Endocrine disrupters

Developing criteria


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Content

Preface

During the past 20 years, concerns have increased that many of the chemicals that we are surrounded by every day, may affect our health via effects on the endocrine system. Today, we know that not only effects on the reproductive system but also thyroid hormonal altering effects of endocrine disruptors (ED) are evident in both humans and wild animals. Furthermore, recent research indicates that endocrine disruptors may also influence other hormonal systems or pathways (cf. e.g. John M. Greally[1] (2010).

Even though there are examples of the existence of a link between exposure to certain chemicals and effects caused by disruption of the endocrine system in humans and wildlife, the extent of the problem of endocrine disrupting chemicals (EDC) still needs to be established. There are still a lot of gaps in our knowledge about the effects of endocrine disruptors: What are the levels of exposure and most importantly, which EDs are most likely to entail a risk to humans and the environment? Are there certain vulnerable populations, which are especially at risk? How are they currently identified? How can methods for identification of hazards, risks and risk reduction measures be improved? And further, how do we prevent adverse effects on health and the environment caused by exposure to endocrine disruptors?

For this purpose, we need internationally agreed definitions and criteria for identification of chemicals with endocrine disrupting properties. The criteria need to be specific but should also enable us to take into account endocrine effects that we are only now starting to recognise. It should also be considered whether the criteria should be identical for different relevant areas of legislation, e.g. industrial chemicals, plant protection products, biocides and cosmetic ingredients and perhaps also veterinary and human medicine.

Therefore, it is important to consider whether existing test methods and endpoints are sufficient for the identification of endocrine disrupting properties. The work on developing internationally accepted test methods for chemicals with hormone modulating and disrupting properties is on-going under the OECD TGPs EDTA Advisory Group, which is currently in charge of developing a comprehensive OECD Guidance Document on the assessment of chemicals for endocrine disruption. In that context, it is foreseen that a

[1] John M. Greally, 17 December 2010, Working paper to the OECD TGP: DRP on new endpoints. Chapter on: Endocrine disruptors and the epigenome (Draft outline).

revision of the so-called Conceptual Framework for testing and assessment of ED chemicals will be discussed in near future.

The current WHO/IPCS definition of endocrine disrupting chemicals is as follows:

- *An endocrine disrupter* is an exogenous substances 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

Today, ED are covered by the REACH, authorisation process on a case-by-case assessment. Under REACH, EDs can be identified as substances where the concern can be equivalent to that of PBT/vPvBs and CMRs, and such substances could be identified as candidates for authorisation (art. 57(f) of REACH). However, REACH neither defines EDs nor does it propose criteria or testing strategies for their identification. The EDs that until now have been proposed for authorisation (e.g. DEHP, BBP, DBP and DiBP) have all been identified on the basis of their reproductive toxic properties (classification as reproductive cat. 1B) and not their endocrine disrupting properties.

Until now, lack of the use of the equivalent concern clause for EDs under REACH may thus be due to lack of criteria for ED identification. It should be mentioned that substances classified as reproductive toxicants in category 2 (in accordance with the CLP Regulation) can only be included on the candidate list if identified under Article 57(f). This could, e.g., apply to some substances whose reproductive toxicity is caused by ED effects.

However, also due to the obligations under the new pesticides regulation (Regulation (EC) No 1107/2009), the development of criteria for endocrine disrupting chemicals has become an urgent issue – and contrary to REACH – the regulation requires that the Commission by 14 December 2013 presents a draft to scientific criteria for the determination of endocrine disrupting properties. In relation to REACH, there will be a review of the scope of REACH in 2012, and in 2013 of the scope of Article 60(3). This article deals with limited possibility of granting authorisation to non-threshold effect substances amongst which EDs are not explicitly mentioned. However, EDs may be included depending on whether EDs should be regarded as threshold or non-threshold effect chemicals. Furthermore, in 2014, it should be considered whether endocrine disruptors should be regulated in cosmetics.

In conclusion, the process leading to more specific regulation of endocrine disrupting chemicals has now been started within the EU and with the adoption of Council Conclusions on Combination effects of

Chemicals during the Swedish EU Presidency in 2009, a track for the further work in relation to the strategy on EDs and especially for addressing combination effects of EDs has been laid down. The European Commission reports are planned to be completed by 2011/2012.

The aim of the workshop was to give delegates from the Nordic Countries an opportunity to express and address current and concrete challenges, to propose criteria for endocrine disruptors and facilitate discussions on the way forward to identify, test, assess and regulate endocrine disrupting chemicals.

Summary and conclusions

The workshop was held on 11 and 12 October 2010 in Copenhagen, Denmark, with participants from the national authorities in Finland, Sweden, Norway and Denmark. It was arranged and chaired by the Danish Environmental Protection Agency and supported by the Nordic Council of Ministers.

The main agenda items were:

- Methods and data interpretation
- How to set up criteria
- The way forward in terms of regulation

Invited speakers presented their views and observations on the scientific status within these topics. Effects of ED on laboratory animals and in the environment were addressed by Ulla Hass (National Food Institute, Technical University of Denmark) and Poul Bjerregaard (Southern University of Denmark), respectively. Anna-Maria Andersson (Copenhagen University Hospital) presented studies on reproductive effects observed in humans that are suspected to be related to exposure to EDs. The available test methods and the OECD conceptual framework were outlined by Ulla Hass covering methods on human health and Jukka Ahtiainen (SYKE, Finland) gave an overview of the ecotoxicological methods. Finally, Agneta Ohlsson (KEMI Sweden) and Pia Juul Nielsen (Danish EPA) addressed the current regulatory practice and the challenges that lie ahead.

The discussions were held in two break-out groups, one discussing the human health perspective and the other discussing the same items but from an environmental point of view. The outcome of these discussions were presented and discussed in plenary sessions.

Methods and data interpretation

The discussion of test methods and data interpretation clarified that the current definition of ED requires at least one *in-vivo* study providing clear evidence of endocrine disruption in order to *confirm* a substance as having ED properties. In the environment group, the view was that sufficient proof could be provided by observed endocrine modulating effects in an intact animal in combination with adverse effects. It was discussed that, ideally, the adverse effects should be relevant at the population level but that, normally, adverse effect response variables in standard

ecotoxicological tests used for environmental hazard and risk assessment such as decreased survival, growth inhibition or inhibition of reproduction would be sufficient. Identification of a substance as a *potential* ED would require, e.g. *in-vitro* or *in-vivo* data indicating potential for endocrine disruption in intact organisms. Both types of information should include evidence for modulation effects on the endocrine system of an organism but not necessarily adverse effects.

With respect to the OECD Conceptual Framework, it was the view that even at the higher levels 4 and 5,[2] data may be insufficient for excluding ED effects in humans. At Level 4, the endpoints do not allow exclusion of ED effects on humans on their own and, at Level 5, the limitations of the methods were pointed out, e.g. lack of certain relevant and sensitive ED endpoints. The human health group concluded that only the new OECD Extended One-Generation Reproductive Toxicity Study (EOGRTS) TG can be used to conclude that a substance is not an ED (based on the endpoints studied in this assay); however, this would also require support by other relevant information.

It was suggested that relevant sensitive ED endpoints should be included in additional guidelines addressing human health endpoints, e.g. the Two-Generation Reproduction Toxicity Study. Moreover, there is a need to improve the environmental test methods, e.g. in relation to fish tests introducing a prolonged and enhanced TG 229 fecundity test and a full fish life cycle test with relevant endocrine-related response variables.

