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**WORKSHOP REPORT ON OECD COUNTRIES ACTIVITIES REGARDING TESTING,
ASSESSMENT AND MANAGEMENT OF ENDOCRINE DISRUPTERS**

SERIES ON TESTING AND ASSESSMENT

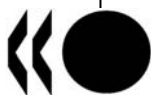
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OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 118

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(Appendices including individual contributions from countries/region and stakeholders to the case study report prepared for the workshop, and workshop presentations are included in Part II)

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INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNEP, UNIDO, UNITAR, WHO and OECD

**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2010**

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No. 118 *Workshop Report on OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters Part I*

No. 118 *Workshop Report on OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters Appendices 1-10 Part II*

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This document is the report of the Workshop on OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters, which was held in Copenhagen (Denmark) on 22-24 September 2010. It comprises a Part I and a Part II.

The Working Group of National Coordinators of the Test Guidelines Programme approved the submission of the report to the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology on 23 November 2009. The Joint Meeting agreed to the declassification of the report on 11 January 2010.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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INTRODUCTION

Background

1. At the request of member countries and the international industry, OECD initiated in 1997 the activity on Endocrine Disrupters Testing and Assessment with the objectives to provide a set of internationally recognised and harmonised Test Guidelines (TGs), and testing and assessment strategies for regulatory use that would avoid duplication of testing and, thus, save resources and animals.
2. Managed by the Endocrine Disrupters Testing and Assessment Task Force (EDTA) and its three Validation Management Groups on mammalian tests (VMG-mammalian), on ecotoxicity tests (VMG-eco) and on non-animal tests (VMG-NA), several comprehensive test validation projects have been completed and Test Guidelines adopted (see **Annex 1**).
3. The first objective was to identify and prioritize development of new and update of existing Test Guidelines. A Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals was agreed in 2002. It was composed of five levels ranking different types of *in silico*, *in vitro* and *in vivo* assays, to be used according to countries regulatory needs. The validation of a number of test methods on biotic systems and health effects then started under the leadership of the three VMGs. Several Test Guidelines useful for the screening and assessment of endocrine disrupters have already been adopted and a number of projects, included in the current work plan for the Test Guideline Programme, concern the development of new or updated Test Guidelines and related documents on endocrine disrupters testing.
4. In 2008, the EDTA Task Force was replaced with the EDTA Advisory Group (EDTA AG). The Working Group of National Coordinators of the Test Guidelines Programme (WNT) requested that the EDTA AG start working on cross-cutting issues including the assessment of endocrine disrupters and the review of the Conceptual Framework. It was suggested that the first step should consist of gathering information from countries on their approaches for assessing endocrine disrupters. The WNT requested that the Secretariat, in consultation with the Bureau of the EDTA AG, organize the work on these issues.
5. The Bureau agreed that the best way forward would be to: (i) prepare a case study report on endocrine disrupting testing, assessment and management in OECD Countries, (ii) organize a workshop to discuss issues arising from the case study report.
6. A summary of the Case Studies Report on Endocrine Disrupters Testing, Assessment and Management in OECD Member Countries is available as **Annex 2**. This document reports on the outcome of the workshop “OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters”.

Workshop objectives

7. The workshop was held to:

Take note of:

- The current activities on chemicals with endocrine activity or disrupting properties in the different countries (including development and validation of test and non-test methods, gaining more information on chemicals as regards their possible endocrine activity, advisory activities and information to industry and the public, regulations)
- How the Test methods (including OECD Test Guidelines) and non-test methods are used in the different countries for decision making concerning identification and evaluation of chemicals with

endocrine activity/disrupting properties (this should include all relevant TG available at OECD, both included or not in the Conceptual Framework).

Discuss and exchange views and experience regarding current activities and practices regarding:

- current tools/data/ information which are essential for different types and levels of decision making (from: providing advice and making advice to industry and the public to: risk assessment-based decision making/regulations concerning providing information on or restricting production, use and/or emission/exposure)
- How the available tools should be used together in weight of evidence based conclusions?
- How much confidence is needed for different types and levels of decisions?

This includes considerations concerning:

- the potency and nature of endocrine activity
 - the scientific basis for linking such endocrine activity causally to harmful effects for man and/ or the environment
 - if and how such information is used together with other relevant information concerning intrinsic properties of the substance as well as emission/ exposure potential / considerations.
- Make recommendations for further work as a follow-up for the workshop regarding:
 - **OECD work:** identify any possible needs for further development of more Guidance Documents, Detailed Review Papers, Test Guidelines, non method projects, examples and tools etc. Specify subject/topic and scope of each and provide a rationale for each recommendation
 - **Research activities in countries:** Identify if possible research areas/topics where creation of new knowledge would improve the general basis for priority setting, hazard and risk assessment and management of endocrine disrupters.

