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European Centre for Ecotoxicology and Toxicology of Chemicals
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Expert Panel to Better Understand Endocrine Disrupter Low Doses Effects

CONTENTS

SUMMARY

1. This expert panel was convened by ECETOC to discuss the issue of so-called low dose effects of endocrine disrupting chemicals (EDC), and to propose a possible research programme to throw more light on this area.
2. Under the term 'low dose effects', the panel took into consideration subjects such as non-monotonic dose responses, the absence of thresholds for toxicity and the potential adverse effects resulting from the combined exposure of EDCs in the low concentration ranges. The panel considered these subjects and proposed suitable definitions.
3. The existence of "low dose effects" is part of an unresolved debate among scientists, but has nevertheless been taken as a reason why traditional risk assessments of EDCs may be unreliable. It is therefore important that the presence of such effects should be firmly established or refuted and their implications for risk assessments fully investigated in order to provide risk assessors with a sound scientific foundation for their work.
4. On the combined exposure of EDCs it was considered that additive effects, rather than synergistic effects are to be expected in the low dose range and this topic was therefore not considered as a top priority to address in the first instance. The panel therefore, formulated two hypotheses which it considered needed to be tested in order to make progress in this subject.
    a) The lack of a threshold means that the dose response relationships established for endocrine disrupters will show the adverse effect at any dose tested.
    b) Non-monotonic responses in dose response relationships for endocrine active chemicals mean that it is not possible to anticipate adverse events using traditional approaches.
5. It was agreed that the only practical way to support or refute these hypotheses is through the use of mechanistic mode of action (MoA) models focusing on key events, their quantitative description and dose-response characteristic. Empirical or direct experimental approaches to test for thresholds are unlikely to be helpful for resolving this issue due to the unrealistically high statistical power which would be required to detect the very small effect sizes assumed to apply at low dose levels. However, in analogy to the lessons learnt with chemical carcinogens, the question of thresholds can be resolved by considering mechanism of carcinogenicity. Applied to endocrine disrupters, the idea would be to identify the key events for the mode of action of chemicals for which low-dose effects have been reported. These key events would range comprehensively from interaction with the molecular target to the ultimate adverse effect (i.e. the whole adverse outcome pathway). Each key event would then be fully characterised in terms of dose and time responses.
6. The key events will need to be characterised over a large range of doses, and the resulting datasets integrated through the use of mathematical modelling using a system biology approach. Such models would then be interrogated to investigate whether or not low dose and/or non-monotonic responses were indeed feasible.
7. It was agreed that before funding is sought for such a large research programme, it will be important for the control of the programme to be placed under the aegis of a neutral body such as the World Health Organisation (WHO) or the Organisation for Economic Cooperation and Development (OECD). This was considered essential in order to ensure maximum confidence in the finding.

1. WORKSHOP OVERVIEW

In the field of endocrine disruption, so-called 'low dose' effects, non-monotonic dose responses (NMDRs), the existence or otherwise of thresholds for toxicity, mixture effects at low doses, and critical windows of exposure are challenging the current paradigm of toxicity testing and risk assessment of chemicals (Zoeller et al, 2012; Vandenberg et al, 2012, UNEP/WHO report 2013). As a result the European regulation on plant production products (1107/2009), the revisions to the biocides Directive (COM[2009]267) and the regulation concerning chemicals (Regulation (EC) No. 1907/2006 "REACH" only support the marketing and use of chemical products on the basis that they do not induce endocrine disruption in humans or wildlife species. Nevertheless the EFSA Scientific Opinion on the Hazard Assessment of Endocrine Disruptors (EFSA, 2013), and the earlier EFSA Scientific Colloquium on Low Dose Response in Toxicology and Risk Assessment (EFSA, 2012), both suggest that there is no reason why endocrine disrupters (EDs) should not be subject to risk assessment and regulated accordingly. The issues on "low dose" effects listed above imply a possible need to make modifications either to the risk assessment paradigm or to current test methods, or both. Furthermore, neither the EFSA Colloquium nor the more recent Workshop on Low Dose Effects and Non-Monotonic Dose Responses for Endocrine Active Chemicals (Beausoleil et al, in preparation) were able to reach consensus about the importance, or even the existence, of these phenomena (see Zoeller et al, 2012; Vandenberg et al, 2012; UNEP/WHO 1212). This difficulty in reaching consensus is partly due to uncertainty caused by the quality of some of the data used to support these concepts, and partly due to a lack of understanding about putative underlying mechanisms of toxicity.

1.1 Workshop objectives

The aim of this workshop was to agree on a specific research programme which would be needed to achieve such a consensus and develop understanding of the implications for risk assessment of endocrine disrupters.

