessment of pesticides. In Europe, the Acropolis project developed the MCRA tool for this purpose, and this is now at the stage for application in cumulative risk assessment. In addition, EFSA has published guidance on the use of probabilistic methodology for modelling dietary exposure to pesticide residues.

RIVM, in collaboration with EFSA, have now used the MCRA tool to assess cumulative exposure to the EFSA CAGs for neurotoxicity and thyroid effects, in 10 populations of consumers (similar to the diets upon which PRIMo is based). Using the substantial computer power available to RIVM, which can be uprated such that all 52 European diets can be included, the computations took only 6 h.

Amongst gaps identified in conducting such assessments were the lack of some processing factors, absence of data on real agricultural use, and a clear definition of what is meant by co-exposure.

MCRA can be combined with the IPRA (Integrated Probabilistic Risk Assessment) tool developed in the Netherlands to provide an integrated probabilistic assessment of cumulative risk, based on the distribution of MOEs (margins of exposure).

There are a number of approaches and assumptions that can be used in probabilistic assessments. There is little or no harmonisation at present, as there has been no pressing need. However, to compliment harmonisation efforts for the hazard assessment of combined exposures, consideration will need to be given of what needs to be harmonised in probabilistic exposure assessment and how this might be achieved. Currently, DG SANTE is working together with the European Member States on harmonising some of these issues and they will use MCRA for this. In addition to the probabilistic method used, harmonisation of reporting will be important as will the structure of input data, e.g. consumption, to enable inter-regional comparisons and data-sharing. It was agreed that, for implementation on a global scale, there first needs to be recognition and harmonisation of the use of probabilistic modelling, followed then by software harmonisation. The use of probabilistic modelling was explored by the Codex Alimentarius in the period 2000-2005, but at that time consumption data were lacking, underlying assumptions and formats were not fully understood and suitable web-based models were not available. In a number of countries outside Europe, MCRA and other software packages have been explored to generate probabilistic results at the national level (e.g. China, Brazil). Furthermore, the use of probabilistic modelling has been explored by a number of stakeholders. The first probabilistic results were generated by NGOs in the US and in Europe twenty years ago. MCRA and/or other probabilistic software can be used to explore the issues that need to be harmonised in the EuroMix harmonisation workshops.

EuroMix will define templates for data input and links to other web-services, and will provide a computing platform for probabilistic exposure assessment, openly accessible to all stakeholders. Work is ongoing to input data on chemicals, in addition to pesticides, such as food additives, dioxins and PCBs, heavy metals and BPA from the EuroMix partners. These data are similar to those sent to

EFSA by the Member States and stored in the EFSA data warehouse. The preferred option is to work closely with EFSA on data quality and further refinement of the web-services and model platform infrastructure.

As part of the EuroMix project, a proof-of-principle study on 140 subjects will be performed in Norway. Information will be collected on exposure including the diet, biomarkers of exposure and of effect. There are links with other major ongoing exposure projects - EU HBM4ME and the Human Exposome. This study will test the predictions of the various EuroMix models for combined exposure via multiple exposure routes. .

It was noted that the mixture selection functionality in MCRA is a useful addition to probabilistic modelling and helpful in selecting the chemicals for the experimental studies to be conducted within EuroMix. Generally, this helps in setting priorities for testing based on exposure considerations. This might underpin an exposure driven test strategy and would form the starting point to calculate the likelihood of co-exposure. The MCR (Maxium Cumulative Ratio) approach has been included, but use of this approach for human health risk assessment has not yet been explored. This will require further discussion (see above).

Conclusions and next steps

The meeting closed with a brief summary of **conclusions and next steps**. It was agreed that harmonisation of the approach used in assessing the risk from combined exposures to chemicals was highly desirable and in some areas such as pesticides it was essential, to ensure the safe and effective continuation of international trade in food commodities. A number of key issues were identified where harmonisation has yet to be achieved, such as the scope of cumulative risk assessments (which “silos”), the basis for grouping chemicals into assessment groups, and how information on modes of action/adverse outcome pathways would be taken into account in such assessments. These topics will be discussed in more detail at later workshops in this series. The next workshop will include risk managers and will focus on impending and future legislation and how and when the approaches and methods developed by EuroMix can contribute.

It was agreed that the review of relevant legislation prepared as deliverable 9.1 should be circulated to participants as soon as possible, to check on accuracy and to fill any gaps.

Annex

EUROMIX First workshop on international harmonisation on the risk assessment of combined exposures to chemicals

Celia Hensman Suite, W12 Conferences, Imperial College London, Hammersmith Campus, Artillery Lane, 150 Du Cane Road, London W12 0HS

20 -21 October 2016

Program

The designated lead will provide a brief introduction to each topic, followed by discussion on common approaches, identification of gaps and possible ways forward

Day 1

14:00 – 15:00: Problem formulation: what is the objective of risk assessment of combined exposure from an international perspective (e.g. harmonisation of methodology, harmonisation of approach to setting MRLs) (Lead: Dr Bette Meek)

15:00 – 16:00: Definition of exposure combination of concern – which chemicals ("legislative/regulatory silos") (Lead: Prof Alan R Boobis)

16:00-16:30: Break

16:30 – 17:30: What is meant by co-exposure (toxicokinetic and toxicodynamic considerations) (Lead: Prof Alan R Boobis)

Day 2

How should chemicals be combined into assessment groups? (Lead: Prof Angelo Moretto)

09:00 – 10:00: Inclusion versus exclusion approach? Assumptions re additivity? Bases for grouping (e.g. chemistry, common effect, common MOA/AOP, function/target)

10:00-10:30: Break

10:30 – 11:30: Use of information on mode of action/AOP (what is meant by common mode of action)? Minimum information to include or exclude common mode of action?

11:30 – 12:30: Potency considerations: common versus critical effect

12:30 – 13:30: Lunch

13:30 – 14:30: How should possible synergy be addressed?

14:30-15:30: Exposure assessment methodology and assumptions? (Lead: Dr Jacob van Klaveren)

15:30-16:00: Break

16:00-17:00: Conclusions and next steps

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Report of EuroMix Second Workshop on International Harmonisation on the Risk Assessment of Combined Exposures to Chemicals

17 May 2017, Thon Hotel EU, Rue de la Loi 75, 1040 Bruxelles, Belgium

Background

EuroMix organised the second of a series of workshops on the international harmonisation of the risk assessment of combined exposures to chemicals on 17 May, 2017 at the Thon Hotel EU, Brussels, Belgium. The specific objectives of the workshop were to discuss current and impending regulation, across different chemical sectors (e.g. pesticides, contaminants) and regions (e.g. USA, Europe) and how and when new science might impact on future regulation. The necessary steps to implement an internationally harmonised, scientific approach to the risk assessment of combined exposures to chemicals in the diet in relevant legislation were explored. The focus of the meeting was on those policies impacting not only on public health but also on international trade of food commodities. The meeting also sought to identify those topics of most relevance for further consideration at the next workshop in the series. Participants involved experts from North America, Europe and South America, as well as national and international organisations such the European Commission (DG SANTE, DG Environment), EFSA, OECD, Codex Alimentarius, WHO, US FDA and US EPA. The programme of the workshop is provided in the Annex. The following individuals attended the workshop:

Name	Country/Region	Organisation
Alan Boobis	UK	Imperial College London
Annamaria Bruno	International	Codex Alimentarius
Evisabel Craig	USA	US EPA
Jean-Lou Dorne	Europe	EFSA
Eloisa Dutra Caldas	Brasil	University of Brasilia
Suzanne Fitzpatrick	USA	US FDA
Peter Korytar	Europe	DG Environment
Eeva Leinala	International	OECD
Bette Meek	Canada	University of Ottawa
Angelo Moretto	Italy	University of Milan
Paul Price	USA	US EPA
Stefanie Rotter	Germany	The German Federal Institute for Risk Assessment (BfR)
Jiri Sochar	Europe	DG SANTE

Roland Solecki	Germany	The German Federal Institute for Risk Assessment (BfR)
Jacob van Klaveren	The Netherlands	The Netherlands National Institute for Public Health and the Environment (RIVM)
Veerle Vanheusden	Europe	DG SANTE
Philippe Verger	International	WHO
Frans Verstraete	Europe	DG SANTE
Andrew Worth	Europe	The Joint Research Centre of the European Commission (JRC)

The meeting room was arranged in board room style. The meeting was chaired overall by Alan Boobis. Stephanie Rotter served as rapporteur together with Alan Boobis. The meeting started with participants introducing themselves. **Alan Boobis** then provided a brief introduction to the objectives of the workshop, which were: to understand current and upcoming legislative needs for cumulative risk assessment of chemicals (with a focus on the diet); how this varies across chemical sectors (e.g. pesticides, additives, contaminants) and the extent to which this might be harmonised; how this varies across geographical regions and the opportunities for harmonisation; the role that scientific research, and particularly that of EuroMix, might play in the development and implementation of legislation in this area. The meeting was organised into three sessions. Session 1 was on current and impending legislation in the area of cumulative risk assessment. Session 2 was on the potential contribution from EuroMix. Session 3 was on implementation of EuroMix advances. During each session, a number of speakers presented their perspectives, each followed by discussion. Copies of the introductory presentations are available from the EuroMix website.

Session 1: Current and impending legislation

The session opened with an **analysis of legal requirements for mixtures of chemicals** both within and outside Europe, together with a review of current frameworks and research for cumulative risk assessment of chemicals. In general, whilst mixture risk assessment is required in a number of regulatory sectors and geographical regions for intentional mixtures (e.g. formulations), this is not always required. Even where mandated, testing of the mixture itself is not always necessary, but a prediction from the components would be accepted. Where there is a legislative requirement to assess the risks of mixtures, guidance is not always available. In some chemical sectors, assessment of certain unintended/incidental mixtures is required, for example run-off from contaminated sites (Superfund sites in USA) and for pesticides in the USA and in Europe, where suitable methodology is under development. Several different approaches are being used to group chemicals for cumulative risk assessment, and this is an area where ongoing research could be very informative. Several frameworks have been developed for cumulative risk assessment, most utilising a tiered approach. Both OECD and EFSA are developing new, overarching frameworks for cumulative risk assessment. A significant limitation of the tiered approach is the lack of relevant information and hence, it is often not possible to progress to higher tiers. Various possibilities have been discussed, such as use of the threshold of toxicological concern (TTC) and application of an additional uncertainty factor to allow for possible exposure to additional chemicals sharing toxicological effects. Often, exposure from uses of the same chemical in different regulatory sectors and/or by different routes (aggregate exposure) is not taken into account. One approach to this is to reserve a fraction of the health based guidance

value, but a better solution would be more accurate exposure assessment. Identifying the key drivers (active substances) responsible for cumulative risk would enable focussed risk management with most impact.

The session continued with a **summary of the work undertaken by the Joint Research Centre** (JRC) of the European Commission as follow-up actions to the Commission Communication on the combined effects of chemicals (COM(2012)252 final), to support the Fitness check of chemicals legislation (REFIT) and as part of the 7th Environment Action Programme – a strategy for a non-toxic environment. To date, JRC has conducted a review of regulatory requirements and guidance, an expert survey, a review of novel approaches and a review of literature case studies on the assessment of chemical mixtures. Currently, JRC is conducting experimental case studies on mixtures of developmental neurotoxicants and of (anti-)androgenic compounds, a literature review of physiologically-based toxicokinetic (PBTK) models for mixtures, a case study on the use of human biomonitoring data and biomonitoring equivalents and a systematic literature review and evaluation of evidence for interactions between environmental chemicals. In an effort to increase harmonisation of assessment, JRC is developing an uncertainty framework for risk assessment of combined exposures, that will provide a transparent means of documenting the entire workflow, including problem formulation, assumptions, constraints, methodological choices, conclusions, and identification and characterisation of uncertainties.

