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Establishment of Criteria for Endocrine Disruptors and Options for Regulation

1 Introduction

During the latest 10-20 years, the potential threats from endocrine disruptors (EDs) to humans and the environment have received increasing attention worldwide. In Europe, a demand for political initiatives resulted in publication of a Community Strategy for Endocrine Disrupters in December 1999 specifying several actions to be taken in relation to further research, international co-operation, information to the public and policy action for the control and regulation of endocrine disruptors. Activities in relation to hazard identification, risk assessment and risk management including development of new test and assessment methods take place at both international (incl. OECD), Community and national level, incl. in Denmark.

In the EU, EDs are dealt with under various Community legislations concerning different types of chemicals and with different regulatory purposes. Under the REACH Regulation (Reg. (EC) No 1907/2006) general provisions on ensuring safe use of chemicals apply. In addition, endocrine disruptors may be included under the authorisation scheme if identified as Substances of Very High Concern in accordance with Article 57(f) by a case-by-case assessment. However, recognising the limited basic knowledge on EDs at the time REACH was adopted, a review of the authorisation procedure with regard to endocrine disruptors is required by 1 June 2013 (cf. Article 138(7)).

The new Plant Protection Products Regulation (PPPR) (Reg. (EC) No 1107/2009) includes approval criteria for endocrine disruptive substances with impact on human health and environment (cf. Annex II[1], point 3.6.5 and 3.8.2, respectively). Furthermore, the Commission is mandated to present a draft with scientific criteria for endocrine disruptors with impact on human health by 14 December 2013. At the present the new biocidal product regulation (BPR) is under negotiation and this proposal includes exclusion criteria for active substances comparable with the criteria in article 57(f) and 59(1) in REACH. The Commission shall adopt a delegated act specifying scientific criteria for the determination of endocrine disrupting properties.

Within the European Commission, DG Environment is in charge of the process establishing criteria for endocrine disruptors that apply across the different regulations. Several member states have presented proposals in relation to human health and environmental criteria for endocrine disruption according to REACH and PPPR as input to this process. In January 2011, the Danish EPA also presented a paper on regulation of endocrine disruptors under REACH in which we announced the intention to provide a proposal for specific criteria for identification of endocrine disruptors by May 2011.

[1] (see paragraph 6.2 for specific text)

The present document with annexes A-C deals with the establishment of our proposed criteria for endocrine disruptors and includes also some general considerations on how endocrine disruptors could be managed under various EU legislations, in particular REACH and PPPR.

2 Background

Exposure to endocrine disruptors has been shown to cause serious effects in wildlife and laboratory animals and a number of health effects in humans seems to be correlated to exposure to endocrine disruptors. Testing and assessment of chemically induced endocrine disruption is a relatively new discipline within toxicology and ecotoxicology and even though the scientific community has contributed with important new knowledge within this area during the last 20 years, many aspects of endocrine disruption still have to be explored.

A special challenge in relation to regulation is that endocrine disruption is not a single mechanism or even mode of action but relates to many different mechanisms, modes of actions or toxicity pathways. Therefore endocrine disruption may lead to a variety of harmful effects. Until now focus has mainly been on effects related to reproduction and development, however, the latest research indicates emerging new endpoints related to e.g. development of cancer, obesity, diabetes, metabolic syndrome, effects on the immune system and brain development.

Furthermore, there are several indications that it is the time of exposure during a critical time window that matters more in relation to inducing effects than the dose. Even exposure to very low doses of EDs may lead to critical imbalances in the level of endogenous hormones during development of the organism that may result in irreversible effects later on in life.

The basic paradigms and tools of toxicology and ecotoxicology seem inappropriate to fully address the issue of endocrine disruption and new approaches for identification, assessment and management of endocrine disruptors are therefore being developed.

In relation to identification of EDs, new test methods have been developed or are under development under the OECD Test Guideline Programme and more test methods will be developed following the progress of science. However, most of these new test methods are not part of the existing minimum standard information requirements for industrial chemicals under REACH or pesticides under Plant Protection Products (PPP). Furthermore, it is a considerable challenge in relation to identification and subsequent regulation of EDs that the main part of the current database on the existing chemical substances - even for pesticide active ingredients with extensive documentation - is based on test methods that do not include all relevant endocrine endpoints which are needed in order to exclude that a substance possesses endocrine disrupting properties.

All these circumstances should be considered when establishing criteria for endocrine disruptors for regulatory purposes. At the same time it should be possible to use the criteria for identification of EDs both in the existing and recently adopted regulations for chemicals (industrial chemicals, PPPs, biocides etc.) as well as in future legislation.

Some EDs are carcinogenic and toxic to reproduction and fulfil the criteria for these endpoints according to the CLP regulation. EDs are also comprised by Art. 57 in REACH for identification as substances of very high concern (SVHC) in the same manner as Carcinogenic, Mutagenic and Reprotoxic (CMR) substances and therefore it seems logic that criteria for identification of EDs in general should follow a similar approach as that for CMR substances. Likewise, in relation to the approval criteria in the PPPR, it is important that the application of criteria for identification of EDs targets potential EDs in such a way that adequate data will be generated to ensure that such an

identification can take place to ascertain that only EDs that have been confirmed to "*cause adverse effects in humans*" or "*on non-target organisms*" will be excluded from authorisation.

Furthermore, the criteria for identification of EDs should reflect the existing knowledge and database concerning endocrine disrupting properties of chemicals but should also be useful when the database and knowledge increase. In addition, the application of the criteria should allow for both screening and final identification of chemicals with endocrine disrupting properties - or should even provide the authorities with a tool for such a screening and final identification of EDs. The latter would be convenient in relation to REACH in order to compensate for its lack of standard information requirements on EDs and would also reflect the current inadequate database in relation to endocrine disrupting properties of industrial chemicals in general.

Finally, it is of particular importance that criteria for identification of endocrine disruptors have a scientific basis and that they are in general accordance with international definitions of EDs.

In order to establish science based criteria for identification of a substance as being an endocrine disruptor, the Danish Environmental Protection Agency commissioned scientific experts from the Danish Centre on Endocrine Disrupters to prepare a proposal for criteria for identification of endocrine disruptors.

The proposal below for definitions and criteria for EDs is based on the proposal from the scientific experts (annex A). More detailed Danish general reflections on regulation of EDs under REACH from January 2011 (annex B) and Danish comments to inputs from other Members States in relation to assessment of EDs (annex C) further substantiate and explain the background for the present proposal.

3 Definition of endocrine disruptors

A crucial point for a more systematic implementation of provisions on EDs into the EU regulatory framework is the establishment of criteria to identify a substance as being an endocrine disruptor. However, this implies that the term "endocrine disruptor" is clearly defined.

Various attempts to set up a science based definition of an ED have been made since the mid 90'ies. Endocrine disrupting properties is an intrinsic property of a chemical regardless of the area of application. Therefore, it is the general view of the Danish EPA that the definition should be appropriate for protection of both human health and the environment and that the same definition should apply for all types of EU legislation and, if possible, the same definition should also apply at the international level.

There is generally wide acceptance of using the IPCS/WHO definitions (2002):

"***ED***
An ED 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.

Potential ED
A potential ED is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations."

These definitions pertain to both human health and the environment. The two definitions of EDs and potential EDs respectively, imply, however, existence of a very large difference in scientific evidence for categorising a substance as either an ED or a potential ED.

For regulatory purposes it seems relevant to consider that potential endocrine disruptors comprise both substances for which there are substantial even though not confirmatory documentation for endocrine disrupting properties and substances for which there are only indications of potential endocrine disrupting properties. Therefore, it seems appropriate that the definition for "potential EDs" is subdivided according to the existing level of evidence as this would allow a tiered assessment of the endocrine disrupting properties of chemicals based on weight of evidence similar to the way CMRs and PBTs (Persistent, Bioaccumulative and Toxic substances) are identified and assessed.

Such a need for an expansion of the WHO definition of potential EDs was also reflected at the OECD meeting of the Advisory Group on Endocrine Disrupter Testing and Assessment (EDTA AG2) in April 2011 (OECD 2011), which agreed on a new operational definition of a possible endocrine disruptor in the context of the Guidance document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption which is under development:

"*A possible endocrine disrupter is a chemical that is able to alter the functioning of the endocrine system but for which information about possible adverse consequences of that alteration in an intact organism is uncertain*".

Regardless of which definitions of EDs and potential EDs that will end up being decided at EU level, such definitions and subsequent criteria will have to be interpreted relative to the scientific evidence available.

Therefore, it is proposed that EU uses the WHO/IPCS definition of EDs and potential EDs, as a basis; however, in relation to regulatory purposes it is proposed to further divide the potential EDs into 2 subgroups: a) suspected[2] EDs; and b) indicated EDs, reflecting the level of evidence:

WHO/IPCS definition	**EU definition**
ED	Category 1: ED (based on *in vivo* data*)*
Potential ED	Category 2a: Suspected ED (mainly based on *in vivo* data)
	Category 2b: Substances with indications of ED properties (Indicated ED) (mainly based on *in vitro/in silico* data*)*

One issue relating to the definition of EDs is the level of documentation incl. interpretation of test results for categorising substances as EDs or potential EDs. The interpretation of test results in relation to endocrine disruption has been discussed during the years and the nearly finalised OECD Guidance document on Standardised Test Guidelines for Evaluating Chemicals for Endo-

[2] The term "suspected" is introduced in analogy with the terminology applied for Category 2 CMR substances in accordance with the CLP Regulation where there is some evidence from humans or experimental animals of and adverse effect and where the evidence is not sufficiently convincing to place the substance as confirmed in Category 1.

crine Disruption will constitute a very important tool for the future identification of EDs and potential EDs (OECD 2011).

4 Criteria for endocrine disruptors

In addition to definitions, a more operational set of “level of evidence rules” or criteria is needed for allowing industry and authorities to determine whether a substance should be considered an ED or a potential ED.

As indicated above, in our view such level of evidence rules should reflect the WHO/IPCS definition, but also be in accordance with the principles for identification of another group of SVHCs namely the CMRs. Furthermore, they should be usable under REACH and the PPPR and the new BPR. Finally, they should be in general accordance with the OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors (OECD CF) and the OECD Guidance document. In respect to the latter guidance, it is recalled that although the Guidance document and the OECD CF more specifically address OECD Test Guidelines and Guidance Documents for identification of EDs, it does allow - on a case-by-case basis using expert judgement and weight of evidence - to use other relevant data as well.

Based on the scientific proposal for criteria for EDs that is further substantiated in annex A, the following criteria for identification of endocrine disruptors are proposed.

Table 1: Proposed Criteria for Endocrine Disruptors

Category 1- Confirmed ED

Substances are placed in category 1 when they are known to have caused ED mediated adverse effects in humans or animal species living in the environment or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to cause adverse ED effects in humans or animals living in the environment.

The animal studies shall provide clear evidence of ED effects in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effects should be considered not to be a secondary non-specific consequence of other toxic effects. However, when there is e.g. mechanistic information that raises doubt about the relevance of the effect for humans or the environment, category 2a may be more appropriate.

Substances can be allocated to this category based on:

- Adverse *in vivo* effects where an ED mode of action is highly plausible
- ED mode of action *in vivo* that is clearly linked to adverse effects *in vivo* (by e.g. read-across)

Category 2a - Suspected ED

Substances are placed in category 2a when there is some evidence for ED effects from humans or experimental animals, and where the evidence is not sufficiently convincing to place the substance in category 1. If, for example, limitations in the study (or studies) make the quality of evidence less convincing, category 2a could be more appropriate. Such effects should be observed in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effect should be considered not to be a secondary non-specific consequence of other toxic effects.

Substances can be allocated to this category based on:

- Adverse effects in vivo where an ED mode of action is suspected
- ED mode of action *in vivo* that is suspected to be linked to adverse effects *in vivo*
- ED mode of action *in vitro* combined with toxicokinetic *in vivo* data (and relevant non test information such as read across, chemical categorisation and (Q)SAR predictions)

Category 2b – Substances with indication of ED properties (Indicated ED)

Substances are placed in Category 2b when there is some *in vitro/in silico* evidence indicating a potential for endocrine disruption in intact organisms.

The evidence could also be observed effects *in viv*o where there is general but not specific evidence relating those to ED (i.e. that may, or may not, be ED-mediated).

5 Evidence required for fulfilment of criteria

Based on the revised OECD Conceptual Framework (OECD CF) for endocrine testing and assessment, the initial considerations for both human toxicity and environmental effects in relation to the proposed criteria are:

A substance can be considered a confirmed **ED (category 1)** based on data from:

- *In vivo* assays providing data on adverse effects clearly linked to endocrine mechanisms (OECD CF[3], level 5)
- On a case-by-case basis, *in vivo* assays providing data about single or multiple endocrine mechanisms and adverse effects (OECD CF, level 3 & 4) combined with other relevant information
- In special cases, where *in vivo* data on adverse effects are lacking, categorisation or (Q)SAR approaches may provide the necessary data in combination with ADME[4] information and *in vitro* data
- Reliable and high quality evidence from human cases or epidemiological studies.

A substance can be considered a **suspected ED (category 2a)** based on data from:

- *In vivo* assays providing data on adverse effects linked to endocrine or other mechanisms (OECD CF, level 5), but where ED mode of action is suspected but not confirmed
- *In vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD CF, level 3 & 4)
- In some cases, read across, chemical categorisation and/or (Q)SAR approaches may provide the necessary data in combination with in vivo ADME information and *in vitro* data
- Good quality epidemiological studies showing associations between exposure and adverse human health effects related to endocrine systems.

A substance can be considered an **indicated ED (category 2b)** based on data from:

- *In vitro* assays providing mechanistic data (OECD CF, level 2)
- (Q)SAR, read-across, chemical categorisation, ADME information (OECD CF, level 2)
- System biology methods indicating associations between the substance and adverse human health effects related to endocrine systems.

Adverse effects are defined in accordance with the WHO/IPCS definition from 2004:

"A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences".

The evidence needed for fulfilment of the criteria is described in detail in annex A, including discussion of which human data and testing and non-testing methods that would be considered suitable and how the results from such methods can be interpreted and used for the categorisation in relation to the criteria.

[3] As in the draft minutes from EDTA AG2 (OECD 2011)

[4] Absorption, Distribution, Metabolism, Elimination

It should be noted that the current OECD CF covers the following modalities: estrogen mediated activity, androgen mediated activity, thyroid-related activity and steroidogenesis interference. A detailed review paper on emerging new endocrine endpoints is under preparation within the OECD. Thus, for the time being, other potential endocrine disruptive effects are not covered by this proposal because appropriate test methods have not yet been developed and validated.

6 Regulation of endocrine disruptors

The use of the definition and proposed criteria within various regulatory schemes with focus on REACH and PPPR, which is most relevant at the moment, is discussed below.

6.1 Endocrine disruptors under REACH

REACH provides that it is for manufacturers, importers and downstream users to ensure that they manufacture, place on the market and use only such substances that do not adversely affect human health or the environment. REACH further provides that it is for authorities to propose Community measures to address hazards and risks in case industry has not sufficiently ensured the safe manufacture and use of their substances. These basic principles also apply to EDs.

REACH requires manufacturers and importers of substances to obtain and assess all available and relevant data on their substances, to assess the hazards and risks, and to implement or recommend appropriate Risk Management Measures (RMMs) for ensuring that risks are controlled throughout the lifecycle of their substances. These provisions apply to all substances irrespective of their toxicological mode of action. Thus, the Danish EPA expects that registrants under REACH assess the available and relevant information on their substances and consider whether their substances fulfil the criteria for being EDs or potential (suspected and indicated) EDs. For substances identified as EDs, we expect that registrants take this into account in assessing the chemical safety and deciding on appropriate RMMs.

For substances identified as potential EDs, it would be in accordance with safe products stewardship if registrants - in analogy with what is required for potential CMR substances relative to the standard information requirements of REACH - conduct or propose additional testing and perform a confirmatory assessment allowing them to conclude on the ED status, even though REACH does not include specific ED-related standard testing and assessment requirements. Another option would be to treat potential EDs as if they were indeed confirmed EDs, i.e. by introducing appropriate RMMs.

The roles of authorities under REACH are to evaluate registered substances that might cause a risk to human health or the environment and, in case risk management is needed at Community level, to propose appropriate measures.

Substances identified as EDs category 1 are considered to fulfil the criteria (art. 57(f)) for inclusion in the Candidate list. DK suggests that potential EDs (category 2a and 2b) similar to other types of SVHCs should be identified and further evaluated. Depending on the likely use and exposure (based on tonnage, uses, emissions, exposure potential and hazard (e.g. potency) characteristics), the severity of effects and the potential risk, such potential EDs could be subject to regulation or prioritised for Substance Evaluation. The latter would allow that further targeted testing of prioritised potential EDs could be required from registrants.

Another possibility is of course - for substances not prioritised for substance evaluation under REACH - that Member States, academia or NGOs on a voluntary basis perform the necessary further targeted testing for a confirmatory ED evaluation which could then be used by a Member

State for proposing confirmed EDs to the Candidate list and subsequent prioritisation for Authorisation.

For each ED identified as well as for potential EDs of higher priority, an analysis of the need for Community Risk Management should be conducted as for any other substance of very high concern. If it is concluded that Risk Management is required, a Risk Management Options (RMO) analysis should be prepared. The purpose of the RMO analysis is to analyse the benefits and drawbacks of various possible RMOs, incl. identification as a SVHC and eventual inclusion in Annex XIV (the authorisation list), Community restrictions, harmonised Classification & Labelling, or other types of risk management. Based on the RMO analysis conducted, authorities should decide on the need for initiating Community Risk Management.

6.2 Endocrine disruptors in the PPPR

The new Plant Protection Products Regulation (PPPR) (Reg. (EC) No 1107/2009) includes approval criteria for endocrine disruptive substances with impact on human health in point 3.6.5 of Annex II: "*An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned in food and feed do not exceed the default value set in accordance with point (b) of article 18(1) of Regulation (EC) No 396/2005".*

Effects on the environment are covered by point 3.8.2 of Annex II:
"*An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible."*

However, for active substances, safeners and synergists with endocrine disrupting properties in relation to non-target organisms in the environment there are, contrary to substances with endocrine disrupting properties in relation to human health, currently no specified criteria for non approval. Likewise, there is no specified definition of negligible exposure for endocrine disruptors in relation to non-target organisms (in the environment).

It is proposed that confirmed EDs (category 1) should be regulated according to the approval criteria on endocrine disrupters with impact on human health and the environment (paragraph 3.6.5. and 3.8.2 of Annex II).[5]

For suspected EDs (category 2a) additional mechanistic studies *in vivo* and/or *in vitro* are necessary. Additional studies can be required by the authorities according to PPPR (cf. Annex II, point 5.8.2). The default assumption is that the mechanism is endocrine. If no mechanistic data are provided or if the mechanism of toxicity is shown to be endocrine, the substance may be considered as being an endocrine disruptor (Category 1). However, if the mechanistic data clearly show that

[5] An exception is evaluation of human health in cases where the evaluation of a substance as an ED or suspected ED is purely based on ecotoxicological effects and it is shown that this is not of relevance for humans.

the mechanism of toxicity is not based on endocrine modulation, the substance is not considered to be an endocrine disruptor.

If there are data from tests other than OECD CF level 5 tests indicating an ED effect and the available level 5 test investigating the relevant end-point (e.g. ED sensitive end-points according to the extended one-generation reproductive toxicity study (draft TG 443), or an updated 2-generation reproductive toxicity assay (updated TG 416 not yet available) the substance is not categorised as an ED unless other evidence indicated ED related adverse effects in relevant studies (e.g. in chronic or carcinogenicity studies).

