Mixture toxicity
Call-for-Action

The Danish Ecological Council

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The Ecological Council
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Mixture toxicity Call-for-action

The Danish Ecological Council calls upon the EU to:

- **Use the Concentration Addition method as the default mixture prediction model**. The majority of experimental studies support this conclusion as both being the most precise and most protective.
- **Ensure the use of the precautionary principle** with regard to the identification of, and control over, chemicals that contribute to mixture toxicity, without awaiting results of further research.

  However, research must continue in parallel in order to improve our understanding of endocrine disrupting chemicals (EDCs) and other problematic chemicals in regard to mixture effects.
- **Agree on an approach for assessing mixtures in real life**. Single chemical risk assessments underestimate real life toxicity systematically and there is sufficient scientific knowledge to conduct mixture toxicity assessments.
- **Explicitly implement demands for mixture toxicity assessment in REACH, the Water Framework Directive, the directive on pesticide residues in food and other relevant directives**. If it is decided not to make a REACH revision in 2012-13, and instead a review, it must be substantiated that a safe regulation of mixtures can be provided within the present REACH-text - by changing annexes (comitology), guidelines and procedures.
- **Initiate the construction of an addendum to the central EU chemicals database**, containing knowledge of chemicals already present in the environment - and the possible use of Concentration Addition. Subsequently this database should be used to make realistic mixture exposure scenarios. Also knowledge about synergistic interactions should be gathered and stored in the database - in order to take this into account for specific combinations of substances.
- **Not only focus on the endocrine disruptors**, which are currently the main focus of mixture toxicity assessment, but also be aware of other types of chemical mixtures.

It is well known and recognized that exposure to chemicals is not from one chemical at a time, but rather simultaneous exposure to a mixture of different chemicals[1,2]. This fact calls for regulatory measures that aim at controlling the risk from such mixtures. Without risk assessment for mixture toxicity it is not possible to ensure proper protection of the environment and human health. However, current EU chemicals legislations mainly consider toxicity of single chemicals, without taking sufficient account of mixture toxicity. In order to ensure a high level of protection from hazardous chemicals, it is thus important to consider how mixtures can be accounted for in EU chemicals legislations.

With the thousands of chemicals on the market it is unfeasible to aim at evaluating all possible mixtures experimentally. However, it is generally recognized that estimation of mixture effects based on knowledge from single chemicals is a feasible approach in most scenarios[3,4]. Using a modeling approach, and especially the Concentration Addition model, to assess mixture toxicity, has been considered well suited by experts such as described in the State of the Art Report on Mixture Toxicity from 2009 (Kortenkamp et al.)[4]. The overall scientific foundation for mixture toxicity assessment is therefore in place and it is now vital that political actions ensure that mixture toxicity is properly accounted for.

At present, the actual implementation into the various legislations in EU still remains. There is an array of relevant EU legislations regarding mixture toxicity. The initial focus should be on ensuring implementation in some of these legislations. When these implementations are in place the principles can be spread to other relevant legislations. Implementation should primarily focus on: REACH/CLP, the Water Framework Directive (WFD), the Cosmetics Directive as well as the pesticide legislation, including pesticides in food, where mixture assessment is already included.

The Danish Ecological Council therefore recommends the following actions:

Concentration Addition should be accepted as the default mixture toxicity prediction model.

Two different models have been proposed for prediction of mixture toxicity. These two models, called Concentration Addition and Independent Action, predict mixture effects of chemicals

1 Deneer, J. W. (2000). Toxicity of mixtures of pesticides in aquatic systems. Pest Management Science, 56, 516-520

2 Carpenter, D.O., Arcaro, K., Spink, D.C. (2002). Understanding the Human Health Effects of Chemical Mixtures. Environmental Health Perspectives Supplements 110:S1

3 Syberg, K., T.S. Jensen, N. Cedergreen, J. Rank (2009) On the use of mixture toxicity assessment in REACH and the water framework directive: a review, Hum. Ecol. Risk. Assess., 15, pp. 1257-1272