A key issue is how emerging methods in toxicology, such as high throughput screens, will be used in cumulative risk assessment. This will likely be linked to key events in AOPs, and ongoing work within EuroMix should establish proof of principle, but harmonisation on the application of such methods in cumulative risk assessment will need further discussion. Similarly, the incorporation of information on systemic exposure, including the use of PBTK models, will require further discussion.

Problem formulation in cumulative risk assessment is critical. It is therefore important that the frameworks used are flexible and there is a suite of tools to deal with range of policy needs.

EU approaches to the **assessment of the cumulative exposure to contaminants** in food were reviewed, following an introduction by DG SANTE. EFSA is already addressing the risk from mixtures of contaminants, to a certain extent, through the scientific advice provided by the CONTAM Panel to DG SANTE. There are no *a priori* criteria for grouping. This is case-by-case, based on exposure, structural and toxicological considerations; the criteria used being clearly explained in the advice provided. Examples include dioxins and dioxin-like compounds (the Toxic Equivalency Factor or TEF approach), non-dioxin-like PCBs (6 markers substances out of 197 possible congeners), polycyclic aromatic hydrocarbons (marker substance approach), brominated flame retardants (e.g. PBDEs, PBBs) and perfluorinated alkylated substances. In addition, several groups of related mycotoxins have been assessed for their respective combined risks. These include aflatoxins, fumonisins, zearalenone and related toxins, ergot alkaloids, pyrrolizidine alkaloids and tropane alkaloids. There are considerable difficulties in such assessments due to the lack of data on toxicity and occurrence, analytical issues and other uncertainties. Presently, the risk assessment and risk management of mixtures of structurally and toxicologically “similar” contaminants are being addressed to some extent, albeit with considerable difficulties and uncertainty. However, the risk assessment and risk management of mixtures of "non-similar" contaminants (e.g. different mycotoxins, different metals) is not yet being addressed.

One of the difficulties is that only those contaminants that are monitored can be controlled, and the choice of which contaminants to monitor is based on feasibility and the relevance of individual compounds to health. Risk management is based on a pragmatic view of the relative importance of exposure to related compounds, e.g. among fumonisins it was decided to address exposure to only

fumonisins B1 and B2, as B3 is only a minor constituent. Work is ongoing to address dual use of veterinary drugs and pesticides, where there may be co-exposure to residues from both uses.

Current approaches in the EU to the **assessment of combined exposure of food additives** were then discussed following an introduction by DG SANTE. At present, there is only limited consideration of the risk from such combined exposures, and there is no consideration of the risk in combination with chemicals from other uses. Within the EU, there is a very specific definition of "food additives", which are substances added to food for technological purposes. Other substances, such as flavours and vitamins, are excluded. In some other parts of the world, the term is used more broadly and in some cases, applies to any substance added to food. Food additives require approval (authorisation) before marketing, part of which includes their safety assessment. Substances that are classified as CMR (carcinogenic, mutagenic or toxic to reproduction) will not be approved (but this excludes impurities). Some combination effects are taken into account, for example, certain food colourings, mixtures of benzoate and ascorbate, which can lead to UV-catalysed formation of benzene, and group ADIs for compounds that share a mode of action, e.g. phosphates, sorbates, benzoates. In the case of caramel colours, three of these have been combined for risk assessment and one other has been considered separately, due to differences in their characteristics. In general, substances with completely different structures and toxicological effects would not be considered together, though if there some reason for concern this is permitted within the legislation. Examples would be when mechanistic consideration of toxicokinetics or toxicodynamics indicates some potential for interaction. Some food additives contain secondary food additives, which will enter the food chain. The possible risk from such chemical combinations is not currently assessed by EFSA.

Previous and ongoing **work at EFSA on generic approaches to cumulative risk assessment** were reviewed. The Panel on Plant Protection Products has published a number of opinions on the risk assessment of combined exposure to residues of pesticides and is currently compiling information on assessment groups based on phenotypic endpoints. The Scientific Committee of EFSA published an opinion in which a generic approach to cumulative risk assessment was described. These outputs were discussed at a scientific colloquium in 2014, which served to inform a new activity of the Scientific Committee, the development of guidance on harmonised risk assessment methodologies for human and ecological risk assessment of combined exposure to multiple chemicals. A tiered approach will be used. Areas where harmonisation is not possible will be identified. Information and models are being developed to improve toxicokinetic assessments, which will also help identify the possibility of interactions.

DG Environment's perspective on cumulative risk assessment and ongoing activities within the EU were next discussed. A key focus is the EC Communication of 2012 on the Combined Effects of Chemicals (COM(2012)252 final). Whilst methodology already exists for assessing the risks from combined exposures to chemicals, a substantial limitation is the paucity of available data, particularly on occurrence of the chemicals. In addition, there is currently no systematic process for assessing combined risks across the range of chemicals to which humans are exposed. To help address this, an inter-service group has been established to promote cross-sector activity, but progress to date has been somewhat limited. Nor has guidance across regulations yet been developed. However, the issue of mixtures more generally is currently under review.

Horizon 2020 is supporting a number of research projects, e.g. EuroMix, to expand the tools and approaches necessary for cumulative risk assessment. In addition, efforts are underway to improve the availability of occurrence data through IPCheM (EU Information Platform for Chemical Monitoring). All relevant EU databases have been connected to this portal and information on chemicals in food, the environment, indoor and outdoor air and from human biomonitoring studies is

available. Both monitoring data and research data on chemical occurrence should be available from IPCheM.

Whilst REACH does not address all possible incidental/unintentional mixtures routinely, this is undertaken if required, e.g. phthalates. The risk management of industrial substances comprising intentional mixtures already considers possible combined effects of the constituents. However, whilst a whole mixture approach is taken to the registration of multi-component substances of unknown composition, environmental monitoring is problematic, as the most toxicologically relevant compounds are often not known.

In the Water Framework Directive, the cumulative risk of groups of structurally-related chemicals is assessed, analogous to the approach taken for contaminants in food. Effect-based tools can also be used on the whole mixture (water sample). If positive, identification of the chemical(s) contributing most to the effect would enable risk management.

A number of additional activities relevant to the cumulative risk assessment and risk management of chemical mixtures are underway. In the Fitness Check of Chemicals Legislation (Regulatory Fitness and Performance Programme, REFIT), the fitness-for-purpose of current frameworks, including those for mixture risk assessment, are being assessed. REFIT is due for completion by the end of 2017. Also, under the 7th Environment Action Programme, to help achieve the objective of a non-toxic environment, the European Commission should, by 2018, develop a strategy to minimise exposure to endocrine disrupting chemicals; and to chemicals in products; to address the safety of nanomaterials; and combination effects of chemicals and minimise exposure. The strategies proposed will need to be agreed by Member States.

Harmonisation across chemical sectors will not be possible overnight, and is best achieved step by step. Risk assessment in the European Union is science-based. The legislation reflects the state of the science. Hence, scientists need to understand the frameworks, for example for risk assessment of combined exposures to chemicals. To facilitate the implementation and utilisation of such frameworks, researchers should develop suitable tools for this purpose. This is one of the key objectives of EuroMix.

In mixture risk assessment, profiling of chemicals is important. This may be for toxicology, but also for exposure, depending on the framework. One possibility is exposure banding, or worst-case exposure estimates (cf TTC). A case study of an incidental/unintended mixture where the chemicals are regulated under different legislative mandates (sectors) would be of value. The default assumption would be concentration/dose addition for chemicals with a similar mode of action.

The approach being implemented in the EU for **the cumulative risk assessment of dietary exposure to pesticides residues** was outlined by DG SANTE and discussed. The need for cumulative risk assessment of pesticide residues as part of the approval process is mandated by European legislation (Reg. (EC) No. 1107/2009 and Reg. (EC) No. 396/2005), with the proviso that the methods used must be scientifically acceptable by the Authority. It is envisaged that once developed, cumulative risk assessment of pesticide residues will be used for several different purposes: approval of active substances, MRL setting, authorisation of PPPs, assessment of high residue events and annual reviews of monitoring data.

EFSA is currently finalising cumulative assessment groups (CAGs) for hazard assessment of combined exposures to pesticides. These are based on grouping for common target organ/system effect (pathological outcome). The first target organs addressed were the nervous system and the thyroid. In additional CAGs for effects on the liver, reproduction and development, the adrenal and the eye are

being prepared. There are several (up to 16) CAGs for each target organ/system, because of the number of distinct toxicological/pathological outcomes. Some of the CAGs are quite large, comprising over 100 chemicals.

EFSA decided to group pesticides that could plausibly act in combination, causing a common specific adverse effect, rather than on mode of action. In part, this was because modes of action are often unknown. There is also concern that compounds acting by different modes of action might still contribute to the common adverse effect and use of common effect for grouping would be precautionary. However, this would give rise to the potential to overestimate the risk for acute exposure. It is envisaged that further refinement might be possible when more detailed information on toxicological modes of action becomes available. In addition, PBTK and PBTD modelling might be utilised as a further development

Relative potency factors (RPFs) will be added to updated annexes on the cumulative assessment groups (CAGs) for effects on the nervous system and the thyroid by the end of 2017. This will be followed by RPFs for the CAGs for effects on the liver, reproduction and development, adrenal and eye. The approach used for determining RPFs for members of a CAGs was discussed. If this is to be based on the common effect (use of the critical effect would be very conservative), agreement will be needed on how the points of departure for the common effect are to be determined. Since this POD will not have been discussed in establishment of the ADI/ARfD, separate consensus will be needed, which could be very resource intensive.

Exposure assessment will be performed probabilistically, using the ACROPOLIS on-line IT tool, which is referred to as the Monte Carlo Risk Assessment (MCRA) software, for this purpose. The assumption is that each component of the CAG contributes to the combined effect in proportion to its exposure and potency for the common effect, based on the assumption of dose addition.

Once the assessment groups have been agreed, the methodology will first be applied to the risk assessment of consumers, based on the exposure assessments in the annual report (the European Union Report on Pesticide Residues in Food, prepared by EFSA). Longer term, the intention is to use the methodology for regulatory purposes, i.e. for pesticide approvals and MRL setting. However, this will depend on the demonstration of the fitness-for-purpose of the methodology, development of detailed procedures, completion of the establishment of all CAGs by EFSA, and an assessment of the new methodology for its impact on health, agriculture and international trade.

There are a number of risk management decisions involved in final implementation of the methodology. These include the assumptions to be made, e.g. on non-detects; imputation of missing values; variability factor to be used; information on use, processing, etc; which toxicological values to use; consumption and occurrence data; exposure distribution confidence interval. These issues have been discussed by a working group of DG SANTE and the Member States and many of them were resolved. The working group also agreed that the combined margin of exposure should be used for expressing the risk, with a probabilistic assessment, rather than an ADI or ARfD for the CAG (the margin of exposure is the ratio between the estimated exposure and a relevant toxicological endpoint taken from an animal study). In addition, a threshold for regulatory consideration should be identified: X^{th} percentile of the population should have a combined margin of exposure above Y. The working group proposed a two-stage approach, in which a conservative scenario would first be assessed, followed by a less conservative scenario, should a potential risk be identified with the first scenario. If a potential risk is identified in the second scenario, risk management decisions will need to be taken as to whether regulatory action is necessary, taking into account the uncertainties in the assessment, or whether further, refined analyses should be undertaken.