Regarding the use of non-test data, the general view was that none of the presently available QSARs can predict ED overall, but that they seem to be able to identify various relevant ED-related effects such as hormone receptor binding and activation and that such tools could be used to prioritize substances for further investigation.

How to set up criteria

It was noted that care should be taken not to restrict the criteria to currently available endpoints and it should be considered how new knowledge and endpoints can be included. Furthermore, an operational categorisation of ED was proposed: Category 1 – confirmed ED; Category 2a: Suspected ED and Category 2b: Potential ED, each level referring to specific data requirements.

It was agreed that different mechanistic groups of ED would not require different criteria as the definition of ED itself is quite general. Furthermore, it was a common view that the same criteria should apply

[2] Level 4 of the OECD conceptual framework: The second highest level, which includes *in-vivo* assays aiming at multiple endocrine mechanisms and effects, e.g. by enhanced OECD 407 (endpoints based on endocrine mechanisms). Level 5 of the OECD conceptual framework: Is the highest level and includes *in-vivo* assays aiming at effects from endocrine and other mechanisms, e.g. 1 and 2 generation assays (OECD TG 415 & 416).

across different legislation and that exposure and potency of ED should not be elements of the criteria but are relevant when considering risk management within various legislative frameworks.

The way forward

The discussion of introducing a specific hazard classification system with categories for ED effects led to a split view where the human health group felt that ED could not be sufficiently accommodated within the existing classification system, realizing, however, that changing the current classification system (CLP) may take many years as it would require agreement at the UN level. The environment group questioned the feasibility and effect of introducing a specific classification for ED considering the resources needed.

It was agreed, however, that the EDs are already covered by Article 57(f) of REACH and that this alone is not enough reason to open a scope discussion of REACH. It may, however, be relevant in order to strengthen the focus on ED to include characterisation criteria on ED in the REACH legal text. For this purpose, the IPCS criteria on confirmed and potential EDs could be used as a starting point.

1. Presentations

1.1 Effects in the environment

Professor Poul Bjerregaard,
University of Southern Denmark
Poul Bjerregaard presented information gained from wildlife related to endocrine disrupters and discussed this in relation to environmental regulation and criteria settings.

The aim of an environmental risk assessment (ERA) is basically to provide a basis for risk management measures appropriate to maintain ecosystem function and structure, and normally the aim is to maintain around 95% of species in an ecosystem. Thus, in a regulatory sense, populations and not individual species are important but if a key species (e.g. seals) is affected, the 95% aim may not be sufficient to maintain the ecosystem. It is known that chemicals with endocrine effects may have an effect on the size of a population and, therefore, the mechanism behind such effects is of interest. Examples of chemicals with an endocrine disrupting effect include TBT, of which concentrations below 1 ng/L has introduced imposex in gastropods, DDT that has induced feminisation of male gulls and terns, feminisation in alligators as well as eggshell thinning in birds, and PCB that has been shown to introduce reproductive disturbance in seals in the Baltic and Wadden Sea. Other well-known observations of endocrine disruption are vitellogenin (VtG) induction in male fish, observation of eggs/oocytes in male testis and skewed sex ratios. In polar bears, bone formation and thyroxine regulation are influenced by EDs.

When deriving criteria for EDs in the environment, the challenges will include: Which hormonal systems should be considered? Are endocrine mechanisms entirely or only partly responsible for effects observed? How detailed information do we need about the mechanism responsible for the observed effects? Are only population effects relevant or may biomarkers be applied?

1.2 Effects in laboratory animals

Ulla Hass,
Research Manager, Research Manager, DTU National Food institute.
The presentation focused on effects on laboratory animals (rats) after exposure to endocrine disrupting chemicals. The following subjects were reported: Definition of EDs, sexual differentiation, sensitive end-

points (e.g. anogenital distance (AGD) and nipple retention (NR)) and study design. Several studies were described focusing on the effects of anti-androgens including phthalates and pesticides, but also effects of thyroid disrupting chemicals were mentioned. Furthermore, effects on parturition after combined exposure to low doses of pesticides were mentioned. The OECD test guidelines on reproductive toxicity were described (including the new draft Extended One-Generation Reproductive Toxicity Study – EOGRTS) with a focus on dosing period and endpoints as well as strength and weaknesses. Finally, it was addressed how existing test guidelines have been – or could be improved. The main conclusion was that a number of studies have shown effects after exposure to EDs, but several of the test guidelines do not cover endocrine sensitive endpoints. This gap should be covered by new test methods such as the EOGRTS.

1.3 Human effects that may be related to endocrine disrupting effects

Anna-Maria Andersson,
Research Manager Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Denmark

The presentation focussed on possible effects of endocrine disruptors in humans. The speaker reported that, with the exception of effects of certain drugs, no proof but only indications of effects in humans due to exposure to EDs are available today, e.g. increase in testis cancer incidences and decrease in semen quality. Furthermore, correlations between exposure to phthalates and effects on AGD have been reported and it is also documented that the content of chemicals in breast milk reflects the exposure of the mother. Moreover, the presentation focussed on puberty timing, age at breast development and the link between breast cancer and earlier puberty onset. Furthermore, it was mentioned that EDs seems to play a role in the increasing prevalence of metabolic syndrome (e.g. obesity, diabetes 2). The conclusion was that there are indications of such effects of ED in humans. Moreover, further challenges exist within the evaluation of epidemiological studies, e.g. possible mixture effects, variation in susceptibility of humans due to genetic factors as well as timing of exposure.

1.4 Overview of test methods and data interpretation

Ulla Hass,
Human Research Manager, DTU National Food institute.
The OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals was presented. Testing and assessment can been conducted on various levels (levels 1–5).

- Level 1 "sorting and prioritisation" is based on existing information, e.g. physical-chemical properties, information on human and environmental exposure or existing hazard data incl. non-test information (read across, chemicals categorisation and QSAR predictions)
- At level 2, "*In-vitro* assays providing mechanistic data", receptor binding affinity is considered, e.g. binding to the oestrogen receptor (ER), androgen receptor (AR) or thyroid receptor (TR)
- At level 3, "*In-vivo* assays" data on single or multiple endocrine mechanisms are evaluated in studies such as: Uterotrophic Bioassay in Rodents (UT) TG 440 (screening for oestrogenic properties and effects), the Hershberger bioassay in rats TG 441 (short-term screening assay for (anti)androgenic properties and effects)
- At level 4, *in-vivo* EM (endocrine modulator) and related adverse effects are obtained but the tests may not cover all aspects or not be very sensitive on all aspects. Examples are the newly revised repeated dose 28-day oral toxicity study in rodents TG 407 (updated, endpoints related to endocrine mechanisms)
- At level 5, "*in-vivo* assays" high tier adverse effect data from endocrine and other mechanisms are evaluated, e.g. in the generation assay such as EOGRTS

The possible conclusions that can be drawn at the different levels of the OECD conceptual framework as well as from the specific test methods were discussed and it was considered if further tests are needed to avoid false positive or false negative results. As an example, it was mentioned that *in-vitro* assays (level 2) can cause both positive and negative results. A negative result does not mean that *in-vivo* ED activity can be excluded but positive data may be used to prioritize or trigger further testing *in vivo*. It was mentioned that *in-vivo* screening at level 3 of e.g. DEHP predicted some developmental toxicity in the higher level tests (which also include the developmental stage) but they underestimated the potency. The conclusion was that there is a need for studies that include the developmental period.