6.3 Endocrine disruptors in the BPR

For the time being the new BPR is under negotiation. The proposal includes exclusion criteria for active substances comparable with the criteria in REACH and also included a direct link to article 57(f) and 59(1) in REACH.

The active substances can only be approved under strictly controlled conditions or if the substance is essential to prevent or control serious danger to public health or the environment or the negative impacts for society will be disproportionate compared to other suitable alternative substances or technologies. The data requirements for biocides are comparable to PPPs. When the new biocides regulation is adopted, a more detailed approach should be developed.

6.4 Endocrine disruptors under other relevant regulation

It is our general view that the definitions and criteria for endocrine disruptors proposed here can directly be used for identification of endocrine disruptors under other relevant EU regulations e.g. in relation to regulation of food, cosmetics, toys, pharmaceuticals, medical devices etc.

7 Conclusion

The proposed definitions and criteria for EDs are science based and tailored to the existing EU regulatory demands. In addition, the proposed approach is also able to pick up new endpoints as science moves forward. Lastly, the proposed approach can generally be used across different EU regulations.

Below, a schematic outline of the proposed definitions and regulatory options based on the proposed approach is summarised.

WHO/IPCS definition	**EU definition**	**REACH – possible regulatory actions**	**PPPR – regulatory action**
ED	Category 1: ED (*in vivo* data*)*	- Identification as SVHC (and possible inclusion in the authorisation list, Annex XIV) - Restriction - Harmonised C&L	No approval unless negligible exposure
Potential ED	Category 2a: Suspected ED (mainly *in vivo* data)	- Development of list of potential EDs - For prioritised potential EDs, development of RMO analysis followed by regulation, if appropriate - Prioritisation for substance evaluation where more data on ED specific properties can be required from industry *	Approval requires further data from industry
	Category 2b: Indicated ED (mainly *in vitro/in silico* data*)*	**	Depending on the case, flag for generation of further data

* For suspected EDs (category 2a): Generation of further ED specific data can be conducted by industry, Member States and research communities on a voluntary basis.

** For indicated EDs (category 2b): Generation of further data to be prioritised depending on exposure potential by industry, Member States and research communities on a voluntary basis.

8 Recommendations for the further process

Based on the above consideration, the Danish EPA would recommend that:

- the Commission facilitates the agreement at EU level of a general definition of endocrine disruptors and potential endocrine disruptors as well as equivalent criteria for identifying endocrine disruptors across regulations, and further

- the obligation of registrants under REACH to assess whether their substance has endocrine disrupting properties is clarified by issuing appropriate guidance (and revising REACH, Annex I, if appropriate),
- a group of interested Member States in collaboration with the Commission and ECHA screen substances (including substances registered under REACH) for endocrine disrupting properties,
- identified potential endocrine disruptors, if meeting priority criteria, are selected for substance evaluation with the aim of obtaining sufficient data allowing a conclusion on their endocrine disrupting properties,
- interested Member States analyse risk management options for relevant identified endocrine disruptors with the aim of deciding on appropriate risk management, and post these analyses on the CIRCA Annex XV IG for commenting before taking a final decision on the most appropriate risk management option.

9 References

OECD (2011). Draft Guidance Document on the Assessment of Chemicals for Endocrine Disruption, Version 10 March 2011. Paris: organisation for Economic Cooperation and Development.

WHO (2002). Global assessment of the state-of-the-science of endocrine disruptors. Eds: Damstra, T., Barlow, S., Bergman, A., Kavlock, R. and Van der Kraak, G., WHO/PCS/EDC/02.2, World Health Organisation, Geneva. 180 pp.

Annex A: Report on Criteria for Endocrine Disrupters. Danish Centre on Endocrine Disrupters, May 2011.

Annex B: Regulation of endocrine disruptors under REACH, DK input to the EU process, 31 January 2011.

Annex C: Danish comments to input from other regulatory bodies/member states in relation to assessment of endocrine disruptors.

Annex A: Report on Criteria for Endocrine disrupters, Danish Centre on Endocrine Disrupters, May 2011

Centre on Endocrine Disrupters

Report on Criteria for Endocrine Disrupters

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GROWTH REPRODUCTION

Table of Contents

Terms of reference and scope

This report has been prepared by the Danish Centre on Endocrine Disrupters as a project contracted by the Danish Environmental Protection Agency. The Danish Centre on Endocrine Disrupters is an interdisciplinary scientific network without walls. The main purpose of the Centre is to build and gather new knowledge on endocrine disrupters with the focus on providing information requested for the preventive work of the regulatory authorities. The Centre is financed by the Ministry of the Environment and the scientific work programme is followed by an international scientific advisory board.

The overall aim of this project is to provide a science based proposal for criteria for endocrine disrupters. The terms of reference for the project specify elements to be included and/or addressed when developing the criteria (Annex 1). Also, several international reports and papers dealing with assessment of endocrine disrupters were provided by the Danish Environmental Protection Agency as background information for the project (Annex 2).

1. Background and aim

Endocrine disruption is not a single effect, but relates to many different mechanisms or toxicity pathways that may lead to multiple harmful effects. Today, focus is mainly on disturbance of the reproductive system and functions controlled by the thyroid hormone system. However, new data indicate that endocrine disrupters may also affect other hormone systems. Several activities like development of new test methods and assessment methods take place at both international (incl. EU, OECD) and national level (incl. in Denmark).

During the recent 10-20 years, the potential adverse effects of endocrine disrupters (EDs) on humans and the environment have received increasing attention. Reproductive changes have been seen in wildlife for example in alligators (Guillette et al. 1994), female polar bears (Wiig et al. 1998) and male gulls (Fry 1995). In humans it is well documented that the incidence of testicular cancer has increased over the last decades (Giwercman et al. 1993; Skakkebaek et al. 2001). The incidences of cryptorchidism and hypospadias also seem to be rising, at least in Denmark, where studies have been performed (Boisen et al. 2005). Furthermore, semen quality of young men in some parts of Europe is generally quite poor (Jørgensen et al. 2006). These effects seem to be related to environmental factors and exposure to endocrine disrupters is suggested to contribute to the development of these effects.

The above-mentioned effects (except testicular cancer) have been observed in several studies in experimental animal models showing reproductive malformations in male animals exposed to endocrine disrupters in the embryonic period (Gray et al. 1994; Gray et al. 1999; Foster 2006; Welsh et al. 2008; Sharpe and Skakkebaek 2008).

The consequences of exposure to EDs can be adverse and irreversible because of the crucial role that hormones play in controlling development (Gray and Kelce 1996). The intra-uterine development is a very delicate process as the reproductive and endocrine systems undergo complex organisation in foetal life. Foetuses and newborns are therefore considered to be uniquely susceptible and vulnerable to influences of EDs whereas similarly exposed young adults are only transiently affected (Gray 1992; O'Connor and Chapin 2003).

In the EU, EDs are dealt with under various Community legislation concerning different types of chemicals and with different regulatory purposes. However, even though EDs are mentioned in the legislative texts, EDs are in general not covered by the existing criteria for chemicals and at the moment there are no agreed criteria for EDs. However, some effects of endocrine disrupters may be identified by the existing criteria for carcinogenicity and toxicity to reproduction.

In line with CMRs and PBTs, substances with ED properties may be identified as Substances of Very High Concern (SVHC) in accordance with REACH, Article 57(f) and included in the candidate list of substances for eventual inclusion in Annex XIV (the authorisation list). Substances with ED properties are referred to as examples of substances of equivalent level of concern as CMRs and PBTs/vPvBs (cf. art. 57(f) "...such as...."). Thus, substances with ED properties are covered by the authorisation scheme under REACH by a case-by-case assessment. Substances included in Annex XIV may be authorised by the authorities for continued manufacture and use provided risks are adequately controlled or the socio-economic benefits outweigh the risks. Basically REACH contains by its standard information requirements a strategy for obtaining data on CMR properties and criteria for classifying CMR into different categories are given in the CLP Regulation. Information requirements and criteria for EDs have not been developed, but would be useful for this regulatory purpose in REACH.

The new European Union Plant Protection Products (PPP) Regulation (1107/2009) includes an exclusion criterion for approval which explicitly states: *"An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible …".* and further in relation to environment *"An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible",* however, negligible exposure is only defined in relation to human health.

Similar provisions are proposed for the new Biocidal Products Regulation currently under negotiation.

Thus, the continued manufacture and use of EDs may be authorised under REACH, while EDs of relevance for human health will be prohibited for pesticides and biocides under respectively the PPP and BP Regulations.

The **aim** of this report is to propose **scientific criteria for the identification of ED substances of concern for human health and the environment.** The use of these criteria for the above mentioned regulatory purposes will also be considered. The overall purpose is to provide input for the ongoing EU work within this field.

2. Criteria for ED

A number of issues potentially relevant for the development of criteria for EDs such as definition of EDs, specificity of effects etc. are considered below followed by the proposed criteria.

2.1 Definition of ED

Various attempts to set up a science based definition of the term "endocrine disrupter" have been put forward since the mid 90'ies. In the draft OECD Guidance Document on the Assessment of Chemicals for Endocrine Disruption (OECD, 2011), reference is made to the widely used definitions of EDs and potential EDs according to WHO (2002):

***"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.*

*A **potential endocrine disrupter** is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations".*

These definitions may at first glance seem to be based on the same concept as to the two categories used for classification of CMR in the CLP Regulation. CMR substances are classified according to the CLP Regulation in two distinct categories related to the level of evidence: CMR cat. 1 and CMR cat. 2, where category 1 is used when the CMR effects are documented (known), and category 2 is used when the effects are suspected. Given a closer look, however, the definitions of ED and potential ED seem to represent the two "ends of" the spectrum of knowledge on ED properties and effects, i.e. the situations where there is extensive documentation for adverse health effects, ED mode of action and a cause-effect relationship and those situations where there is only limited knowledge on "properties that might lead to". Consequently, we find that there is a need for separating the potential EDs into two groups consisting of suspected EDs and substances with indications for EDs. Also, the definition of an endocrine disrupter may - especially due to the use of the term "consequently causes" - signal that it is a requirement that very detailed information on the relationship between the altered function of the endocrine system and the adverse effect has to be provided. To have such knowledge detailed and extensive cause-effect mechanistic studies would be necessary – and that will rarely, if ever, be the case. There is, however, a great deal of scientific knowledge in general on the relationship between alterations of the endocrine system and adverse health effects and we find that the definitions should signal that such knowledge can be used for defining a substance as an ED. We have, therefore, in the present report slightly revised the WHO definition of an endocrine disrupter, i.e. deleted the word "consequently", and included a definition of suspected ED. Thus our definitions that give the background for our proposed criteria are:

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

Potential endocrine disrupter:
***A suspected endocrine disrupter** is an exogenous substance or mixture that may alter function(s) of the endocrine system and consequently may cause adverse health effects in an intact organism, or its progeny, or (sub)populations."*

*A substance with **indication of endocrine disrupting properties (called indicated ED)** is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.*

The establishment of CMR categories has made it possible in a consistent way to require new and targeted information/testing for suspected/potential substances in order to obtain new information or testing which allows a definitive decision/judgement, i.e. as appropriately to confirm or reject the suspicion by placing the substance in "the confirmed category" or to remove it from "the suspected category", respectively. It is furthermore as an alternative possible - if e.g. the cost of generating the required new information or testing is large - to assume that the substance is a confirmed CMR, and thus implement appropriate risk management measures without new data generation/testing. Finally, it is possible - while waiting for the new information/testing to be made available - to implement some preliminary/interim risk management measures in accordance with the precautionary principle.

In line with the approach employed for the CMR substances, it is also considered appropriate to operate with different categories of substances with ED properties, i.e. "suspected and indicated EDs" and "confirmed EDs". This is especially important for substances with ED properties because neither REACH nor the PPP and the new Biocides Directive operate with standard information/targeted testing requirements specifically related to ED properties in contrast to what is the case for CMR substances. The reason is most probably that suitable regulatory testing methods for ED properties were very limited at the time of development, negotiation and adoption of REACH. Such test - and non-test – methods are however continuously being developed, validated and standardized in particular in the context of the OECD Test Guidelines programme and under the OECD QSAR management group. Now several standard test methods, non-testing approaches and guidance documents exist and can be used for generating targeted ED related information if such is requested in relation to certain substances undergoing substance evaluation under REACH or specific needs for evaluating ED are indicated and therefore targeted testing for ED is required under the PPP or the Biocides Directive.

It is the general view in this report that the same scientific definition of criteria should apply for all types of regulatory use within EU, and ideally, the same definition should apply at international level as well.

In addition, we consider it appropriate that the definition comprises a confirmed group as well as potential EDs, as this would allow a tiered assessment of the ED properties of chemicals similar to the way that CMRs are identified and assessed.

2.2 Use of environmental data for evaluation of human toxicity and vice versa

For ED identification, both environmental vertebrate test data and human toxicity data are useful in the overall evaluation, because the endocrine systems are closely related.

One potential issue when comparing findings from *in vivo* mammalian studies using oral exposure to studies in fish is the differences in metabolism that may occur depending on the route of exposure (e.g. oral dosing in mammals and aqueous exposure in fish). Aqueous exposure of fish, via uptake through the gills and skin, essentially bypasses metabolism in the small intestine and liver, through direct entry to the blood stream. Therefore, it is possible to visualize a scenario in which endocrine activity of a substance can be markedly decreased by metabolism after oral exposure. This could lead to no endocrine related findings in the oral studies in mammalians, whereas endocrine activity may manifest itself in fish where the substance is not metabolised before reaching the site of action or relevant organ. However, such effects could be manifested in mammalian studies if using dermal or inhalation exposure. The opposite situation may also be seen

if metabolisation is needed to produce endocrine disrupting metabolites; some chemicals may be metabolised by mammals after oral exposure but not by fish.

The use of environmental test data for evaluation of human toxicity can be differentiated into ED identification on one side and risk assessment on the other side. For ED identification, environmental vertebrate test data can often be included in the overall evaluation. Possible different metabolic pathways should though be taken into account. Concerning risk assessment, environmental test data for evaluation of human toxicity can be complicated because of the differences in both exposure scenarios and the differences in test designs also discussed in the beginning of paragraph 3.5.

The use of human toxicity data for evaluation of wildlife ecotoxicity can also be differentiated into identification and risk assessment. For identification of ED, data from both sources should be taken into account as described above. For risk assessment, the differences discussed in paragraph 3.5 should be considered, but the human data incl. data from mammalian studies in e.g. rodents can be useful for (especially) mammalian wildlife.

2.3 Adverse effects

The definition of EDs and suspected EDs both include the term "adverse". The WHO/IPCS definition of the term "adversity" is used in this report:

"*A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences*." (WHO/IPCS 2004)

2.4 Potency considerations

An option for identifying EDs could be to include potency for causing adverse effects, i.e. defining an effect level below which a substance can be identified as an ED substance (and consequently above which it would not be identified as an ED).

However, the internationally agreed WHO definition of EDs does not include potency considerations or requirements. Furthermore, the new draft OECD Guidance Document on identification of EDs (OECD 2011) does also not contain potency criteria or considerations. Also, CMR substances are not identified based on their potency for causing effects but rather on the level of evidence of their hazard. Thus, it would not be consistent to introduce a different approach for identifying EDs as substances of an equivalent level of concern to CMR substances than the approach used for CMR effects. Indeed many EDs are causing reproductive toxic effects and they should therefore be identified by use of the same approach. Furthermore, there are several indications that it is the time of exposure during pregnancy and/or early life-stages that matters (exposure during critical time windows) rather than the dose. In the light of this, potency considerations are not included as part of the criteria for identification of EDs.

2.5 The most sensitive or lead effect

It has in relation to human health been proposed that in cases where ED-induced effects are not the lead toxic effect but are seen at dose levels significantly higher than those causing other toxic effects; the substance is not an ED of regulatory concern. This is clearly in contrast to the CLP criteria for classification of (CM)Rs, where only specificity of effects is required and such

substances are considered of regulatory concern. Also, the WHO definition of EDs does not include considerations of this.

The argument given for this proposal is that the most sensitive/lead toxic effect of a substance will generally drive the risk assessment and be used to determine appropriate risk mitigation measures. This argumentation does not include considerations of how to derive a DNEL and the appropriate use of uncertainty/assessment factors for this. Using this argumentation would mean that for example a substance causing histopathological kidney effects at the lowest dose level and malformations of male reproductive organs due to anti-androgenicity at the next dose level should not be regarded as an ED of regulatory concern. Furthermore, dismissing the ED properties in a regulatory context if there are other more sensitive toxic effects for the individual chemical would also invalidate the evaluation of mixtures of chemicals with similar types of ED effects, but differences in lead toxic effect. Consequently, we do not support this approach and have not included this in the proposed scientific criteria for EDs.

2.6 Specificity of effects

Effects on the endocrine system or effects potentially linked to ED mode of action seen at dose levels causing marked generalized toxicity may or may not be non-specific secondary consequence of the marked generalized toxicity. The ED effects should only be considered relevant if they are not a secondary non-specific consequence of other toxic effects. Otherwise, the substance should not be considered a specific hazard to the endocrine system This is in line with the CLP criteria for classification of (CM)Rs and also included in the proposed criteria for EDs in this report

2.7 Relevance of experimental mammalian data for humans

The relevance for humans of effects observed in mammalian animal studies is an important issue, but in most cases there will be insufficient data for evaluating this. The conservation of the endocrine system through the animal kingdom and especially in vertebrates support that effects in mammalians such as rodents are relevant for humans. Actually, studies have shown that the basic events of reproductive development are homologous in mammalian species, and that rodent models have great utility for evaluating the potential of xenobiotics to alter human reproductive development (Gray 1992). Therefore and in line with the practice for other areas of toxicology, the default assumption is relevance. Consequently, only if an ED mechanism of toxicity in animals is identified that is clearly not relevant to humans, can the effects in animal studies be considered irrelevant for humans.

Endocrine disruption may in rare cases induce toxic effects in rats that are not likely to occur in humans due to specific detailed endocrine differences between rats and humans. However, the endocrine disruption seen in the rats can certainly be of relevance for humans, because the same spectrum of hormones is important for rats and humans. In humans, a severe effect on hormones is therefore likely to cause other adverse effects.

2.8 Route of exposure in experimental studies

Investigations on substances administered by subcutaneous, intravenous, or intraperitoneal injections or other application routes may demonstrate endocrine activity, but these experimental conditions may affect absorption, metabolism and/or excretion in comparison to e.g. oral exposure. As a consequence, observed effects may or may not be clearly predictive for especially the risk for these effects. The effects observed may, however, provide data of relevance for the identification of suspected or potential EDs. When evaluating the relevance of such findings knowledge on the

toxicokinetics of the substance from e.g. animal studies or modelling, internal dose levels etc. is useful and should be taken into account.

2.9 Criteria for EDs

Based on all of the above consideration, the proposed ED criteria shown in table 1 have been developed. They are inspired by the criteria for reproductive toxicity and as those include specificity as an important issue.

The substances can be allocated to one of the three groups defined in section 2.1. Appropriate grouping should always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach. Individual datasets should be analysed case-by-case using expert judgement.

Table 1 Proposed criteria for EDs

Group 1- Endocrine disrupter

Substances are placed in group 1 when they are known to have produced ED adverse effects in humans or animal species living in the environment or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to cause ED effects in humans or animals living in the environment.

The animal studies shall provide clear evidence of ED effect in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effects should be considered not to be a secondary non-specific consequence of other toxic effects. However, when there is e.g. mechanistic information that raises doubt about the relevance of the adverse effect for humans or the environment, Group 2a may be more appropriate.