Following this discussion of EU approaches to cumulative risk assessment, the meeting addressed some of the approaches in use internationally, starting with the **US EPA approach to the cumulative risk assessment of pesticides**. EPA defines cumulative risk as "the risk of a common toxic effect associated with concurrent exposure by all relevant pathways and routes of exposure to a group of chemicals that share a common mechanism of toxicity." To date, cumulative risk assessment has been performed on five common mechanism groups (CMGs): organophosphates, N-methyl carbamates, triazines, chloroacetanilides, and pyrethrins/pyrethroids. Establishing such CMGs is very data and resource intensive. Hence, EPA has recently introduced a screening framework for cumulative risk assessment to assist in identifying potential candidate CMGs and conducting screening-level assessments (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework). The framework follows the same principles as the WHO/IPCS framework. All pesticides undergoing registration review are assessed for membership of a candidate CMG (or an existing CMG), on the basis of pesticidal MOA, structural similarity, target organ toxicity and apical outcomes, and MOA for mammalian toxicity, on the basis of submitted data and published information. It is envisaged that high throughput screening in ToxCast will be of value in candidate CMG construction. If screening indicates that the evidence is against a group of pesticides sharing a common mechanism, no cumulative risk assessment is necessary. If there is evidence for a common mechanism, the candidate CMG is subject to screening level toxicology and exposure assessment. If the margin of exposure is not adequate, further refinement of exposure and/or toxicity is needed. If sufficient evidence is available for a MOA/AOP and the causal key events, a CMG is established and assessed. Screening level assessments are currently being performed for multiple candidate CMGs, including the mectins.

In the US, substances that are not detected in monitoring for residues are excluded from cumulative risk assessments and chemicals must share a common mechanism of toxicity to be included in a CMG, which contrasts with the approach used/proposed in the EU. It is apparent that there are significant differences between the EU and the USA in the approaches being taken to group pesticides for cumulative risk assessment. If the European and US criteria lead to very different group sizes, cumulative risk assessment is likely to result in different conclusions on human health protection. Ideally, common criteria for grouping chemicals should be developed and applied, based on fundamental scientific principles.

A brief explanation of how the **Codex Alimentarius Commission addresses risks from exposure to chemicals in food** was provided. Codex Alimentarius develops food standards that, whilst not mandatory, are the benchmark for international harmonisation to protect human health and ensure fair practices in the food trade. These standards are based on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, in order that they assure the quality and safety of the food supply. Codex Alimentarius is responsible for risk management advice, relying on input from WHO/FAO scientific advisory committees for risk assessment, i.e. JECFA, JMPR, JEMRA, JEMNU, and ad hoc expert consultations on emerging issues. Lower tier assessments are often very conservative, but the lack of data make it difficult or impossible to refine the assessment. Hence, balancing protection of human health whilst ensuring fair trading practices is complex.

To date, cumulative risk assessment has not been a major consideration by Codex Alimentarius or any of the WHO/FAO committees, although there are some instances where this has been undertaken, for example assessment of dioxins and dioxin-like compounds by JECFA. In addition, the topic has been discussed by the committees on a number of occasions. For example, JMPR noted in 2008 that it would continue to monitor ongoing activities in the field and eventually advise on the need for cumulative risk assessment for certain groups of pesticides. In 2014, following a request from the 46th

session of the Codex Committee on Pesticide Residues (CCPR), JMPR reviewed the various approaches for assessing cumulative risk of chemicals in food that are currently under development or in use worldwide. JMPR recommended that the Secretariat identify relevant developments in cumulative risk assessment and place them on the agenda for discussion at the next appropriate JMPR. Generic issues in the area of cumulative risk assessment are being explored by the WHO Chemical Risk Assessment Network Coordinating Group on Combined Exposures. At Codex Alimentarius level, discussion has started within the Codex Committee on Contaminants in Food on the need for cumulative risk assessment of certain groups of mycotoxins and the issue has been identified as an emerging one by the coordinating committee for Europe.

Session 2: Potential contribution from EuroMix/Session 3: Implementation of EuroMix advances

The session started with an outline of **approaches being developed within EuroMix for the assessment of combined exposure to chemicals**. EuroMix is developing a tiered approach to exposure assessment, comprising: screening tier, deterministic tier and Hazard Index approach, probabilistic approach, probabilistic approaches including likelihood of co-exposure. In the screening tier, chemicals are grouped based on QSARs, e.g. all food additives predicted to cause liver steatosis; worst case for hazard (e.g. TTC); rough estimate of exposure, e.g. worst case from deterministic assessment. If the MOE exceeds specified (high) value, e.g. 10,000, there might not be a need for testing, depending on the risk managers decision. This would be a highly unrealistic and conservative scenario. In the first tier, deterministic exposure models, such as those from EFSA, are used for each regulatory sector only. Examples include PRIMo for pesticides, the Food Additive Intake Model and the GEMS Food diets, which models are based on different conservative data and assumptions. There is no overarching deterministic approach covering all regulatory sectors and this will raise many practical challenges and/or extremely conservative outcomes. In higher tiers, probabilistic assessments of exposure are used, with random sampling from distributions of both consumption and occurrence. Guidance on the conduct of probabilistic exposure assessment has been published by EFSA and suitable software (MCRA) has been developed; access is freely available through a partnership between EFSA and RIVM for the member states involved in the in the implementation of cumulative risk assessment of pesticides. All consumption and monitoring data from all EU member States held by EFSA can be utilised in the modelling since EFSA has harmonised the formats. This will help in combining assessments over different regulatory sectors. Case studies on probabilistic assessments of combined exposure are currently being conducted within and across chemical sectors. A key factor is the available of relevant data, which varies markedly from chemical sector to chemical sector. This may necessitate imputation of missing values, the consequences of which are being explored. EuroMix is investigating a number of possible refinements, among which are: exposure driven approaches, inclusion of toxicokinetic information (e.g. is co-exposure likely?), use of information on AOPs to refine CAGs, integration of exposure and hazard estimates (deterministically and/or probabilistically), aggregate and combined exposure, comparison between calculated intake and observations in humans.

The final topic discussed was EuroMix research on **how to group chemicals for cumulative risk assessment**. The default assumption is that exposure to each individual compound in a CAG is below its respective health based guidance value (risk management considerations will apply to each chemical) and the combined effect of the group is a consequence of dose addition, unless there is good evidence otherwise. However, prior to such an assessment, consideration needs to be given to what is meant by common toxicity, the basis for grouping. For some chemicals, there is a wealth of information, data-rich compounds, whereas for others there is a dearth of information, data-poor

compounds. Approaches to formation of CAGs should be sufficiently flexible to recognise this, and take account of the available information in the various assessment tiers.

The approach adopted by EFSA for formation of CAGs for pesticides (data-rich compounds) comprises four levels: target organ (level 1), phenotypic effect (level 2); common MOA/AOP (level 3), common mechanism (level 4). In practice, the distinction between level 3 and level 4 has not been clearly defined, and it is likely that robust evidence for level 3 would make level 4 redundant. Inclusion in a CAG is independent of whether the common effect is the critical (i.e. basis of health based guidance value) effect or not. Currently, EFSA is working on constructing level 2 CAGs for pesticides. Those of the nervous system and the thyroid have been published and work is advanced on another 4 target organs/systems. Eventually, CAGs will be created for 15 different target organs/systems. As an example, over 200 pesticides have been identified that affect the liver. Eleven different level 2 CAGs have been created to cover these effects, for example hypertrophy (189 members), fatty change (steatosis) (106 members), cell degeneration/cell death (139 members). There is appreciable overlap in CAG membership. A key question is how/if information on AOPs can help refine the CAGs. EuroMix is working on several AOP-based case studies, one of which is liver steatosis. The different AOPs, with associated key events (KEs), responsible for steatosis have been mapped and methods for determining key event involvement are being developed and applied to selected compounds. An important question that EuroMix is seeking to address is whether effects on different MIEs/KEs "cumulate" at environmentally relevant doses (exposures). This is being investigated both in vitro and in vivo, over an appropriate range of exposures.

For data-poor compounds there are fewer options. For such compounds, in silico (QSARs and/or molecular docking simulations) and in vitro approaches may be necessary. EuroMix is investigating how/if to combine QSAR models that address adverse outcomes or specific KEs. It is likely that QSARs will be more specific for KEs (particularly the molecular initiating event, MIE) than for adverse outcomes, as there may be competing structural requirements for the MIEs leading to the same adverse outcome. In silico approaches could be used qualitatively, to assess the likelihood of (different levels of) CAG membership. Confidence in this approach can be enhanced by the use of multiple models.

Similarly, EuroMix is investigating how best to utilise in vitro information on KEs and/or MIEs to develop CAGs. Such information can be used to assess probability of belonging to level 3 CAGs and, *inter alia*, the probability that compounds will exhibit dose additivity.

Each predicted or measured data value and conclusion (e.g. CAG level 2 membership) has associated with it a degree of uncertainty. This needs to be addressed and quantified to the extent scientifically possible (e.g. EFSA framework, WHO/IPCS framework, Codex Alimentarius framework).

The most scientific approach to CAG membership would be the "retain and refine" method, in which broad criteria are used to identify CAG membership but members are then weighted (refine) for a number of probabilities (e.g. common AOP). However, the practicality of such an approach needs to be weighed against problem formulation. In some scenarios, the number of compounds involved and/or the time available to provide advice/take action, may mean that there are insufficient resources to pursue this approach in full (or even in part). However, in order to determine which pragmatic assumptions (e.g. exclusion of chemicals with different AOPs for the same adverse outcome) are still health protective, comparison with the 'full' model will be necessary. In this way, information on the degree of conservatism associated with different options can be obtained, and EuroMix will include such comparisons in its research programme. This will lead to the development of

a framework for cumulative risk assessment that is flexible and feasible, enabling a balance between precision and pragmatism, according to the problem formulation.

Once CAGs have been created, it will be necessary to develop relative potency factors. The utility of in silico and in vitro approaches for this purpose is also being investigated by EuroMix. Possible in silico approaches include use of the appropriate TTC value for the structural class of compound and distribution of the (predicted) point of departure within those for the CAG. In vitro, qualitative concentration-response data for KEs or the MIE, with appropriate in vitro to in vivo extrapolation, could be used.

Final discussion and conclusion

Currently, there is no overarching approach to cumulative risk assessment (CRA), either within the EU (across regulatory sectors) or internationally. Approaches to CRA vary across regulatory sectors and geographies vary, sometimes markedly. In some areas, CRA is currently not a significant consideration, whereas in others there is appreciable concern. However, even in the latter case, approaches utilised in different regions show appreciable differences. The most common approach to date for developing cumulative assessment groups is use of common structure and/or co-occurrence and/or designed function (e.g. pesticidal mode of action). EuroMix is exploring implications of different exposure and toxicology cut-offs for human health protection, both experimentally and by simulation.

Work is underway both within and beyond the EU to explore harmonisation of approaches to cumulative risk assessment within and across chemical sectors. Case studies will be invaluable here.

The next workshop will explore in more detail how the results of EuroMix can help further the international harmonisation of cumulative risk assessment.

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Second EuroMix workshop on international harmonisation on the risk assessment of combined exposures to chemicals

Program

The objective of the second workshop is to explore the necessary steps to implement a harmonised scientific approach to the risk assessment of combined exposures to chemicals in the diet in relevant legislation. The focus of the meeting should be on those policies impacting not only public health but also on international trade of food commodities.