1.5 Overview of test methods and data interpretation, Environment

Jukka Ahtiainen,
Senior Scientist, SYKE, Finland
The existing methods for testing the ecotoxicological effects of chemicals (including OECD TGs: 229, 230, FSDT-draft, 206 and 231) as well as data interpretation were discussed. This included presentation of available methods, final as well as drafts, and their adequacy for testing of EDC. The OECD conceptual framework covers several modalities of ED (androgenic receptor mediated, estrogenic receptor mediated, thyroid receptor mediated and steroidogenesis interference (new)). However, the invertebrate hormone system is not covered, e.g. juvenile hormones, but a few OECD test guidelines for reproductive toxicity effects on invertebrates, which may also include effects mediated via hormone interference, are available (e.g. the TG on Reproduction of Springtails (TG 231), the Daphnia Reproduction Test (TG 211)).

The OECD Endocrine Disrupters Testing and Assessment Advisory Group (EDTA AG) has agreed on the following objectives of guidance documents:

- Support to regulatory authorities' decision on the potential human health and ecological hazards of chemicals when they receive test results from a (draft) TG related to ED screening/testing. Give guidance on how to interpret the outcome of individual tests and how to increase evidence on whether or not a substance may be an EDC
- To minimize animal testing globally through a two-step process: 1) Using a harmonized framework and application of the recommendations for assessing test results together with existing information; and 2) recommending test methods that may be performed if authorities need more evidence, while avoiding unnecessary testing

1.6 Overview – criteria and relevant EU legislation. Where do we go from here? How to establish criteria?

Agneta Ohlsson,
KemI, Sweden
Pia Juul Nielsen,
Deputy Head of Chemicals Division, Danish EPA
Agneta Ohlson addressed in her presentation the use of cut-off criteria in PPP regulation 1107/2009 for approval of active substances in plant protection products (PPP). The regulations requires that active substances, safeners and synergists that in accordance with Regulation (EC)

No 1272/2008 have to be classified as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties. Furthermore, substances that are classified as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties. The advantages with using the criteria based on classification rules are that no complete risk assessment is needed, which means less workload for the Member States (MS). Moreover, in practice, this approach may lead to a higher level of protection of humans and the environment than today.

Results from an investigation in Sweden of the impact of the "cut-off" criteria show that 4% of the active substances in Annex I and from stage 3 pending for decision (a total of 271 substances) fulfil the "cut-off" criteria adopted in Regulation (EC) No 1272/2008. The approval criteria in combination with the traditional risk assessment will result in a more satisfactory level of protection of human health and the environment than today.

The definition of endocrine disrupters (EDs) used by WHO is: "exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations".

In this context, WHO/IPCS defines an adverse effect as a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population, which results in impaired functional capacity or impaired capacity to compensate for additional stress, or increased susceptibility to the harmful effects of other environmental influences. The speaker concluded that such substances are, or have to be, classified as "toxic for reproduction" (category 2).

Pia Juul Nielsen presented an overview of the Danish activities on knowledge building, research and regulatory possibilities in the area of ED. She identified some main discussion items and challenges for the criteria setting and also addressed the challenges with respect to guideline development, test methods and the issue of interpretation of test results against criteria. Furthermore, the issue whether to address endocrine disrupting effects under the current repro-classification versus the need for a new hazard category was brought up. With respect to REACH, she highlighted that the current REACH standard information requirements are not sufficient for ED and that the provisions in Article 57(f) need interpretation, which has not been agreed between EU MS to cover the regulatory needs. This was discussed in the scope of the review of REACH in 2012 and 2013 as well as the PPP revision in 2013. On the way forward, Pia Juul Nielsen outlined an approach for an evidence-based categorisation of EDs and finally gave some additional ideas to a pragmatic approach for regulatory priority setting of testing, assessment and regulation of endocrine active/ disruptive substances.

2. Report from workshop sessions 1 and 2: Test methods and data intepretation

2.1 Summary and conclusions

2.1.1 OECD Conceptual Framework

The human health group discussed what could be concluded on the basis of the test results from each specific test method at the various levels in the OECD conceptual framework. Summarizing the discussion, it was agreed that, in some cases, a substance can be categorized as an ED based on *in-vitro* tests (level 2) supported by weight of evidence and read across. Furthermore, considering level 2 of the conceptual framework (*in-vitro* data), the group was of the opinion that a negative *in-vitro* result cannot exclude ED effects and that it would be necessary to apply a broader panel of *in-vitro* tests and have good knowledge on toxicokinetics to make even preliminary conclusions at this level. Most participants agreed, however, that it would be possible to classify for ED properties on the basis of positive test results at level 3 (*in-vivo* data providing evidence of modulation of the endocrine system but where there is generally not agreement about whether/which types of effects should be regarded as adverse effects (AE)) although some participants felt more information would be needed. At level 4, most of the ED endpoints are optional and the mandatory endpoints do not allow a final conclusion on their own if only negative responses are obtained. It was noted that a negative result at this level (obtained with adult animals) cannot exclude ED effects during e.g. other developmental stages. The discussion of the level 5 focussed on the limitations of the methods, e.g. lack of sufficient endpoints and the risk of false positive results due to use of high dose levels. It was concluded that only the Extended One-Generation Reproductive Toxicity Study (EOGRTS) can be used to exclude ED effects if it is supported by other relevant data.

The environment group discussed the EU and WHO/IPCS definition of EDs. It was pointed out that the definition of a (confirmed) ED requires evidence of endocrine disruption, and that 100% proof of causality and adversity is in reality only possible in a few cases. It was also mentioned that such absolute proofs are not required for using ecotoxi-

cological data in environmental hazard and risk assessment as the basis of regulation of chemicals in general. Furthermore, it was the opinion that sufficient proof could be provided by observation of endocrine modulating effects in combination with traditional adverse effects relevant at the population level, e.g. effects on mortality, growth, development or reproduction.

The group further agreed that in order to *confirm* a substance as having ED effects, at least one *in-vivo* study providing clear evidence for endocrine modulation and adverse effects caused by the substance in an intact organism would be needed. Identification of a substance as a *potential* ED would require documentation for endocrine disruption, e.g. *in-vitro* data indicating potential for endocrine modulation in an intact organism, *in-vivo* data providing evidence for endocrine modulation (e.g. VtG induction in male fish) or an observed adverse effect *in vivo*, which may be ED-mediated but where there is some doubt about whether this is the case in the particular case in question.

The group further noted that the criteria for ED should not include assessment of the *risks* of effects (i.e. potency or exposure-related information).

2.1.2 Test guidelines

On the discussion of test guidelines, the human health group supported that sensitive ED endpoints should be included in the EOGRTS test as well as in the two-generation reproduction toxicity study (TG 416). Furthermore, improvements to the reproduction/developmental toxicity screening test (TG 421) were proposed. Finally, it was suggested to include the pubertal assays (USEPA TG) as an OECD TG.

As a general remark, the human health group stated that several relevant male endpoints were covered in the OECD conceptual framework tool box whereas female endpoints are not as extensively covered. It was suggested to let the screening tests cover more broadly the different endpoints and the group suggested some additional endpoints to be included.

The environment group concluded that the TG 230 (fish screening assay) alone was not a sufficient screening test for the environment to determine ED properties. Nevertheless, the group felt that sex hormone mediated effects in fish may be covered by the test or the TG 229 (short-term fish reproduction assay) could be used to identify potential EDs. It was also mentioned that TG 229 cannot be used for derivation of a NOEC or LOEC value on fecundity with sufficient certainty for a definitive risk assessment due to the limited test design of TG 229. It was mentioned that it was proposed in the draft fish testing strategy document of OECD that a prolonged and enhanced version of TG 229 (VtG & fecundity test) is needed to complement the draft FSDT (covering VtG and development in relation to impact on sex ratio). Finally, it was stated that ED effects in

invertebrates are not really covered by the existing guidelines even though some TGs exist, covering reproduction of different pelagic and soil-dwelling taxa.

