Key scientific issues relevant to identification and characterisation of endocrine disrupting substances.

Report of ED Expert Advisory Group

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Human Health and Environmental Risks of Endocrine Active Substances – EFSA meets Stakeholders

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Endocrine Disrupters Expert Advisory Group (ED EAG)

- Established Nov 2011 as a sub-group of the ad hoc group of Commission Services, EU Agencies and Member States for the Community Strategy for Endocrine Disrupters
- To provide detailed reflections on scientific issues relevant to endocrine disrupting substances (EDs), not specific to any regulatory framework, including advice/orientation on scientific criteria for the identification of EDs
- Composed of (eco)toxicologists with regulatory and/or endocrinology background nominated by members of ad hoc group
- JRC-IHCP led, supported by planning group (drawn from expert group)

First task of ED EAG

- Produce a report capturing opinions of experts on key scientific issues relevant to identification of EDs
- Not required to produce a consensus report, may present differing opinions and options
- Report target date March 2013

Scope of report

- Capture the experts' opinions on key scientific issues relevant to the identification of endocrine disrupting substances
- State of the Art report (Kortenkamp A, et al., 2011) first 3 stages of the decision criteria used as a starting point for the discussions:
  - Stage 1: Adversity and Mode of Action
  - Stage 2: Human and wildlife relevance
  - Stage 3: Toxicological Evaluation (potency, lead toxicity, specificity, irreversibility and severity)
- Some of abovementioned factors connected to the identification of endocrine disrupting substances, while other factors connected to a further characterisation of the hazard. Thus report covers both identification and characterisation of EDs

Background Material

- State of the Art Assessment of Endocrine Disrupters, Kortenkamp et al. 2011
- OECD Detailed Review Paper on State of the Science on novel *in vitro* and *in vivo* screening and testing methods and endpoints for evaluating endocrine disruptors. No.178, 2012
- OECD Conceptual Framework and Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, GD 150, 2012
- IPCS/WHO 'Global Assessment of the State-of-the-Science of Endocrine Disruptors Report', 2002

Definitions and understanding of basic terms

- IPCS/WHO used as a working definition

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." (IPCS/WHO, 2002).

Definitions and understanding of basic terms

Scope of endocrine system

- Keep it broad and simple to provide flexibility for future developments and increased understanding of endocrine perturbations linked to disease outcomes of serious concern in either humans or wildlife

- Don’t restrict to specific aspects of endocrine system currently of concern (EATS) however in reality only able to detect endocrine disrupting modes of action where we have well enough developed assays permitting investigation of a specific endocrine modality

Definitions and understanding of basic terms

- IPCS definition for **Adversity** not specific to endocrine disrupters "*A change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences*." (IPCS/WHO, 2009).

- This IPCS definition of an adverse effect includes consideration of a population level effect. Since the protection level is set at the population level for environmental assessments, for an effect to be considered adverse it should have the potential to impact at the population level.

Definitions and understanding of basic terms

In the context of the IPCS Mode of Action and Human Relevancy Framework mode of action is defined as follows:

"The biologically plausible sequence of key events , starting with the interaction of an agent with a cell, through functional and anatomical changes leading to an observed effect supported by robust experimental observations and mechanistic data" (Boobis et al, 2009).

Definitions and understanding of basic terms

Proof of causality

According to the IPCS/WHO definition of an endocrine disrupter a causal association between an alteration of the function of the endocrine system and the adverse health effect is provided by the term 'and consequently causes'.

The ED EAG acknowledged that absolute proof of causation might be too high a requirement in establishing a substance as an endocrine disrupter and proposed instead convincing evidence of a biologically plausible linkage between the activity of the chemical in producing the alteration of the endocrine system and an observed adverse effect

Factors for ED Identification

- Demonstration of an Adverse effect for which there was convincing evidence of a **biologically plausible link** to an endocrine disrupting mode of action were considered the 2 main elements for the scientific identification of an ED

- Specificity also considered part of ED identification, since the primary mode of action of a substance should be disruption of the endocrine system rather than the disturbance of the endocrine system being a secondary consequence of other non endocrine-mediated systemic toxicity

- Relevance of the data to humans should be assumed in the absence of appropriate data demonstrating non-relevance. For wildlife data on all species relevant. Relevance applied in context of identified effects being relevant at population level

Adversity v physiological modulation

i) when can an observed change be considered as adverse (considering the IPCS definition of adversity)

- Fluctuations within the normal limits of homeostasis may be considered as physiological modulation without adverse consequence.
- At what point these fluctuations may become significant in the absence of an accompanying observable adverse effect on function could not be defined and would always be a case-by-case decision.
- If the stimulus is constant the fluctuation may be maintained/re-set (high or low compared to normal) indefinitely
- Important to evaluate the possible impacts of such a maintained change of state on an organism's capacity to compensate for additional stress (re. IPCS def of adversity)

Endocrine-mediated Adversity

ii) what types of adverse effects may be endocrine-mediated

- Depends on definition of endocrine system
- Mode of action not known for many substances
- So with a broad definition of endocrine system many types of adverse effects may be potential candidates

Endocrine mode of action and causal link to adversity

Endocrine disruption not one mode of action but many

Including:

- interference with production, transport and metabolism of hormones
- disrupting target receptor function by
  - inappropriately activating the receptor (hormone receptor agonist)
  - inhibiting the action of the receptor (hormone receptor antagonist)

Endocrine mode of action and causal link to adversity

- Endocrine activity shown through *in vitro* mechanistic assays, e.g. receptor binding/(in)activation or interference with hormone production, however, such activity may not be expressed *in vivo* and the link to an adverse outcome is not provided by evidence of such endocrine activity alone.
- Evidence of endocrine activity *in vitro*, along with evidence of an *in vivo* biomarker and adverse effect coupled with **a biologically plausible relationship** between the measured parameters maybe sufficient to conclude on endocrine disruption.
- The type and amount of information needed at the different levels depends on the mode of action considered as well as the type of effect observed

Factors for further characterisation of identified EDs

- Factors such as potency, severity, irreversibility and lead toxicity were considered not part of the identification but could inform on further characterization of the hazard of such substances.