08:30-17:15, 17 May 2017 Thon Hotel EU, Rue de la Loi 75, 1040 Bruxelles, Belgium

08:00-08:30	Welcome coffee and registration	
SESSION 1: Current and impending legislation		
Rapporteurs	Stefanie Rotter and Alan Boobis	
08:30-08:45	Introduction and objectives of meeting ***Alan R Boobis, Imperial College London***	
08:45-09:45	What legislation would have to be addressed? ***Roland Solecki, BfR, Germany***	20 min + 40 min discussion
09:45-10:30	Ongoing work on harmonisation ***Andrew Worth, JRC, Italy***	20 min + 25 min discussion
10:30-11:00	Refreshment break	
11.00-13.00	Perspectives of risk managers on: - need for cumulative risk assessment - difficulties in implementing management of combined exposures to chemicals - precautionary principle in current and future approaches - what do risk managers need from science	Input from DG SANTE on pesticide risk management contaminant risk management and additive risk management, DG Environment on environmental contamination, Codex Alimentarius on chemicals in food and US-EPA on mixture risk management
12:30-13:30	Lunch	
SESSION 2: Potential contribution from EuroMix		
14:00-14:45	What can be offered by exposure and hazard assessment and scientific progress to achieve harmonisation - Introduction (tiered assessment, examples of how uncertainties are covered in the current approach and in a future approach, how hazard data can be used…) ***Jacob van Klaveren, RIVM, The Netherlands***	30 min + 15 min discussion

14.45-15:30	AOP wise testing and how to reduce uncertainties in grouping pesticides and/or chemicals in cumulative assessment groups and how to use computational tools to identify which chemicals should be grouped ***Angelo Moretto, University of Milan, Italy***	30 min + 15 min discussion
15:30-16:00	Refreshment break	
SESSION 3: Implementation of EuroMix advances		
16:00-17:00	General discussion with the focus on harmonisation and MRL setting - timeline for implementation - possible risk management strategies when there is a potential concern and how the risk assessor could contribute - other issues relevant for harmonisation such as precautionary principle and costs for testing	
17:00-17:15	Conclusions and next steps	

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Report of EuroMix Third Workshop on International Harmonisation on the Risk Assessment of Combined Exposures to Chemicals

25 October 2018, Hammersmith Campus, Imperial College London, London W12 0NN, UK

Background

EuroMix organised the third of a series of workshops on the international harmonisation of the risk assessment of combined exposures to chemicals on 25 October, 2018 at the Hammersmith Campus of Imperial College London, London, UK. The specific objectives of the workshop were to explore ways in which the EuroMix toolbox can contribute to harmonised scientific approaches to the risk assessment of combined exposures to chemicals in the diet, in relevant legislation. In support of this objective, illustrative case studies were presented and discussed, and used to inform guidance for consideration at the final workshop. Participants involved experts from North America, Europe and South America, as well as national and international organisations such EFSA, JRC, OECD, WHO and US EPA. The programme of the workshop is provided in the Annex. The following individuals attended the workshop:

Name	Country/Region	Organisation
Alan Boobis	UK	Imperial College London
Stephanie Bopp	Europe	The Joint Research Centre of the European Commission (JRC)
Eloisa Dutra Caldas	Brazil	University of Brasilia
Jean-Lou Dorne	Europe	EFSA
Takaaki Ito	International	OECD
Jacob van Klaveren	The Netherlands	The Netherlands National Institute for Public Health and the Environment (RIVM)
Anna Lowit	USA	US EPA
Bette Meek	Canada	University of Ottawa
Luc Mohimont	Europe	EFSA
Angelo Moretto	Italy	University of Milan
Emiel Rorije	The Netherlands	The Netherlands National Institute for Public Health and the Environment (RIVM)
Roland Solecki	Germany	The German Federal Institute for Risk Assessment (BfR)

Name	Country/Region	Organisation
Philippe Verger	International	WHO
Hilko van der Voet	The Netherlands	Wageningen University & Research (WUR)
Johanna Zilliacus	Sweden	Karolinska Institutet

The meeting was chaired by Alan Boobis. Angelo Moretto served as rapporteur, together with Alan Boobis. The meeting started with participants introducing themselves. **Alan Boobis** then provided a brief introduction to the objectives of the workshop, which were: to review the outcome of the first two workshops; to review ongoing work on harmonisation elsewhere; to explore ways in which the EuroMix toolbox can contribute to the risk assessment of combined exposures to chemicals; to compare and contrast different approaches to the risk assessment of combined exposures to chemicals in the diet, in relevant legislation by means of illustrative case studies; to consider how the EuroMix toolobox might contribute to the different needs to risk assessors and promote greater harmonisation in the approaches used. The meeting was organised into three sessions. Session 1 was on Conclusions from the first two workshops and EuroMix contribution. Session 2 was on Illustrative case studies. Session 3 was on Conclusions and next steps. During each session, a number of speakers presented their perspectives, each followed by discussion. Copies of the presentations are available on the EuroMix website.

Session 1: Conclusions from the first two workshops and EuroMix contribution

The session opened with a review of **What have we learned from the first two workshops (A Boobis).** In general, an overall objective of the workshops was to identify to what extent the process of the assessment of combined exposures to multiple chemicals can be harmonized across geographical regions and regulatory domains. How can this be done assuring consumer safety without restricting international trade unnecessarily, on the basis of sound science? The scope of the assessment needs to include exposure sources and routes, the bases for grouping substances, and the chemical sector(s) to be considered.

At the first Workshop (London, 20-21 October 2016), it was concluded that, in general, problem formulation for the assessment of combined exposures to chemicals is not well developed, and often lacks transparency in a number of elements. For example, the chemical scope (which sectors and chemistries) is often not explicitly identified, and not all of the factors used as a basis for grouping chemicals are always explicitly stated. Problem formulation should include chemical sector/space to be covered, regulatory context, timescale, resources available, acceptable level of uncertainty; percentiles of concern when using probabilistic approaches.

There was general agreement that a tiered approach should be used, which was likely to vary depending on the chemical sector and available information. Areas in which further discussion was considered necessary included the scope (e.g. which sectors/“silos”) of the assessment; criteria for grouping chemicals for assessment; how information on MOA/AOP should be used in assessments. All agreed that further harmonization was desirable, and indeed was necessary in some areas, including pesticides (international trade).

At the Second workshop (Brussels, 17 May 2017), participants discussed the legislative needs for the assessment of combined exposures to chemicals in different chemical sectors within the EU and across different geographical regions for the same sector. Participants also considered the role that scientific research plays as a determinant of future legislation. Perspectives of risk managers were presented in

the areas of pesticides, contaminants, additives, industrial chemicals and chemicals in general. It was agreed that the work of EuroMix could contribute in a number of areas, such as a tiered approach to grouping; and the assessment of uncertainty.

It was concluded that there is currently no overarching approach available for the assessment of combined exposures to chemicals. Different approaches are in use across chemical sectors and geographical regions. It was noted that the most common approach for grouping chemicals is a combination of structure, co-occurrence, and designed function. EuroMix will explore the consequences of different choices and assumptions in conducting such assessments.

The session continued with an **Update on ongoing work on harmonisation** (T Ito). OECD activities in the area of Environment, Health and Safety aim at the development of harmonized, high quality instruments, work-sharing to avoid duplication, prevent unnecessary non-tariff trade barriers, and to shorten time to market. The combined exposure assessment project started in 2014, following up on a WHO/OECD/ILSI HESI International Workshop in 2011. The goal of the project is technical convergence between member countries in the assessment of combined exposures to multiple chemicals. The expected deliverables include an outline of considerations for assessing combined exposures to multiple chemicals, which is addressed primarily to regulatory authorities and should not be considered strict guidance. The composition of the expert group, the structure of the document and the approach adopted were described. Key components are problem formulation; use of a tiered approach; hazard and exposure assessment; and risk characterisation. It is hoped that the document will be published by the end of 2018.

As of the present, there is no specific plan for follow-up, but one of the expectations is for the sharing of case studies amongst countries and organisations and that the OECD will continue to gather experience and knowledge on CRA activities. Possible follow-up activity will depend on suggestions of expert group members and feedback from countries

It was agreed that an inventory of case studies would be useful, identifying lessons learnt and those areas (chemical sector, geographical, regulatory) that are not covered by the developed case studies. Different groups have requested case studies (EFSA/OECD/EuroMix/WHO, etc), and there are clearly opportunities for sharing these, ideally using a common platform. OECD may discuss the possibility of devloping a standard template for problem formulation and possibly for other aspects of CRA such as uncertainty analysis and weight of evidence. In addition, to share case studies optimally, data would need to be organized using a common template. There may be a role for EuroMix here.

The session continued with several presentations on the EuroMix toolbox. The first of these was on **Retain and refine based on expert opinion and applied to pesticides using the EuroMix model and data platform** (H van der Voet). The final version of the EuroMix toolbox will provide an open web-based platform enabling integration of all data types and sources necessary for CRA. The toolbox includes modules for exposure, hazard and risk, and provides for data input and derived calculations. Visualisation includes use of the “RISK21 matrix” and it is proposed that boundaries for variability and for uncertainty should be included. Participants ageed that some further consideration needs to be given to the implementation of this feature.

The “Retain and Refine” approach was described. This includes consideration of the following in creating CAGs

- uncertainty on membership;
- missing hazard data;
- missing exposure data.

With this approach "Refine" indicates that a probability of membership of a CAG is given to all compounds and none is dropped from the calculation. An analysis is then conducted for potential risk drivers (e.g. using mixture selection).

Uncertain of membership is estimated by a combination of:

- expert elicitation
- QSAR
- Molecular docking
- Any combination

Uncertainty for missing hazard data, including that of the RPFs, is also estimated. EuroMix proposes use of the TTC, generic or specific, to estimate the POD/RFP, with associated uncertainty, in the absence of chemical-specific information.

While technically feasible, this approach should tested by applying it to realistic case-studies to determine its practicality and conservatism. Key assumptions used in applying the probabilistic approach proposed should be clearly identified and a sensitivity analysis conducted to determine which are the risk drivers. The generation of the data required for input for this approach is resource intensive and, therefore, this has to be balanced with pragmatism and feasibility. It was suggested that the EuroMix toolbox could be used to determine the contribution of each factor or assumption to the final outcome, to determine whether its inclusion was necessary. While the "Retain" approach is recognized as scientifically sound, it might be that a number of retained substances contribute so little to the total risk that they may be safely ignored in the CRA, and criteria should be developed for such a decision.

E Rorije next described EuroMix **In silico tools for lower tier CAG membership and potency estimates**. The approaches being developed were illustrated using hepatic steatosis as an example. Several methods can be used to assess CAG membership in the absence of higher tier data. These include:

- Generic QSAR models based on apical endpoints. This has been applied to over 600 compounds

  These are not very specific and CAG membership is rather inconclusive. There are no QSAR models for the prediction of MOA, but EuroMix is developing some models for this purpose

- Molecular Docking (MD)

  MD is useful in predicting MIEs, however it is assumed that binding results in activation. The models cannot differentiate between agonism and antagonism. MD does enable activity (binding) to different nuclear receptors (MIEs) to be distinguished.

Low tier for Potency estimation:

- NOAEL, if available;
- Read-across;
- TTC (generic or CAG specific);
- Docking (use binding energy).

Potency estimates based on binding energy are generally very conservative, with estimates almost always lower than the 5th percentile NOAEL for steatosis (i.e. the threshold for a CAG-specific TTC]

All of the data and calculations are available in the EuroMix toolbox.

The session continued with a presentation on **Examples of multiple routes of exposure and how these might affect the Margin of Exposure** (J van Klaveren). The EuroMix toolbox now includes considerable information on exposure. Consumption data (diet) from different EU countries, organized in EFSA is available. A concentration (of pesticides) database is also available in which processing factors are provided.

The impact of using relative potency factors based on different data sets, including the EFSA reports on CAG groups, in vitro studies and in silico (QSAR) predictions to calculated MOEs has been assessed. The consequences of using different approaches for RPF determination and of the breadth of chemical space used in the exposure assessment were also assessed. The MOEs for the CAG group of pesticides causing steatosis, as an example, were all > 500. In general, MOEs were lower, sometimes much lower, when additives and, particularly, contaminants were considered together with pesticides. This is, to a large part, a consequence of how poor/missing exposure data are addressed.

The calculations were performed assuming dose-additivity for CAG members, based on phenotypic effects (EFSA level 2) and including compounds for which this effect was not the critical effect (i.e. the effect used for the Point of Departure/Reference Point for establishing the health-based guidance value).