1.2 Workshop structure

Day 1 consisted of two sessions:

- Session 1: 5-minute presentations by each of the members, giving initial reactions to these Terms of Reference. How can they be addressed? Have you got any concerns, views, …
- Session 2: Brainstorm: Agreement of definitions of terms. What would this research programme look like? What studies are crucial to resolve these issues?

Day 2 consisted of two sessions:

- Session 3: To frame the proposal: Outline skeleton of the research programme.
- Session 4: Workshop conclusion: Main message. Next steps: Funding possibilities, implementation of research programme, need for further workshops.

1.3 Plenary presentation

Summary of main themes from participant's short presentations and discussion:

Any experimental approaches in this area of science need to take into account and control for the known and well described potential confounders of test outcomes such as the test diet, migration of chemicals from cage and water bottles etc.

Any work should be hypothesis-driven meaning that well-constructed hypotheses are required and need to be agreed.

Work should be statistically robust and include power calculations for the endpoints of concern and the degree of change required to be detected.

Work in this area is likely to have the best chance of success in addressing hypotheses around thresholds and the nature of low dose responses if it is focused on chemicals where the MoA is known or can be inferred.

Focus should be on effects known to be adverse, or predicted to be markers of adverse outcomes from a known MoA.

It is intrinsically not possible to investigate threshold events where effects sizes are small using traditional approaches that employ apical adverse outcomes as the endpoint. It is much more likely that focusing on key events in a MoA will be informative

Non-monotonic dose responses for adverse events in vivo are rarely seen and, if seen, the underlying mechanisms have not been described.

Synergistic effects at low dose are rarely if ever seen in vivo for endocrine active chemicals or those that work through other MoAs.

Adverse effects occur when homeostatic mechanisms are overcome.

There is therefore a need for reproducibility of the findings to be established for these low-dose effects in order to provide more convincing evidence.

1.4 Terms of reference

Invited experts who agreed to be part of the workshop were from the E.U., U.S. and Japan and represented academia, regulatory authorities and industry. Wider participation from regulatory authorities would have been welcome for addressing this controversial "low dose" issue.

The purpose of this group was to discuss, review and if appropriate amend the following hypotheses and to propose and agree a course of action whereby these or amended hypotheses could be investigated. It should be recognised that the starting draft hypotheses were devised in order to stimulate workshop discussion and do not represent the views of the organising committee or of workshop participants.

1. Endocrine active chemicals do not have a threshold below which adverse effects are not seen and should be regarded and tested in a different way to chemicals acting through other modes of action.
2. At low dose / exposure levels, endocrine active chemicals exhibit non-monotonic dose responses.
3. When mixed at low doses, endocrine active chemicals produce effects greater than those which may be predicted from simple dose addition.

Before considering the hypotheses in detail the group thought it important to review and agree on some key definitions of terms commonly used in this area and as they apply to the hypotheses.

'Adverse'. The definition as described by IPCS/WHO supplemented by the further definition contained in the IPCS Mode of Action (MoA) document were agreed and it was recognised that this definition would cover events that may be initiated by exposure during sensitive windows.

'Non-Monotonic dose response'. This can be defined as a change in direction of the relationship between response and changing dose; it should not be confused with a plateau of response.

'Low Dose'. For the purposes of this workshop low dose was agreed to mean dose levels within the range of known or predicted human exposure levels. However some participants felt that consideration should also be given to dose levels associated with small effects independent of their numerical values.

'Threshold'. An exposure level below which there are no adverse health effects and no such effects can be predicted based on knowledge of key events and MoA.

1.5 Review of the terms of reference and hypotheses

The group provided constructive challenge to the draft hypotheses, resulting in extensive revision.

Original Hypothesis 1

"Endocrine active chemicals do not have a threshold below which adverse effects are not seen and should be regarded and tested in a different way to chemicals acting through other modes of action."

Group consensus

As written the statement was not supported by the group as an hypothesis and was considered essentially meaningless.

The hypothesis as written cannot be tested or demonstrated by observational means for chemicals acting through any mode of action including endocrine. A key obstacle is the statistical power needed to detect low effect sizes that may be assumed at low threshold dose levels.

Proposal

The hypothesis, derived from studies with very potent oestrogenic chemicals, may not be relevant for low potency oestrogens or chemicals acting via other MoAs (e.g. prostaglandin synthesis inhibitors). The hypothesis needs to be re-formulated and focussed on specific areas for investigation:

- Questions need to be posed on a case by case basis.
- These should come from an understanding of the MoA and key events for a specific chemical or class.
- The dose response relationship for key events in a MoA or in an Adverse Outcome Pathway (AOP) will need to be understood to enable it to be investigated as will any dose response relationships for effects that may be considered adverse.
- Adverse outcome needs to be integrated using an understanding of relationships between the ultimate adverse effect, key events, the site of possibly multiple action and the exposure, and such integration is likely to require a longer term initiative.