Substances can be allocated to this group based on:

- Adverse *in vivo* effects where an ED mode of action is highly plausible
- ED mode of action *in vivo* that is clearly linked to adverse *in vivo* effects (by e.g. read-across)

Group 2a - Suspected ED

Substances are placed in Group 2a when there is some evidence from humans or experimental animals, and where the evidence is not sufficiently convincing to place the substance in Group 1. If for example limitations in the study (or studies) make the quality of evidence less convincing, Group 2a could be more appropriate. Such effects should be observed in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effect should be considered not to be a secondary non-specific consequence of other toxic effects.

Substances can be allocated to this group based on:

- Adverse effects *in vivo* where an ED mode of action is suspected
- ED mode of action *in vivo* that is suspected to be linked to adverse effects *in vivo*
- ED mode of action *in vitro* combined with toxicokinetic *in vivo* data (and relevant non test information such as read across, chemical categorisation and QSAR predictions)

Group 2b – Substances with indications of ED properties (indicated ED)

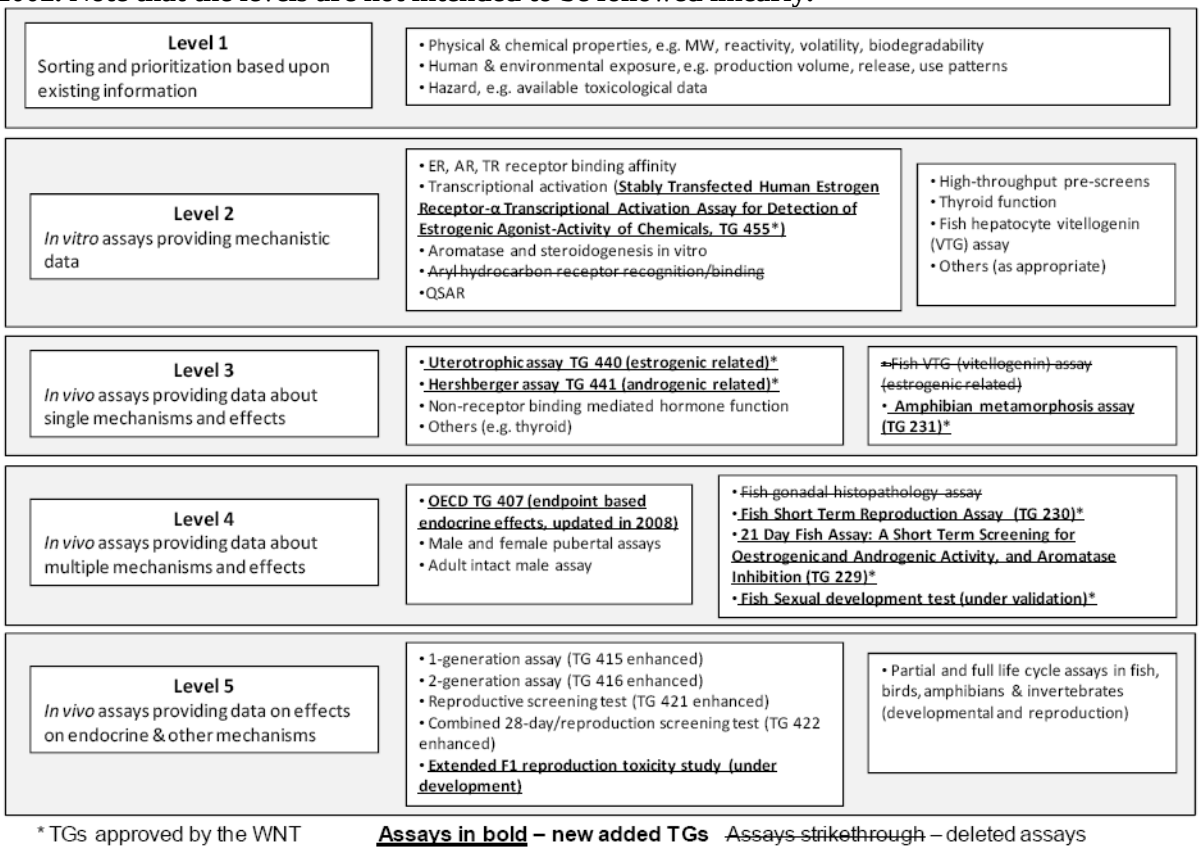
Substances are placed in Group 2b when there is *in vitro*/in silico evidence indicating potential for endocrine disruption in intact organisms. Evidence could also be observed effects *in vivo* that could be ED-mediated.

3. Evidence needed for fulfilment of criteria

3.1 OECD Conceptual Framework

The OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters was agreed at a workshop held in Tokyo in 2002. It is a tool box including screening and testing methods that were considered useful for the assessment of oestrogen receptor (ER), androgen receptor (AR) and thyroid mediated effects. At an OECD Workshop held in Copenhagen in September 2009, a proposal for revising the CF (Figure 1) was submitted by the OECD Secretariat and it was recommended to revise the CF further within a few years where more experience and scientific progress has been gained (OECD 2011). This CF revision was further discussed and revised at the EDTA meeting held in Paris in April 2011and also updated with newly approved test guidelines (see Annex 3).

Figure 1 OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (as revised in 2009), including tests which were unavailable when the CF was first proposed in 2002. Note that the levels are not intended to be followed linearly.



Based on the revised OECD Conceptual Framework for endocrine testing and assessment, the initial considerations for both human toxicity and environmental effects in relation to the proposed criteria are:

A substance can be considered an ***ED (group 1)*** based on data from:

- *In vivo* assays providing data on effects clearly linked to endocrine mechanisms (OECD, level 5)
- On a case-by-case basis, *in vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, level 3 & 4) combined with other relevant information

- In special cases, categorisation or QSAR approaches may provide the necessary data in combination with *in vivo* ADME information and *in vitro* data
- Reliable and good quality evidence from human cases or epidemiological studies.

A substance can be considered a ***suspected ED (group 2a)*** based on data from:

- *In vivo* assays providing data on effects linked to endocrine or other mechanisms (OECD, level 5), but where ED mode of action is suspected
- *In vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, level 3 & 4)
- In some cases, read across, chemical categorisation and/or QSAR approaches may provide the necessary data in combination with *in vivo* ADME information and *in vitro* data
- Good quality epidemiological studies showing associations between exposure and adverse human health effects related to endocrine systems.

A substance can be considered an ***indicated ED (group 2b)*** based on data from:

- *In vitro* assays providing mechanistic data (OECD, level 2)
- QSAR, read-across, chemical categorization, ADME information (OECD, level 2)
- System biology methods indicating associations between the substance and adverse human health effects related to endocrine systems.

In the following section, it will be discussed which human data and testing and non-testing methods that would be considered suitable and how the results from such methods can be interpreted and used for the grouping in relation to the criteria.

3.2 Data from human studies

In epidemiology direct and unequivocal evidence of a causal link between human exposure to chemicals, including EDs, and health effects only rarely exists. Examples include cases of chemical accidents or heavy occupational exposures, where a certain chemical agent has been identified as a disrupter of an endocrine organ. One example is the Seveso disaster in 1976, where a chemical manufacturing plant accident resulted in very high dioxin exposure in the residential population. Another example is the pesticide DBCP (Dibromochloropropane), which caused severe hypospermatogenesis and sterility in workers in a banana farm - effects disappearing after withdrawal of exposure. A third example is premature puberty (gynecomastia) in children exposed to oestrogenic agents in food; again the symptoms disappeared after change in food intake. Where such evidence exists that can link exposure to a specific substance or mixture to adverse effects related to human endocrine systems, this evidence should be sufficient to categorize the substance as an **ED (group 1)**.

Such direct evidence is, however, uncommon in human populations. Important indirect evidence of human exposure to EDs is secular trends in hormone dependent pathologies, such as those observed for hormone dependent cancers (e.g. increased incidences of prostate cancer, breast cancer and testicular cancer), and dysfunctions of the reproductive organs (e.g. reduced semen quality, increased prevalence of cryptorchidism and hypospadias). An increase in disease incidence over few decades provides strong evidence that non-genetic factors are important for the aetiology - and notably factors, which have changed markedly over few generations. In contrast, no direct evidence is provided on which specific factors or combinations of factors are to blame. However, the fact that the diseases are closely linked to hormonal function suggests that external factors disturbing hormonal balance and function are involved. In addition, the fact that similar diseases can be found

in animal experiments in which the animals are exposed to EDs suggests that humans may also be affected.

Like secular trends, heritability studies and migration studies are capable of providing strong and direct evidence, that external factors (non-genetic factors) are important for some disease aetiologies. For testis cancer, for example, strong evidence is provided from all these three types of studies, that 1) non-genetic factors are essential for disease development, and 2) the origin of the disease is already established in foetal life and thus the factors causing the disease act in utero. Unfortunately, no useful animal or *in vitro* models are available for testis cancer. The consequence of this should be that regulation can be based on indirect evidence as e.g. associations found in epidemiological studies (rather than no regulation being made due to lack of direct evidence).
In terms of associating human health outcome to certain exposure scenarios, epidemiological studies are essential. Especially, large and prospective cohort studies are useful, in that relevant information during sensitive periods, for example during pregnancy and early childhood can be obtained and biological samples (e.g. serum and urine) can be stored for later analysis of EDs. In that way, case-control studies can be nested within the cohorts when relevant health outcomes appear (such as early breast development, cancers etc.). These studies are extremely time consuming and costly, but nevertheless a crucial and necessary instrument. In the analysis of epidemiological data, various known confounders can be taken into account, minimizing the risk of false associations. Evidence of linkage between exposures and outcomes from epidemiological data sets will always be indirect but clear associations observed in epidemiological studies (including heritability and migrations studies) should still be considered sufficient to categorize a substance or a mixture as a **suspected ED (group 2a).**

Lastly, the real life scenario of people being exposed to hundreds of potential EDs in relatively low doses, the plausibility that combined exposure is the actual factor disturbing the hormonal systems, and the additional fact that in utero exposure often does not manifest until decades later, makes it extremely difficult to provide evidence of human health effects of EDs exposure. In this respect, new types of data management, such as systems biology methodologies are needed to approach an understanding of which factors or combination of factors contribute significantly to the disease trends observed in the population. Substances being flagged in system biology methods as associated with adverse human health effects related to the endocrine systems should be considered as ***indicated*** **EDs (group 2b)**.

3.3 Field studies

Due to the complexity of the exposure history in field studies, only very specific cases, with well defined exposure scenarios or a well defined relationship between biological effect and chemical exposure, could be used to define a chemical as an **ED (group 1).** An example of the latter is the case with the antifouling agent tributyltin (TBT) which caused the TBT-specific effect called imposex in female molluscs. The field studies were major factors in the detection of TBT as an endocrine disrupter and in the decision of banning TBT worldwide as an antifouling agent. A chemical spill in a lake could also be an example where a known chemical could be linked to an ED effect in the field and thereby be categorized as an endocrine disrupter through a field study.

Generally, observations of ED effects in field studies would trigger *in vitro* and *in vivo* studies to confirm causal relationship between effect and substance(s). However, the confirmation of casual linking between chemical exposure and effect requires that the species normally used in laboratory tests are sensitive to the observed effect. This is not always the case as for example in egg shell thinning caused by DDE where galliforms used in tests are almost insensitive to the effect.

3.4 Experimental *in vivo* studies, mammalians

During recent years, a number of OECD guidelines have been developed or updated for identification of ED activity and/or effects, incl. some *in vitro* tests, *in vivo* screening assays (Hershberger TG 441 and Uterotrophic assay TG 440), Repeated Dose 28 Day Oral Toxicity Study in Rodents (TG 407) and the extended one generation reproductive toxicity study (draft TG 443, EOGRTS).

Among the existing OECD Test Guidelines for mammalian reproductive toxicity, exposure during all vulnerable periods of development is performed in the two-generation reproductive toxicity study design (TG 416). This was updated in 2001 with inclusion of some endocrine disruption sensitive endpoints such as onset of puberty (VO, PPS), oestrous cyclicity, qualitative evaluation of primordial follicle counts and anogenital distance in F2 (only if triggered by altered sex ratio in F1). The updated TG 416 does, however, not include endocrine disruption-related sensitive endpoints such as areola/nipple retention, anogenital distance at birth, measurement of thyroid hormones and TSH levels. Reproductive toxicity studies that lack sensitive endpoints cannot fully exclude the possibility that chemicals testing negative may still be EDs. The new extended one generation reproductive toxicity study (draft TG 443, EOGRTS) includes more endpoints sensitive to endocrine disruption than TG 416, i.e. areola/nipple retention, anogenital distance at birth, measurement of thyroid hormones levels. Also, quantitative evaluation of primordial and small growing follicles and corpora lutea is included in contrast to the TG 416 that includes only a qualitative evaluation of primordial follicle counts. Full histopathology of mammary gland (males and females) is also performed for all high-dose and control adult animals. Research has shown the mammary gland, especially in early life mammary gland development, to be a sensitive endpoint for oestrogen action. Consequently, it is recommended in the EOGRTS that endpoints involving pup mammary glands of both sexes be included in this Test Guideline, when validated. In addition to the markedly increased endpoints sensitive to EDs, the EOGRTS is also expected to have greater sensitivity than TG 416 as it requires an increased number of pups to be examined (OECD 2011).

The effects observed in reproductive tests with rodents may be due to endocrine disruption or other mechanisms and it is important to consider the weight of all available evidence. However situations in which a single assay provides conclusive evidence that a chemical is an ED do exist. For example, feminized AGD in male offspring (observed in EOGRTS, TG 416 and possibly in TG 421/422) can be considered as conclusive evidence of an adverse ED effect. OECD Guidance Document 43 on reproductive toxicity (OECD 2008) states "*A statistically significant change in AGD that cannot be explained by the size of the animal indicates effects of the exposure and should be used for setting the NOAEL*." Also, some other effects or pattern of effects in male rat offspring, *e.g.* decreased AGD, increased nipple retention and malformations of reproductive organs, clearly indicate that adverse effects mediated via impact on the endocrine system are involved. Similarly, in female offspring a pattern of adverse effects including early vaginal opening, effect on oestrus cyclicity and longer AGD signal that the effects are most likely due to oestrogenic effects of a substance. In such cases, this is considered sufficient to categorize the substance as an **ED (Group 1).**

Some adverse effects which are indicative of endocrine disruption activity can also be observed in reproductive toxicity studies (TG 416, EOGRTS). These include for example prolonged gestation period, dystochia, effects on semen quality etc. Combined with mode of action data for the substance (from QSAR, *in vitro* or *in vivo* screening assays) such data can - depending on the weight of evidence - be used to categorize a substance as an **ED or a suspected ED (Group 1 or**

2a). If placement in group 2a as a suspected ED is considered most relevant based on the existing data this should trigger further testing to elucidate the ED suspicion. If the mechanism of toxicity is shown to be endocrine, the substance is considered as being an ED (Group 1). However, if the mechanistic data clearly show that the mechanism of toxicity for the observed effect is not based on endocrine effects, the substance is presumably not an endocrine disrupter (and excluded from the ED groups).

The reproduction/developmental screening tests TG 421 and 422 using a reduced number of animals and dose levels are included in Level 5 as supplemental tests because they may give limited but useful information on interaction with endocrine systems. EDs may be detected by effects on reproduction (e.g. increased gestation length, dystochia, and implantation losses), genital malformations in offspring, marked feminized AGD in males, changes in histopathology of sex organs or effects on the thyroid gland. Such results can - depending on the weight of evidence - be used for categorizing a substance as an **ED or a suspected ED (Group 1 or 2a)**. If placement in group 2a as a suspected ED is considered most relevant this should trigger further testing to elucidate the ED suspicion.

In vivo screening assays (such as Hershberger TG 441 and Uterotrophic assay TG 440) can show that a substance can interfere with the endocrine system in animals, i.e. the substance has an ED mode of action *in vivo*. They do, however, only provide limited or no data on adverse effects. Also, these assays may use intact weanling animals or ovariectomised or castrated animals. In the latter case, the physiological homeostasis of the whole organism has been altered to maximise the sensitivity of the test to identify endocrine activity. It can be argued that the results from tests with ovariectomised or castrated animals cannot be taken as evidence of real adverse effects in intact animals (c.f. the WHO definition of ED). However the same cannot be argued when these tests have been conducted with weanling animals. Furthermore, the OECD validation of these assays is based on data for EDs and the validation results show that EDs with the mode of actions studied in these assays were positive in these assays. For the Hershberger assay, the dose levels causing effects generally seem to be similar to or even higher than those causing ED effects in generation studies (Hass et al 2004). Consequently, a positive response in these assays can be used for categorizing a substance as a **suspected ED (Group 2a).** In cases, where there is supporting evidence from read-across demonstrating a clear link between the ED mode of action *in vivo* and adverse *in vivo* effects categorizing as **ED in group 1** is relevant. In such situations, more complete data may – depending on e.g. regulatory need and exposure - have to be obtained from a higher tier test, which will then be evaluated in conjunction with the screening data. Note, that negative data from a higher tier test should generally be given more weight than positive data from a lower tier screen, but only if the same class of vertebrates has been employed at both tiers, the quality of the data is good, the suspected mechanism or mode of action is adequately covered by the endpoints, and a sensitive life stage has been used in the higher tier negative test (OECD 2011).

Repeated dose toxicity studies in adult animals such as TG 407, TG 408 contain some endocrine relevant endpoints (e.g. weights and histopathology of sex organs). Some addtional endpoints sensitive to ED were added when updating the TG 407, however, these are mainly optional. The OECD validation showed that strong and moderate EDs were detected in this test, whereas other EDs showing effects during sensitive developmental periods were not detected.

3.5 Experimental *in vivo* studies, non-mammalians

In contrast to human health, where adverse effects are linked to the level of the individuals, adverse ecotoxicological effects are related to effects at the population level (Van Leeuwen & Vermeire

2007). This is generally reflected in the endpoints of concern, which are related to mortality, growth, development or reproduction. Effects on these endpoints are regarded relevant for the maintenance of wild populations. An endpoint such as for example sex ratio in fish, which may be impacted by certain sex hormone interfering EDs, is directly relevant for population level effects be

cause diminution or extinction of one of the sexes will impact the reproductive maintenance of that population. The above mentioned difference in protection goal for human health and the environment is not directly related to the criteria for EDs but it is anyway relevant in this context because several of these ecotoxicological endpoints will not detect an ED effect which is affecting only the most sensitive specimens of a population whereas toxicological endpoints would detect these effects. Also, in ecotoxicology testing related to non mammalian species, the types of adverse effects addressed are all relevant to population level effects. In addition to this it should be realised that ED related adverse effects recorded in mammalian toxicity tests are also relevant to wildlife mammals.

The following OECD guidelines have been developed with ED specific biomarkers/endpoints:

TG 229 (Fish Short Term Reproduction Assay (FSTRA)) is a 21 d screening test with fish (fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*) or Japanese medaka (*Oryzias latipes*)) where the endocrine specific biomarkers/endpoints are: 1) Changes in the concentration of the estrogenic induced yolk protein vitellogenin. 2) Secondary sex characteristics. Fecundity is included as an apical (adverse effects related) endpoint. It is not ED specific but can in combination with the other endpoints indicate ED related apical effects on reproduction. Observed changes in vitellogenin concentrations and/or in secondary sex characteristics would lead to categorizing the substance as a **suspected ED (Group 2a).** If fecundity is also affected in combination with one of the other endpoints, the substance would be categorized as an **ED (Group 1)** if supporting evidence from read-across demonstrates a clear link between the ED mode of action and the adverse *in vivo* effects.