Non-food exposures (e.g. farmers, applicators, bystanders, residents) cannot presently be estimated using the EuroMix toolbox itself, and hence to conduct a full aggregate exposure assessment requires the appropriate estimates to be imported. The toolbox has provision for this. For example, it is possible to link the BROWSE model (Bystanders, Residents, Operators and WorkerS Exposure models for plant protection products) from the toolbox.

The consequences of the different assumptions used in these calculations should be explored by EuroMix, for both combined and aggregate exposure to chemicals.

Discussion of the EuroMix toolbox continued with a presentation on **Kinetics and IVIVE (In vitro to in vivo extrapolation)** (E Rorije). EuroMix has developed a generic physiologically-based toxicokinetic (PBTK) model, based on that developed by the EU COSMOS project. Chemical specific parameters are estimated in silico, using QSAR and from physicochemical properties. This has been undertaken for all of the substances in the EuroMix inventory.

The approach was illustrated using cypermethrin as an example. Most parameters were predicted within a factor of 10 but some, particularly plasma protein binding, were not well predicted. The reasons for this need to be explored. Prediction of metabolic rates is under development, using read across from an existing QSAR model for fish metabolism. The possibility of developing a similar model for rat or human could be considered. The current model assumes that the parent is the toxic moiety.

The final presentation in this session was on the **Applicability of EuroMix tools for other regulatory sectors** (B Meek). The Canadian Government has mandated the evaluation of a large number of industrial chemicals. Therefore, the approach adopted has to be tiered and very pragmatic, both for prioritization and for assessment. In general, many of these chemicals are data poor, yet prioritization and, if necessary, assessment is required using the data available. Hence, read across from within chemical categories is of considerable importance. Criteria are needed for grouping chemicals. Characteristics that can be used are:

- Structural similarity;
- MOA;
- Physicochemical properties, environmental fate, human/environmental effects;

- Qualitative/quantitative comparison.

OECD IATAs (Integrated Approaches to Testing and Assessment) provide a pragmatic means to integrate available data and target testing strategies. OECD is developing case studies, using defined templates to increase the collective experience. In reviewing case studies to date, read across was the most frequently used approach in the assessments.

Further guidance is necessary, and the EuroMix toolbox could be of value in addressing some of the existing needs. Such areas include:

- the definition of analogues/category boundaries, and uncertainty analysis and reporting;
- assessment of industrial chemicals (not occupational), in data-poor situations;
- exposure assessment;
- contaminated sites, where predictive application to data poor situations is necessary;
- development of case studies for industrial chemicals (e.g. PBDEs/phthalates);
- application to the OECD IATA case studies on CRA and on chemical categories

Session 2: Illustrative case studies

The first case study was on the **Implementation of CRA of pesticide residues by EFSA** (L Mohimont). The CAGs for pesticides used by EFSA are based on phenotypic effect, dose-addition is applied to all members of the CAG, and it is assumed that there is no interaction among members of the CAG (indeed, none is expected). Suitable methodology was developed by the PPR Panel from 2007-2013. All relevant data for assessing combined exposure to members of such a CAG are available (i.e. on toxicology, consumption, residues) and the necessary tools have been developed, i.e. MCRA (RIVM) and an internal EFSA model (SAS-based).

Initially a retrospective CRA will be conducted. During 2019, in a pilot phase, CAGs for the nervous system and the thyroid will be assessed. From 2019-23, CAGs for eight other organs/systems (adrenals, development, eyes, haematopoietic system, kidneys, liver, reproduction and testes) will be assessed. Prospective CRA awaits kick-off by the EC and member states.

There will be four reports on the nervous system CAG. One report will be on the CAGs, describing the identification and characterisation of the common effects. There will be two reports on combined exposure assessment (from RIVM, using MCRA and from EFSA, using SAS). The fourth report will be on characterization the risk from combined exposure to CAG members. Considerations included will be identification on the index compounds, and analysis of uncertainties associated with CAG membership and the assumption of dose-additivity.

General criteria have been identified for CAGs for the nervous system and for thyroid effects. Criteria for identification of index compounds have been defined, which include potency. RPFs have been calculated for all CAG members, based on NOAELs (or adjusted LOAELs, if necessary), where the lowest NOAEL from acceptable studies was used. 420 Active Substances (AS) have been assessed for effects on the nervous system and the thyroid. Seven effects of relevance were identified (five for nervous system, two for thyroid) and seven CAGs were created. The CAG for hypothyroidism included changes in T3/T4/TSH and induction of adenomas/carcinomas. There was no exclusion based on human relevance (e.g. thyroid adenomas due to increased T4 elimination).

Within a CAG, some of the members had a known (or presumed) MOA, whilst others did not, but shared at least one of the indicators identified for the common effect. For example, within the nervous system - motor effects CAG there are 85 AS with a known MOA and 35 for which the MOA was not

known. Within the thyroid – hypothyroidism CAG, the percentage of active substances with known MOA is much lower.

An assessment of the uncertainty in grouping compounds into the CAG for hypothyroidism was undertaken, as a case study. This was based on weight of evidence and expert knowledge elicitation. First, possible lines of evidence were identified. Each line of evidence was then weighted for its contribution to determining CAG membership. An overall score for each AS was calculated by multiplying the scores for all lines of evidence. Based on these scores AS were sub-divided into 7 sub-groups, members of which had an approx. similar level of evidence. Expert knowledge elicitation was then used to assess what percentage of members in each of the sub-groups caused hypothyroidism (true positives). The estimated probabilities of true CAG membership could then be taken into account when conduct CRA. Key risk drivers will be identified, and if there is concern (low margin of safety (MOS - ratio of reference value for index compound to exposure, or low MOE), then a more detailed analysis on the probability of CAG membership will be undertaken.

The appropriateness (uncertainty) of assuming dose-addition for CAG members will be assessed. Considerations upon which this assessment will be based include empirical information on the combined effects of the AS, MOA and toxicokinetics.

Risk managers have agreed that the threshold for regulation is the 99.9th percentile of the population with a protection goal of a combined MOE of at least 100. Uncertainty analysis of hazard characterisation, exposure assessment and model uncertainties will form an essential part of the assessments.

A number of points were noted with regard to the approach described.

Inclusion of all members of a CAG in an assessment, based only on hazard, implies an assumption of co-occurrence/exposure. Information on actual co-occurrence would be of value (work on this is ongoing within EuroMix). In addition, inspection of the tail of acute exposure distributions reveals that this comprises a very small number of compounds, usually only one. Such information could be used in a refined assessment.

It was noted that compounds can fall into more than one CAG (e.g. for nervous system), and that the effect of an AS on which CAG membership is based is not necessarily the critical effect on which its reference value is based. Since CRA will be performed only after assessment of each compound has shown that there is no concern for the compounds taken individually, this could be a consideration in refinement of the methodology.

The question of whether and how account will be taken of the human relevance of certain effects, such as some of those on the thyroid in rodents, in CAG membership was raised.

The appropriateness of basing CAG membership on a single indicator showing a statistically significant change in one study was questioned. Additional issues where further consideration might be merited include the use of the NOAEL of the most sensitive indicator as POD for CRA, the way in which the lines of evidence are used to assess weight of evidence, and the aspects addressed in expert elicitation.

The second illustrative case study was on the **Cumulative risk assessment of pesticides in the US** (A Lowit). EPA cumulative risk assessments for pesticides are risk based. The mechanism of pesticidal activity is used as an indicator of potential mode of action for human health effects.

Key principles in a refined CRA are:

- integration of toxicity and exposure data; i.e. time-frame
- realistic assessment, e.g. use monitoring data, avoid compounding conservativism (especially in CRA vs individual compound assessment)
- maintain geographical, temporal and demographic specificity
- be able to "track back" sources of exposure for sensitivity analysis

To date, refined CRA has been performed for five CMGs: organophosphates (OPs), N-methyl carbamates, pyrethroids, triazoles, and choloroacetanilides. In each case, members of a CMG were shown to share the same MOA. In some cases, for example for carbamates, EPA conducted specific studies to confirm dose-additivity. For each CMG, an index compound was selected, based on the quality and quantity of data available. Uncertainty in the POD of the IC propagates throughout the CRA. Potency is not a consideration in the choice of IC by EPA.

Temporality is a key consideration in CRA. It is important to consider biological time, i.e. toxicodynamics. This helps determine the relevant dose metric, e.g. C_{max}, AUC and likelihood of co-exposure. For example, N-methyl carbamates show peak toxicity at 30 min and recovery by 2 hours. Hence, whilst 24-hour exposure estimates would be sufficient in most cases, if necessary, refinement would be possible, based on eating pattern.

Models are available to estimate aggregate exposure from multiple sources.

Compounds may be excluded from a CAG because of low hazard potential, e.g. some pyrethroids show no effects up to a limit dose of 5000 mg/g bw. Similarly, pyrethroids with no residues in any crop were excluded from the dietary assessment. For residential uses, only those uses likely to give rise to significant exposure were included in the assessment. For hazard characterization of pyrethroids, severity scores in animal studies for behavioural and other signs were used.

In the triazine assessment of 2018, a PBPK model was developed for the IC, atrazine, and this was used to determine PODs for all of the triazine herbicides, including chlorotriazine metabolites, in the CAG. The model was used to allow for different age groups and different exposure scenarios (routes of exposure).

EPA has recently developed a screening framework to identify candidate CMGs. This uses the same principles as in the previously published guidance for CMG creation, i.e. chemical structural similarity, hazard profile, pesticidal mode of action and mammalian MOA/AOP. Shared chemical structure is not sufficient on its to support a candidate CMG. Rarely is apical outcome used as the sole basis for determining a candidate CMG, e.g. OPs and pyrethroids would not be considered in the same CMG. In the absence of good evidence for a common mechanism of action, no CRA would be necessary (Option 1 in the Framework), e.g. sulfonylureas. Where a candidate CMG supports a common mechanism of action, but there are insufficient data to define the key events in the MOA, a screening level tiered exposure assessment is conducted (Option 2 in the Framework), e.g. anilinopyrimidines. If this assessment gives rise to no concern, the CRA can be concluded. If there is potential concern, the CMG would be refined, to enable a higher tier assessment to be undertaken (this has not been necessary to date). EPA is preparing a publication on use of ToxCAST data to support identification of candidate CMGs.

Session 3: Conclusions

The workshop closed with a brief presentation on **Integration with other activities such as WHO/EuroMix workshop and EuroMix guidance** (J Zilliacus). WHO is organising an expert consultation within the frame of EuroMix. This will be held 16-19 April, 2019 in Geneva. The workshop

will comprise a series of case studies, in which different organisations will have assessed combined exposure to the same group of chemicals, using their own choice of methodology and inputs. One option will be to use the tools and data available in the EuroMix toolbox. Based on the outcome of this exercise, guidance will be prepared on when and how a risk assessment of combined exposure to multiple chemicals should be undertaken within an international context, for example by JMPR. The guidance will be produced according to WHO procedures and will not be complete until after EuroMix has ended.

A EuroMix Handbook is being prepared describing the approach for mixture risk assessment developed by EuroMix. This will provide practical guidance for the implementation of Euromix tools in risk assessment of combined exposure to multiple chemicals under a variety of problem formulations, and will cover both data-rich and data-poor situations. The aim is to ensure that the Handook is aligned with the OECD document and (draft) EFSA framework, and to avoid unnecessary repetition. There will be further training sessions for stakeholders in early 2019 on the EuroMix toolbox and Handbook. Finally, EuroMix is organising a joint stakeholder workshop with the sister H2020 project, EDC-MixRisk. This will be 26-27 March, 2019 in Brussels.