Based on the above discussions and proposals a revised hypothesis was drafted and agreed:

"The lack of a threshold means that the dose response relationships established for endocrine disrupters will show adverse effects over a very wide range of dose levels."

Original Hypothesis 2

"At low dose / exposure levels, endocrine active chemicals exhibit non-monotonic dose responses."

Group consensus

As written, the statement is not a compelling hypothesis for valid scientific investigation. However if it were to be made more specific then there is some scope for empirical investigation.

Proposal

The hypothesis needs to be re-formulated, made more specific and to explicitly include the concept of adversity:

- Focus on addressing the question for chemicals where there is sufficient understanding of the MoA that leads to adverse effects. Consequently, for completeness, this work could involve studying several different MoAs that are presently relevant in the field of endocrine disruption.
- Focus on chemicals where there are claims about the existence of NMDRs.

- Model established key events and use selected in vivo approaches. Well defined in vitro studies using dose-response for markers related to adversity can also complement the in vivo approach.
- Ensure the MoA understanding is able to distinguish adverse events from homeostatic effects.
- Ensure the approach is statistically robust; understand and control sources of variation.
- Understand the dose range and ensure a sufficient number of dose levels is used to provide refined knowledge in line with the hypothesis.
- Ensure that the level of change constituting a real change in direction of the dose response is understood and agreed.

Based on the above discussions and proposals a revised hypothesis was drafted and agreed:

"Non-monotonic responses in dose response relationships for endocrine disrupters mean that it is not possible to anticipate adverse events using traditional approaches."

Original Hypothesis 3

"When mixed at low doses, endocrine active chemicals produce effects greater than those which may be predicted from simple dose addition."

Group consensus

This is not a priority area in the first instance and any further work should take into account and learn from the numerous extensive reviews and position papers already in the public domain – EU State of the Art Report (Kortenkamp et al, 2011), (Boobis et al, 2011), EU (2011), ECETOC Report (2012).

Proposal

- There is some potential to investigate the hypothesis but activities should be focussed in areas where synergy may be predicted from existing knowledge of MoA.
- Need to agree and use definition and understanding of low dose.
- Modelling and prediction should be based on MoA data.
- Need a clear agreement of what level of change / effect would be considered greater than that predicted by dose addition alone.

Based on the above discussions and proposals a revised hypothesis was drafted and agreed but it was not considered a priority area for further work at this time.

"MoA understanding can be used to predict 'synergy' when chemicals are mixed and when tested these mixtures will show 'synergy'."

In summary:

- The group provided clear guidance on how the hypotheses and terms of reference should be revised.
- Further consideration of thresholds and non-monotonic dose responses were clearly identified and agreed as the priority areas. Although, not identified as a top priority, Mixture effects should be addressed at a later stage in the research programme.

- The group agreed that traditional effect-led approaches to further study low dose and likely low effect responses was scientifically inappropriate, technically highly challenging with respect to experimental design and the number of experimental subjects required to achieve a robust outcome. For these reasons the group unanimously supported a MoA/AOP led approach, with the focus on key events, their quantitative description and dose response characteristics, as best way to understand threshold and low dose effects.

