IDEA
International Dialogue for the Evaluation of Allergens

# Report on the IDEA Workshop on the
# Characterisation and Categorisation of allergens

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The aim of this Workshop was to discuss some of the issues raised by the previous non -animal hazard assessment methodology workshop in May 2018. The May workshop had concentrated particularly on approaches to the combination of in vitro assays for hazard assessment for the purpose of the identification of a NESIL as a basis for risk assessment.

Participants were encouraged to think 'outside the box' and therefore a wide ranging enthusiastic discussion ensued. The topics covered can be broadly summarised as follows:

- Objectives
- Use of existing data
- Non-animal test framework
- Approaches to data analysis
- Next steps for IDEA.

## i) OBJECTIVES OF A NON-ANIMAL TEST FRAMEWORK

There are two aspects to potency, namely potency of induction of sensitization and intensity of the elicitation response in the subjects who react.
It was agreed to focus IDEA's work on the potency of induction of sensitization keeping in mind the potential opportunity to look at approaches to gain potency information based on the vigour of the allergic reaction in sensitized people.

## ii) USE OF EXISTING BENCHMARK DATA

Since several chemical datasets are available its selection should include the consideration of clear rules and guidelines. However, it was noted that an increase in the quality criteria for entry of a chemical to the data base inevitably results in a smaller data set, whereas in real life one often has to use imperfect data to make a reasonable prediction.
The question was raised whether the data indicate that separation of fragrance ingredients into separate chemical classes would be useful for risk assessment purposes. It was stated that there might be 'chemical classes' where the prediction is good, but some others are not likely to be useful. Nonetheless, it was agreed that there was a need for

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new thinking about what classes could be constituted. This could be based on a paradigm of reaction mechanistic domains.
RIFM has identified that, in 90% of the cases looked at, the LLNA predicted a no-effect level well. However, caution is needed in validating against human data; e.g. the RIFM data needs to be used with care as the HRIPT values cannot be interpreted as directly related to the actual no effect level in humans.

*A new database of aggregate evidence was proposed involving both animal and human data, which must allow full transparency. It should not be solely focus on fragrance ingredients but might be fragrance heavy.*

### iii) NON-ANIMAL TEST FRAMEWORK

GENERAL CONSIDERATIONS
Potency is an intrinsic property of a sensitizing substance. It is likely to be a chemical, biological continuum, not discrete steps, which varies very widely between chemicals. There are a lot of interesting approaches for the assessment of potency but as yet no agreement on the best approach.
*IDEA needs to consider its further input to this important issue*

BENCHMARKS
At recent OECD meetings there have been discussions about good benchmark materials and whether the LLNA and Human data included in the current CosEU database should be curated further. Consequently, workshop participants suggested to extract some materials from the CosEU database where there is good data (and define criteria for those) and to use this as a starting point to build a benchmark database. It was pointed out that many of the in vitro test systems appear to rely on animal data as the ' gold standard'. On this basis if one can predict the LLNA, one is close to predicting potency.
*The criteria for acceptance as 'gold standards' needs to be revisited.*

TEST SYSTEM SELECTION
A substantial number of test systems have already been developed. This raises the issue of what the priorities for further test development should be and how to select the most utilisable existing ones. In moving forward it is important to be specific on where the gaps in our knowledge that are key to better risk assessment and would benefit from further test development are.
Discussion ensued on whether a suitable assay for the first key event (KE1) is the most important since all the tests for subsequent events (KE2, KE3) are an indirect measure of key event 1. One recommendation was to enlarge the DPRA to look into the aspect of the strength of the reaction. The alternative view expressed is that relationship between the key steps is not so simple, that is, while the interaction with the protein is necessary, it does not adequately define the more complex process. An important question in this context is whether there are thresholds for each step or all are continuous variables. Measures of hazard do not automatically turn into candidates for measures of potency. In terms of further involvement in this area it was proposed *that IDEA should look at possible ways forward to facilitate feasibility studies rather than launch larger (and expensive) research programmes.*

ENDPOINTS
A major challenge is that markers of potency should be causally and quantitatively associated with the relevant endpoint – which is the acquisition of allergy.
Skin sensitization potency probably depends on the vigour of T-lymphocyte responses, the quality of the responses and the breadth of T-lymphocyte responses, meaning the number of clones. The problem is that these are difficult to measure in vitro and to understand what are the events that influence the behaviour of the T-lymphocytes. From this perspective the maybe most promising one would be the activation of dendritic cells.
Other endpoints discussed included:

- antibody responses, could the vigour of antibody production provide a measure for potency?;
- holistic assessment of biological responses to sensitizing chemicals (gene expression / epigenetic / proteomic signatures);
- Detailed qualitative evaluation of protein haptenation (kinetics/amino acid selectivity / orientation of hapten expression);
- More exhaustive characterization of the response of cutaneous dendritic cells to sensitizing chemicals (quality and quantity);
- Qualitative /quantitative aspects of danger signals responses.

It was expressed to be cautious to only rely on a few samples to reach conclusions. *To make the data more manageable, a signal noise ratio estimation (meaning a more probabilistic characterization of the effects) was suggested.*

**iv) METHODS FOR DATA ANALYSIS**

In using the data to determine a NESIL it needs to be clear that in the QRA it is a human threshold. A better analysis of existing animal data was advocated. It was agreed that we are *lacking a good weight of evidence guidance for the AAT 'world'*. There was a brief discussion on whether different weighting factors should be used for human and animal data, but no conclusion was reached. For evaluating combination of existing data, it was suggested that a competition based on the assessment of a set of chemicals by the various approaches could be set up to judge on the outcome of prediction, applicability and perhaps also the costs.
There is a variety of approaches that could be used to combine data from different tests, etc.
The use of artificial intelligence in the assessment of toxicology of skin sensitization was proposed (the implication of this is a willingness to accept the outcome despite not fully understand the system behind). This raises the issue of transparency.

**v) NEXT STEPS FOR IDEA**

This aspect was not formally discussed in any detail (although possible areas for IDEA are set out above in italics). However two additional topics were specifically mentioned.

GENETIC ASPECTS
It was questioned to which extent current in vitro tests are able to catch up the variation in genetic polymorphism, as most are working with single cell lines.
Research in Lyon's University using minimal invasive human skin biopsies was described which enabled the differentiation between allergy and irritation. Allergens have an impact on nearly 2000 genes, 255 are specific to the irritant effect, 453 are positively correlated genes (common effects). Based on the work done so far they assume that all allergens have a specific signature in the gene profile. Another challenge is to distinguish between a weak irritant reaction and real allergic reaction for the same molecule. It was mentioned that work on to dose response has started in the clinic in Malmö (Magnus Bruze and Cecilia Svedman) on Amerchol.
*IDEA is already involved with the University of Lyon activities in this area. Whether it should also look at other aspects too needs to be discussed.*

EXPOSURE DATA BASE
Developing the criteria for such a database would be a useful activity for an IDEA Task Force, similar to the hydroperoxide TF. The group would need to ensure that there is no overlap with work already done or going on at CosEU or OECD. Once such a database is available*, IDEA could check how different approaches deliver with regard to developing a meaningful NESIL (point of departure in a risk assessment).*