Conclusions

There is considerable alignment of the principles for assessment of combined exposure to multiple chemicals in the guidance of IPCS, OECD, EFSA and other organisations. These all emphasise the importance of problem formulation, including specification of the objectives and acceptable degree of uncertainty for assessment, and the basis for grouping and the selection of assessment approach. There was general agreement on the need for tiered approaches for both hazard and exposure assessment, to avoid overly conservative assumptions. The use of mode of action information in refining assessment groups has also been broadly incorporated, as has been transparent delineation of uncertainties at each tier. In a number of chemical sectors, there is common application of these principles. However, in the area of pesticides, there are significant differences between the proposed approach in Europe and that which is in use in the USA.

The EuroMix toolbox has potential application, regardless of the approach used in different sectors or geographical regions. Whilst harmonisation of the specific risk assessment methodology might not be possible, at least in the short term, it should be possible to harmonise the principles used, the standard of reporting and data templates. The EuroMix guidance will seek to provide best practice for the range of problem formulations that might concern risk managers and will encourage further harmonisation, to the extent possible.

Third EuroMix workshop on international harmonisation on the risk assessment of combined exposures to chemicals

Program

The objective of the third workshop is to explore ways in which the EuroMix toolbox can contribute to harmonised scientific approaches to the risk assessment of combined exposures to chemicals in the diet, in relevant legislation. In support of this objective, illustrative case studies will be discussed, and used to inform guidance for consideration at the final workshop.

09:00-17:00, 25 October 2018: Hammersmith Campus, Imperial College London, London W12 0NN

08:30-09:00	Welcome coffee and registration	
Chair/Rapporteurs	**Alan R Boobis *(Imperial College London)*/TBD**	
SESSION 1: Conclusions from the first two workshops and EuroMix contribution		
09:00-09:15	Introduction and objectives of meeting ***Alan R Boobis (Imperial College London)***	15 min
09:15-10:00	What have we learned from the first two workshops ***Alan R Boobis (Imperial College London)***	25 min + 20 min discussion
10:00-10:30	Update on ongoing work on harmonisation ***Takaaki Ito (OECD)***	20 min + 10 min discussion
10:30-11:00	Refreshment break	
11.00-13.05	Outline of the EuroMix toolbox 1. Retain and refine based on expert opinion and applied to pesticides related to pesticide regulation ***Hilko van der Voet (WUR)*** 2. Approaches for less extensive CAGs based on mode of action using tools such as QSARs/molecular docking and cost-effective and reliable *in vitro* assays ***Emiel Rorije (RIVM)*** 3. Examples of multiple route exposure and how these might affect the Margin of Exposure ***Jacob van Klaveren (RIVM)*** 4. Kinetics and IVIVE ***Emiel Rorije (RIVM)*** 5. Applicability of tools for other regulatory sectors/chemical classes/grouping principle ***Bette Meek (University of Ottawa)***	5 x (15 min + 10 min discussion)

13:05-14:00	Lunch	
SESSION 2: Illustrative case studies		
14:00-15:45	- EFSA: Implementation of CRA of pesticide residues ***Luc Mohimont (EFSA)*** - EPA: Organophosphates; carbamates; pyrethroids ***Anna Lowit (EPA)***	2 x (30 min + 20 min discussion)
15:45-16:15	Refreshment break	
SESSION 3: Conclusion		
16:15-16:45	Integration with other activities such as WHO/EuroMix workshop and EuroMix guidance ***Johanna Zilliacus (Karolinska Institute)***	20 + 10 min dicussion
16:45 -17:00	Conclusions and next steps	15 min

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Report of EuroMix Fourth Workshop on International Harmonisation of the Risk Assessment of Combined Exposures to Chemicals

10.00-17.00, 15 April 2019
WHO HQ, Avenue Appia 20, 1202 Geneva, CH

Background

EuroMix organised the fourth of a series of workshops on the international harmonisation of the risk assessment of combined exposure to multiple chemicals on 15 April, 2019 at WHO HQ, Avenue Appia 20, 1202 Geneva, CH. The specific objective of the workshop was to explore the potential of the EuroMix Handbook to contribute to harmonised scientific approaches to the risk assessment of combined exposure to multiple chemicals in the diet and more generally, in relevant legislation. Perspectives on the Handbook (and the Toolbox) were invited from representatives of international organisations. Issues that might arise in utilising the Handbook and the Toolbox at international level were identified, for consideration at the Expert Consultation, 16-18 April, 2019. Participants involved experts from Europe, North America, South America and Asia, as well as national and international organisations such EFSA, JRC, OECD, WHO and FAO. The programme of the workshop is provided in the **Annex**. The following individuals participated in the workshop:

Name	Country/Region	Organisation
Janis Baines	Australia	Food Standards Australia New Zealand (retired)
Alan Boobis	UK	Imperial College London
Stephanie Bopp	Europe	JRC (The Joint Research Centre of the European Commission)
Eloisa Dutra-Caldas	Brazil	University of Brasilia
Amelie Crépet	France	ANSES (Agency for Food, Environmental and Occupational Health & Safety)
Jean-Lou Dorne	Europe	EFSA (European Food Safety Authority)
Vittorio Fattori	International	FAO (Food and Agriculture Organization of the United Nations)
Natalie Von Gotz	Switzerland	Federal Office of Public Health
Jacob van Klaveren	The Netherlands	RIVM (National Institute for Public Health and the Environment)
Eeva Leinala	International	OECD (Organization for Economic Cooperation and Development)
Soren Madsen	International	WHO (World Health Organization)
Bette Meek	Canada	University of Ottawa

Angelo Moretto	Italy	University of Milan
Roland Solecki	Germany	BfR (Federal Institute for Risk Assessment)
Philippe Verger	International	WHO (World Health Organization)
Gerrit Woltering	The Netherlands	RIVM (National Institute for Public Health and the Environment)
Liu ZhaoPing	China	CFSA (China National Center for Food Safety Risk Assessment)
Johanna Zilliacus	Sweden	Karolinska Institute, Stockholm

1. Introduction

The Meeting was the fourth, and last, of a series of workshops organised by EuroMix to explore options and potential limitations in the international acceptance of harmonised approaches to the risk assessment of combined exposure to multiple chemicals. The previous meetings were as follows:

- First Workshop: 20-21 October 2016, Imperial College London, London W12 0HS
- Second Workshop: 17 May 2017, Thon Hotel EU, Brussels, Belgium
- Third Workshop: 25 October 2018, Imperial College London, London W12 0NN

The meeting was chaired by Alan Boobis. Angelo Moretto served as rapporteur. Participants introduced themselves. **Alan Boobis** then briefly outlined the aims of the EuroMix project and specifically the outcomes of the previous workshops. While the meetings involved mainly risk assessors, the second workshop also included a number of risk managers, whose involvement proved to be very informative.

The main conclusions from the previous workshops were:

- Currently there is no agreed approach to the risk assessment of combined exposure to multiple chemicals in Europe (or elsewhere), although there is alignment of the general principles
- Approaches to the risk assessment of combined exposure to multiple chemicals vary across sectors and with geography, reflecting the needs of the risk manager
- The most common approach for grouping chemicals is based on structural similarity and/or co-occurrence (in products) and/or designed function
- There is agreement that dose-addition is an appropriate default assumption and that synergy is rare at human-relevant exposures
- There is currently no general agreement on how information on mode of action (MOA)/adverse outcome pathway (AOP) should be used in the risk assessment of combined exposure to multiple chemicals
- There is potential for the EuroMix Handbook and Toolbox to contribute to harmonisation of the risk assessment of combined exposure to multiple data rich and/or data poor chemicals

The aim of the present workshop was to evaluate the EuroMix Handbook, section by section. On this basis, a number of risk assessment bodies were asked to provide (i) their perspectives on the potential utility of the Handbook in the risk assessment of combined exposure to multiple chemicals by their own organisations and (ii) their feedback on how the Handbook could be improved.

Participants were also asked to consider which sections of the Handbook would be most relevant for discussion at the Expert Consultation, 16-18 April 2019 and to identify any potential limitations.

The presentations are available on the EuroMix website.

2. EUROMIX HANDBOOK

Johanna Zilliacus then presented in detail the content of the EuroMix Handbook.

2.1. INTRODUCTION

The EuroMix Handbook describes approaches to the risk assessment of combined exposure to multiple chemicals, drawing upon tools in the Euromix Toolbox. The EuroMix Toolbox provides a suite of web-based tools and data, which can be used to perform such an assessment. The EuroMix Handbook consists of a main text with a description of the methodology and tools, and a number of Annexes that cover the methodology in detail and provide templates, examples and training material for the EuroMix Toolbox. The EuroMix Toolbox comprises a number of modules, includes a data repository, and there is a separate Toolbox user manual.

The EuroMix Handook took into consideration and is aligned with recent activities in CRA (note CRA (cumulative risk assessment) is used here to mean "risk assessment of combined exposure to multiple chemicals"), particularly the OECD's Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals and EFSA's Guidance on Harmonised Methodologies for Human Health, Animal Health and Ecological Risk Assessment of Combined Exposure to Multiple Chemicals.

The main features of the Handbook include:

- Component-based approach
- Grouping based on toxicological considerations
- Dose addition as default model
- Relative potency factors (RPF) approach
- Probabilistic exposure assessment
- Mainly, but not exclusively, dietary exposure

Whilst the Handbook is currently focused on grouping based on toxicological considerations, it is possible to input groupings based on other parameters and tools, such as co-exposure or both co-exposure and toxicological considerations, with modules in the Toolbox. The Handbook is applicable to a wide range of problem formulations because it can be applied using different grouping principles (structure, exposure…), to any chemical, (relative) potency of chemicals can be derived using different approaches (e.g. using acceptable daily intake (ADI)/tolerable daily intake (TDI), or no observed adverse effect level (NOAEL)/benchmark dose (BMD) for critical or specific effect), and provides methodology to handle data poor substances.

The Toolbox is based on previous software (MCRA) and allows the uploading of the user's own or other data, as necessary, to perform CRA.

The software will be made freely available to users and a web-based manual and training will be provided. The software includes many modules that address the different aspects of CRA (hazard characterisation, exposure assessment, risk characterisation, uncertainty analysis), which can be used as needed.

The Toolbox includes food consumption data, from 11 European countries, and food monitoring data, mainly on pesticides, from the same countries. These data are owned by individual countries and can be used in web-based assessments, but they cannot be downloaded (unless specific permission of the data owner is provided). Users can upload their own data, provided the data are in the format required by the Toolbox, and templates are available to assist in achieving this. The EuroMix Toolbox will initially

include data on the toxicological endpoints: hepatic steatosis, craniofacial malformation and feminization.

COMMENTS from the participants

The question of verification of the data in the Toolbox was raised. Verification and peer review of data are needed, even if the data are proprietary. Currently the data provided in the Toolbox are "as is". Users need to satisfy themselves as to their reliability, as appropriate. For example, some of the data, such as the results of national food consumption surveys, will have been verified by the owner. The use of the Toolbox for regulatory risk assessment will further require that the modules/models meet certain requirements, such as transparency. Not all of the code is publicly available, but it might be possible to rely on performance verification by EuroMix, but this will depend on the type and quality of information in the Toolbox. There is a need to follow guidance, such as on the use of quantitative structure-activity relationships (QSARs) (perhaps from EFSA or JRC) and on AOPs (OECD) to ensure the acceptability of such information in the Toolbox. Whilst guidance on these topics exists, it is not always suitable for the areas addressed by EuroMix.

Data in each module should be described and made publicly available (e.g. in Zenodo) with a description of the metadata that feed the module. This will greatly enhance the prospects of the tool being used in practice.

The toxicological data in the Toolbox needs to be expanded. Currently it contains *in vitro* and *in vivo* data generated by EuroMix as well as *in vivo* data from the literature collected by EuroMix, only on the three endpoints, hepatic steatosis, craniofacial malformation and feminisation used in the EuroMix exploratory studies. It was noted that OECD has a template to harmonise data collection of intermediate effects and adverse outcomes (OECD OHT 201). It was recommended that this be included in the Handbook.