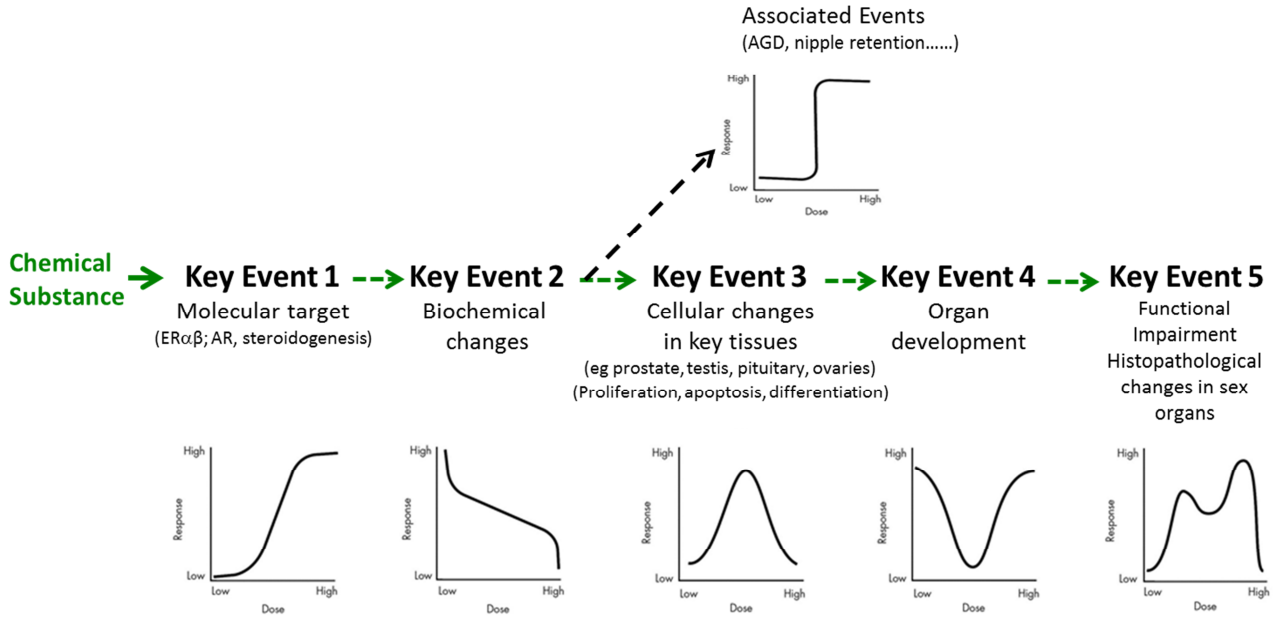
2. OUTLINE OF THE RESEARCH PROGRAMME

It was agreed to approach testing of the two primary hypotheses through mechanistic MoA models:

Chemical – primary interaction (quantifiable) – second change (quantifiable) – cell proliferation, apoptosis, etc. (quantifiable) – changes in organ structure and function (quantifiable) – adverse effect (quantifiable) (see Figure 1).

Experimental models to be used need to be further discussed but it was considered critical that peri-natal exposure containing specific windows of sensitivity during development has to be taken into consideration.

Figure 1: Recommendation for targeted studies to address 'low dose' effects and threshold of toxicity: to assess dose-response / time course for each critical key event of the MoA and evaluate overall point of departure



The concept is to first identify the key events (and associated events) of a mode of action which starts from the interaction of a chemical with a biological molecular target and eventually ends up with an adverse effect (e.g. histopathological lesions) . Each key event needs to be fully characterised in terms of dose and time responses. As the objective is to identify any low dose effects, should they exist, a large range of dose levels should be investigated. Particular attention needs to be paid at the low end of the dose-response curve and at potential point(s) of departure on this curve. It is anticipated that mathematical modelling will be needed to integrate all the information generated on the measurements of each key and associated events.

Although fragmented parts of these experiments have already been performed and reported in the literature this concept introduces a system biology approach to the field of 'low-dose effects'.

3. CONCLUSIONS AND RECOMMENDATIONS

Next steps:

1. It was agreed to perform a scientific evaluation on the application of the agreed concepts. ECETOC is looking into funding possibilities for this task.
2. A second meeting of the Scientific Expert panel will take place in Q4 2013 when the results of the scientific evaluation are available.
3. It was agreed to place any such initiative under the umbrella of an international organisation in order to demonstrate complete impartiality: the preferences were WHO/IPCS or OECD. European Science Foundation (ESF) and the National Academy of Sciences were discussed as possibilities for funding of the next meeting as well as for possible funding of the (to be developed) research program.

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APPENDIX A: LIST OF PARTICIPANTS

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APPENDIX B: WORKSHOP PROGRAMME

Monday 22 April 2013

12.15 – 13.00 Registration and lunch

Moderator: **Peter Matthiessen**

13.00 – 13.20 Tour de Table

Session 1 – KEY RESEARCH ISSUES:

5-minute presentations of each of the members, reactions to the Terms of Reference. Key research issues, concerns, views…

13.20 – 14.40 Rapporteur: **Peter Matthiessen**

Rémi Bars, Bayer CropScience, France

Marie-Noëlle Blaude, Scientific Institute of Public Health (WIV-ISP), Belgium

Alan Boobis, Imperial College London, UK

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Richard Sharpe, MRC Edinburgh, UK

14.40 – 15.00 Coffee break

Session 2 – REVIEW / BRAINSTORM:

The main themes from Session 1: ideas, concerns, proposals to address the issues raised.

Rapporteur: **Dick Lewis**

15.00 – 18.00 **Expected outcome: Agreement of definitions of terms.**
(open ended) **What studies are crucial to resolve these issues?**
What would this research programme look like?

18.00 End of Day 1

20.00 Dinner

Tuesday 23 April 2013

Session 3 – TO FRAME THE PROPOSAL:

Synthesise themes from Sessions 1 and 2 to come up with the outline of the research programme.

Rapporteur: **Peter Matthiessen**

09.00 – 12.30 **Outline of the research programme**

12.30 – 13.30 Lunch

Session 4 – WORKSHOP CONCLUSION

13.30 – 14.30 **Main message**

14.30 – 17.00 **Next steps: Funding possibilities, implementation of research programme, need for further workshops, ...**

17.00 Adjourn

Close of Workshop

APPENDIX C: ORGANISING COMMITTEE

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