Workshop preparation

8. The workshop was prepared by a small group including experts from Canada, Denmark, Germany, Japan, the United States, the European Commission, and industry. This group had regular conference calls and a progress report was submitted to the WNT meeting in March 2009.

WORKSHOP PROGRESSION

9. The workshop *OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters* was held in Copenhagen, Denmark, from 22 to 24 September 2009. It was hosted by the Danish EPA. Sixty-five participants, including representatives from governments, industry and environmental NGOs attended the workshop. The participants list is attached as **Annex 3**.

10. Mr. Claus Torp, director of the Danish EPA, welcomed the participants. Betty Hakkert (Netherlands) chaired the workshop. The workshop consisted of plenary and breakout sessions. Four breakout groups were established to address specific questions prepared before the workshop. The groups received the same questions, but discussed them from angles of human health (group 1), environment (group 2), and human health and environment (groups 3 and 4). Activities on testing, assessment and management of endocrine disrupters in the US, the EU and Japan were presented, as well as the views of an Environmental NGO, industry, and the International Council on Animal Protection in OECD

Programmes on the use of TGs and other tools for the assessment of endocrine disrupters (See Agenda in [Annex 4](#) and presentations in [Appendix 10, Part 2](#)).

11. The questions for the breakout sessions are attached as [Annex 5](#). The questions were used as support to the discussions. On the second day, the breakout groups were requested to provide recommendations on the basis of the first day discussions. After the conclusion of each breakout session, the participants reconvened in the plenary to hear and discuss the summaries of the breakout session outcomes.

12. On the last day, the workshop reviewed, revised and adopted the conclusions and recommendations as presented below. The Chairperson and the Secretariat thanked the hosts for their substantial support and contributions towards the success of the meeting.

WORKSHOP OUTCOME

13. The workshop exchanged information on countries and stakeholders' activities regarding testing, assessment and management of endocrine disrupter chemicals (EDCs) and agreed on recommendations for further work, considerations/recommendations, and research needs related to EDCs. As recommendations were proposed by the four breakout groups, and are reproduced as agreed at the workshop, a few of them overlap.

RECOMMENDATIONS FOR FURTHER OECD WORK

Conceptual framework

The Conceptual Framework (CF) for EDTA, adopted at the Workshop held in Tokyo in 2002 (see [Annex 6](#)), is a tool box including screening and testing methods that were considered useful for the assessment of estrogen receptor (ER), androgen receptor (AR) and thyroid mediated effects.

- More Guidance for using the CF and its test methods in a weight of evidence is recommended. This guidance should set out the coverage of the CF: which Test Guidelines, endpoint data, and (to the extent possible) modes of action data are sufficient to inform each type of decision concerning EDCs, i.e. a decision which integrates the data available from the various guidelines? The three main types of decision include 1) prioritisation decisions; 2) decisions about whether there is potential to interact with the hormone system; 3) definitive decisions on whether a substance is an EDC. Such a document would be of great value to many chemical regulators who are currently unsure how the various Test Guidelines can be applied in practice.
- The CF should be revised within a few years where more experience and scientific progress have been gained. In view of the revision of the CF, detailed recommendations from some breakout groups for revising the CF are available in [Annex 7](#).

Assessment

- A document on weight of evidence approaches for the assessment of suspected EDCs should be developed to identify and encourage use of consistent, transparent approaches within the particular regulatory contexts. Case studies presented in the current report and further contributions could be useful for preparing such a document. This work should recognize and build on existing harmonised methods/guidance and on the activities of other agencies in member countries on this subject of weight of evidence assessment.

- OECD should encourage opportunities to share case studies on chemicals that potentially disrupt endocrine systems on an international basis, e.g. the results of the initial USEPA screening programme, which begins this year. We recommend that OECD should investigate the feasibility and potential value of a case study sharing activity because it may influence the content and/or interpretation of future data packages on possible EDCs, and may be helpful in developing guidance on weight of evidence approaches.
- Data from non-mammalian species should be used to inform mammalian hazard identification and vice versa unless there is clear evidence that the mode of action is not relevant. It is recommended to analyse whether, how and why to do this, to consider scientific basis and take also account of its usefulness / role in regulatory contexts.
- Recognizing the role of the CF as a toolbox and the first step taken in the case study report in the context of this workshop, it is recommended to continue with exchanging information about and experience and practices of the role of EDC related test results and data in various OECD regions/countries and regulatory frameworks and consider using the OECD EDTA Advisory Group for this activity.