TG 230 (21 Day Fish Assay) is like TG 229 a 21 days screening test. Observed changes in vitellogenin concentrations or in secondary sex characteristics would lead to categorizing the substance as a **suspected ED (Group 2a).** The difference from TG 229 is that fecundity is not included so this test is without apical endpoints and cannot foresee adverse effects.

The androgenised female stickleback screen (AFSS) is accepted as an OECD GD (Guidance Document) and is in principle a TG 230 where androgenised female thee spined sticklebacks (*Gasterosteus aculeatus*) are exposed to potential anti-androgens. A change in the androgen induced protein spiggin is the ED specific endpoint, and observed induction of spiggin would lead to categorizing the substance as a **suspected ED (Group 2a)**. No apical endpoints are included.

TG 231 (Amphibian Metamorphosis Assay (AMA)) is a screening test for thyroid activity in amphibians. The ED specific endpoint is thyroid gland histopathology and apical endpoints are hind limb length, snout-vent length, developmental stage and wet weight. A TG 231 with effect on thyroid gland histopathology categorizes the substance as a **suspected ED (Group 2a)** with thyroidal disrupting properties. Regardless that it includes both endocrine and adverse effects, it is debatable due to its test design whether it is sufficient for also categorization as an **ED (group 1**). At the moment no adopted higher tier amphibian standard tests exist, but a Larval Amphibian Growth & Development Assay (LAGDA) is under development. This test is the amphibian equivalent to TG 234 and would be placed at CF Level 4 when validated. The test includes endpoints that indicate ad verse effects so it might also be able to identify group 1

EDs but it will not be qualified to reject ED effects because reproduction is not included in the test proposal.

TG 229, 230, AFSS and 231 are all screening assays and negative results from these tests should not be used to overrule positive *in vitro* or QSAR data (**indicated ED (Group 2b**)) because of the small statistical power of the measurements of the endpoints in these screening tests and because these tests do not include exposure during either sensitive life stages or reproduction.

The FSDT is a Fish Sexual Development Test and newly accepted as an OECD Test Guideline (TG 234). TG 234 would be an alternative to a full life cycle test when reproduction is not expected to be the most sensitive endpoint. TG 234 is a partial life-cycle test covering sexual development and differentiation in small fish species as fathead minnow, zebrafish, Japanese medaka and three spined stickleback.ED specific biomarkers include vitellogenin concentration changes and phenotypic sex reversal (changes in sex ratio). The latter is also an apical endpoint. When sex ratio is affected, TG 234 can be used for hazard categorization to group a substance as an **ED (group 1**). If only vitellogenin concentration is affected, the substance would be categorized as a suspected ED (group 2a). A negative FSDT cannot provide evidence of lack of ED unless combined with a study including reproduction.

Three longer term fish assays with endocrine relevant endpoints are under development within the OECD test guideline programme:
1. A fish reproduction partial life cycle test, that can categorize a substance as an **ED (Group 1)**, when reproduction is affected. A negative test could in combination with a negative TG 234 be used as the highest tier of information concerning lack of endocrine disruption in fish unless other equivalent *in vivo* data (mammalian as well as non-mammalian) show adverse ED effects.
2. A Medaka Multi-Generation Test (MMGT) using Japanese medaka. This test includes several endocrine relevant apical endpoints such as reproduction and sex ratio. Positive effects on the ED related apical endpoints would categorize a substance as **ED (group 1**) and a negative test would categorize a substance as non ED unless other equivalent *in vivo* data (mammalian as well as non-mammalian) show adverse ED effects.
3. A Fish Life-Cycle Toxicity Test with endocrine relevant endpoints included. As the MMGT, This test includes several endocrine relevant apical endpoints such as reproduction and sex ratio. Positive effects on the apical endpoints would categorize a substance as **ED (group 1**) and a negative test would categorize a substance as non ED unless other equivalent *in vivo* data (mammalian as well as non-mammalian) show adverse ED effects.

The Avian Reproduction Assay (TG 206) is a Level 4 test where a positive outcome either categorize the substance as a **suspected ED (Group 2a)** if for example egg shell thinning is an effect, or as an **ED (Group 1)** if for example egg production is affected. However, both categorizations need supporting evidence concerning ED for the substance because TG 206 does not include endpoints solely specific for EDs.
The Avian Two Generation Test (ATGT) is a Level 5 draft proposal including several endocrine biomarkers (e.g. sex and thyroid hormone levels) as well as apical endpoints (sex ratio, fertile egg numbers, reproduction etc) and a substance can be considered an ED (**group 1**) based on positive ED specific data from this test. When no counter evidence exists, negative results for this test can also be used as the highest tier of information concerning lack of endocrine disruption in birds.

For invertebrates, no OECD TGs with ED specific endpoints have yet been finalised but several TGs and draft TG proposals may include endocrine disruption related effects. These tests include

inhibition of reproduction and production of male neonates in *Daphnia magna* (enhanced TG 211), Enchytraeid (worm) Reproduction Test (TG 220), Earthworm Reproduction Test (*Eisenia fetida/Eisenia Andrei* (TG 222)), Predatory mites (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (TG 226), developmental toxicity in Dung flies (*Scathophaga stercoraria* (TG 228)), Collembolan reproduction test in soil (TG 232) and Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment (TG 233) and test guidelines under development such as the mollusc partial and full life-cycle tests, the copepod reproduction and development test, daphnia multi-generation assay and mysid life-cycle toxicity test. It is noted that generally due to insufficient information about the endocrinology of the taxa mentioned, none of these tests include known ED specific endpoints.

Extrapolation of data from invertebrate tests to vertebrates and vice versa will often be difficult due to the differences of the endocrine systems and because the endocrine system of several invertebrate classes is more or less unknown. For example, the moulting hormones (ecdysteroids) are specific for insects and crustaceans and ED effects on this system would not be expected to affect vertebrate endocrinology. When developed and validated, invertebrate life-cycle tests with ED specific biomarkers and apical endpoints may, however, in some cases be used for identification of a substance as an **ED (Group 1)** but currently only placement as a suspected ED (Group 2a) would be possible in case of positive results in the existing tests.

3.6 *In vitro* studies

Many *in vitro* assays exist for detection of chemicals with potential endocrine disrupting activity and for elucidation of their mechanisms of action. Assays have been developed for detection of receptor binding, transactivation or inhibition of nuclear receptors (ER, AR, TR, PPAR, AhR etc), to detect effects on synthesis of sex steroid hormones (H295R steroidogenesis assay (draft TG 456), aromatase assays) as well as assays developed to investigate effects at more functional levels like proliferation of human breast cancer cells. Some of these methods have been validated by the OECD test programme, a few has approved OECD test guidelines and more assays are underway. In general, a pronounced and potent AR antagonistic effect and/or a testosterone lowering effect in the H295R assay indicates strongly that the chemical may cause *in vivo* anti-androgenic effects.

Overall, positive *in vitro* data should categorize the substance as an **indicated ED (group 2b)**, whereas positive *in vitro* data together with toxicokinetic *in vivo* data showing systemic exposure may in some cases allow to categorize the substance as a **suspected ED (group 2a)**.

Also, *in vitro* data can provide useful information in cases where the modes of action of observed *in vivo* effects are uncertain.

3.7 Read-across and (Q)SAR studies

Overall based on convincing read-across, chemicals categorisation and/or positive (Q)SAR data it relevant to consider a substance as an **indicated ED (group 2b)** until other data are evident. Furthermore, reliable and positive non-test data together with *in vivo* ADME information indicating systemic exposure may in some cases allow to consider the substance as a **suspected ED (group 2a)**. Reliable positive non- test information such as read across may in specific cases together with positive *in vivo* ED data on structural analogs be sufficient for categorization of the substance as an **ED (Group1).**

Finally, just like *in vitro* data may be used for providing information on endocrine modes of action also reliable positive non-test information may provide such information.

4. Examples and new knowledge

4.1 Examples of available ED data and relevant placement in groups

In the following some theoretical examples are given including examples where there is ED data related to the environment or human toxicity or a combination of this:

1. Reliable QSAR prediction suggests binding and activation of a substance to a steroid receptor as e.g. the androgen receptor (AR). The substance is categorized as an **indicated ED (group 2b)**. *In vitro* assays confirm AR binding and activation. A TG 229 is showing induction of nuptial tubercles (medaka), an androgenic induced secondary sex characteristic. The substance is categorized as a **suspected ED (group 2a)**. The FSDT (zebrafish or Japanese medaka) results in a skewed sex ratio toward males, with a no female population at the highest exposure level. The substance is defined as an **ED (group 1)**.

2. TG 440 (Uterotrophic assay) is positive and a TG 229 is confirming oestrogenicity by induction of the yolk protein vitellogenin. The substance is **suspected ED (group 2a)**. An *in vivo* test with an endpoint that can confirm adverse oestrogenic effect (e.g. TG 234, FSDT) can provide basis for defining the substance as an **ED (group 1)**

3. A FLCTT is positive with a change in reproduction and fecundity. The test should be followed up by relevant non-test information, ADME information, *in vitro* and/ or an *in vivo* biomarker testing (TG 229 or 230 or 234) to inform whether this is an ED related effect and whether the substance should be placed in **ED group 1**.

4. *In vitro* assays show AR-antagonism. The substance is categorized as **indicated ED (group 2b)**. *In vivo* toxicokinetic information indicates that the *in vitro* dose level is relevant for internal exposure *in vivo* and non test information provides a link to adverse effects. The substance is categorized as **suspected ED (group 2a)**. TG 416 or EOGRTS show anti-androgenic effects. The substance is placed in **ED group 1.**

5. Oestrogenic specific *in vitro* assays are positive. The substance is categorized as **indicated ED (group 2b)**. TG 440 (Uterotrophic assay) and *in vitro* assay for AR-antagonism is negative. The substance remains an **indicated ED (group 2b),** because the Uterotrophic screening assay at CF Level 3 cannot exclude ED effects due to lack of exposure during sensitive life stages and reproduction. TG 416 or EOGRTS show anti-androgenic effects and the substance is **categorized as ED (group 1)**. Targeted mode of action studies show decreased testosterone levels during foetal development and explain mode of action for the anti-androgenic effects.

6. *In vivo* study shows decreased testosterone levels in male foetuses on GD 21. The substance is categorized as **suspected ED (group 2a)**. Read-across to similar substances (e.g. phthalates) provide a clear link to adverse *in vivo* effects. The substance is categorized as **ED (group 1)**.

7. *In vitro* assays show oestrogen agonism. TG 440 is positive and TG 229 is positive with effect on the apical endpoint fecundity. The substance is categorized as an **ED (Group 1)** because of the combination of adverse apical effects and MOA specific test results. This is an example of use of a combination of human toxicity data and wildlife tests.

8. A positive result in ER-based *in vitro* assays in combination with a positive TG 440 (Uterotrophic assay) is strong evidence for (anti)estrogenic activity leading to at least **suspected ED (group 2a)**. Effects on endocrine endpoints in TGs 407, 408, 453 or 421/422, 416 may provide sufficient evidence to conclude concern for endocrine disruption, i.e. **ED (group 1)** and therefore no need for further testing. Negative existing *in vivo* effects data should, however, be interpreted with caution as they may either indicate that the tests used do not have sufficient power to detect ED effects or alternatively that the endocrine activity does not lead to adverse effects and hence does not present a concern for ED. In such cases, further testing in the confirmatory EOGRTS which includes sensitive endpoints for ED effects could clarify the situation.

9. *In vivo* studies show effects where ED mode of action is suspected leads to **suspected ED (group 2a)**. If data *in vitro* or *in vivo* support that an ED mode of action for the observed effects is plausible, the substances is considered an **ED (group 1).**

4.2 Possibility for including new knowledge/ new methods

The proposed criteria and the examples have been developed mainly based on the current knowledge on endocrine effects related to the sex and thyroid hormones. However, it is proposed that the principles in the criteria are used also for novel ED mode of actions as well as new available ED related endpoints.

5. Proposed regulatory use of the criteria

5.1 Identification as a Substance of Very High Concern

As mentioned in the DK EPA document to CARACAL (January 2011) under REACH, a substance fulfilling one or more of the criteria specified in Article 57 may be identified as a Substance of Very High Concern (SVHC) in accordance with the procedure in Article 59. Article 59(8) specifies that if ECHA's Member State Committee (MSC) reaches a unanimous agreement on the identification of a substance as a SVHC, then this substance shall be included in the candidate list. Thus, to the extent that no specific criteria are available for identification of SVHC substances as for identification of EDs under Article 57(f), then it is up to the MSC to take the decision on a case-by-case basis.

Article 57(a)-(c) specifies which substances that are of very high concern for human health, namely substances classified as CMR, category 1A or 1B. Article 57(d)-(e) specifies which substances that are of very high concern for the environment, namely PBT and vPvB substances. Thus, it is clear that only hazardous substances of particular concern can be identified as SVHC and included on the candidate list.

In addition to the criteria in Article 57(a)-(e), Article 57(f) contains a "safety net" which on a case-by-case basis can be used for identifying other substances that give rise to an equivalent level of concern to those listed in Article 57(a)-(e) if they are "likely to cause serious effects on human health or the environment". As examples of the type of substances which could be identified as SVHC substances in accordance with the Article 57(f) criteria are substances with ED properties.

Considering that Article 57 is intended to identify only those hazardous substances which are of very high concern to be included on the candidate list, a similar prioritisation scheme could be anticipated for EDs. Various options for prioritisation of EDs for inclusion on the candidate list according to Article 57(f) include:

5.1.1 Seriousness of effects

According to the definition of an ED (WHO 2003), it is a "*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.* Article 57(f) refers to "probable serious effects to human health or to the environment". Due to the difference in terminology between Article 57(f) and the working definition of EDs, a question could be whether it is possible to distinguish between "adverse effects" and "serious effects" of EDs.

However, reflecting on our current knowledge of effects of EDs, we hardly ever see adverse effects of EDs that are not serious. They include for example most obviously a range of endocrine related development/reproductive toxicity endpoints as well as endocrine related carcinogenicity. But in addition, emerging evidence seems to suggest that the increasing prevalence of obesity and diabetes as well as development of metabolic syndrome and effects on the immune system may in certain cases be related to endocrine disruption even though a clear proof of its relation to exposure to ED chemicals may not have been established with high certainty at present. Regarding environmental effects, the effects recorded in relevant ecotoxicity tests related to endocrine activity/modulation all address ecological relevant parameters related to development, growth or reproduction. Hence, these types of adverse effects are all serious. Thus, the difference between the two possible interpretations provided above seems to be non-existent for EDs in practical terms.

In conclusion, it is from a scientific point of view neither relevant nor reasonable to distinguish between "adverse" and "serious" effects of EDs when identifying a SVHC.

5.1.2 Potency

An option for prioritising amongst identified EDs could be to include only EDs with a high potency for causing adverse effects, i.e. by identifying an effect level below which an ED could be identified as an SVHC substance (and consequently above which an ED would not be identified as a SVHC).

Based on the arguments given in section 2.4, potency considerations are considered irrelevant as criteria for identification of a SVHC.

5.1.3 Evidence for identification of SVHC under article 57(f) in REACH

As mentioned above, CMR substances of an equivalent level of concern to EDs can be identified as SVHC substances and included in the candidate list, if they fulfil the criteria for classification in category 1A or 1B. Category 1A substances are substances that are known to cause effects in humans, while category 1B substances are substances that based on animal studies are presumed to cause effects in humans. CMR category 2 substances are not fulfilling the Article 57 criteria as the evidence for the serious effects of these substances is insufficient.

Using the same approach for EDs, it is suggested that group 1 EDs should be identified as SVHC substances under Article 57(f), while suspected and indicated EDs (Group 2a and 2b) would not be automatically identified as SVHC substances due to insufficient evidence, but could trigger further testing.

5.2 Identification as an ED substance in PPP

The approval criteria in relation to human health set out in point 3.6.5 of Annex II in the new EU pesticide regulation states: *"An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible ….*
By 14 December 2013, the Commission shall present to the Standing Committee on the Food Chain and Animal Health a draft of the measures concerning specific scientific criteria for the assessment of endocrine disrupting properties to be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 79(4).
Pending the adoption of these criteria, substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.
In addition, substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties " (EC 1107/2009, Annex II).

The approval criteria in relation to environment set out in point 3.8.2 of Annex II in the new EU pesticide regulation states:
"*An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible".*

This approval criteria deals with potential impact on human health due to ED properties, whereas approval criteria relating to ecotoxicology follows a risk-based approach.

It is proposed that the scientific criteria for EDs developed in this report are used both for human health and ecotoxicological effects of EDs. EDs in group 1 and in some cases also suspected EDs in group 2a are relevant. An exception may, however, be evaluation of human health in cases where the evaluation of a substance as ED or suspected ED is purely based on ecotoxicological effects and it is shown that this is not of relevance for humans.

For suspected EDs (group 2a) additional mechanistic studies *in vivo* and *in vitro* would be useful. The default assumption could be that the mechanism is endocrine. If no mechanistic data are provided or if the mechanism of toxicity is shown to be endocrine, the substance may be considered as being an endocrine disrupter in animals (group 1). However, if the mechanistic data clearly show that the mechanism of toxicity is not based on endocrine effects, the substance is presumably not an endocrine disrupter.

6. Summary and conclusions

This report provides a proposal for scientific criteria for the identification of substances with endocrine disrupting properties for humans and the environment (see table 1, page 9). A number of issues relevant for the development of criteria for EDs are discussed and include definition of ED, specificity, potency and others. The criteria include 3 groups, i.e. ED (group 1), suspected ED (group 2a) and indicated ED (group 2b). The evidence relevant for the 3 groups is discussed and described based on the OECD test methods including the OECD Conceptual Framework. Also, some theoretical examples illustrate the use of the criteria. Furthermore, the regulatory use of these criteria in relation to REACH article 57(f) and the new PPP regulation is considered. It is proposed that EDs in group 1 should be identified as SVHC in REACH article 57/f) and as ED substances under PPP. For suspected and indicated EDs (group 2a and 2b), further data may be necessary to evaluate whether the substances is an ED (group 1).

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8 Abbreviations

ADGRA: Amphibian Development, Growth and Reproduction Assay
ADME: Absorption, Distribution, Metabolism, and Excretion
AFSS: Androgenised Female Stickleback Screen
AGD: Anogenital Distance
AhR: Aryl hydrocarbon Receptor
AMA: Amphibian Metamorphosis Assay
AR: Androgen receptor
ATGT: Avian Two Generation Test
BfR: Federal Institute for risk assessment
CF: Conceptual Framework
CLP: Classification, Labelling and Packaging
CMR: Carcinogenic, Mutagenic, and toxic to Reproduction
DNEL: Derived No Effect Level
ECHA: European Chemicals Agency
ED: Endocrine disrupter/disrupting
EDs: Endocrine disrupters
EOGRTS: Extended one-generation reproductive toxicity study
ER: Oestrogen receptor
EU: European Union
FLCTT: Fish Lifecycle Toxicity Test
FSDT: Fish Sexual Development Test
FSTRA: Fish Short Term Reproduction Assay
H295R: Human adrenocarcinoma cells, steroidogenesis assay
IPCS: International Programme on Chemical Safety
MMGT: Medaka Multi-Generation Test
MSC: ECHA's Member State Committee
NOAEL: No Observed Adverse Effect Levels
SVHC: Substance of Very High Concern
OECD: Organisation for Economic Co-operation and Development
PBT: Persistent Bioaccumulative and Toxic
PPAR: Peroxisome Proliferator-Activated Receptor
PPP: The new European Union Plant Protection Products Regulation
PPS: Preputial Separation
QSAR: Quantitative Structure-Activity Relationship
REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals
TG: Test Guideline
TR: Thyroid receptor
VO: Vaginal Opening
vPvB: very Persistent, very Bioaccumulative
WHO: World Health Organisation

Annex 1 Terms of Reference

The overall aim of the project is to provide a science based proposal for criteria for endocrine disrupters. The terms of reference for the project specify the following elements to be included and/or addressed when criteria are developed:

Definition

- Proposal of a definition for endocrine disrupters; if appropriate the definition should be based on existing definitions and possible adjustment of these
- Assessment of the possibility for evidence based categorisation of endocrine disrupters in ”confirmed” and ”suspected” in line with the categorisation of carcinogenic, mutagenic and reprotoxic substances, including specification of level of documentation from OECD/EU test methods or the scientific literature
- Considered if subcategorisation of the two mentioned categories is appropriate and if this is the case, indication of the criteria for subcategorisation (e.g. level of evidence or art/nature of available information)

Criteria

- The criteria should cover both human health and environment
- The criteria are intended to be used across different existing regulations
- Data from both non-test methods, test methods, epidemiology and field studies should be considered
- The proposal for criteria should be discussed in relation to relevant OECD test methods and the OECD Conceptual Framework for testing and assessment of EDs.
- When defining criteria it should be considered to make it possible taking new scientific information, experience and knowledge into consideration when available.
- Discussion of the criteria in relation to REACH standard information requirements and REACH article 57 (f) should be included
- Assessment of the possibilities for using the criteria in specific regulations, such as the new PPP regulation and if possible the biocides regulation currently under negotiation should be included

Annex 2 Background papers

BAuA: Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of ED substances as SVHC, 20 October 2010 (incl. Danish comments to BAuA document, 30 November 2010).