Regarding use of non-test information in the assessment of EDs, the human health group agreed that none of the presently available QSARs can predict ED overall but QSARs cover some of the ED mechanisms and such results could be used to prioritize substances or used together with other types of data by weight of evidence (WoE).

2.2 Discussion item 1

- How much confidence is needed for different types and levels of decisions for ED assessment?
- What can be concluded on the basis of test results from each specific test method at the various levels in the OECD Conceptual Framework? And what cannot be concluded?

2.2.1 Human health

Referring to the OECD Conceptual Framework, the discussion in the group can be summarized as follows:

- A positive result from level 2 *(in-vitro*) in the OECD Conceptual Framework could lead to a conclusion of potential or suspected ED and indication of a clear anti-androgenic effect; however, these test systems do not include a metabolic system. It was the opinion of the group that in some cases substances can be categorized at this level based on *in-vitro* tests supported by weight of evidence and read across
- The group was of the opinion that a negative result at level 2 cannot exclude ED effects as only some mechanisms are covered and it would be necessary to apply a panel of tests in order to conclude at this level. It was further noted that although methods are available, definite Test Guidelines are needed. Finally, it was considered important that future methods will include metabolism to prevent false positive and negative substances even though it was recognized that inclusion of metabolisation systems in *in-vitro* assays had its limitations
- Most participants agreed that it would be possible to classify for ED on the basis of positive test results at level 3 of the OECD conceptual framework (i.e. not included in REACH). For example, OMC (a sunscreen) is weakly positively estrogenic in the uterotrophic (UT) (TG 440) test but was found negative in a two-generation study (TG 416). The reason can very well be that TG 416 does not always identify weak oestrogens because the current test guideline lacks a

range of ED-related parameters now included in the EORGTS. Some of the participants felt that more information was needed to classify on the basis of positive level 3 results. Furthermore, in case of negative results at this level of the conceptual framework, one can only conclude that a substance is not active with respect to the response variable in question, i.e. not an ER or AR agonist or antagonist, but, on this basis, it is not possible to conclude that the substance is not an ED because the substance may disturb other hormone system(s)

- At level 4 of the OECD Conceptual Framework, most of the ED endpoints are optional and, in some cases, the mandatory endpoints do not allow a final conclusion on ED on their own. It was discussed when and on which indications, optional endpoints need to be tested, and when the testing of all endpoints should be required. In case of a positive result at level 4, it may be possible to conclude on ED effects; however the possibility of interference from general toxic effects of the substance with the ED test results should be considered. It was further noted that a negative result at this level (obtained with adult animals) cannot exclude ED effects during development
- At level 5, some limitations were discussed: Among the existing guidelines, only the extended one-generation reproductive toxicity study (EOGRTS) includes sufficient endpoints for detecting ED effects of a substance. The existing test guidelines do not cover effects on senescence and menopause and it was of concern that often only high dose levels are studied. It was discussed if and how it can be concluded at this level that a substance is an ED based on positive test results. It was agreed that an effect on AGD (anogenital distance) and NR (nipple retention) clearly indicates anti-androgenic effects while effects on oestrus cycles and mammary gland development indicate estrogenic effects. Finally, it was agreed that a negative result in the EOGRTS test based on the current knowledge (and the endpoints studied in this assay) can be used as evidence of no ED effects if it is also supported by other relevant information

2.2.2 Environment

It was pointed out that the definition of an endocrine disruptor requires full evidence of ED effects, which is only possible in a few cases. It was the opinion that it should be possible to provide sufficient proof based on observed endocrine modulating effects in combination with a sufficient level of proof of effects on endpoints that have implications at population level like death, growth, development or reproduction.

The group further noted that the criteria for ED should not include assessment of the *risks* of effects (i.e. potency and exposure).

It was agreed that there is a need to discriminate between adverse effects (AE) and biomarkers for endocrine activity or modulation in the

discussion of EDC. Biomarkers seem a useful way forward to predict ED effects. However, there is a need to know the natural variability of the biomarkers to determine their significance. The ecological significance of the biomarker itself is another discussion. It was noted, however, that AE and biomarkers could be seen as a continuum and that the relevance of a biomarker depends on the relation between the biomarker and an AE. Sex ratio change in fish was mentioned as a useful parameter for identifying an effect related to interference with sex hormones as well as for providing evidence of adverse effects of ED.

It was agreed that, in order to confirm a substance as having an ED effect, at least one *in-vivo* study providing clear evidence for endocrine disruption in an intact organism would be needed. This should include evidence of adverse effects (AE) plus evidence for endocrine activity or modulation (EA), e.g. FSDT test with VtG (EA), plus an observed effect on the sex ratio (AE). A difficult borderline case could be if effects of a synthetic androgen on fish sex ration in FSDT were observed but without concurrent evidence of effect on VtG. However, effects on male VtG are only expected from anti-androgens and oestrogens and lack of detectable decrease in the female ratio can be explained by the test design of FSDT. Hence, also in this case, it was felt that FSDT would be able to definitively identify ED effects (in this case androgenicity), also because non-hormone-mediated effects on the sex ratio in fish, which would be detectable in FSDT, are currently not known. A third example could be a positive result from an extended TG 229 test with VtG (as ED endpoint) supplemented with an observed effect on fecundity (as AE endpoint).

Categorisation of a substance as having a potential for ED effects would require documentation for endocrine disruption, e.g. *in-vitro* data indicating potential for endocrine disruption in intact organisms such as evidence of binding or gene activation activity relative to fish hormone receptors, VtG induction in male fish (oestrogens, anti-androgens) but with no measure of AE (e.g. in FSA, TG 230). The evidence could also be observed effects *in-vivo* that may, or may not, be ED-mediated but where there is substantiated evidence that the effect with a reasonable level of certainty may indeed be caused by EA.

2.3 Discussion item 2

2.3.1 Human health

Should sensitive ED-related endpoints (cf. extended one-generation reproductive toxicity study, EOGRTS) like anogenital distance (AGD), nipple retention (NR) and mammary gland development be a mandatory part of the test requirement? Do we need inclusion also in TG 416 (two generation reproduction toxicity study)? What about TG 421/422 (reproduc-

tion/developmental toxicity screening test)? Should the pubertal assays (USEPA TG) be included as an OECD TG?

The direct answer from the group to the first question regarding EOGRTS was yes!

It was also agreed that the sensitive ED endpoints also need to be included in the Two-Generation Reproduction Toxicity Study (TG 416).

Regarding the Reproduction/Developmental Toxicity Screening Test (TG 421/422) it was agreed that inclusion of the endpoint AGD and possibly NR (if the study is extended to day 13) as well as of mammary gland development (whole mount at day 4) could presumably enhance the sensitivity of the test substantially in relation to EDs.

Furthermore, it was generally felt to be a good idea to include the pubertal assays (USEPA TG) as an OECD TG also.

2.3.2 Environment

Is the FSA (TG 230) a sufficient screening test for the environment? Or should other TGs be considered instead: TG 229 (Fish reproductive toxicity screen) or the (draft) FSDT (on juvenile developing fish: VtG but includes also sex ratio (on all three recommended species) and secondary sex characteristics on fathead minnow and medaka). How should various types of results from these tests be used?