Monitoring and incorporating new science

- OECD should consider monitoring progress on EDC topics to be considered in relation to current and future Test Guidelines development and/or where there is uncertainty on how assessment should be made (e.g. combined exposure to multiple chemicals with a similar mode of action, low dose outcomes) and how to respond accordingly, e.g.:
 - By exchanging experience/approaches and outcomes of activities concerning these issues in various OECD regions/countries
 - By adding references to these issues in the explanatory notes of the CF.

Mammary glands

- Endpoints involving mammary glands of both sexes should be considered and when validated included in mammalian studies such as the extended one-generation study. A review of mammary gland assessment methods should be made so that the most appropriate age of animals and methods, and their placement in existing assays can be made. It is already an optional endpoint in TG 407 (see the histopathology guidance document). Research has shown the mammary gland to be a sensitive endpoint for estrogen action. At least some forms of breast cancer have been linked to estrogen and other endocrine mechanisms.

Endpoints not in existing guidelines

- A Detailed Review Paper (DRP) should be drafted to explore the incorporation of established *in vitro* test methods for additional hormonal systems (such as the glucocorticoid system) into OECD TGs. The DRP should also include linkages with *in vivo* endpoints. The rationale of this recommendation is to cover other hormone pathways that could potentially lead to developmental effects.
- A DRP should be drafted to explore *in vitro* and *in vivo* test methods for additional key signalling systems important for endocrine toxicity (e.g. glucocorticoid receptors, AhR, peroxisome proliferator-activated receptors (PPARs), and other endocrine related nuclear receptors) to be

considered for incorporation into existing or as new OECD TGs. The DRP should ideally evaluate the known or potential interactions of chemicals with these signalling pathways, including *in vitro* and *in vivo* methods designed to evaluate such interactions, and the likelihood that chemicals can induce adverse outcomes (e.g. obesity, metabolic syndrome, dyslipidemia, etc.) through these mechanisms of action. The DRP should identify data gaps when considering human health and ecotoxicological effects. This work should take note of and build on the activities of the OECD molecular screening working groups (e.g. thyroid and reproductive screening working groups), the information captured in the OECD DRP on the use of metabolizing systems for *in vitro* testing of endocrine disruptors¹, and the recommendations in the US National Academy of Sciences report on Toxicity Testing in the 21st Century. This activity recognizes growing evidence of the significant health and population impacts caused by disruption of signalling of these systems during critically sensitive life stages. For example, impairment of the cortisol response to stress in fish has been demonstrated in the field and the laboratory with a large range of apparently unrelated chemicals. A DRP would need to review this field, especially developments subsequent to a review published 6 years ago (Pottinger TG. 2003. *Pure Appl. Chem.* 75, 2321-2333), and recommend whether further research is needed, or Test Guidelines should be developed.

- It is recommended to develop a document describing useful endocrine endpoints not currently in existing guidelines, but which might need to be validated and included in future TGs or TGs updates. This document would be helpful to the Test Guidelines Programme. Examples of such endpoints might include measurement of vitellogenin in the fish life cycle test; behavioural endpoints; control of photosynthetic activity in multicellular plants; ecdysteroid and juvenile hormone titres in arthropods; and interference with control of symbiotic relationships (e.g. Fox JE et al. 2007. PNAS 104, 10282-7).
- A DRP on endocrine induced effects not currently covered by TG battery should cover the following issue: Are there simple markers that are currently measured or that can be incorporated in higher level tests to detect these? The rationale is that there are important endpoints/systems that can be adversely influenced by EDCs that are currently not robustly evaluated in higher level tests (e.g. metabolic disease, cardiovascular disease, diseases of senescence).
- OECD should prepare a document to explore the need for incorporating delayed toxicity in ageing animals due to exposure during development into level 5 protocols. The rationale for this recommendation is that exposure during development has been shown to lead to latent pathological changes. For example, existing mammalian TGs at Level 5 do not evaluate some parameters that have been shown to be influenced by EDCs and could be predictive of adverse outcomes in aging humans. In particular, some outcomes are not expressed until approximately 7 months (failure of ovarian cyclicity; prostate inflammation): well after the animals are terminated based on the current reproductive toxicity guidelines.

Dose/Concentration setting in screening tests

- Guidance for ecotoxicity concerning the setting of doses/concentrations in screening tests should be developed. One issue is the need to ensure that the dose is as high as possible (to maximise the chances of seeing an effect) without incurring the risk of systemic toxicity confounding the results.