4 Kortenkamp, A., Backhaus, T. & Faust, M (2009) State of the Art Report on Mixture Toxicity, European Commission, Brussels, Belgium

with similar and dissimilar mode of action (MOA), respectively. The concepts were first described in the 1920s-1930s by Loewe & Muischnek[5] and by Bliss[6], respectively. Both models allow for mixture toxicity prediction based on knowledge regarding the toxicity of single chemicals, which is a major advantage in a risk assessment context. There is, however, one major problem with the application of the models. In order to group chemicals based on similar or dissimilar MOA, we need to know the specific MOA of the chemicals. Such knowledge is available for some pesticides, and possibly also some pharmaceuticals, but is generally lacking for most anthropogenic chemicals that are used today. It is an unrealistic task to determine specific MOAs for all these chemicals. One reason for this is that for most of these chemicals we know little about their toxicity. Another reason is that not only one specific MOA, but rather an array of toxic MOAs, could be environmentally relevant (e.g. for different species, exposure concentrations etc.)[7]. Assuming it is necessary to group chemicals in order to select the best suited model, it is not realistic to assess mixture toxicity in a broad scale. Much effort has therefore been directed toward discussing whether one of the models can be used as default prediction model. In summary, growing scientific evidence suggests Concentration Addition as a well suited default model[8], because the Concentration Addition model is precise, precautionary and often can be readily applied (e.g., using summed risk quotients). We therefore strongly recommend that Concentration Addition is used as the default mixture prediction model. How to deal with different MOAs will be further developed in the next section below.

Assessing mixtures in real life

Even though Concentration Addition should be applied for all mixtures, it is not readily implementable to all types of data. It is beyond the scientific foundation of the model to group very different types of data (e.g., mutagenic potency with estrogenic activity). It is important to recognize that this is not in contradiction with applying Concentration Addition to mixtures of chemicals with both similar and dissimilar MOA. Data measured with one endpoint can be caused by different MOAs (death is the logical example) even though some endpoints measure effects due to a specific MOA. It is therefore important to develop an approach that ensures proper protection from chemical mixtures and at the same time is scientific sound and feasible. There are several options for such a real life implementation:

i) The simplest solution would be to apply a mixture safety factor to all risk assessments. The magnitude of this factor could be based on the number of chemicals in the mixture. Even though this is easily implementable it is a solution that is not based on the scientific knowledge within the field, and we recommend that more scientifically verified approaches are addressed prior to solely applying safety factors.

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5 Loewe, S., Muischnek, H., 1926. Effect of combinations: mathematical basis of problem. N-S. Arch. Ex. Path. Ph. 114, 313-326

6 Bliss (1939) The toxicity of poisons applied jointly, Ann. Appl. Biol., 26, pp. 585-615

7 Syberg, K. et al. (2008). Mixture Toxicity of Three Toxicants with Similar and Dissimilar Modes of Action to Daphnia magna. Ecotoxicology and Environmental Safety 68: 428-36

8 Kortenkamp, A., Backhaus, T. & Faust, M (2009) State of the Art Report on Mixture Toxicity, European Commission, Brussels, Belgium

ii) The most comprehensive approach would be to agree upon a number of assays that combined ensures testing of all effects considered to be the most problematic (e.g. carcinogenic, endocrine disruption, bioaccumulation). Data from these assays should then be collected for all chemicals relevant to a specific mixture toxicity scenario, and added with Concentration Addition for each assay. That will produce a number of different mixture toxicity assessments. The most protective should subsequently be used. Since not all chemicals might have an effect in a single assay, there should furthermore be added an additional safety factor of one, for each chemical that is not included in the assessment that is used (so that the final safety factor would equal the number of chemicals not assessed specifically). This approach would however imply that a substantial amount of data for each chemical has to be produced - possibly more than within the current system. Whether this is realistic is questionable since it would require the industry to produce significantly higher amounts of data than currently (within REACH). Furthermore, the tonnage tiers would have to be changed, since chemical contributions from smaller productions should be assessed for the above mentioned problematic effects.

iii) Another approach could be to construct risk quotients (RQs) for each chemical, and then add the RQs numerically. In doing so, we would go beyond the fundamental principles of Concentration Addition. However, the scientific consensus regarding how to group chemicals has already moved beyond the original theoretical concept, since the strict definition of similar and dissimilar MOA used by Bliss (1939) only holds true for few groups of chemicals under specific conditions. Furthermore, the Independent Action theory was developed only for binary outcomes (e.g. death/no death) but is applied much broader today. It can thus be argued that the verification of applying both Concentration Addition and Independent Action is based on empirical rather than theoretical observations. A similar empirical approach could thus be used to verify how RQs can be used in assessment of mixtures.

The Danish Ecological Council believes that adding RQs would be a good pragmatic approach, ensuring that all the relevant chemicals are taken into account. In favor of this approach is the fact that mixture toxicity assessment in existing legislations such as CLP does not group chemicals in very specific MOA-based categories. The inclusion of summed RQs could possibly be done in a tired approach where summed RQs would be a first precautionary tier 1 assessment. Production of more specific data for Concentration Addition prediction would only be required if the tier 1 assessment indicate risk, as discussed in a recent publication by Backhaus & Faust (2012)[9]. It should furthermore be considered to apply a safety factor for those chemicals that are present but lack toxicity data, as discussed above.