BfR, Draft Concept for the Development of Criteria for Assessment of Endocrine Disrupting Properties with Human Relevance According to the Plant Protection Products Regulation (Reg. (EC) No 1107/2009).

UBA discussion paper on interpretation of Art. 57 (f) REACH with respect to substances having endocrine disrupting properties hazardous to the environment, 26. May 2010 (+ Danish preliminary input/comments to the UBA discussion paper, 12 July 2010).

UK, Regulatory definition of an endocrine disrupter in relation to potential threat to human health, 2010 discussion paper

DK EPA paper: Regulation of endocrine disruptors under REACH, 31 January 2011

ECETOC: Bars R, Broeckaert F, Fegert I, Gross M, Hallmark N, Kedwards T, Lewis D, O'Hagan S, Panter GH, Weltje L, Weyers A, Wheeler JR, Galay-Burgos M. 2011. Science based guidance for the assessment of endocrine disrupting properties of chemicals. Regulatory Toxicology and Pharmacology 59:37-46.

OECD (2011): Draft Guidance Document on the Assessment of Chemicals for Endocrine Disruption, ENV/JM/TG(2011)4, 11 Mar. 2011.

Annex 3: OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (from draft minutes for EDTA meeting in April 2011, however with a footnote 5 added in accordance with the discussions at the EDTA meeting)

The Conceptual Framework lists the OECD TGs and standardized test methods available, under development or proposed that can be used to evaluate chemicals for endocrine disruption. The Conceptual Framework is intended to provide a guide to the tests available which can provide information for assessment of endocrine disrupters but is not intended to be a testing strategy. This Conceptual Framework does not include evaluation of exposure data in accordance with the scope of the OECD GD 150. Further information regarding the use and interpretation of these tests is available in OECD Guidance Document 150.

Mammalian and non mammalian Toxicology

Level 1 Existing Data and Non-Test Information	• Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability • All available (eco)toxicological data from standardized or non-standardized tests. • Read across, chemical categories, QSARs and other *in silico* predictions, and ADME model predictions	
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s)	• Estrogen or androgen receptor binding affinity • Estrogen receptor transcriptional activation (TG 455) • Androgen or thyroid transcriptional activation (If/when TGs are available) • Steroidogenesis in vitro (draft TG 456) • MCF-7 cell proliferation assays (ER ant/agonist) • Other assays as appropriate	
	Mammalian Toxicology	**Non-Mammalian Toxicology**
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s)[1]	• Uterotrophic assay (TG 440) • Hershberger assay (TG 441)	• Xenopus embryo thyroid signalling assay (When/if TG is available) • Amphibian metamorphosis assay (TG 231) • Fish Reproductive Screening Assay (TG 229) • Fish Screening Assay (TG 230) • Androgenized female stickleback screen (GD 140)
Level 4 *In vivo* assays providing data on adverse effects on endocrine relevant endpoints [2]	• Repeated dose 28-day study (TG 407) • Repeated dose 90-day study (TG 408) • 1-generation assay (TG 415)	• Fish sexual development test (Draft TG 234) • Fish Reproduction Partial Lifecycle Test (when/If TG is Available)

	• Male pubertal assay (See draft GD 150, Chapter C4.3)[3] • Female pubertal assay (See draft GD 150, Chapter C4.4)[3] • Intact Adult Male Endocrine Screening Assay (See draft GD 150, Chapter Annex 2.5)[3] • Short Term Development Assay (TG 414) • Chronic toxicity and carcinogenicity studies (TG 451-3) • Reproductive screening test (TG 421 if enhanced) • Combined 28 day/reproductive screening assay (TG 422 if enhanced) • Developmental Neurotoxicity (TG 426)	• Larval Amphibian Growth & Development Assay (when TG is available) • Avian Reproduction Assay (TG 206) • Mollusc Partial Lifecycle Assays (when TG is available) [4] • Chironomid Toxicity Test (TG 218-219) [4]
Level 5 *In vivo* assays providing *more comprehensive* data on adverse effects on endocrine relevant endpoints over *more extensive parts of the life cycle of the organism* [2]	• Extended one-generation reproductive Toxicity Study (draft TG 443)[5] • 2-generation assay (TG 416 most recent update)[5]	• FLCTT (Fish LifeCycle Toxicity Test) (when TG is available) • Medaka Multigeneration Test (MMGT) (when TG is available) • Avian 2 generation reproductive toxicity assay (when TG is available) • Mysid Life Cycle Toxicity Test (when TG is available)[4] • Copepod Reproduction and Development Test (when TG is available)[4] • Sediment Water Chironomid Life Cycle Toxicity Test (TG 233) [4] • Mollusc Full Lifecycle Assays (when TG is available) [4] • Daphnia Reproduction Test (with male induction) (TG 211) [4] • Daphnia Multigeneration Assay (if TG is available) [4]

Notes to the OECD Conceptual Framework

[1] Some assays may also provide some evidence of adverse effects.

[2] Effects can be sensitive to more than one mechanism and may be due to non-ED mechanisms.

[3] Depending on the guideline/protocol used, the fact that a substance may interact with a hormone system in these assays does not necessarily mean that when the substance is used it will cause adverse effects in humans or ecological systems.

[4] At present, the available invertebrate assays solely involve apical endpoints which are able to respond to some endocrine disrupters and some non-EDs. Those in Level 4 are partial lifecycle tests, while those in Level 5 are full- or multiple lifecycle tests.

[5] The new EOGRT study (draft TG 443) is more desirable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints including hormone measures not included in the current 2-generation study (TG 416).

Note 1: Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information and needs for testing and assessment.

Note 2: The assessment of each chemical should be on a case by case basis, taking into account all available information, bearing in mind the function of the framework levels

Note 3: The framework should not be considered as all inclusive at the present time. At levels 2, 3, 4 and 5 it includes assays that are either available or for which validation is under way. With respect to the latter, these are provisionally included.

Annex B: Regulation of endocrine disruptors under REACH, Danish EPA input to the EU process, 31 January 2011.

Chemicals
J.nr. MST-621-00011
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31 January 2011

Regulation of endocrine disruptors under REACH

1 Introduction

During the latest 10-20 years, the potential serious effects of endocrine disruptors (EDs) on humans and the environment have received increasing attention. Activities like development of new test methods and assessment methods take place at both international (incl. EU, OECD) and national level (incl. in Denmark). In the EU, EDs are dealt with under various Community legislation concerning different types of chemicals and with different regulatory purposes. The present document deals with EDs and how these could be managed under the REACH Regulation.

2 Definition of endocrine disruptors

2.1 Definitions

Various attempts to set up a science based definition of the term "endocrine disruptor" (ED) have been made since the mid 90'ies. It is the general view of the Danish EPA that the same definition should apply for all types of EU legislation and, if possible, the same definition should also apply at international level.

In addition, we consider it appropriate that the definition concerns both (confirmed) EDs and potential or suspected[6] EDs, as this would allow a tiered assessment of the endocrine disrupting properties of chemicals similar to the way CMRs and PBTs are identified and assessed. In the draft OECD Guidance Document on the Assessment of Chemicals for Endocrine Disruption (OECD, 2010), reference is made to the widely used definitions of EDs and potential EDs according to WHO (2003):

[6] It might be better to use the term "suspected" instead of "potential" in analogy with the terminology applied for Category 2 CMR substances in accordance with the CLP Regulation. However, for convenience only the term "potential" is used in this document.

ED
An ED 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.

Potential ED
A potential ED is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.

It is noted that the Commission intends to report on the implementation of the "Commission strategy on endocrine disruptors" later this spring. Furthermore, the Commission is funding a project on the state of science on EDs which should be finalised after summer 2011. Regardless of which definitions of EDs and potential EDs that will end up being decided at EU level, such definitions and criteria will have to be interpreted relative to the scientific evidence available. One issue relating to the definition of EDs is which level of documentation that is required for establishing a causal link between the impact on the endocrine system and the effects in the organism, its progeny or (sub)populations. Another interpretation issue may relate to the level of evidence for adverse effects observed really being caused by or related to endocrine disruption or modulation and not by other Modes of Actions.

Thus, the proposed definitions need to be further discussed. If possible, however, the Danish EPA would be in favour of agreeing on definitions that apply not only in the EU but also internationally.

2.2 Criteria

In addition to definitions, more operational sets of criteria should be developed allowing industry and authorities to determine whether a substance should be considered an ED or a potential ED. These criteria could primarily be based on the OECD Conceptual Framework for testing and assessment of endocrine disrupting chemicals (OECD, 2010[7]); however, other valid test and assessment methods than those adopted in the OECD may also be available in future. Therefore, on a case-by-case basis other relevant test and assessment methods will have to be considered as well.

Initial considerations on how to use the OECD Conceptual Framework for endocrine testing and assessment could be as follows:

A substance should be considered an ED based on data from:

- *In vivo* assays providing data on effects from endocrine and other mechanisms (OECD, level 5 as definitive testing)
- On a case-by-case basis, *in vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, level 3 & 4) combined with other relevant information
- In special cases, categorisation or QSAR approaches may provide the necessary data in combination with ADME[8] information and *in vitro* data

[7] The OECD Conceptual Framework will probably be revised following the development, validation and adoption of new OECD TGs, revision of existing OECD TGs and creation of various GDs, DRPs etc.
[8] Absorption, Distribution, Metabolisation, Excretion.

A substance should be considered a potential ED based on data from:

- *In vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, level 3)
- *In vitro* assays providing mechanistic data (OECD, level 2)
- QSAR, read-across, grouping predictions (OECD, level 2)

It is recognised that details on which testing and non-testing methods that would be suitable and how results of such methods should be interpreted and concluded need to be further elaborated. The Danish EPA has initiated work with this purpose and intends to provide suggestions for that in May 2011.

3 Endocrine disruptors under REACH

The REACH Regulation provides that it is for manufacturers, importers and downstream users to ensure that they manufacture, place on the market and use only such substances that do not adversely affect human health or the environment. REACH further provides that it is for authorities to propose Community measures to address hazards and risks in case industry has not sufficiently ensured the safe manufacture and use of their substances. These basic principles also apply to EDs.

However, it is recognised that our basic knowledge on EDs regarding mode of action, test and assessment methods and their link to adverse effects of high concern is not yet developed to the same level as for other types of hazards relating to SVHC (i.e. CMRs and PBTs). Furthermore, for endocrine disruption REACH does not include standard information requirements to the same extent as for CMR and PBT properties. Also available guidance related to data interpretation and testing strategies is in general less developed for endocrine disruption than for CMRs and PBTs. Finally, decision rules for deciding on CMR and PBT/vPvB properties relative to endocrine disruptors are also significantly different. For CMRs and PBTs defined rules and criteria are available under CLP and REACH, respectively. This is currently not the case for endocrine disruptors. Hence, in practice industry might have difficulties in identifying and managing endocrine disruptors even though of course industry in principle is responsible for safety of chemicals also in relation to endocrine disruption.

In conclusion, it is likely that the identification of EDs under REACH may require an even greater level of expert case-by-case scientific judgement than will be the case for CMRs and PBT/vPvBs. It is therefore foreseeable that the authorities in practice might have a greater role in identifying and assessing EDs and providing new guidance to industry for such identification and assessment work than will be the case for CMRs and PBTs/vPvBs.

3.1 Industry obligations

REACH requires manufacturers and importers of substances to obtain and assess all available and relevant data on their substances, to assess the hazards and risks, and to implement or recommend appropriate Risk Management Measures (RMMs) for ensuring that risks are controlled throughout the lifecycle of their substances. These provisions apply to all substances irrespective of their toxicological mode of action. However, as mentioned above, industry might have particular difficulties in fulfilling their obligations with regard to EDs without more detailed guidance, support and active contributions from authorities.

If the exposure to an ED is not negligible, serious health and/or environmental effects are likely and need to be addressed by manufacturers, importers and downstream users of substances. Although not directly specified in REACH that registrants of substances shall consider whether a substance is an ED, it is clearly indicated under Human Health Hazard Assessment that "based on all the available information, other effects [than the traditional ones] shall be considered when necessary" (REACH, Annex I, 1.0.2), and that the Environmental Hazard Assessment shall comprise "the hazard identification based on all available information" (REACH, Annex I, 3.1.1).

Thus, the Danish EPA expects that registrants under REACH assess the available and relevant information on their substances and consider whether their substances fulfil the criteria for being EDs or potential EDs (criteria for both to be defined). For substances identified as EDs, we expect that registrants take this into account in assessing the chemical safety and deciding on appropriate RMMs. For substances identified as potential EDs, registrants should, in analogy with what is normally required for potential CMR substances, conduct or propose additional testing and assessment allowing them to conclude whether the substances are EDs. It may, however, be considered whether it in certain cases in analogy with the provisions for PBT and vPvB substances (cf. REACH, Annex I, 4.1), would be appropriate that industry instead of conducting further testing may treat the substances as if they were EDs and implement appropriate RMMs and operational conditions.

3.2 Authority provisions

The roles of authorities under REACH are to evaluate registered substances that might cause a risk to human health or the environment and, in case risk management is needed at Community level, to propose appropriate measures.

With regard to substances registered under REACH, we could foresee the following procedure:

1. Identification of EDs

The registered substances should be screened for EDs and potential EDs by checking not only the registrants' Chemical Safety Assessments, but also checking against other available information (e.g. existing information from various open data sources including lists of EDs and potential EDs (DG ENV list), test and non test information including QSAR predictions not reflected in the registration dossier, etc.). This activity could be done by a group of interested MSCAs with technical support from ECHA (having full access to the registration data).

2. Substance evaluation

Potential EDs identified should be further evaluated and eventually prioritised (taking into account exposure (based on tonnage, uses, emissions, exposure) and hazard (e.g. potency) characteristics) for Substance Evaluation, which would allow that further targeted testing[9] of prioritised potential EDs could be required from registrants, if appropriate. Substance evaluation is conducted by MSCAs.

[9] In relation to testing strategies for potential EDs, existing endpoint specific REACH Guidance as well as relevant OECD Guidance, such as guidance currently being drafted by OECD (i.e. draft Guidance Document on the Assessment of Chemicals for Endocrine Disruption and the draft Fish Testing Strategy Guidance) could be used and in addition more Guidance should be developed.

3. Establishment of a list of EDs
Based on step 1 and, if needed for some substances, step 2, working lists of (confirmed) EDs and potential EDs should be established. This activity could be done by a group of interested MSCAs.

4. Analysis of Risk Management Options
For each ED identified, an analysis of the need for Community Risk Management should be conducted as for any other substance of very high concern. If it is concluded that Risk Management is required, a Risk Management Options (RMO) analysis should be prepared. The purpose of the RMO analysis is to analyse the benefits and drawbacks of various possible RMOs, incl. identification as a SVHC and eventual inclusion in Annex XIV (the authorisation list), Community restrictions, harmonised Classification & Labelling, or other types of risk management. In concluding on the most appropriate RMO for best ensuring control of risks, issues such as use pattern, emissions and exposure versus type and potency of possible effects could be taken into account.

Although it is at the discretion of any individual MSCA to conclude on both the need for Community risk management and on which RMO to chose, it is considered beneficial and appropriate to post the RMO analysis on the CIRCA Annex XV IG for discussion with other MSCAs before taking the final decision on drafting a proposal on the most appropriate RMO.

Depending on the outcome of the RMO analysis, various options exist:

5a. Identification as a Substance of Very High Concern
If the RMO analysis shows that authorisation of the use of an ED is the most appropriate option, the first step would be to develop a proposal for identification as an SVHC in accordance with REACH, Article 57(f). Note, however, that if an ED fulfils the criteria for classification for reproductive toxicity and/or carcinogenicity, a proposal for harmonised C&L should be prepared in accordance with the CLP Regulation. Following harmonised C&L and inclusion in Annex VI of the CLP Regulation, the substance may be identified as an SVHC in accordance with Article 57(a) or (c) (when classified in categories 1A or 1B). Thus, identification in accordance with Article 57(f) would probably only be relevant for endocrine properties not leading to such classifiable effects (cf. examples of such effects on page 7).

If the ECHA Member State Committee (MSC) concludes that the Article 57 criteria are fulfilled, the substance will be included in the candidate list of substances for eventual inclusion in REACH, Annex XIV (the authorisation list). Substances on the candidate list will be prioritised by ECHA (in particular tonnage and wide dispersive use are important prioritisation criteria, but also the potency of the effect could be taken into account) and, based on an opinion of the MSC, ECHA will submit its recommendation for inclusion in Annex XIV to the Commission. The legislative proposal for inclusion in Annex XIV is made by the Commission and the final decision is taken in accordance with the REACH Regulatory Committee procedure with scrutiny (Article 133(4)).

It is noted that a proposal for inclusion of a substance in the candidate list could in practice have another purpose than a subsequent inclusion in REACH, Annex XIV, as in accordance with REACH, Article 7(2) producers and importers of certain articles containing substances on the candidate list are obliged to notify ECHA about this fact. Thus, new information on use in articles may become available that may end up changing the conclusion of the initial RMO analysis on which type of regulation is the most appropriate, what the scope should be, etc.

5b. Proposal for Community restriction

If there is an unacceptable risk to human health and/or to the environment and the RMO analysis shows that restriction of the use or certain uses of an ED is the most appropriate option (e.g. if the substance is mainly found in imported articles and/or that it is beforehand evaluated that a total ban of certain or all uses of the substance is reasonable), a restrictions proposal should be developed. The proposal should be submitted to ECHA.

5c. Proposal for harmonised Classification & Labelling

If scientific evidence documents that the ED fulfils the criteria for classification for reproductive toxicity and/or carcinogenicity, a proposal for harmonised C&L should always be developed and submitted to ECHA in accordance with the CLP Regulation and irrespective of the outcome of the RMO analysis. It is noted that such a harmonised C&L will in itself trigger a range of "downstream regulations" with the aim of minimising exposure.

5d. Other Risk Management

Other Community or national risk management options may be considered as, e.g., the setting of Occupational Exposure Levels or Environmental Quality Standards. It is not very likely that SVHC substances such as confirmed EDs will primarily be regulated by such means, but it cannot be excluded that in certain cases and/or for some uses this may be a realistic and reasonable option.