It was stated that TG 229 (includes response variable relating to EA: VtG and AE: (effects on fecundity) and can thus be used to identify EA and when positive even signs of ED effects. But in the draft OECD fish testing strategy document, the test does not seem to be generally acceptable for derivation of a robust NOEC or LOEC value on fecundity due to the limited test design (few exposure concentrations and replicates and inherent variable AE response).

The group felt that the TG 230 alone was relevant for detection of EA but not sufficient to determine ED properties as TG 229 could be used to identify potential EDs for further testing or further consideration when evaluated together with other ED-related evidence. It was noted that the fecundity endpoint of the TG 229 is quantitatively weak due to the variability of the controls but when interpreted qualitatively together with other ED-related endpoints and compared with a full life cycle test, it may be sufficient as documentation in certain cases.

It was in accordance with the mentioned draft OECD fish testing strategy document that a prolonged and enhanced version of the TG 229 fecundity test is needed and, furthermore, that a test of androgenic properties is lacking (it was mentioned that FSA and FSDT detect antiandrogens, that the latter seems to be significantly more sensitive than the former but that a particularly sensitive test seems to be the stickleback test, on which an OECD guidance document is currently being developed).

A member of the group noted that behavioural endpoints as an alternative to sexual development seem to be missing from the discussion of testing EDC. Group members responded that test of behavioural effects is very labour-demanding although sensitivity can be high. However, practicality has to be considered as well.

It was stated that particular tests focussing exclusively on ED-related effects in invertebrates are not fully covered by current OECD TGs. It was, however, also mentioned that several OECD TGs exist relating to inhibition of reproduction (covering annelids, enchytraeids, daphnids, spring tails and mites) and that they are likely to cover effects on reproduction mediated by interference with the endocrine system of the test organism. Furthermore, it was mentioned that lack of TGs specifically focussing on ED-mediated effects in invertebrates was caused by a general lack of sufficient knowledge about invertebrate endocrinology with the exception of perhaps insects and perhaps to some extent gastropods. Finally, it was mentioned that the endocrinology of amphibians is relatively well known and that one OECD TG based on thyroid disruption in frogs is available and another is under validation.

2.4 Discussion item 3

Are there other relevant endpoints which should be mandatory /recommended or triggered parts of the tests? Are the parameters measured in current tests sufficient?

2.4.1 Human health

As a general remark, the group stated that male endpoints were covered by the OECD Conceptual Framework whereas female endpoints are not as extensively covered. It was suggested to let the screening tests cover more broadly the different endpoints (see item 2). It was agreed to suggest the following endpoints as mandatory or recommended:

- Hormone levels (oestrogen, androgen, thyroid)
- Sexual dimorphic behaviour (e.g. activity) and sexual dimorphic areas in the brain in Developmental Neurotoxicity Test (the optional DNT module of EORGTS & TG 426)
- Some of the participants wanted the EOGRTS by default to include also the second generation as it was stated that the F2 could be more sensitive than F1 in relation to EDs. Based on the outcome of two comprehensive retrospective analyses of existing two-generation test data on the importance of the second generation made during the development of the EORTGS, other participants were not convinced that available evidence substantiated this view
- It was discussed if epigenetic investigations should be included

- It was stated that the test parameters tested in current tests are not sufficient for determining al types of ED-related effects

2.4.2 Environment

The discussion of this question was covered under Discussion item 2 above.

2.5 Discussion item 4

Include in the discussion how non-test information (read across, grouping of chemicals and QSAR predictions) may be used for assessment of ED (OECD Conceptual Framework).

2.5.1 Human health

The group stated that non-test information could give some preliminary information, e.g. if a compound was similar to vinclozolin, it could be relevant to make a read across. It was agreed that no QSAR can predict all types of ED-related effects but that QSARs cover some of the ED-related mechanisms and it was mentioned that QSAR results can be used to prioritize substances, e.g. when considering inclusion of substances in the CORAP-list for substance evaluation under REACH (REACH Commission Rolling Action Plan). In general, it was agreed that indications from non-test data could be used as motivation for requesting more information from industry.

2.5.2 Environment

The discussion of this item was postponed to the workshop sessions 3 & 4 on criteria setting.

3. Report from workshop sessions 3 and 4: How to set up criteria

3.1 Summary and conclusions

The human health group agreed that it was important that criteria for ED should be focussed on hazards and not on risks (potency and exposure) and noted that care should be taken not to restrict the criteria to only rely on currently available endpoints but that new available endpoints should be evaluated for relevance to endocrine disruption on a continuous basis.

The group further recommended that, as a mandatory requirement, relevant TGs should include assessment of AGD, NR and EAT hormones, which can indicate effects on the endocrine system.

Furthermore, the group proposed to change the definition of an endocrine disruptor by supplementing the term "causes" with "is linked to": An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes or *is linked to* adverse health effects in an intact organism, or its progeny, or (sub)populations.

The environment group suggested the following criteria for categorisation of EDC:

- Category 1 – confirmed EDC: Based on positive evidence from FFLCT; FSDT. Furthermore, it was suggested to propose an extended OECD TG 229 aiming to provide basis for assessment at this level
- Category 2a – suspected EDC: Based on OECD TG 229 and TG 230
- Category 2b – potential EDC: Based on *in-vitro*/*in-silico* evidence, e.g. fish receptor binding assays; mammalian *in-vitro* assays, YES, YAS and QSAR models

It was realized that the examples provided above relate only to existing OECD fish TGs or such TGs under development.

On the question whether different mechanistic groups of ED require different regulatory approaches, the human health group felt that the definition itself is quite general and, therefore, the criteria can also be general and cover different mechanistic groups. The environment group agreed to this and, furthermore, it was the opinion of both groups that the same basic criteria should apply across different legislations but that

implementation on how those criteria are used to prioritize, test, assess and regulate may differ.

Finally, both groups took the view that only evidence-based criteria would be appropriate and hence that potency of ED should not be part of the criteria but of relevance when considering prioritisation and later in the process, also when considering risk management options. Both groups also agreed that exposure levels should not be an element of the criteria.

3.2 Discussion item 1

Which endpoints for endocrine disrupting effects should be part of the criteria (for potential EDs and for EDs) for human health effects/environmental effects?

3.2.1 Human health

The group agreed that it was important that criteria for ED should be focused on hazards and not on risks. Care should be taken not to restrict the criteria to the currently available endpoints but consider that new endpoints may become available. The relevance of new scientific developments within endocrine disruption should be evaluated continuously.

Relevant existing endpoints may include assessment of AGD, NR and EAT hormones, which can indicate effects on the endocrine system. Number of receptors was also mentioned as a possible endpoint.

The endpoint "delayed parturition in rats" was considered to be connected to an effect of a disrupted endocrine system. The exact mechanism is not known; however, it could be aromatase inhibition and the effect may be rat-specific.

Furthermore, it was proposed to change the WHO/IPCS definition and add the words "linked to": An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes or *is linked to* adverse health effects in an intact organism, or its progeny, or (sub)populations.

It was stressed that effects are often interpreted as ED effects but without knowing the mechanism. Care should be taken not to confuse ED effects with general toxicity.