[9] Backhaus T. & Faust M. Predictive Environmental Risk Assessment of Chemical Mixtures: A Conceptual Framework. Environmental Science and Technology. 2012. 46 (5), pp 2564-2573

Mixture toxicity assessment in REACH/CLP

Mixture toxicity should be assessed under REACH. Under the current regulatory regime mixture toxicity is only assessed when substances are regulated under the Classification and Labeling of product and mixtures Directive (CLP). It is important to develop approaches that enable mixture toxicity prediction when assessing all phases in the chemical life cycle. The major obstacle for broader inclusion of mixture toxicity in REACH seems to be the fact that REACH aims at regulating single productions rather than regulating chemicals in regard to a more holistic environmental/human health concern, where exposure to chemicals comes from different productions. Such productions will often be separated both in time and space. There are thus two major problems that should be addressed in regard to REACH. First, it is vital to ensure that the regulation, from a legal perspective, demands mixture toxicity assessment with the chemicals relevant to the actual exposure scenarios (e.g. those from other productions of relevance and those already present in the environment), rather than just the chemicals in a specific preparation. Secondly, it is important to initiate surveys to determine what chemicals are actually present in the European environment, in order to enable an inclusion of these chemicals in the exposure scenarios.

Implementation of mixture toxicity assessment in the Water Framework Directive

The overall objective of the Water Framework Directive is to ensure proper ecological quality in European waters. It is beyond doubt that hazardous chemicals present in the environment can have an effect on ecological quality, and it is therefore important that demands for mixture toxicity assessment is explicitly implemented in the directive. There are no current demands for such assessments and it should be of high priority to ensure such an implementation. It is furthermore important that approaches to select chemicals in specific scenarios are developed. These approaches should be constructed as general methodologies that can be used for future assessments of mixture effects in the aquatic environment.

Expansion of a database for chemicals in the environment in Europe

Collected knowledge of chemicals already present in the environment should be gathered in an addendum to the central database at ECHA, where it should be used to make realistic exposure scenarios in regard to all relevant chemicals legislations. Inspiration on how such a database (/addendum) can be constructed can be found in the Italian DESC database (DESC 2010)[10], in the European Pollutant Release and Transfer Register (PRTR)[11] and in the Danish NOVANA project (NST 2011)[12]. One additional mixture-specific type of information that could be stored in the

[10] DESC (2010) Ecotoxicological database on Chemical substances, IRSA-CNR, Rome, Italy. Can be accessed at: **www.irsa.cnr.it/Docs/Perso/BarraC_DESC_en.pdf**

[11] European PRTR: **http://prtr.ec.europa.eu/**

[12] NST (2011) NOVANA 2011 - 2015. Danish Nature Agency, Copenhagen, Denmark. Can be accessed at: **www.naturstyrelsen.dk/Naturbeskyttelse/National_naturbeskyttelse/Overvaagning_af_vand_og_natur/NOVANA/** (in Danish)

database is knowledge about observed synergistic[13] interaction. Since Concentration Addition assumes additivity[14] by default, any synergistic interactions will be underestimated. For most scenarios synergistic and antagonistic[15] interactions are not relevant, but in cases where few chemicals dominate the overall toxicity of the mixture, synergism might be important. It is therefore relevant to gather all knowledge about synergistic interactions. Such information can be used to evaluate whether synergy must be addressed in specific scenarios where chemicals with known synergistic interactions are present.

Effort dedicated the understanding of non-EDC mixtures

Finally, it is important not to forget mixture effects of chemicals with toxic effects other than endocrine disruption. The endocrine disruptors are currently the main focus of mixture toxicity assessment, since experiments have shown alarming mixture effects at low doses[16]. However, other types of chemicals can also elicit severe mixture effects. It is therefore important that other types of chemical mixtures are considered. For example, carcinogenic and mutagenic chemicals are two very important groups of chemicals where little is known about the mixture effects.

[13] The overall biological effect of two or more chemicals taken together is greater than the sum of their separate effect at the same doses

[14] The overall biological effect of two or more chemicals acting together which is the simple sum of the effects of the chemicals acting independently

[15] The overall biological effect of two or more chemicals is actually less than the sum of the effect of the chemicals taken independently of each other

[16] Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes et al.: Hormones and endocrine-disrupting chemicals: low-dose effects and non-monotonic dose responses. Endocr Rev 2012, 33(3):378-455. Epub 2012 Mar 14.

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