4 Identification as a Substance of Very High Concern

Under REACH, substances fulfilling one or more of the criteria specified in Article 57 may be identified as SVHC substances in accordance with the procedure in Article 59. Article 59(8) specifies that if ECHA's Member State Committee (MSC) reaches a unanimous agreement on the identification of a substance as a SVHC, then this substance shall be included in the candidate list. Thus, to the extent that no specific criteria are available for identification of SVHC substances, then it is up to the MSC to take the decision on a case-by-case basis.

Article 57(a)-(c) specifies which substances that are of very high concern for human health, namely substances classified as CMR, category 1A or 1B. Article 57(d)-(e) specifies which substances that are of very high concern for the environment, namely PBT and vPvB substances. Thus, it is clear that only hazardous substances of particular concern can be identified as SVHC and included on the candidate list.

In addition to the criteria in Article 57(a)-(e), Article 57(f) contains a "safety net" which on a case-by-case basis can be used for identifying other substances that give rise to an equivalent level of concern to those listed in Article 57(a)-(e) if they are "likely to cause serious effects on human health or the environment". As examples of the type of substances which

could be identified as SVHC substances in accordance with the Article 57(f) criteria are substances with endocrine disrupting properties.

Considering that Article 57 is intended to identify only those hazardous substances which are of very high concern to be included on the candidate list, a similar prioritisation scheme could be anticipated for EDs. Various options for prioritisation of EDs for inclusion on the candidate list according to Article 57(f) include:

Seriousness of effects
According to the above mentioned definition of EDs, these are substances that alter the functions of the endocrine system and consequently cause adverse effects in the intact organism, its progeny or (sub)populations. Article 57(f) refers to "probable serious effects to human health or to the environment". Due to the difference in terminology between Article 57(f) and the working definition of EDs, a question could be whether it is possible to distinguish between "adverse effects" and "serious effects" of EDs.

However, reflecting on our current knowledge of effects of EDs, we hardly ever see effects of EDs that are not serious. They include for example most obviously a range of endocrine related development/reproductive toxicity endpoints as well as endocrine related carcinogenicity. But in addition, emerging evidence seems to suggest that the increasing prevalence of obesity and diabetes as well as development of metabolic syndrome and effects on the immune system may in certain cases be related to endocrine disruption even though a clear proof of its relation to exposure to endocrine disrupting chemicals may not have been established with high certainty at present. Regarding environmental effects, the effects recorded in relevant ecotoxicity tests related to endocrine activity/modulation all address ecological relevant parameters related to development, growth or reproduction. Hence, these types of adverse effects are all serious. Thus, the difference between the two possible interpretations provided above seems to be non existent for endocrine disruptors in practical terms.

In conclusion, it is the view of the Danish EPA that from a scientific point of view, it is neither relevant nor reasonable to distinguish between "adverse" and "serious" effects of EDs.

Potency
Another option for prioritising amongst identified EDs could be to include only EDs with a high potency for causing adverse effects, i.e. by identifying an effect level below which an ED could be identified as an SVHC substance (and consequently above which an ED would not be identified as an SVHC).

However, comparing with the provisions for identifying CMR substances as SVHC substances, CMRs are not identified based on their potency for causing effects but rather on the level of evidence of their hazard. Thus, it would not be consistent to introduce a different approach for identifying EDs as substances of an equivalent level of concern to CMR substances than the approach used for CMR effects (indeed many EDs are causing reproductive toxic effects and they should therefore be identified by use of the same approach). Furthermore, there are several indications that it is the time of exposure during pregnancy that matters (exposure during critical time windows) more than the dose. In the light of this, potency considerations seem irrelevant as criteria for identification.

Thus, the Danish EPA does not support using potency as an approach for identifying EDs for inclusion on the candidate list. Instead, potency considerations could be an inherent part of the RMO analysis preceding the decision to propose an ED for inclusion on the candidate list via the Article 57(f) procedure (as it could be for any other types of SVHC referred to in article 57, cf. the above discussion of analysis of RMO).

Evidence
As mentioned above, CMR substances of an equivalent level of concern to EDs can be identified as SVHC substances and included in the candidate list, if they fulfil the criteria for classification in category 1A or 1B. Category 1A substances are substances that are known to cause effects in humans, while category 1B substances are substances that based on animal studies are presumed to cause effects in humans. CMR category 2 substances are not fulfilling the Article 57 criteria as the evidence for the serious effects of these substances is insufficient.

If the same approach should be used for EDs, it is suggested that confirmed EDs would be identified as SVHC substances under Article 57(f), while potential EDs would not be identified as SVHC substances due to insufficient evidence[10].

5 Recommendations

Based on the above consideration, the Danish EPA would recommend that:

- the Commission facilitates the agreement at EU (and if possible at international) level of a general definition of endocrine disruptors and potential (or suspected) endocrine disruptors as well as equivalent criteria for identifying endocrine disruptors,
- the obligation of registrants under REACH to assess whether their substance has endocrine disrupting properties is clarified by issuing appropriate guidance,
- a group of interested Member States in collaboration with the Commission and ECHA screen substances (including substances registered under REACH) for endocrine disrupting properties,
- identified potential endocrine disruptors, if meeting priority criteria, are selected for substance evaluation with the aim of obtaining sufficient data allowing a conclusion on their endocrine disrupting properties,
- interested Member States analyse risk management options for relevant identified endocrine disruptors with the aim of deciding on appropriate risk management, and post these analyses on the CIRCA Annex XV IG for commenting before taking a final decision on the most appropriate risk management option.

6 References

EC (1997). European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife: Report of the Proceedings 2-4 December 1996, Weybridge, UK (EUR 17549), Brussels, Belgium, European Commission.

[10] Potential EDs could be prioritized for substance evaluation with the aim of requesting confirmatory data allowing a final conclusion on the endocrine disruption status.

OECD (2010). Guidance Document on the Assessment of Chemicals for Endocrine Disruption, Version 9 (17 November 2010).

WHO (2003). Global assessment of the state-of-the-science of endocrine disruptors. Eds: Damstra, T., Barlow, S., Bergman, A., Kavlock, R. and Van der Kraak, G., WHO/PCS/EDC/02.2, World Health Organisation, Geneva. 180 pp.

Annex C: Danish comments to input from other regulatory bodies/member states in relation to assessment of endocrine disruptors

DK EPA Chemicals Division
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17 May 2011

Danish comments to inputs from other regulatory bodies/ Member States in relation to the assessment of endocrine disruptors.

During the last year, various regulatory bodies from some member states have made proposals in relation to the assessment on endocrine disruptors. The Danish EPA appreciates the discussions raised, and shares many of the views, considerations and interpretations presented in the working papers concerning this subject. However, in relation to some important issues we do not agree to the presented approaches as reflected in the following comments.

In this document, we present our comments to the following documents:

1. BfR document on PPPR, human health criteria, "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)", 5th May 2010

2. DE-UK document on human health criteria, "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health", 13th May 2011

3. UK document on ecotox criteria, "Definition of an Ecotoxicological Endocrine Disrupter for Regulatory Purposes", April 2011

4. BAuA document on REACH, human health criteria, "Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of EN substances as SVHC", 20th October 2010

5. UBA document on REACH, ecotox criteria, "Discussion paper on interpretation of Art. 57(f) REACH with respect to substances having endocrine disrupting properties hazardous to the environment", May 26th 2010.

We have not previously in a written form made comments to the first three documents. Our new comments are placed first. It should be noted that our views on this subject to some extent have developed as a result of various discussions during the last year. For each proposal, our comments are presented according to the specific issue dealt with. Some issues might be commented in more or all of the proposals. The most comprehensive line of argumentation will in that case often be found in the first comments presented here, but excep-

tions might occur in relation to whether the proposal concerns legal framework (REACH vs. PPPR) or endpoints (human health vs. ecotoxicology).

This document should be seen in close relation to the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation from 17 May 2011 and the Danish input to the EU process in relation to Regulation of endocrine disruptors under REACH from 31 January 2011.

1. Comments to the BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)", 5th May, 2010.

The Danish EPA has not previously commented this document. Our point of view on a number of the general issues is reflected in the comments to 4: BAuA document "Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of EN substances as SVHC". In the following we address some of the more specific key issues in this document:

- ***Definition of ED***

The Danish EPA is not in favour of having separate definitions of endocrine disruptors for human health and environmental effects. In our proposal the same definition and criteria apply for both human health and environmental effects (see the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011).

The Danish EPA agrees that (point 2.2) the WHO/IPCS definitions is a good starting point for the discussion:

"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.

A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations".

However, given a closer look, the definitions of ED and potential ED seem to represent the two "ends of" the spectrum of knowledge on ED properties and effects, i.e. the situations where there is extensive documentation for adverse health effects, ED mode of action and a cause-effect relationship and those situations where there is only limited knowledge on "properties that might lead to". Consequently, the Danish EPA finds that there is a need for including an additional sub-categorisation of potential EDs. (see further in our proposal of 17 May 2011)

- ***Definition of adversity***

The Danish EPA agrees (point 2.2) to use the WHO/IPCS definition of the term "adversity":

"A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences." (WHO/IPCS 2004)

- ***Endocrine modulation as a hazard per se***

The Danish EPA agrees that (point 3) "…endocrine modulation is not a hazard per se, but rather concerns mode of actions (MOA) of toxicity that may cause adverse effects in humans". We would, however, add: "or wild organisms in the environment" to this sentence. Furthermore, endocrine disruption, i.e. clear effects on the endocrine system of a certain magnitude is clearly related to potential adverse effects in humans and wildlife.

- ***Consideration of mechanisms of action***

The Danish EPA agrees that (point 3) "…an evaluation of the mechanism/mode of action (MOA) …is also required to discriminate endocrine disrupters of high concern from those of lower concern".

The Danish EPA agrees that (point 4.2) when observing effects potentially related to ED, "The default assumption…is that the mechanism is endocrine. If no mechanistic data are provided or if the mechanism of toxicity is shown to be endocrine, the substance may be considered as being an endocrine disruptor in animals. However, if the mechanistic data clearly show that the mechanism of toxicity is not based on endocrine effects, the substance is presumably not an endocrine disruptor…".

We are of the opinion that for categorising a substance as a confirmed ED (category 1), either adverse *in vivo* effects where an ED mode of action is highly plausible or ED mode of action *in vivo* that is clearly linked to adverse *in vivo* effects (by e.g. read-across) must be observed. However, our proposal of 17 May 2011 also contains two sub-categories (suspected and indicated EDs), for which less evidence (in *vivo*, *in vitro* or from (Q)SAR models) is needed for the link between indications of ED activity and adverse effects. The regulatory use of the categories is different, since substances in category 1 (confirmed EDs) should be identified as SVHC substances under REACH and meet the criteria for non-approval in the PPPR, whereas for category 2a or 2b substances, the WoE together with other priority considerations (e.g. exposure potential and risk management options) may trigger further testing/data generation.

- ***Consideration of relevance to humans***

The Danish EPA agrees that (point 3) "…an evaluation of…its relevance for humans…is also required to discriminate endocrine disrupters of high concern from those of lower concern".

The Danish EPA agrees that (point 4.3) "The default assumption…would be relevance. Consequently, only if a mechanism of toxicity in animals is identified that is clearly not relevant to humans, the decision tree might be left at this step". (i.e. the substance will be considered an ED only in animals).

Endocrine disruption may in rare cases induce toxic effects in rats that are not likely to occur in humans due to specific detailed endocrine differences between rats and humans. However, the endocrine disruption seen in the rats may certainly nevertheless be of relevance for humans (e.g. causing other adverse effects in humans than those caused in the rat), be-

cause the same spectrum of hormones is important for rats and humans. In humans, a severe effect on hormones is therefore likely to cause other adverse effects. Hence a cautious weight of evidence scientific analysis must be performed and generally very convincing and comprehensive evidence is required before it in specific cases my be concluded that substances causing endocrine disruption in the rat does not do so in humans.

- ***Exposure considerations***

The Danish EPA does not agree that (point 4.4.1) identification of EDs should be based on exposure considerations (option 1 in the proposal of BfR). It is noted that neither the WHO definitions nor the new Guidance Document on the Assessment of Endocrine Disruption (OECD 2011) operate with exposure considerations in relation to criteria for identification of EDs.

In our view, exposure related priority setting considerations may be relevant when considering whether to obtain further information or testing data on potential EDs, as well as in relation to risk management options for confirmed EDs, but exposure considerations should not be part of the criteria for the identification of EDs.

Furthermore, the proposal of a science based definition of negligible exposure to humans is not, to our understanding, in line with Reg. (EC) No 1107/2009.

- ***Potency considerations***

The Danish EPA do not agree that (point 4.4.2) potency considerations should be part of the criteria for EDs. The internationally agreed WHO/IPCS definition of EDs does not include potency considerations or requirements. The new draft OECD Guidance Document on the Assessment of Endocrine Disruption (OECD 2011) does also not contain potency criteria or considerations.

Furthermore, there are several indications that for EDs it is the time of exposure during pregnancy and/or early life-stages that matters (exposure during critical time windows) rather than the dose. In the light of this, potency considerations should in our view not be included as part of the criteria for identification of EDs.

Therefore, as for CMR substances, The Danish EPA proposes the categorisation of EDs to be based on the level of evidence. Indeed many EDs are causing reproductive toxic effects and they should therefore be identified by use of the same approach. In our proposal for criteria of 17 May 2011, a high level of evidence is needed for categorisation as a confirmed ED in category 1, whereas less strong evidence will lead to categorisation in either category 2a (suspected ED) or category 2b (indicated ED) (see also the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011).

Also, the use of potency for identification of EDs could result in a situation with lower protection of human health and environment as potent EDs with only very limited exposure would be categorised as EDs whereas moderate or weak EDs with extensive exposure would not be identified as EDs.

Just like exposure considerations may be relevant to use in relation to whether to require more information / test data on potential EDs, potency considerations may also play a role. It also has to be considered further whether potency of confirmed EDs in certain specific cases

may be relevant to consider in relation to risk management options. However, potency should not be part of the criteria for the identification of EDs.

- ***ED identification using required standard OECD TG studies***

The Danish EPA agrees (point 3) that some EDs might be identified in some standard toxicology tests (e.g. OECD Test Guidelines and EU Test Methods) that are routinely performed to fulfil the data requirements for plant protection products. However, The Danish EPA disagrees (point 3) that "the required studies are expected to be able to provide evidence for endocrine effects". We disagree that the required studies in all cases will be able to identify all types of EDs. It should be acknowledged that many endpoints linked to ED modes of action are insufficiently covered by the existing standard test methods, including the standard test methods mentioned in the proposal of BfR (i.e. the recently amended short-term toxicity study in rats (OECD TG 407), the chronic toxicity/carcinogenicity studies (OECD TG 451-453), the (two-generation) reproduction study (OECD TG 416) and the prenatal developmental toxicity study (OECD TG 414), even though some of them represent the current highest tier tests for detecting endocrine disrupting properties in mammals. The extended one-generation assay (EOGRTS, draft TG 443) presently under adoption procedure in the OECD will provide some substantial improvements regarding sex hormone related ED endpoints in mammalian organisms. However, it is very likely that data from this test method may only become available for a very limited number of active ingredients of PPPs such as new active ingredients, since the draft amended data requirements of the PPPR currently do not contain a requirement for conducting an extended one generation test when data from a two-generation reproductive toxicity test (OECD TG 416) is available.

This issue illustrates the importance of operating with more categories of EDs, so that also potential (indicated and suspected)[11] EDs will be identified (by use of e.g. (Q)SAR predictions, *in vitro* assays and *in vivo* screening studies). For the potential EDs, the WoE together with other priority considerations (e.g. exposure potential and risk management options) may trigger further testing/data generation.

In addition, emerging evidence suggests that the increasing prevalence of obesity and diabetes as well as development of metabolic syndrome and effects on the immune system may in certain cases be related to endocrine disruption. Since these endpoints are not covered in any of the established OECD test guidelines, whole classes of endocrine disruptors inducing such effects will not be identified by use of OECD test guidelines. Therefore the Danish EPA would propose that triggered by evidence, improvement of testing methods with regard to those emerging endpoints should be considered (when possible) on a case-by-case basis, in order not to dismiss effects on the endocrine system which might be of relevance to human health.

- ***Non-monotonic response curves***

The Danish EPA agrees that (point 3) "…triggered by evidence (provided that so-called low dose effects are further substantiated concerning robustness and reproducibility), improvement of testing methods with regard to the low dose range should be considered on a case-by-case basis, in order not to dismiss effects on the endocrine system which might be of relevance to human health".

[11] See our proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011

- ***ED effects of CMR substances***

The Danish EPA agrees that (point 4.1) "…it is recommended to also include substances proposed to be labelled CMR cat.1A or 1B into the analysis of endocrine disrupting properties, and to clarify their mechanism of toxicity". We would, however, add "if scientifically possible" to this sentence.

- ***Use of 2 ED categories***

a) The Danish EPA agrees (point 4.4.2) to use 2 main categories for EDs. In the Danish proposal of 17 May 2011, category 1 is the confirmed EDs, and category 2(a and b) are the potential (suspected and indicated) EDs. However, the Danish EPA does not agree on basing the criteria on severity of effects and potency considerations. In our view, the inclusion criteria should be evidence based (se the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011).

b) The Danish EPA agrees that only confirmed EDs (category 1 in the Danish proposal of 17 May 2011) should be regulated (not approved) in the PPPR, whereas for substances in category 2 further testing might be necessary.

2. Comments to the DE-UK document: "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health", 13th May 2011.

The Danish EPA has not previously commented this document. Our point of view on a number of the issues is reflected in the comments to 1: BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)" and/or in the comments to 4: BAuA document "Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of EN substances as SVHC". In the following our comments to some of the more specific key issues in this document are reflected (due to the tight time-frame between receiving this document (13th May) and submitting the Danish proposal (17th May), it cannot be excluded that more comments to this document might be developed later on):

- ***Definition of ED***

Please see our comments to 1: BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)."

- ***Adversity***

a) The Danish EPA agrees to use the WHO/IPCS definition of the term "adversity":

"A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences." (WHO/IPCS 2004)

b) The Danish EPA also agrees that (point 15) a change in circulating levels of a particular hormone should not automatically be considered an adverse effect in itself. However, a change in levels of a particular hormone should be taken into consideration in a weight-of-evidence approach by use of scientific expert judgement, and could, together with indications from e.g. (Q)SARs or *in vitro* assays, in the Danish proposal of 17 May 2011 for Criteria for Endocrine Disruptors and Options for Regulation, lead to a categorisation of the substance as a "suspected ED" or "indicated ED" (ED category 2a or 2b). According to the draft OECD Guidance Document on the Assessment of Endocrine Disruption, such a change is an indicator of hormonal activity and should be considered with caution. The reason is that although it is possible that such a change may not lead to adverse effects in the study used, adverse effects may be detected in other, e.g. longer-term and more comprehensive studies.

- ***Intact organisms***

The Danish EPA agrees that (point 16) "…observations from screening tests in ovariectomised or castrated animals cannot be taken as evidence of adverse effects in the intact animal".
However, *in vivo* screening assays (such as Hershberger TG 441 and Uterotrophic assay TG 440) can show that a substance can interfere with the endocrine system in animals, i.e. the substance has an ED mode of action *in vivo*.
It should be acknowledged that these assays may use intact weanling animals instead of ovariectomised or castrated animals. In that case, the observations should be taken as evidence of real adverse effects in intact animals.
Furthermore, the OECD validation of these assays is based on data for confirmed EDs and the validation results show that EDs with the mode of actions found in confirmatory studies were actually positive in these assays. For the Hershberger assay, the dose levels causing effects generally seem to be similar to or higher than those causing ED effects in generation studies. Consequently, using the criteria proposed by Denmark, a positive response in these assays can be used for categorising a substance as a suspected ED (Category 2a) (see the Danish proposal of 17 May 2011).