- Factors may be used in combination to rank EDs according to "level of concern"

- ***No agreement*** on use of the factors together or alone to differentiate EDs into categories of higher or lower concern

Factors for further characterisation of identified EDs

- Factors important for characterising EDs are normally used in context of exposure and risk assessment for predicting safe levels
- In absence of risk assessment some experts considered that:
  - not to use the information on the factors was to ignore available data
  - factors could be used together to differentiate high from low concern EDs, respecting that policy decisions would need to be introduced in defining categories particularly with respect to potency cut-offs.
- Other experts considered that level of concern should be ultimately based on a risk assessment, i.e. including exposure considerations, since low exposures to highly potent EDs may be less of a concern than high exposure to less potent EDs

Scheme for evaluation of EDs

- Demonstration of an **adverse effect** for which there was convincing evidence of a **biologically plausible link** to an **endocrine disrupting mode of action** were considered the 2 main elements for the identification of an ED

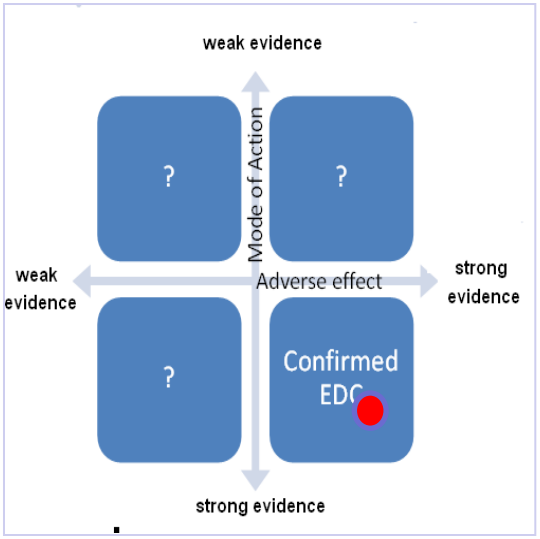
- **Specificity** also considered part of ED identification, since the primary mode of action of a substance should be disruption of the endocrine system rather than the disturbance of the endocrine system being a secondary consequence of other non endocrine-mediated systemic toxicity.

- **Relevance** of the data to humans should be assumed in the absence of appropriate data demonstrating non-relevance. For wildlife data on all species relevant. Relevance applied in context of identified effects being relevant at population level

SoA Report Matrix

- Matrix potentially useful visualisation tool to show how MoA and adversity should be considered in parallel and not in sequence

- To consider a substance as a confirmed ED, evidence for a biologically plausible causal relationship between the endocrine activity and the observed adverse effect(s)has to be incorporated



Scenarios represented by SoA matrix

strong evidence for adverse effects/weak evidence for endocrine disrupting MoA

e.g. evidence of endocrine activity *in vitro* but no plausible link to the pattern of adverse effects observed

weak evidence for adverse effects/strong evidence of endocrine disrupting MoA

e.g. evidence of endocrine activity *in vitro* and confirmed *in vivo* via appropriate biomarkers but for which adverse effects coherent with the type of endocrine activity not observed

Further investigations required in both cases

Weight-of-Evidence Considerations

- Weight of evidence approaches need to be applied in both evaluating adverse effects and endocrine activity, particularly in capturing the weight of evidence establishing the relationship between endocrine activity and adverse outcomes
- IPCS MoA and human relevancy framework highlighted as existing weight of evidence approach for evaluating modes of action
- Although the expert group considered that the level of evidence required by the framework might be too high a requirement for the identification of an ED

Testing Needs

- The Expert Group in the time available were unable to fully evaluate the adequacy of current test methods

- The SoA report could form the initial basis of an evaluation of existing regulatory test methods.

Availability and adequacy of current tools and methods for assessment of EDs

- Reference to OECD Conceptual Framework which includes assays to identify adverse effects and mechanistic/mode of action assays
- Currently available OECD tests only allow a conclusion to be reached for EATs modalities, for mammals, fish and possibly amphibians, but not for birds or invertebrates
- In some cases it may be possible to reach a conclusion based on a single test (*e.g.* TG234 for fish, TG416 or TG443 for mammals)
- Specific data gap - lifetime exposure from *in utero* to old age, early life exposure on cancer incidence, impact on menopause.

Further research needs

- Further work to identify relevant *in vivo* biomarkers indicative of endocrine activity to augment existing assays was recommended
- Knowledge of the endocrine system in invertebrates, birds, amphibians, as well as plants and microbes was currently lacking
- The OECD DRP No.178 points to the lack of certain mechanistic assays for the investigation of non EATS modalities
- It was recommended that priority areas for further development of assays to investigate specific endocrine pathways should be informed by emerging human health issues or observed negative impacts on wildlife populations and hypothesised link to endocrine-related causes

Further work of ED EAG

- Review of threshold, low dose effects, non-monotonic dose responses for EDs, in view of REACH review under 138 (7) and authorisation of EDs
- Generic guidance for application of horizontal criteria
- Further evaluation of adequacy/availability of test methods

Thank you for your attention

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