3.2.2 Environment

The group suggested the following categorisation of EDC:

- Category 1 – confirmed EDC: Based on evidence from FFLCT; FSDT. Furthermore, it was suggested to propose an extended OECD 229 aiming to provide basis for assessment at this level
- Category 2a – suspected EDC: Based on OECD 229 and 230

- Category 2b – potential EDC: Based on *in-vitro/in-silico* evidence, e.g. fish receptor binding assays; mammalian *in-vitro* assays, YES, YEAS and QSAR models

It was suggested that, based on a weight of evidence approach, it should be possible to upgrade substances from Category 2a or b to Category 1 by WoE justification, employing supplementary information from read-across to close structural analogues and/or reliable (Q)SAR predictions and/or mammalian data concerning ED.

3.3 Discussion item 2

Do different mechanistic groups, such as anti-androgens, oestrogen-like, thyroid-disrupting and other endocrine disrupting activities, require different regulatory approaches and/or can they be covered by the same definition of criteria?

3.3.1 Human health

The immediate answer to this question was that as the definition itself is quite general, the criteria can also be general and cover the different mechanistic groups. However, it was discussed what to do with thyroid toxicants that lead to altered T4 levels, resulting in non-specific effects such as decreased body weight, behavioural effects (if observed), and possible testicular effect.

3.3.2 Environment

The group agreed that the different mechanistic groups of ED substances do not require different regulatory approaches and can be covered by the same definition even if they have different modes of action. It was noted in the discussion that anti-androgens/thyroids are not covered by the currently available fish assays but such effects are included in the definition.

3.4 Discussion item 3

Discuss if/how the same set of criteria can be applied for all legal purposes, e.g. industrial chemicals, PPPs, biocides and cosmetics.

3.4.1 Human health

It was agreed that the same criteria can be used by the different legal acts. Further it was stressed again that the criteria should be hazard-based.

3.4.2 Environment

It was agreed that the same criteria should apply across different legislation.

3.5 Discussion item 4

Should potency of endocrine–active substances be part of the criteria?

3.5.1 Human health

It was the opinion of the group that potency of endocrine-active substances is only relevant for priority setting for further testing and risk assessment and should not be part of the criteria for hazard categorisation as an EDC.

3.5.2 Environment

It was agreed that potency should not be part of the criteria. However, very potent ED substances may need more strict regulation.

3.6 Discussion item 5

Should the exposure level to EDs be part of the criteria (as proposed by BfR)?

3.6.1 Human health

It was the group's opinion that exposure levels should not be part of the criteria. However, exposure levels could be taken into account in different risk management options.

It was further noted that, at high dose levels, ED effects could be a result of general toxicity. If, for example, all organ weights have decreased and not only organs related to ED effects, these effects should be evaluated against the exposure and general toxicity endpoints.

3.6.2 Environment

The group stated clearly that exposure should not be an element of the criteria.

4. Workshop session 5: The way forward in terms of regulation

4.1 Summary and conclusions

The human health group had an extensive discussion on classification and labelling of EDs. Although the group realized that changing the current CLP system may take many years, it felt that ED could not be sufficiently accommodated within the existing system. The group discussed different regulatory options for adapting the current classification and regulatory systems:

- Introduce a new hazard category for ED. According to REACH, a hazard classification including a new ED category would trigger a requirement for an exposure and risk assessment of the substance in question
- Change the existing classification criteria for reprotoxicity in order to accommodate also the ED effects
- Introduce criteria for ED under Article 57(f) and standard information requirements on ED effects in REACH Annex VII–X. This would make it easier to identify ED substances as SVHC but would in itself not trigger an exposure and risk assessment under Article 14 of REACH

Finally, the group identified a number of examples of substances with ED effects that are not currently classified under the existing regulation.

The environment group agreed that screening and definitive criteria on ED should be applied and referred to the suggestion made under Sessions 3 & 4, Item 1.

The members of the environment group questioned the feasibility and effect of introducing a specific classification for ED effects as it will be difficult to come to an agreement and that they felt that there may be a high risk that invested resources could be wasted.

The environment group, however, supported the idea that criteria for ED identification could be included in REACH, e.g. by using the IPCS criteria on confirmed and potential EDs. This was discussed in details in Section 2.2.2.

It was the view of the groups that the EDs are already covered by Article 57(f) of REACH and that this alone is not enough reason to open a scope

discussion of REACH. However, if, for other reasons, a scope discussion is initiated, it could be relevant to discuss definition and criteria for EDs.

4.2 Discussion item 1

For other groups of chemicals, e.g. PBT and CMR substances, substances are categorized as either suspected or confirmed. Would such an approach be appropriate also for the regulatory considerations of endocrine disrupters (screening criteria/definitive criteria)?

4.2.1 Human health

The human health group discussed some of the questions briefly while others were extensively debated. The group did not discuss Item 1 specifically but concentrated on the related discussion under Item 2 on classification of EDs.

4.2.2 Environment

The group agreed that it would be good to have screening and definitive criteria. Please refer to the proposed categorisation scheme under Section 3.2.2.

4.3 Discussion item 2

Should chemicals be classified and labelled for endocrine disrupting effects? What is the added value if we make a classification and which types of ED responses cannot be covered by the existing classification scheme?

4.3.1 Human health

It was noted that introducing a classification for ED effects into the CLP system would probably require as many as 10 years. It was further argued that if there are no information requirements then industry is not likely to provide the data. REACH Article 57(f) includes ED effects but there are examples of ED substances that may not be classified as CMR under the current system. The group further discussed different options of changing the current classification system:

- Introducing a new hazard category for EDs. The implications of this would be triggering of exposure and risk assessment under REACH as well as increased possibilities of identifying EDs (which are not already CMRs) as SVHCs, and possible inclusion in REACH Annex XIV (substances that require authorisation)

- The second option would be to change the existing classification criteria for reprotoxicity in order to better accommodate also ED effects
- A third option will be to add criteria for ED under Article 57(f) and as a consequence include additional information requirements in REACH Annex VII–X. The suggested change of Article 57 should include all optional endpoints in TG 407 (e.g. starting in Annex VIII of REACH). This would ensure identification of suspected ED but unfortunately only the very potent EDs will be identified. It was discussed why we should classify for ED effects and a number of examples of substances with ED effect, which are currently not classified were mentioned:
  - The ED mechanism should be considered in the same manner as Germ cell mutation, which can trigger a classification based on the mechanism or mode of action. Why not do the same with the EDs and regard them as constituting a similar hazard?
  - Decreased T4 level – should this trigger a classification? Decreased AGD – should this trigger a classification? AGD is recognized as an adverse effect in the OECD reproductive toxicity GD 43 (Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment; OECD 2008c)[3]

Consequently, the group felt that if ED substances as those exemplified above should be captured by the classification system, then a new hazard category will most likely be needed.

4.3.2 Environment

Members of the group questioned the feasibility and effect of introducing a specific classification for ED effects. It was the feeling that it would be difficult to come to an agreement on a classification for ED effects and that the resources invested in this may well be wasted. This view is supported by the fact that usually classification is not mechanism-based.

Under the current system, data from fish tests for ED (FSDT, FFLCT) can be applied as the basis of classification of chronic toxicity in the environment and are often more sensitive than endpoints for general toxicity. The effect of a possible ED classification was further questioned in the light that soft measures outside the classification system may be needed for consumer communication even for substance that are not categorized as Cat 1 ED, e.g. parabens.

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[3] In this GD it is stated "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".
Ref. OECD (2008). Guidance document on mammalian reproductive toxicity testing and assessment. OECD Series on Testing and Assessment No. 43. Organisation for Economic Cooperation and Development, Paris. 88 pp.

4.4 Discussion item 3

Should exactly the same subdivision of EDs (Cat 1A, 1B, and 2) be made as for CMRs – and should the type of evidence for each be similar?