- ***CMR cat 1A and 1B***

The Danish EPA does not agree that (point 18) "in most cases there is no additional value in pursuing the ED issue for CMR 1A and 1B substances". As it is stated in the DE-UK document (point 18): "…it should be noted that in the context of REACH, as the Authorisation process address only the hazard property for which inclusion on the SVHC list was proposed, it may still be appropriate to assess whether the CMR 1A or 1B substance is also an ED".
Furthermore, firstly identification of e.g. a carcinogenic substance as having endocrine disruptive effects, could have importance for the future risk management of combined exposures to more EDs. Secondly, future changes in legislation may change the way EDs and/or CMRs are handled in a way that make the knowledge of endocrine disruptive effects of CMRs more important than it may seem today.

- ***Identification of EDs in standard toxicological tests***

The Danish EPA does not agree that (point 19) "EDs can be indentified in standard regulatory tests that are routinely performed to fulfil the requirements of various regulatory programmes".
Please see our comments to the BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)."

- ***Plausibility of mode/mechanism of action***

The Danish EPA agrees that (point 22) “In order to conclude that a substance is a (confirmed) ED there must be a reasonable evidence base for the supposition that there is a plausible/coherent link between the induced endocrine perturbation/activity and the adverse effects seen in the intact organism studies”. However, we disagree that (point 15): “Crucially, to consider that a substance might require attention for regulatory purposes, any endocrine perturbation must result in adverse effects, such as pathology or functional impairment”.
In the Danish proposal of 17 May 2011, it is proposed to use more categories of EDs, and for EDs in category 2, less evidence is needed for the link between indications of ED activity and adverse effects. Please see also our comments regarding considerations of mechanisms of action in 1: BfR document: “Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009).”

- ***Study design***

In the DE-UK document, it is stated that (point 20) all studies “… must be conducted to an acceptable protocol and to good standards and be well reported”.
The Danish EPA agrees that data quality needs to be considered but do not agree that only data from standard test methods can be used, since this would lead to an exclusive use of studies conducted according to OECD test guidelines and similar standardized test methods. Other well conducted and reported studies should, also be used in the WoE assessment. As it is also stated in the DE-UK document (point 22): “Such a mechanistic link could be established, for example, using information from the *in vitro* and *in vivo* screening assays (levels 2 and 3) of the current OECD conceptual framework for testing and assessment of EDs (Appendix 3) or from more ad-hoc studies”.

The importance of this is underlined by the fact that new endocrine disruptive endpoints emerge, these will not all be covered or identified by use of the established standard test methods such as the OECD test guidelines. This issue underlines the importance of operating with more categories of EDs, so that also potential (indicated and suspected) EDs will be identified (by use of e.g. (Q)SAR predictions, *in vitro* assays, *in vivo* screening studies and non-guideline studies). For the potential EDs, the WoE together with other priority considerations (e.g. exposure potential and risk management options) may trigger further testing/data generation.

- ***Relevant routes of exposure***

The Danish EPA does not agree that (point 20) only studies using relevant exposure routes (oral, dermal or inhalation) can be accepted for identification of EDs. Even though absorption, metabolism and/or excretion may differ between studies using administration by subcutaneous, intravenous, intraperitoneal injections or other application routes compared to e.g. oral exposure, such studies may provide data of relevance for the identification of suspected or indicated EDs. Therefore, they may be used for hazard identification, especially in weight-of-evidence approaches by the use of scientific expert judgement by making appropriate ADME considerations.
However, observed effects may or may not be clearly predictive for the risk for these effects, and it is often difficult to derive a DNEL from such studies for use in risk assessment.

- ***Considerations when requesting vertebrate studies***

The Danish EPA does not agree that (point 24) “Before studies involving testing in vertebrates are requested, considerations should be given to the dose levels at which the adverse effects potentially related to ED were first seen, or that if these dosages are relatively high (the substance being of low potency for the potentially ED-related effect), then it may be justifiable not to conduct such additional studies”.
We would rather base a decision on whether to request further testing or not on a weight-of-evidence approach taking into concern all available (Q)SAR predictions, *in vitro* and and *in vivo* results. One potential problem in basing the judgement on potency *in vivo* only is that the *in vivo* study used might have a low power or low sensitivity for the endpoints investigated, and that more sensitive ED-related endpoints might detect effects at much lower doses than observed in the first place.

- ***Potency***

The Danish EPA agrees that (point 28) “In general terms, toxic effects are only of regulatory relevance when they occur at relevant dose levels”.
However, for EDs there are several indications that it is the time of exposure during pregnancy and/or early life-stages that matters (exposure during critical time windows) rather than the dose. Furthermore, when taking combined exposures (to many different substances from many different sources for substances with similar effects) into concern, it become obvious that toxic effects at dose levels higher than those observed for humans or in wildlife become much more relevant.
In the light of this, potency considerations should in our view not be included as part of the criteria for identification of EDs, but rather be a part of the considerations - where also other priority considerations like exposure potential might be included - for the choice of appropriate risk management measures.

This is supported by the fact that the internationally agreed WHO/IPCS definition of EDs does not include potency considerations or requirements. The new draft OECD Guidance Document on the Assessment of Endocrine Disruption (OECD 2011) does also not contain potency criteria or considerations.

Please, see also our comments regarding potency to 1: BfR document: : “Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009).”

- ***Use of STOT-Re guidance values as cut-off criteria***

The Danish EPA disagrees to use cut-off values in the identification of EDs. The reasoning is given above in our comments regarding potency considerations.

The Danish EPA agrees that (point 36) “CMR 1A and 1B substances posses serious, well-established and specific hazard properties”, and that (point 36) “only EDs of equivalent level of concern to carcinogens, mutagens or reproductive (CMR) toxicants Category 1A and 1B (under the CLP regulation) may be included in the list of SVHC as possible candidates for Authorisation under REACH and be considered for non-approval under the draft BPR”.
However, CMR substances are not identified based on their potency for causing effects but rather on the level of evidence of their hazard.
Thus, it would not be consistent to introduce a different approach for identifying EDs as substances of an equivalent level of concern to CMR substances than the approach used for

CMR effects. Indeed, many EDs are causing reproductive toxic effects and they should therefore be identified by use of the same approach.

In the light of this, thresholds and potency considerations are not included as part of the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011.

- ***Relevance to humans***

The Danish EPA agrees that (point 25) "The default assumption of any adverse effect seen in regulatory toxicity studies is that the effect is relevant to humans".

Please see also our comments to 1: BfR document: : "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)."

The Danish EPA also agrees that (point 27) "…even when effects are not relevant to humans, they could still be relevant to non-target species in the environment".

3. Comments to the UK document: "Definition of an Ecotoxicological Endocrine Disrupter for Regulatory Purposes", April 2011.

The Danish EPA has not previously commented this document. Our point of view on a number of the possible discussion points is reflected in the comments to 1: BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)", 2: DE-UK document: "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health" and/or 5: UBA document: "Discussion paper on interpretation of Art. 57(f) REACH with respect to substances having endocrine disrupting properties hazardous to the environment". In the following our comments to some of the more specific key issues in this document not specifically addressed elsewhere in this paper are reflected:

- ***Criteria in different pieces of legislation***

The Danish EPA is not in favour of having separate definitions of endocrine disruptors for different pieces of EU legislation. However, Denmark proposes to have more categories of EDs, and that the different categories can be handled differently in different types of EU legislation (see also the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011).

- ***Definition of ED***

Please see comments to 1: BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)".

- ***Adversity***

Please see comments to 2: DE-UK document "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health".

- ***Link between endocrine perturbation and adverse effects***

The Danish EPA is not in favour of having separate definitions of endocrine disruptors for human health and environmental effects.

In the UK document (point 16), it is stated that "to designate a substance as an ecotoxicological ED, any endocrine perturbation must result in, or be plausibly connected with, observed adverse ecotoxicological effects in intact organisms that can impact detrimentally on the population of one or more environmental species" and (point 27) that "in order to conclude that a substance is an ecotoxicological ED there must be a reasonable and coherent line of evidence for a link between adverse population-related effects seen in intact organism studies and en endocrine-disrupting mode-of-action".

The Danish EPA agrees that for categorising a substance as a confirmed ED (category 1) either adverse *in vivo* effects where an ED mode of action is highly plausible or ED mode of action *in vivo* that is clearly linked to adverse *in vivo* effects (by e.g. read-across) must be observed. However, our proposal of 17 May 2011 also contains two sub-categories of the potential ED category (suspected and indicated EDs), for which less evidence (*in vivo* screening data, *in vitro* data or predictions from (Q)SAR models) is available for the link between indications of hormonal activity and adverse effects. The regulatory consequences in relation to the categories are different, since substances in category 1 should be identified as SVHC substances, whereas category 2a or 2b substances should trigger further testing/data generation depending on exposure potential.

- ***Use of ecotoxicological studies in HH assessment***

A) In the UK proposal (point 25) it is stated that in most cases, "if the substance is considered to be an EDs from a human health perspective it is unlikely to need consideration from an ecotoxicological perspective". This is not the case under REACH, since the information will be relevant for proper risk management. For regulation under the PPPR, there could be cases where human exposure is negligible, and it therefore still will be relevant to consider the ecotoxicological perspective.

In general the Danish EPA is not in favour of having separate definitions of endocrine disruptors for human health and environmental effects. According to our proposal of 17 May 2011, both information from ecotoxicological and from "human health studies" (rat studies in particular) would be used in a weight of evidence approach in order to identify EDs. In this context it may be relevant to recall that data from laboratory rat studies may be as relevant for wild mammalian species (environment) as for humans (human health).

B) In the UK document it is stated (point 22) that the OECD TG 231 "…might have value in relation to the assessment of endocrine-disrupting potential in relation to human health considerations".

The Danish EPA is in favour of using both data from traditional ecotoxicological tests (on invertebrates, fish, birds, amphibians) and "human health relevant studies" (on rodent species in particular) for identification of EDs on environmental relevance.

One potential issue when comparing findings from *in vivo* mammalian studies using oral exposure to studies in fish is the differences in metabolism that may occur depending on the route of exposure (e.g. oral dosing in mammals and aqueous exposure in fish). Aqueous exposure of fish, via uptake through the gills and skin, essentially bypasses metabolism in

the small intestine and liver, through direct entry to the blood stream. Therefore, it is possible to visualize a scenario in which endocrine activity of a substance can be markedly decreased by metabolism after oral exposure. This could hypothetically, if the ED is caused by the parent compound only, lead to no endocrine related findings in the oral studies in mammalians, whereas endocrine activity may manifest itself in fish where the substance is not metabolised before reaching the site of action or relevant organ. However, it is likely that ED effects could also be manifested in mammalian studies if exposure were by dermal route or inhalation. The opposite situation may, furthermore, be seen if metabolisation of the parent compound is needed to produce endocrine disrupting metabolites. In that case some chemicals may be metabolised to EDs by mammals after oral exposure but not by fish.

The use of environmental test data for evaluation of human toxicity can be differentiated into ED identification on one side and risk assessment on the other side. For ED identification, environmental vertebrate test data can often be included in the overall evaluation because the endocrine systems of different vertebrate taxa are closely related. Knowledge about possible different metabolic pathways should though be taken into account. Concerning risk assessment, environmental test data for evaluation of human toxicity can be complicated because of the differences in exposure scenarios, metabolic capacity, differences in test designs etc.

The use of human toxicity data for evaluation of wildlife ecotoxicity can also be differentiated into identification and risk assessment. For identification of ED, data from both sources should be taken into account. For risk assessment, the differences above should be considered, but the human data incl. data from mammalian studies in e.g. rodents can be useful for (especially) mammalian wildlife.

- ***Study design***

Please see our comments to 2: DE-UK document: “Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health”.

- ***Potential endocrine disrupters***

The Danish EPA agrees that it will be useful to operate with more categories of EDs, so that also “potential” (in the Danish proposal for Criteria for Endocrine Disruptors and Options for Regulation of 17 May 2011, sub-categorised into “suspected” and “indicated”) EDs will be identified.

- ***ED effects on invertebrates***

The UK document (point 30) states that “where a substance is considered to a potential ED towards invertebrate species due to its mode of action on the target pest species (e.g. substances that change ecdysteroid and juvenile hormone systems), it is proposed that the potential population effects on invertebrates should be determined at the field scale (or equivalent”.
We would like to consider this suggestion further.

- ***Use of concentration/dose/potency***

The Danish EPA does not agree that there should be a consideration of the concentration/dose/potency in the identification of endocrine disrupters.
Se also our comments to about potency considerations in 1: BfR document: “Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC)

No 1107/2009)" and 2: DE-UK document "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health".

4. Comments to the BAuA document: "Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of ED substances as SVHC", 20th October 2010.

The following comments were submitted by the Danish EPA 30th November 2010.

"The Danish EPA welcomes and appreciates the initiative of the German authorities (BAuA and UBA) to initiate discussions on criteria for identification of substances as endocrine disruptors and the application of such criteria for REACH, Article 57(f). We have already provided some general comments to the UBA ED discussion paper following the CARACAL meeting in June 2010 and many of these comments are relevant also for the present BAuA document (attached here for information as Annex 1). Thus, there will be some overlap between our previous comments and the comments below.

Definition of endocrine disruptor
Basically, we note that discussions on a common definition of the term "endocrine disruptor" are ongoing and have been so since the 90'ies (see, e.g., the report of the OECD workshop on endocrine disruptors in 2009; OECD Series on Testing and Assessment 118, 2010). We are currently discussing this issue also in Denmark and plan to provide our input to the discussion in the beginning of next year. Nevertheless, we would like to already now provide a number of initial and general thoughts on a future definition:

- The same definition should apply both in the EU and at international level, which calls for international collaboration preferably within the OECD EDTA programme.
- The same definition should apply for all types of chemicals including industrial chemicals, pesticides, biocides, cosmetics, etc.
- The same definition should apply for both human health and environmental effects.
- In parallel to the other groups of Substances of Very High Concern (SVHC), i.e. CMRs and PBT/vPvBs, two sets of definitions for (i) endocrine disruptors and (ii) suspected or potential endocrine disruptors, respectively, should be considered.
- The definition should cover other types of long term toxic effects besides cancer and reproductive toxicity[12], if links between ED and such effects become scientifically justified in future.
- When applying the definition in relation to regulating the use of pesticides, biocides, medicine and veterinary medicine it should be considered whether to exclude certain types of non-vertebrate endocrine properties or mediated effects, such as plant and insect hormone properties.

A good starting point for discussing and further developing a definition would be the proposal by the Commission from 1999:

[12] Reproductive toxicity: fertility, mating behaviour and developmental toxicity

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

And similarly the basis for agreeing on a definition of suspected or potential endocrine disruptors could be:

A potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism (cf. OECD 2010, which here makes reference to EU 1996).

However, irrespective of how the term "endocrine disruptor" will end up being defined at international or EU level, we also need to discuss and agree on how to address endocrine disruptors under REACH. This issue is being dealt with in the rest of this document. In our view, to the extent a registrant concludes that his substance is an endocrine disruptor (confirmed or potential), this should be addressed when developing and implementing risk management measures ensuring control of risks. For authorities, the most obvious way to address endocrine disruptors is under the authorisation provisions, in particular the identification of SVHC substances for inclusion in the candidate list, but substances with such properties may also be addressed under the evaluation title (substance evaluation) or under the restrictions title of REACH as appropriate. Nevertheless, in the present document we will concentrate on the provisions for identifying SVHC substances in accordance with Article 57(f).

Interpretation of REACH, Article 57(f)

Under REACH, Article 57(f), substances with endocrine disrupting properties[13] can be selected for inclusion in the candidate list, if there in addition to the endocrine disrupting properties is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern as CMRs and PBT/vPvBs. The practical interpretation of these conditions is not further specified in REACH and neither in available guidance. Nevertheless, some initial considerations on how to understand these conditions could be:

- ***Endocrine disrupting properties***

The term "endocrine disrupting properties" is neither defined in REACH nor in any REACH guidance. Substances with "endocrine disrupting properties" must refer to substances fulfilling the definition of an "endocrine disruptor" or "a potential endocrine disrupter", i.e. that it "alters" functions of the endocrine system and consequently causes adverse effects in the organism, or that it is likely to do so.

However, it could also be understood to more specifically relating to the mode of action, i.e. that the substance has the intrinsic potential to disrupt functions of the endocrine system of an organism. Such an interpretation would mean that there is no requirement that a substance having such an intrinsic potential would actually adversely affect the organism as a consequence of exposure to such a substance. Nevertheless, we have difficulties in seeing that a substance that is disrupting the endocrine system would not cause adverse effects, so we would not favour such an interpretation.

[13] As well as substances with PBT or vPvB properties, but these are outside the scope of this document.

- ***Probable serious effects***

It is worthwhile noting that the text of Article 57(f) is different from that in the provisional definition of the Commission for endocrine disruptors, as the latter definition refers to "adverse effects" and not as in Article 57(f) to "serious effects".

Two alternative interpretations are possible here:

One is that "serious effects" mean effects that are more serious than "adverse effects". According to this interpretation this would essentially mean that not all endocrine disruptors would fulfil the Article 57(f) provisions for inclusion on the candidate list. This interpretation makes it hence necessary to distinguish between adverse and serious effects, that such a distinction is scientifically justifiable and that it can be operationalised.

A slightly different interpretation is that "serious effects" in Article 57(f) are the same as "adverse effects" in the Commission working definition from 1999.

However, reflecting on our current knowledge of effects of endocrine disruptors, we hardly ever see effects of endocrine disruptors that are not serious. They include for example most obviously a range of endocrine related development/reproductive toxicity endpoints as well as endocrine related carcinogenicity. But in addition, emerging evidence seems to suggest that the increasing prevalence of obesity and diabetes as well as development of metabolic syndrome and effects on the immune system may be related to endocrine disruption even though a clear proof of its relation to exposure to endocrine disrupting chemicals has not been established at present. Regarding environmental effects, the effects recorded in relevant ecotoxicity tests which are including adverse effect parameters besides parameters related to endocrine activity/modulation all address ecological relevant parameters related to development, growth or reproduction. Hence these adverse effects are all serious. Thus, the difference between the two possible interpretations above may in practical terms not be significant.

- ***Equivalent level of concern***

The third condition of Article 57(f) is that the probable serious effects caused by a substance with endocrine disrupting properties are of equivalent level of concern to CMRs and PBT/vPvBs. So what are the concerns of these types of substances?

- Carcinogens: A carcinogen is of very high concern because it has the potential to induce the development of tumours in the body that can cause severe effects that may even threaten the survival of the individual.
- Mutagens: A mutagen is of very high concern because it has the potential to cause a permanent damage to the amount or structure of genetic material in a cell, which may affect the survival or reproduction of the individual (somatic mutations) or its progeny (due to inheritable changes in the genes caused by germ cell mutations in the parents).
- Reproductive toxicants: A reproductive toxicant is of very high concern because it has the potential to cause adverse effects on sexual function and fertility of adults and/or adverse effects on development of the offspring.
- PBT/vPvBs: A PBT or vPvB substance is of very high concern because it due to its persistency and bioaccumulative properties has the potential to cause long-term adverse effects in humans or in organisms in the environment which are very difficult to predict and which are also very difficult and/or slow to reverse.