Currently, the Toolbox includes information on toxicity only when it is available in English.

Many of these points are subject to discussion with EFSA and in the EuroMix follow-up. EFSA will assess the performance of the EuroMix Toolbox using its own software. RIVM has an agreement with EFSA for data collection. EFSA is currently collecting toxicological data using the OECD harmonised templates (OHTs) that include AOP and kinetic data (OECD OHT 58). Linkage to this information from the Toolbox would be very helpful.

2.2. PROBLEM FORMULATION

The template for problem formulation, which includes provision for an analysis plan, and an example, which are provided as annexes to the Handbook, were described. These are not included in the Toolbox, but the template was developed based on the methodology implemented in the Toolbox.

COMMENTS from the participants

It was suggested that the template for problem formulation and the example should be included in the Toolbox.

It was noted that the template includes a mixture of risk management and risk assessment elements. In conventional risk analysis, problem formulation is the responsibility of the risk manager, albeit in

dialogue with the risk assessor, to avoid a mismatch between what is asked by the risk manager and what is feasible or scientifically sound, from the point of view of risk assessment. In addition, existing legislation will dictate some aspects of problem formulation. It was suggested the template should clarify what is the responsibility of risk management and of risk assessment (i.e. scientific issues) in the analysis plan.

Both the problem formulation template and the Toolbox should be sufficiently flexible to allow a variety of questions to be addressed, depending on the needs of the risk manager. Suitable problem formulation is an essential prerequisite for CRA to be useful and, hence, to enable an appropriate answer to the problem to be provided.

The template includes a field for description of the mixture (components of potential concern), but there is no explicit mention of a gatekeeper step as such, which is included in all of the recent frameworks, including those from WHO, EFSA and OECD.

2.3. IDENTIFICATION AND ASSESSMENT OF AOP NETWORKS

This section covers grouping of compounds based on toxicity data (CAGs) and determination of relative potency factors (RPFs). The Handbook focuses on the use of data on AOPs both for grouping and for RPF determination. The methodology for identification and assessment of AOPs (AOP networks) is based on the OECD AOP handbook (2018).

If AOPs are not available in the AOP wiki, they should be developed, if possible. Such AOPs should then be coded (according to the AOP wiki) and uploaded to the Toolbox. When this is done, the information can be used to conduct CRA because individual quantitative KE data will be automatically connected in the relevant modules. In this respect, it is important to be very clear about the reliability of the KE data and the AOP used.

Modules are available in the Toolbox for key event (KE) quantitative data (note: to make more general, the term “effect” is used for a KE in an AOP, and “response” is used for what is measured experimentally to assess a KE). Such data can be used for potency estimates, if data on adverse outcomes per se are not available.

The default assumption is dose-addition for a common adverse outcome, independent of differences in molecular initiating event (MIE) and some of the KEs.

The Toolbox allows CRA to be performed for a single AOP or for a network of AOPs (e.g. similar and dissimilar acting compounds). This is in recognition that in different regulatory domains, the criteria for grouping may differ.

COMMENTS from the participants

There is a need to clarify how information on AOPs can be used in the grouping of multiple chemicals for risk assessment of their combined exposure. It is possible to perform CRA in the absence of information on AOPs or even RPFs. Some explanation of how to perform such an assessment, and the use of the Toolbox, in such situations, should be provided.

Some of the terminology used in the Handbook could be more explicit. For example, MOA and AOP are sometimes used synonymously and sometimes differently. It would be helpful if such terms were defined, consistent with international usage.

2.4. COLLECTION OF TOXICITY DATA

The Handbook recommends use of systematic review and weight of evidence for data collection. Any type of data can be used or uploaded to the Toolbox. The Handbook provides a template for data collection (but see above).

COMMENTS from the participants

It was suggested that the section on data collection could perhaps be shortened and the emphasis on systematic review should be removed, as systematic review is often not necessary. Instead, the emphasis should on the need for transparency in the approaches, assumptions and data used in the assessment, whatever they are. The approach to data collection will be driven by problem formulation. Some guidance on when systematic review might be needed and other possible options would be helpful.

The templates are very KE/AOP focused. It might be helpful to summarise the assays used in different tiers in a CRA in a table for use in the Toolbox.

2.5. TIERED TESTING STRATEGY

Tiered testing based on an AOP network is also described. This requires selection of suitable assays (*in vitro*).

The methodology suggested in the Handbook includes:

- Identification of KEs in the AOP network that can provide information for grouping or for determination of RPFs
- Identification of *in silico*, *in vitro* and *in vivo* assays for the KEs or AOs
- The need to assess the
    - relevance of the assays
    - reliability of the assays
    - availability and feasibility in terms of costs and resources
    - information provided for grouping, RPFs, prioritisation for further testing
- Selection of assays to be included based on the assessments
- Description of the assays (test systems and responses) in the tables for use in the Toolbox

A template for description of a tiered testing strategy when used is provided in the Handbook.

COMMENTS from the participants

The Handbook could perhaps be clearer that the Toolbox can be used with any data for hazard characterisation (e.g. raw data to assess RPFs using the Toolbox or independently (externally) derived RPFs which are then uploaded; the use of AOPs is not a prerequisite).

As noted above, when developing new AOPs, these should be assessed according to the OECD process, but one option is to indicate different levels of reliability/confidence in an AOP depending on its maturity. There is some concern about the reliability of some of the AOPs currently in the Toolbox, as these have not yet been verified according to the OECD procedure. Hence the need for a formal process, and also for providing a very clear explanation of the confidence in these AOPs.

It was noted that confidence in the output of a CRA will differ depending on the confidence in the AOP, and its acceptance, especially if the AOP has not been independently verified. This should be clearly reported in the uncertainty analysis. It should also be made clear that, as the AOPs currently in the Toolbox are not verified, Toolbox outputs using such information should be considered as preliminary examples of proof-of-principle of the Toolbox and the proposed approaches. EuroMix examples/case studies are really for illustrative purposes. All methods will need to have been suitably characterised/verified before use in risk assessment for regulatory purposes. Concern was expressed that, despite this, CRA using non-validated AOPs could lead to unreliable conclusions, and once the conclusions are available, it will be difficult to counter their interpretation. When using the Toolbox, this should be made clear as appropriate, particularly in the output.

The Handbook should be clearer on the role of AOPs, since these are not currently being used even in the risk assessment of individual chemicals. Specific for CRA is that AOPs are of potential value in refining assessment groups. It should be born in mind that EuroMix is an innovation project, which tried to anticipate the evolution of risk assessment. That is why an AOP-based approach has been included. However, the Handbook should make clear what is applicable now and what might be possible in the future.

New approach methodologies (NAMs) (*in silico* and *in vitro*) used for KE event characterisation in CRA should be appropriately verified before such application. The Handbook should cross-reference existing guidance on approaches to verification of these methods for individual chemicals, as this would be relevant to their use in CRA.

2.6. GROUPING

The methodology proposed in the Handbook includes a number of considerations such as:

- Level of grouping (target organ, common effect/AO, common specific mode of action /AOP)
- AOP network
- Substance category
- Collect toxicity data (*in silico*, *in vitro*, *in vivo*, human epidemiology)
- Organise data in lines of evidence
- Assess data for relevance and reliability
- Decide on group membership using weight of evidence approach
- Report group membership in table for use in EuroMix toolbox (either 0 (not included) or 1 (included) or a value between 0-1 indicating the probability for belonging to the assessment group)

Different methodological considerations are possible for grouping off-line, using essentially any criteria as appropriate. A filtered (include/exclude) list of chemicals can then be uploaded to the Toolbox. It should be noted that with respect to OECD guidance on grouping, the Handbook addresses also the use of QSAR for this purpose.

COMMENTS from the participants

There should be clear information in the output of the CRA of what criteria were used as the basis for grouping.

Another key issue is the harmonisation of grouping. Some of the approaches outlined in the Handbook might not be generally accepted internationally.

There is a need to harmonise terms used in the Handbook (and the Toolbox) to the extent possible, to facilitate understanding and uptake of the Handbook. For example, the term “active substances” is understood by many to refer to pesticides. If a more general meaning is implied, perhaps a different term could be used, such as “chemicals”.

The Handbook should be checked to ensure that the OECD considerations for grouping have been adequately addressed.

2.7. RELATIVE POTENCY FACTORS (RPFs)

A number of options for determining RPFs are described on the Handbook. *In vivo* data should be used if available, for the time being, until reliable extrapolation of *in vitro* data becomes routinely possible (for both toxicokinetics and toxicodynamics). The RPF can be calculated using the NOAEL/LOAEL or BMD_x. Any value can be used for the BMR to determine the RPFs, depending on the shape of the dose-response curves. Normally it should be between 20 and 80%, as this is the statistically most reliable part of the curve.

If the BMD is to be used as a point of departure, the EFSA guidance on BMD should be used to decide on an appropriate BMR.

The choice of index compound should be based on the quality of the data, and this should be from *in vivo* studies.

If there is more than one point of departure (POD) for a compound, some basis for the choice of the POD will need to be decided, e.g. the lowest, the mean value. Is it for an upstream of a downstream key event? Guidance on this would be helpful.

IVIVE should be used to convert an *in vitro* POD to an external POD *in vivo*.

If toxicological information is not available, the threshold of toxicological concern (TTC) or a variant, such as an endpoint-specific TTC, can be used.

The Toolbox enables calculation of RPFs for multiple compounds based on their respective dose-response curves, and designation of one of the compounds as the index compound.

The Handbook explains how to design mixture studies based on RPFs to select various dose proportions for the chemical combinations. The methodology described in the Handbook includes:

- The use of equipotent doses/concentrations of substances
- The need to derive RPFs of individual substances
- The use of several doses of individual substances and binary mixtures
- The analysis of the results using the benchmark dose method

2.8. EXPOSURE

The Handbook proposes probabilistic exposure assessment, based on the previous MCRA tool and in line with EFSA guidance. The Toolbox provides distributions for both acute and chronic exposure with uncertainty estimates. Food consumption data are available from consumption surveys in 11 countries while concentration data are available from monitoring (i.e. measurement of levels of substances in raw agricultural commodities and conversion of food-as-eaten) with application of processing factors, in the same countries.

In the absence of measured concentrations of a specific chemical, either extrapolation from other foods or legal limits in food can be used.

Estimates of non-dietary exposures can be imported and used for aggregate exposure assessment in the Toolbox.

The results should be expressed as a margin of exposure (MOE), relative to the index compound.

Uncertainty in the assessment should be described and quantified to the extent possible. Templates are provided for this.

The Toolbox enables identification of substances commonly found together in the exposure (from the diet) and to which the studied population is mainly exposed. The approach is based on individual exposure correlations estimated from individual food consumption patterns, concentration data and RPFs. This information can be used for grouping, prioritisation, refinement of the risk assessment and prioritisation of mixtures to be tested. The statistical method implemented in the Toolbox for this purpose is the Sparse Nonnegative Matrix Underapproximation (SNMU). This appears to be a unique implementation in the EuroMix Toolbox and was considered by the participants to be a valuable contribution to CRA methodology.

COMMENTS from the participants

Consideration of exposure vs toxicology criteria for grouping chemicals should be addressed up-front in the Handbook, since refinement of exposure is an essential tier in a number of problem formulations. At present, grouping is considered largely in the section on hazard assessment. Grouping based on exposure can sometimes be easier than that based on hazard, since there are usually not that many driver compounds and it might be possible to decide *a priori,* based on MOE, whether to even consider including a compound in an assessment group. If the margin of safety (i.e. exposure/health-based guidance value (HBGV)) (based on the critical effect) is less than an agreed percentage (i.e. saturation of HBGV is < x%), should the substance even be considered for inclusion in the assessment group?