4.4.1 Human health

Please refer to the discussion in Section 4.3.1.

4.4.2 Environment

The answer is covered by the discussion in Section 3.2.2. The group flagged an issue for later discussions: Would separate conclusions on ED for human health and environment be needed?

4.5 Discussion item 4

Should criteria for identification of endocrine disruptors (specific definition of EDC) be included in REACH, e.g. in a new Annex in the 2013 review (corresponding to the PBT/vPvB criteria in REACH, Annex XIII)?

4.5.1 Human health

This item was not discussed; however, see the discussion in Section 4.3.1.

4.5.2 Environment

It was agreed that it would be logic to include ED characterisation criteria in REACH, e.g. by using the IPCS criteria on confirmed and potential EDs (see Section 2.2.2.).

4.6 Discussion item 5

Is the substantiated, high suspicion of reproductive health effects so serious that this calls for use of the precautionary principle?

4.6.1 Human health

This question was not discussed due to lack of time.

4.6.2 Environment

The group answered that the use of cautious assessment approaches and even sometimes of the precautionary principle case by case should be

decided on the basis of the degree of evidence for ED effects, their potency and the exposure to the chemical in question.

4.7 Discussion item 6

Discuss the way forward in terms of regulation – which processes to foresee? What are the process/possibilities in the EU from now on; review of REACH in 2012; review of ED Article 60.3 in 2013 – what should we suggest and at what time? How can we use the OECD Conceptual Framework in the REACH review?

4.7.1 Human health

This question was not discussed due to lack of time.

4.7.2 Environment

It was the view of the group that the ED is already covered by Article 57(f) of REACH and that this alone is not enough reason to open a scope discussion of REACH. However, if, for other reasons, a scope discussion is initiated, it could be relevant to discuss EDs.

The group discussed if thresholds for ED effects exist. It was stated that thresholds were observed at low levels and that part of the discussion of thresholds is linked to analytical and test methodology. If thresholds for ED effects can be proved, the current legal text says that EDs can be authorized under the adequate control route (Article 60(2)).

The question of combined exposure to different EDs was raised in this context but was postponed to the following workshop on combination effects.

4.8 Discussion Item 7

How do we deal with gaps and uncertainties in available data?

4.8.1 Human health

This question was not discussed due to lack of time.

4.8.2 Environment

This question was not discussed due to lack of time.

Abbreviations

2gen	Two generation study
AE	Adverse effects
AGD	Anogenital Distance
AR	Androgen receptor
BBP	Benzyl butyl phthalate
BfR	Federal Institute for Risk Assessment (Germany)
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, Mutagenic or Toxic to Reproduction (Hazard classification)
DBP	Dibutyl phthalate
DEHP	Di(2-ethylhexyl phthalate)
DiBP	Di-isobutyl phthalate
DNT	Developmental Neurotoxicity Test
EA	Endocrine Activity
EAT	Estrogen Androgen Thyroid
ECHA	The European Chemicals Agency
ED	Endocrine Disruptor or Endocrine Disruption
EDC	Endocrine Disrupting Chemical
EDTA AG	Endocrine Disrupters Testing and Assessment Advisory Group
ENV	Environmental
EOGRTS	Extended One Generation Reproduction Toxicity Study
ER	Oestrogen Receptor
ERA	Environmental Risk Assessment
EU MS	EU Member State
FFLCT	Fish Full Life-cycle Test
FSA	Fish Screening Assay
FSDT	Fish Sexual Development Test
FSTRA	Fish Short-Term Reproduction Assay
HH	Human Health
IPCS	International Programme on Chemical Safety
MS	Member States
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration, Lowest Observed Effect Concentration
NOEL	No Observed Effect Level, Lowest Observed Effect Level
NR	Nipple Retention
OECD TG	Organisation for Economic Co-operation and Development Test Guidelines
OECD TGP	Organisation for Economic Co-operation and Development Test Guideline Programme
OECD TG 206	Avian Reproduction Test
OECD TG 211	Daphnia magna Reproduction Test
OECD TG 229	Fish Short Term Reproduction Assay
OECD TG 230	21-day Fish Assay
OECD TG 231	Amphibian Metamorphosis Assay
OECD TG 407	Repeated Dose 28-day Oral Toxicity Study in Rodents
OECD TG 415	One-Generation Reproduction Toxicity Study
OECD TG 416	Two-Generation Reproduction Toxicity
OECD TGP	Organisation for Economic Co-operation and Development Test Guidelines Program
PBT/vPvBs	Persistent, Bioaccumulative and Toxic/ very Persistent and very Bioaccumulative
PPP	Plant Protection Products
QSAR	Quantitative Structure Activity Relationship
TG	Test Guideline
USEPA TG	United States Environmental Protection Agency Test Guidelines

UT	Uterotrophic Test
VtG	Vitellogenin
WHO	World Health Organization
WoE	Weight of Evidense
YAS	Yeast Androgen Screen
YES-assay	Yeast Estrogen Screen assay

Resume og konklusioner

Workshoppen blev afholdt den 11. og 12. oktober 2010 i København med deltagelse fra myndighederne i Finland, Sverige, Norge og Danmark. Den blev arrangeret og ledet af Miljøstyrelsen med støtte fra Nordisk Ministerråd.

Hovedpunkterne på dagsordenen var:

- Metoder og fortolkning af data
- Hvordan opstiller man kriterier
- Vejen frem udtrykt ved lovgivning

Inviterede foredragsholdere præsenterede deres synspunkter og observationer med hensyn til den videnskabelige status for disse emner. Hormonforstyrrende stoffers effekter på laboratorieorganismer og miljøet blev præsenteret af henholdsvis Ulla Hass (DTU Fødevareinstituttet) og Poul Bjerregaard (Syddansk Universitet). Anna-Maria Andersson (Rigshospitalet) præsenterede studier om effekter på forplantningsevnen observeret hos menesker, som mistænkes for at have forbindelse med eksponering for hormonforstyrrende stoffer. De nuværende metoder og OECD's begrebsramme blev skitseret af Ulla Hass, inklusiv metoder vedr. menneskers sundhed, og Jukka Ahtiainen (SYKE – Det finske Miljøinstitut) gav et overblik over økotoksikologiske metoder. Afslutningsvis talte Agneta Ohlsson (KEMI – Kemikalieinspektionen, Sverige) og Pia Juul Nielsen (Miljøstyrelsen) om den nuværende lovgivningsmæssige praksis og om de fremtidige udfordringer.

Diskussionerne foregik i to undergrupper, hvor den ene diskuterede det sundhedmæssige perspektiv, mens den anden diskuterede de samme emner men ud fra et miljømæssigt synspunkt. Resultatet af disse diskussioner blev præsenteret og diskuteret i plenum.

Metoder og fortolkning af data

Diskussionen om testmetoder og fortolkning af data gjorde det klart, at den nuværende definition af hormonforstyrrende effekter kræver mindst et *in-vivo* studie, der klart beviser hormonforstyrrende effekter, for at *bekræfte* at et stof har hormonforstyrrende effekter. Det var miljøgruppens holdning, at det vil være tilstrækkeligt bevis, hvis der i en ubeskadiget organisme observeres forandrende effekter på hormonsystemet kombineret med alvorlig skadevirkning. Det blev diskuteret, at de alvorlige skadevirkninger ideelt set burde være relevante på popula-

tionsniveau, men at responsvariabler for alvorlige skadevirkninger i de økotoksikologiske standardtest, som anvendes til miljøfare- og -risikovurderinger, fx fald i overlevelse, væksthæmning eller hæmning af forplantningsevnen, vil være tilstrækkelige. Bestemmelse af et stof som *potentielt* hormonforstyrrende vil kræve fx *in-vitro* eller *in-vivo* data, der viser et potentiale for hormonsforstyrrende effekter i ubeskadigede organismer. Begge typer informationer bør indeholde bevis for forandrende effekter på en organismes hormonsystem, men ikke nødvendigvis alvorlige skadevirkninger.