Thus, the general concerns of substances identified through REACH, Article 57(a)-(e) CMRs and PBTs are that they have the potential to cause severe long-term effects which may be irreversible and/or difficult to predict and/or reverse. The identification of a substance as a CMR is fully hazard driven and only the level of evidence for presence of the properties is used in relation to the assignment to categories of CMR whereas potency (e.g. expressed as a dose or concentration limit) of the substance is not considered.

Identification of substances for the candidate list in accordance with Article 57(f)
Following the above discussion on how to understand REACH, Article 57(f), and considering that the same type and level of concern should apply, the following conclusion can be drawn in relation to substances with endocrine disrupting properties:

> A substance fulfils the Article 57(f) criteria for a substance with endocrine disrupting properties for inclusion in the candidate list, if the substance can cause severe long-term effects as a consequence of its endocrine disrupting properties.

It is noted that in particular many substances classified for reproductive toxicity (human health) would fall under this category. If only the same parameters as used in testing for reproductive toxicity are measured and used in relation to substances with endocrine disrupting properties highly probable to cause serious effects in the organism, this would only affect the current level of protection for the human health in those cases where the ED properties form the basis for raising the classification from Repr. Cat. 2 to Repr. Cat. 1B. Similar considerations apply to classification for ED related carcinogenic effects. But in future depending of scientific development and generation of new data ED may perhaps also be shown relate to certain other types of long-term toxic effects or syndromes, which may be caused by chemicals. Furthermore, in relation to ecotoxicity of non PBT/vPvB substances conclusive evidence of endocrine disrupting properties may trigger authorisation as the most appropriate risk management approach which would not otherwise be possible.

Therefore, parameters of specific relevance for defining endocrine disrupting properties of chemicals should be identified and be the primary parameters to consider for identification of endocrine disrupting substances under Article 57(f). The Danish EPA has initiated analyses of this issue and expects to provide input to the discussion in the beginning of next year.

Specific comments to the BAuA document
Following the considerations above, we have a number of more specific comments to the BAuA document on the German approach to identification of endocrine disruptors.

- ***Definition of 'endocrine disrupting properties'***

As mentioned above, we are not in favour of having separate definitions of endocrine disruptors for human health and environmental effects.

For clearly distinguishing endocrine active substances which have not (yet) been shown to lead to adverse effects from endocrine disruption, from substances where such effects have been shown, we agree with a proposal made by BIAC to refer to the former as endocrine modulators (or endocrine active substances or suspected endocrine disruptors)

As a consequence, we consider that endocrine modulating substances can be identified through not only *in vivo* vertebrate toxicity studies, but also from *in vitro* studies, read-across

and (Q)SARs in which various parts of the functioning of the endocrine system are studied. In any case, results of non-animal studies can be used for identifying endocrine modulating substances that are "suspected" endocrine disruptors.

- ***Evidence of serious health effects***

On page 5-6, it is mentioned that "serious effects" is closely linked to "adverse effects" and further that a significant part of these can be linked to hazard classification in accordance with the CLP Regulation. However, the intention of the REACH authorisation regime is not that all classified substances should be addressed by the authorisation provisions. Rather, the intention is that only substances with a potential to cause serious effects giving rise to an equivalent level of concern as CMR and PBT/vPvB substances should be included in the candidate list. It is in our view generally accepted that EDCs (where the adverse effects are related to endocrine disruption) are considered as substances of very high concern. It is for such substances likely that serious effects may be caused to human health and/or to organisms in the environment.

- ***Equivalent level of concern***

On page 6, the condition on equivalent level of concern to CMR and PBT/vPvB substances is proposed to be related to the "potency" or a dose or concentration limit of the substance. However, as also mentioned above, the criteria for identifying another type of SVHC, the CMR substances, do not in general operate with potency or dose limits. Furthermore, as we also suggest above, the term "equivalent level of concern" should be defined by looking at the severity of concern related to substances with CMR or PBT/vPvB properties.

On page 7, it is suggested that in particular substances fulfilling the hazard classification criteria for STOT RE Cat 1, Repro Cat 2 and Carc Cat 2 caused by endocrine disruption should be considered. We do not disagree that such substances may very well qualify for inclusion in the candidate list, when the effect seen in these tests is caused by endocrine disruption, but other types of effects and, in particular, other effect levels may qualify as well.

On page 7-8, the use of a cut-off value of 10 mg/kg bw/day is proposed. The Danish National Food Institute has compiled data for the Danish EPA (see Annex 2) showing that among a group of recognised endocrine disruptors comprising various phthalates, pesticides and chlorinated substances, only two of these (vinclozolin and PCB) would have LOAELs below the proposed 10 mg/kg bw/day limit and these are already classified and severely restricted.

Furthermore, it is also noted that the cut-off value of 10 mg/kg relates to the 90-day repeated dose toxicity studies, while if only a 28-day study is available, the limit would be 3 times higher. Effects on endocrine systems and on, in particular, reproduction are however often seen after shorter exposure periods and often in sensitive time windows. If setting any dose limit or cut off value was relevant - what we dispute - this in itself would call for setting a higher cut-off value.

Generally, it should be underlined that endpoints linked to ED modes of action in general are insufficiently covered by the existing test methods, including the current two-generation assay. The proposed extended one-generation assay will provide some substantial improvements regarding sex hormone related ED endpoints in mammalian organisms, however, data from this test method will only be required for a limited number of chemicals.

On page 8 below the table, it is mentioned that cut-off values would ensure the same level of concern as CMRs. However, the concern with CMR (and PBT/vPvB) substances is not primarily related to their potency but rather to the serious long-term and/or irreversible effects that exposure to these substances may cause.

Therefore, we also cannot agree to the proposal here that if an endocrine effect is only caused at a dose level above a certain cut-off value, then this does not fulfil the Article 57(f) requirements. Essentially, it is not the potency of an endocrine disruptor that has to be of an equivalent level of concern to CMR and PBT/vPvB substances, it is the seriousness of the effects that has to be of an equivalent level of concern. Consequently, we consider it outside the scope of identification of substances for the candidate list to include conditions related to the potency of substances.

In our view the criteria should be strictly hazard based and only related to the seriousness of the effects that potentially could be caused following exposure to such substances with endocrine disrupting properties.

Comments to figure 1

Following the above comments, we disagree with only focusing on the three hazard classes (STOT RE Cat 1, Repro Cat 2 and Carc Cat 2) and the use of the proposed cut-off values". "

Please note that an updated version (of 17 May 2011) of Annex 2 mentioned in these comments are attached to this document.

The following issues were not discussed in the comments from the Danish EPA submitted 30th of November 2011 (or we have elaborated further on the thoughts since then). However, we would like to address these issues, since we in some cases do not fully agree with the interpretation by BAuA:

- *Definition of ED*
- *Use of results from studies with different routes of substance administration*
- *Use of (Q)SAR, in vitro and in vivo screening tests for identification of EDs*
- *Use of results from the Uterotrophic and the Hershberger assay*
- *Use of potency in ED criteria vs. use of potency in combination with exposure for prioritisation of substances for regulation and choice of risk management measures.*

These issues are addressed in our comments to 1: BfR document: "Draft Concept Paper. Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg (EC) No 1107/2009)" and 2: DE-UK document "Regulatory Definition of an Endocrine Disrupter in Relation to Potential threat to Human Health".

5. Comments to the UBA document: "Discussion paper on interpretation of Art. 57(f) REACH with respect to substances having endocrine disrupting properties hazardous to the environment", May 26th, 2010.

The following comments were submitted by the Danish EPA 12th of July 2010.

"We appreciate the discussion paper from UBA on the interpretation of art. 57(f) and share many of the views, considerations and interpretations presented in the paper.

We note that the document is restricted to interpretation of art. 57(f) in relation to:

- chemicals with endocrine disrupting (ED) properties, and
- their environmental effects

We would welcome development of a future discussion document where ED properties of chemicals are dealt with in relation to both environmental and human health effects in this context.

We agree with the discussion paper in that art. 57(f) of REACH only refers to substances with ED properties as examples of substances of equivalent level of concern to CMRs and PBTs/vPvBs (cf. art. 57(f) "...such as...."). Hence, there may well be other types of substances with properties of equivalent concern other than substances with ED properties and PBT like substances.

We also note that basically REACH contains by its standard information requirements a strategy for obtaining confirmatory data on CMR and PBT properties – but not for ED properties. For substances having CMR or PBT properties, REACH conceptually operates with two separate levels of evidence:

- CMR substances are classified according to the CLP Regulation in two distinct categories related to the level of evidence for the substance to posses the C, M or R properties: CMR cat. 1 (1A or 1B) and CMR cat. 2., where category 1 is used when the CMR effects are documented (known), and category 2 is used when the effects are suspected.
- For PBT substances, where no hazard classification system has been established, the proposed new Annex XIII operates with two distinct levels of categories, namely definitive criteria and screening criteria for the P, B and T properties.

Hence two distinct evidence based categories of such SVHC substances have been created, i.e.

- "a confirmed category" and
- "a suspected category"

Establishment of these two categories makes it possible in a consistent way to require new and targeted information/testing for substances in “the suspected category” in order to obtain new information/testing which allows a definitive decision/judgement, i.e. as appropriately to confirm or reject the suspicion by placing the substance in “the confirmed category” or to remove it from “the suspected category”, respectively. It is furthermore as an alternative possible if the cost of generation of the required new information/testing is large, to assume that the substance actually belongs to the confirmed category and thus implement appropriate risk management measures without new data generation/testing. It is finally even possible while waiting for the new information/testing to be made available to implement some interim risk management measures in accordance with the precautionary principle.

In similarity with the approach employed for substances with CMR and PBT properties, it would also be appropriate to operate with two distinct categories of substances with ED properties, i.e. “suspected EDs” and “confirmed EDs”. It is actually especially important for substances with ED properties because, as mentioned above, neither REACH nor CLP operate with standard information/targeted testing requirements related to ED properties contrary to what is the case for substances with CMR and PBT properties. The reason is most probably that suitable standard testing methods for ED properties did not exist at the time of development, negotiation and adoption of REACH. Such test – and non-test – methods are however continuously being developed, validated and standardized in particular in the context of the OECD Test Guidelines programme and under the OECD QSAR management group. Already now several standard test methods, non testing approaches and guidance documents exist and can be used for targeted ED related information requirements if such are requested in relation to certain substances undergoing substance evaluation under REACH.

In relation to these considerations it is interesting to note that in addition to a definition of a confirmed category of endocrine disrupters, to which the UBA discussion document is referring, a definition of as suspected ED category has been proposed in the past:

“A potential ED is a substance that possesses properties that might be expected to lead to ED in an intact organism” (cf. OECD 2010, which here makes ref. to EU 1996).

As mentioned above we find it of particular importance to include also such a definition in the context of defining substances with ED properties.

In relation to the working definition of ED (COM 1999) referred to in the UBA discussion document we note that this definition is more then 10 years old, that science has progressed since then also within this field and that other proposed definitions have been put forward or exist (OECD 2010, p10 a.o).

Anyway in relation to the definition of substances with confirmed ED properties proposed by the Commission in 1999 to which the UBA discussion paper refers:

An endocrine disrupter is an exogeneous substance or mixture that alters function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations.

The following comments can be made:

- Reference is made to *"the endocrine system"* and *"organism"* but it is not specified in the definition which types of organisms the definition relates to. Organisms also include plants, microbes and invertebrate taxa, but in general endocrine disrupters often implicitly refer to the endocrine system of vertebrates because the endocrine system of other types of organisms is in most cases not so well known or when known not regarded as a particular protection target as such.

- The definition covers both individual *substances and mixtures* (and by the latter hence also in reality the effects of simultaneous exposure to individual chemicals).

- The definition requires that the *function of the endocrine system is altered* meaning that not only the extent/dose at which the hormone system is being altered matters, but also that timing matters. It is well known from endocrinology that timing is an important parameter in the endocrine control of the maintenance of the homeostasis, development and reproduction of organisms. Hence endocrine disruption may be caused by disturbance of the right timing of that control e.g. caused by chemicals inhibiting or activating various receptors, enzymes etc. at especially unfortunate points in time for the organism when such interference triggers abnormal reactions, which may be more or less irreversible. Furthermore, it is also known from endocrinology that dose/concentration response curves may not be monotonic and it is noted that discussion is ongoing in relation to ED and "low dose effects", inverted U-shaped dose-response curves etc.

- The definition includes establishment of *causality.* Establishment of such a causal link is in natural science normally done by showing that the independent variable (here extent of endocrine activity) correlates with the dependent variable (extent of adverse effects) backed up by various types of supporting evidence related to "modes of action" or "Adverse Outcome Pathways" and/or well established and/or plausible biochemical, physiological or biological theories/hypothesis.

- The definition refers to *adverse health effects in an intact organism or its progeny, or (sub) populations.* Many different definitions of adverse effects exist which may include increased susceptibility to naturally occurring stress factors. In general in ecotoxicology effects on survival, growth, development and reproduction as recorded in single species laboratory tests are regarded as ecologically relevant types of effects relevant for the maintenance of wild populations, which are the protection target. We agree in principle to the reference made in the UBA discussion paper that relevant effects may also be such that are more or less indirectly related to these types of effects, e.g. mating behaviour. The reference to subpopulations in this definition may then relate to also particular sensitive sub-populations of wild animals, if such are known to exist. This reference to subpopulations may however be especially important in relation to human health where specific subpopulations may be especially sensitive such as the unborn children, children, sensitive genotypes, people with particular habits or life style that implies high exposure to certain EDCs.

We have in our internal discussions of the UBA document been around a range of other more or less detailed issues regarding ED and REACH, but will here restrict ourselves to the above made considerations meant to be supplementary to the UBA discussion paper. Nevertheless, we would be happy to continue the discussions with UBA staff and other interested parties involved in these discussions.

Reference

OECD 2010: “Workshop Report on OECD Countries activities regarding testing, assessment and management of endocrine disrupters Part I”; “Part II: Annexes 3-11 to the case studies report on endocrine disrupting testing, assessment and management in OECD member countries”, ENV/JM/MONO(2010)2, OECD EHS Publication Series on testing and Assessment no. 118, IOMC; Paris 2010. “

Updated version (of 17 May 2011) of the Annex 2 mentioned in relation to comments to 4: BAuA document on REACH, human health criteria, "Human health criteria for endocrine disruption (ED) according to Art. 57 (f) of the REACH regulation: German approach to the identification of EN substances as SVHC", 20th October 2010

Annex 2. Overview of effect levels determined for known endocrine disruptors (provided by DTU Food, the National Food Institute), updated 17 May 2011.

NOAELs and LOAELs for some substances with endocrine disrupting properties and adverse effects, and comparison of LOAELs with STOT RE guidance values for 90-day studies, i.e. 10 mg/kg bw/d for Category 1 and 100 mg/kg bw/d for Category 2. Please note that the LOAELs in many cases are based on decreased AGD and/or increased nipple retention and these endpoints are <u>not</u> included in the current OECD Guidelines.

Substance	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Adverse effect(s) at LOAELs	LOAELs below 10 mg/kg bw/d?	LOAELs below 100 mg/kg bw/d?	References
DEHP	3 100 5	10 300	↓ AGD, ↑ Nipple retention, rat ↓ Testosterone GD 18, rat *Reproduktion (germ cell depletion, ↓ testis weight), developmental tox, rat*	Maybe?	Maybe?	Christiansen et al (2010) (27) Howdeshell et al 2008 (1) *Wolfe and Leyton, 2003 (*)EU RAR, EFSA*
DiNP	750 300 -	900 600 *750*	↓ AGD, rat ↑ Nipple retention, rat ↑ Nipple retention, rat	No	No	Boberg et al (2010) (28) Boberg et al (2010) (28) *Gray et al 2000 (2)* Exxon 1996 (*)
DnBP	- 250 50 10 100 -	250 500 250 50 300 (52) ***2***	↓ AGD, rat ↓ AGD, rat ↓ AGD, rat ↓ Testosterone GD 19, rat ↓ Testosterone GD 18, rat Embryotoxicity, rat *Germ cell development, mammary gland changes,*	No	No	Ema & Miyawaki 2001 (3) Jiang 2007 (4) Zhang 2004 (5) Lehmann et al 2004(6) Howdeshell et al 2008 (1) Wine et al 1997 (7) *Lee 2004 (8)*

Substance	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Adverse effect(s) at LOAELs	LOAELs below 10 mg/kg bw/d?	LOAELs below 100 mg/kg bw/d?	References
			rat			
DiBP	**125** *100*	250 300	↓ AGD, ↑ Nipple retention, rat ↓ *Testosterone GD 18, rat*	No	No	Sallenfait et al 2008 (9) *Howdeshell et al 2008 (1)*
BBP	***50*** 167 100 100 185 182	250 250 500 300 375 910	↓ *AGD, rat* ↓ AGD (GD 21), rat ↓ AGD, rat ↓ Testosterone GD 18, rat Developmental toxicity, mice Developmental toxicity, rat	No	No	*Tyl et al 2004 (10)*Ema et al 2003 (11) Nagao et al 2000 (12) Howdeshell et al 2008 (1) Ema et al 1990 (19) Price et al 1990 (26)
Prochloraz	5 ***3,7***	10 13	↑ Nipple retention, rat *Reproductive toxicity, rat*	Maybe	Yes	Christiansen et al (2009)(29) *Cozens et al 1982 (*)*
Epoxicona-zol	**2,3**	23	Rat, 2-gen study, repro	No	Yes	Hellwig & Hildebrand 1992 (*)
Linuron	***0,8-1*** 10 25	 50	*Reproductive toxicity* Developmental, rabbit ↑ Nipple retention, rat	No	No	McKintyre et al 2000 (13)
Vinclozolin	- **5** *4* 4,9	5 10 - 	↑ Nipple retention, rat ↓ AGD, rat 2 gen, reproductive toxicity, rat Reproductive toxicity, rat	Yes	Yes	Hass et al 2007 (14) Hass et al 2007 (14) *Hellwig et al 1994, BASF (*)* Hellwig et al 1990, BASF (*)
Procymi-don	10 12,5 12,5 2,5	25 37,5 125 12,5	↑ Nipple retention, ↓ AGD, rat ↓ AGD, hypospadia, rat ↓ AGD, hypospadia, rat ↓ *AGD*, hypospadia, testis effekt, rotte	No	Yes	Hass et al 2007 (14) Wickramaratne et al 1998 (*) Hoberman et al 1992 (*) *EFSA scientific report 2009*
PCB's Arochlor 1254 Arochlor	- - - -	30 0,05 0,1 0,01	↓ AGD, ↓ Testosterone (↑ AGD, ↑ prostate weight, mice) ↓ AGD, ↓ organ weights, ↓	Yes	Yes	Lilienthal 2006 (15) Gupta 2000 (16) Faqi 1998 (17) Faqi 1998 (17)

Substance	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Adverse effect(s) at LOAELs	LOAELs below 10 mg/kg bw/d?	LOAELs below 100 mg/kg bw/d?	References
1016 PCB77 *PCB126*			testosterone, rat			
(DDT) pp DDE		*10* 100	↑ Nipple retention, rat ↓ AGD, rat	Maybe	Yes	*You 1998)(18)* You 1998 (18)
Butylpara-ben		10 (100) 100 600 200	↓ sperm production, young rats (↓Testosterone, ↓ epidi-dymis weight) Sperm count Uterotrophic, rat Uterotrophic, rat (dry 200, wet 600)	No or ?	No or ?	Oishi 2001 (21) Kang et al 2002 (22) Hossaini et al 2000 (20) Rout-ledge 1998 (23)
Isobutyl-paraben	100	72 250	Uterotrophic, mouse Uterotrophic, rat	No	No	Darbre et al 2002 (24) Koda et al 2005 (25)

AGD = anogenital distance; GD = gestation day

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