The Handbook could expand on possible options once the key risk drivers have been identified. Should one progress to higher tier assessment, should additional *in vitro* testing be undertaken, should the focus be on risk management options? Some indication of the factors that would help determine the next steps would be helpful (this would presumably depend to some extent on problem formulation).

It was noted that refinement of exposure estimates is essential in CRA. Aggregate exposure can also be relevant when defining the groups.

The section on uncertainty assessment could perhaps be expanded slightly in the Handbook, with cross-reference to relevant guidance.

It was suggested that the text on the SMNU method might fit better in the risk characterisation section.

2.9. RISK CHARACTERIZATION

The dose-addition model is the default model. Response addition and synergy may be considered on a case-by-case basis.

2.10. UNCERTAINTY ANALYSIS

Uncertainties are related to the different steps in the mixture risk assessment and the Handbook provides a template for the uncertainty analysis, where uncertainties should be identified and described, and quantified if and when possible.

COMMENTS from the participants

It is important to distinguish quantifiable variability in model parameter estimates vs "real" uncertainty (unknowns). Much of the current reference to "uncertainty" in the Handbook refers to variability, Hence, the meaning of uncertainty analysis as described in the Handbook should be more clearly explained.

3. PERSPECTIVES OF DIFFERENT RISK ASSESSMENT BODIES

Individuals from a number of risk assessment bodies were invited to provide their comments on the likely utility of the Handbook in the risk assessment of combined exposure to multiple chemicals by their own organisations. Any suggestions as to how the Handbook might be improved would also be welcome.

3.1. OECD

OECD develops methodology but does not carry out risk assessment of single or multiple chemicals. OECD has recently published some "Considerations for risk assessment of combined exposure to multiple chemicals", which includes discussion on points to address in problem formulation and on the risk assessment of exposure to multiple chemicals. It covers a wide range of scenarios.

The EuroMix Handbook is very complimentary to the OECD publication.

There is a need to include more mechanistic data and AOPs in risk assessment, which have considerable potential in the grouping of chemicals. NAMs can help in assessing AOPs, which in turn can help in better use of such data, although these have yet to be validated. There is a good measure of agreement on the general methodological approaches to CRA. However, decisions on how the methodology should be applied take place in different decision contexts (i.e. the problem formulation), so that sometimes the approach used will vary. It is proposed that we should start to use the methodologies now, and then refine the approach as necessary, with experience.

It is important to continue to develop a range of case studies (which is ongoing in different regions), and it would be helpful to increase the number of case studies available. Perhaps the ones developed by EuroMix and by EFSA will inform application.

With respect to data collection and storage, there is a need to harmonise how this is done, building on work already done at the OECD with the OECD Harmonised Templates, to enable data sharing.

3.2. WHO

The need for WHO is to be able to assess chemical risk and to provide robust advice to risk managers. This requires technical guidance that translates the results of research such as from EuroMix into risk assessment practice.

The Handbook and the Toolbox are closely linked, and it is not always easy to distinguish what refers to which.

With respect to the Handbook, some of the recommendations are not very applicable for international risk assessment, i.e. by JECFA/JMPR. But there are some parts of the Handbook that, it is hoped, could be used as the basis of practical guidance.

Regarding the Toolbox, this is a very powerful platform, but it needs to be determined whether it allows sufficient flexibility for the approach necessary in the work of WHO, especially JMPR and JECFA. An important question is how the platform is to be maintained after EuroMix finishes. There are also issues of transparency and of validation if the Toolbox is to be used in international risk assessment.

3.3. FAO

In addition to the views of the WHO, the need for a flexible approach, that covers both data rich and data poor compounds, was emphasised.

Approaches for the risk assessment of dual use compounds (pesticides and veterinary drugs) have recently been harmonised by FAO/WHO. It is important that these can be accommodated in the Toolbox and could perhaps be addressed in the Handbook.

3.4. JRC

JRC has undertaken work on methodology for risk assessment of combined exposure to multiple chemicals, covering both dietary and non-dietary exposure. The flexibility of being able to use and import one's own data into the Toolbox is appreciated.

Use of NAMs and AOPs in such assessments is important.

With respect to the Handbook, it is not always clear what can be done in the Toolbox and what cannot be done at the moment (or at all).

The Handbook is easy to read. But there is some imbalance in the detail, for example the section on the identification of mixtures vs the one on IVIVE, where the latter is quite brief. There could perhaps be a better balance in the Handbook for data rich and data poor chemicals.

The Handbook could provide more guidance on communication and working across silos. This is not always clear, for example on how and when non-dietary exposure should be integrated into assessments.

Most of the documents cited in the Handbook are from EFSA and related to pesticide CRA. Perhaps this should be broadened to other key documents for non-dietary exposure and other chemical sectors.

Overall, it was felt that the Toolbox would contribute to the harmonisation of approaches to the risk assessment of combined exposure to multiple chemicals.

3.5. EFSA

The recent EFSA guidance on risk assessment of combined exposure to multiple chemicals addresses systematically problem formulation, hazard characterisation, exposure assessment, risk characterisation and the reporting summary. This guidance was applied by EFSA in the case study on four compounds for the WHO Expert Consultation.

EuroMix should give careful thought to dissemination of its different outputs and achievements, in scientific journals and in open source platforms, for each individual model developed, to maximise the incorporation of these tools into other platforms and so optimise their use in risk assessment by a range of scientific advisory bodies.

Training in the methodology is important and some thought needs to be given to how this can be accomplished.

With respect to the use of the Toolbox within EFSA, there is a formal process, described in the procedure for new tools, by which such innovations are assessed. There would need to be a critical appraisal of the individual models and methods. This is context dependent. Models used by EFSA need to be open source. A recommended option is to connect relevant EuroMix models and data in the Toolbox to EFSA's OpenFoodTox.

In general, the Handbook and Toolbox were positively received. There are possibilities of applying the Toolbox in the European context. This could be facilitated by submission of a project idea to the EU Risk Assessment Agenda and by integration of the EuroMix tools with other EU tools.

3.6. GENERAL DISCUSSION

There are various mentions of the Handbook, Toolbox and User Manual for the Toolbox, but they are not always clearly distinguished. Each of these needs to be clearly described, particularly the role of the Handbook. How do these all map to each other?

How should AOPs for endpoints not covered during the EuroMix project be addressed?

OECD could perhaps co-ordinate the development of AOPs for endpoints of specific regulatory concern, to ensure that these are adequately covered. Could this be a research focus within Horizon Europe?

Suggested enhancements to the Toolbox include: provision for the generation of transparent summaries of the model outputs, enabling trace-back to the relevant inputs; clear explanations for the selection of parameters, decisions, input data; perhaps link modules to relevant templates (and vice versa). There could be more modules on exposure, for grouping and for lower tier exposure estimation.

P450-specific metabolism of chemicals and physiologically-based kinetic models should also be addressed.

The output of the Toolbox should be independently verifiable and replicable: the Toolbox provides all of the details necessary for this.

4. KEY MESSAGES AND POINTS FOR DISCUSSION AT THE UPCOMING WHO EXPERT CONSULTATION

It is noted that, at present, the format of consumption data used by JMPR and JECFA is not compatible with the Toolbox (summary statistics vs raw data for individuals) because they are not based on individual food consumption data. In addition, since distributions of monitoring data are generally not available to JMPR/JEFCA, usually a full probabilistic assessment cannot be performed. At international level, performing a full probabilistic assessment would require using a distribution of occurrence either from residue trials or from monitoring data. What sources could be used for suitable residue and food consumption data for this?

The presence of several metabolites as residues of a single substance can also be of concern for assessments by JMPR/JECFA. What assumptions can be made about shared and different MOAs, and hence membership or not of an assessment group?

Whilst the focus of JECFA and JMPR is chemical exposure from the diet, WHO also has activities related to Drinking Water, and Indoor Air Quality and Ambient Air Quality. The implications of this may need to be considered at the Expert Consultation.

Problem formulation needs to be clearly defined for JMPR and JECFA.

It is noted that the EU Commission is willing to use the monitoring data as background to assess the saturation of the remaining ADI for a new compound to be authorized. It remains to be determined whether this approach can be applied or is even feasible at JECFA/JMPR.

CRA for pesticide or veterinary drug residues at international level is complicated by national/regional difference in GAP, and by the lack of residue data for some of the compounds belonging to the same assessment group, at a given meeting. Relevant data are usually submitted by sponsors in response to a data call. Ways of addressing these issues will need to be explored.

JMPR/JECFA might prefer to use MOA for endpoints considered relevant as a basis of grouping chemicals. However, experience in recent years has shown that sponsors generally do not provide mechanistic data, despite repeated calls for such data. This makes it difficult to identify MOAs, although data might be available in the literature. A related issue is how data generated using NAMs should be used for this purpose.

Organisational aspects within the JECFA/JMPR process will need to be carefully considered: e.g. flow of data via WHO; standard of reporting; compatibility of data collection. It would be helpful to agree on common templates, to help in harmonisation and to ensure data format compatibility.

How can FAO/WHO facilitate training, if at all?

5. Conclusions

The Handbook was generally well received. Participants felt that it was clearly laid out and that, together with the Toolbox, it should make a significant contribution towards international harmonisation of approaches and methods used in the risk assessment of combined exposure to multiple chemicals.

Participants made a number of suggestions on how the Handbook might be clarified or extended. These included:

- Ensure that the Handbook is "neutral" in the approach and methods used, to provide maximum flexibility for the range of problem formulations that arise in different chemical sectors and regions
- Clarify the difference among the Handbook, the Toolbox and the User Manual for the Toolbox, and their respective roles
- Improve the balance of the text, which is extensive in some areas but relatively short in other, equally important, areas
- Provide guidance on options where AOPs are not available for the chemicals being assessed, including assessing confidence in AOPs developed *de novo* for an assessment and options where AOPs cannot be developed
- Discuss options also for exposure-based grouping rather than only toxicologically-based grouping of chemicals
- Expand the section on exposure to consider approaches in addition to the full probabilistic assessment described
- Ensure that templates are suitable for data exchange, for example are harmonised with the OECD templates for data collection
- Provide some guidance on reporting standards, including suitable summaries for risk assessors and for risk managers

The participants concluded that there were several sections of the Handbook and modules in the Toolbox that could potentially contribute to risk assessment of combined exposure to multiple chemicals by JECFA/JMPR and that would merit discussion at the Expert Consultation. However, potential limitations were also identified, including availability of suitable data on exposure, formats for consumption data, transparency of the models used in the Toolbox, and the need for verification of methods, algorithms and software.

Annex

Fourth EuroMix workshop on international harmonisation on the risk assessment of combined exposures to chemicals

Program

The objective of the fourth workshop is to explore the extent to which the EuroMix guidance serves the needs of harmonised scientific approaches to the risk assessment of combined exposures to chemicals in the diet, in relevant legislation.

15 April 2019: Room D (7th floor), WHO HQ, Geneva, Switzerland

Day 1		
09:00-10:00	Registration	
Chair/Rapporteur	**Alan R Boobis *(Imperial College London)*/Angelo Moretto (University of Milan)**	
10:00-10:15	Welcome and introductions	Alan R Boobis (Imperial College London) and All
10:15-10:30	Background and objectives of meeting	Alan R Boobis (Imperial College London)
10:30-11:30 11:30 -12:30	The EuroMix Handbook General discussion	Johanna Zilliacus (Karolinska Institute) All
12:30-13:30	Lunch	
13:30-15:30	Perspectives of risk assessors (including) - OECD - EFSA - WHO - FAO - JRC	Short presentations (5-10 min) and general discussion
15:30-16:00	Refreshment break	
16:00-17:00	Implications for WHO Expert Consultation	All