Med hensyn til OECD's begrebsramme var holdningen, at selv på de højeste niveauer 4 og 5,[4] kan data være utilstrækkelige til at udelukke hormonforstyrrende effekter i mennesker. På niveau 4 tillader effektmålene i sig selv ikke at hormonforstyrrende effekter i mennesker udelukkes, og på niveau 5 blev metodernes begrænsninger påpeget, fx mangel på visse relevante og sensitive hormonforstyrrende effektmål. Sundhedsgruppen konkluderede at det kun er den nye OECD test guideline Extended One-Generation Reproductive Toxicity Study (EOGRTS) [udvidet én-generations reprotoksicitetsstudie], der kan bruges til at konkludere at et stof ikke er hormonforstyrrende (baseret på de undersøgte effektmål i denne test). Dette vil imidlertid også kræve anden understøttende relevant information.

Det blev foreslået, at relevante, sensitive hormonforstyrrende effektmål bliver inkluderet i supplerende guidelines for sundhedsmæssige effektmål, fx to-generations reprotoksicitetsstudiet. Desuden er der behov for at forbedre de miljømæssige testmetoder, fx at indføre en længerevarende og forbedret TG 229 frugtbarhedstest i forbindelse med fisketest og en fuld livscyklus fisketest med relevante responsvariabler med hensyn til hormonforstyrrende effekter.

Med hensyn til at anvende data, der ikke stammer fra test, var det den generelle opfattelse, at der ikke er nogen af de pt tilgængelige QSAR-metoder, der kan forudsige hormonforstyrrende effekter generelt, men at det ser ud til, at de kan identificere nogle relevante effekter, som kan relateres til hormonforstyrrelser, som fx hormonreceptorbinding og -aktivering, og at sådanne værktøjer vil kunne anvendes til at prioritere stoffer til yderligere undersøgelse.

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[4] Niveau 4 i OECD's begrebsramme: Det næsthøjeste niveau, som inkluderer in-vivo test, der er rettet mod sammensatte hormonforstyrrende mekanismer og effekter, fx den udvidede OECD 407 (effektmål baseret på hormonforstyrrende mekanismer)
Niveau 5 i OECD's begrebsramme: Er det højeste niveau og inkluderer in-vivo test, der er rettet mod effekter fra hormonforstyrrende og andre mekanisker, fx én- og to-generationstest (OECD TG 415 & 416).

Hvordan opstiller man kriterier

Det blev bemærket, at man skulle passe på ikke at begrænse kriterierne til de pt tilgængelige effektmål, og at man skal overveje hvordan ny viden og nye effektmål kan inkluderes. Derudover blev der foreslået en operationel kategorisering af de hormonforstyrrende effekter: Kategori 1 – bekræftede hormonforstyrrende effekter; Kategori 2a – mistanke om hormonforstyrrende effekter og Kategori 2b – potentielt hormonforstyrrende effekter, hvor hver kategori har særlige krav til data.

Man var enige om, at forskellige mekanismegrupper inden for hormonforstyrrende effekter ikke behøver forskellige kriterier, da definitionen af hormonforstyrrende selv er ret generel. Desuden var man enige om, at de samme kriterier skulle gælde på tværs af forskellig lovgivning og at eksponering og potentiale for hormonforstyrrende effekter ikke skulle være en del af kriterierne men er relevante, når det drejer som om risikostyring inden for forskellige lovmæssige rammer.

Vejen frem

Diskussionen om at introducere et specifikt fareklassificeringssystem med kategorier for hormonforstyrrende effekter splittede de to grupper. Sundhedsgruppen følte at hormonforstyrrende effekter ikke kunne få tilstrækkelig plads inden for det eksisterende klassificeringssystem, men var klar over, at en ændring af det nuværende klassificeringssystem (CLP) kunne tage mange år, da det kræver enighed på FN-niveau. Miljøgruppen tvivlede på effekten af, og om det kan betale sig at introducere, en særlig klassificering af hormonforstyrrende effekter, når man samtidig tager de nødvendige ressourcer i betragtning.

Der var dog enighed om, at de hormonforstyrrende effekter allerede er dækket af REACH, Artikel 57(f), og at dette alene ikke var grund nok til at åbne for en diskussion af, hvad REACH omfatter. For at sætte øget fokus på hormonforstyrrende effekter kunne det imidlertid være relevant at inkludere karakteriseringskriterier for hormonforstyrrende effekter i REACH lovtekst. Til dette formål kunne IPCS kriterierne vedr. bekræftede og potentielle hormonforstyrrende effekter bruges som udgangspunkt.

Appendix A – Workshop programme

NORDIC WORKSHOP
Developing criteria for endocrine disruptors
October 11th 2010

Programme

10.00-10.15	**Arrival and registration**	
10.15-10.45	Welcome and introduction to the workshops	Henrik Søren Larsen Pia Juul Nielsen
10.45-11.30	Effects in the environment	Poul Bjerregaard
11.30-12.15	Effects in laboratory animals	Ulla Hass
12.15-13.30	**Lunch**	
13.30-14.15	Human effects that may be related to endocrine disrupting chemicals	Anna-Maria Andersson
14.15-14.45	Overview: Test methods and data interpretation - Human health/tox, incl. OECD Conceptual Framework - Environment/ecotox, incl. OECD Conceptual Framework	 Ulla Hass Jukka Ahtiainen
14.45-17.00	Workshop-sessions (1+2): Test methods and data interpretation - Human health/tox, - Environment/ecotox,	Group moderators: Ulla Hass Jukka Ahtiainen

October 12th 2010

Programme

09.00-09.10	Good morning	Agneta Ohlsson
09.10-09.30	Summing up workshop-sessions 1+2 (both groups)	Rapporteur
09.30-10.15	Overview - Criteria and relevant EU legislation. Where do we go from here? How to establish criteria?	Agneta Ohlsson Pia Juul Nielsen
10.15-10.45	**Coffeebreak**	
10.45-12.00	Workshop-sessions (3+4): How to set up criteria? - Human health/tox - Environment/ecotox	 Group moderators: Christine Bjørge Henrik Tyle
12.00-13.00	**Lunch**	
13.00-14.30	Workshop-sessions (5): The way forward in terms of regulation - Human health/tox - Environment/ecotox	 Group moderators: Christina Ihlemann Finn Pedersen
14.30-15.00	**Coffeebreak**	
15.00-15.30	Summing up workshop-sessions 3+4+5 (both groups)	Rapporteurs
15.30-16.00	Summary and conclusion	Henrik Søren Larsen

Appendix B – Presentations

Appendix B may be downloaded as a separate pdf file

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Endocrine disrupters
Developing criteria

During the 2010 Danish Presidency of the Nordic Council of Ministers environment and health was a prioritized subject. The aim was enhanced Nordic information exchange. To support this aim a number of Nordic workshops were held to strengthen the capacity building and discuss future regulatory aspects in the area of endocrine disruptors, combination effects, and soft regulatory measures and effective risk communication. One of the workshops held in November focused on developing criteria for endocrine disruptors. This report describes the workshop presentations, the ensuing discussions, and the outcome.

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