Combined exposure

¹ OECD Series on Testing and Assessment No 97

- Attention could be given to consideration of combined exposure to multiple chemicals when conducting assessments. Dose/concentration addition seems an appropriate model when a common mode of action /toxicity pathway is involved and should be considered when related toxicities may act similarly
- A Review of the state of the science and regulatory developments in evaluation of combined exposure in context of ED effects testing/evaluation (e.g. additive effects, interactions between substances with similar/complimentary mode of action) should incorporate assessment practices from both human and ecotoxicity assessments. Case studies could perhaps be included. The review should include cumulative risk assessments based on considerations of exposure and similar mechanism and/or mode of action and/or common adverse outcomes. (Note there is recent international work in this field. The key to this exercise is to provide novel and useful information, to explore harmonization of approaches and/or identify best practices).

CONSIDERATIONS/RECOMMENDATIONS

Animal welfare

- When considering new Test Guidelines for *in vivo* test methods or new endpoints for EDCs, especially those with potential for negatively impacting the welfare of animals in laboratories, particular consideration should be given to the added value of these endpoints.

Definition of endocrine disrupter

- In the background documents provided for workshop participants, the WHO definition² of “endocrine disrupter” was provided as a working definition for the workshop. In this context, it was discussed whether the OECD could consider modifying this definition as follows: The addition of the term “or is causally linked to” was proposed to allow for upstream events or another associated endpoint to be used instead of the adverse effect itself as an endpoint for the regulation of chemicals. This is important because it could allow newer toxicological methods to be used in hazard/risk assessment. Upstream events have already been used in risk assessment, but further discussion of this concept is warranted. This issue was discussed by the workshop attendees but no consensus on this recommendation was reached. Concerns were raised about the potential legal implications of the definition proposed given that endocrine disruption is already mentioned in many legal contexts in member countries. Furthermore, care must be used in considering the issue of causal linkage because some upstream events, although causally linked to downstream impacts, do not always result in adverse outcomes.

Use of all available information

- All available relevant and reliable information concerning exposure and hazard (non test data/ test data) should always be used.
- Assessment documents should be transparent in how various data and information were collected, interpreted, weighed and evaluated. Evaluation of data from specific studies developed through OECD TGs, or other protocols, and the integration of data from multiple studies when addressed and presented in a transparent, clear and consistent manner can aid risk assessors, risk managers

² 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.

and stakeholders in evaluating the significance of the information in the context of a particular regulatory decision. The review of specific studies' strengths and limitations in a clear and consistent manner can contribute to transparency in the overall weight of evidence analysis because the underlying evaluation of each contributing line of evidence (i.e. data from each study) is provided. Assumptions used in evaluating specific studies and in formulating a weight of evidence analysis across multiple studies, when clearly documented and consistently applied, also contributes to a transparent presentation of scientific information and interpretations. A clear and consistent evaluation of the impact different assumptions can have in a weight of evidence analysis also contributes useful information to support a transparent discussion of the uncertainty in a weight of evidence analysis, which can be a critical component for some regulatory decisions.

- In relation to the WNT decision regarding new projects related to Test Guidelines, the added value in a regulatory context (e.g. how test results may be used for decision making) should be considered besides scientific issues.
- The OECD CF should not be seen in isolation. It is recommended to consider how to improve the use of other toxicity /ecotoxicity methods and data in relation to the EDTA CF and vice versa (e.g. what does ED findings mean for normally used apical toxicity /ecotoxicity endpoints and vice versa)?
- High quality non-standard test data should be included in the weight of evidence evaluation of a chemical. The weight given to non-standard tests would depend on their design, robustness and quality. In some cases a non-standard study would be more informative or more sensitive than the guideline study, but in other cases it would only supplement the data from TGs. The OECD should consider developing guidance to investigators for conducting non-standard studies that may be used in hazard/risk assessment.
- One logical approach to evaluate chemicals with no data could be to initially subject them to a battery of validated *in vitro* assays and/or relevant *in vivo* screens, however if this is not possible, chemicals can/should first be prioritised on basis of exposure, tonnage, (Q)SARs, chemical type etc for ED.

Molecular screening

- Contributions from member countries to the OECD molecular screening project (based on the ToxCast Program) allow evaluating a large number of substances using a similarly large number of *in vitro* assays. A number of these assays include endocrine related endpoints. Test substances that have robust data sets from higher level tests will be useful to allow the program to evaluate the predictive value of the *in vitro* assays. Results from these activities could inform ED testing and assessment.

Extended One Generation and Two Generations Reproductive Toxicity Studies

- The design of the extended one generation reproductive toxicity study, including developmental neurotoxicity and developmental immunotoxicity, allows examination of exposures during windows of development that are uniquely sensitive to toxicity to reproductive, central nervous system and immune system development. These three systems are potentially vulnerable to substances with ED activity.

RESEARCH NEEDS

Dose/Concentration – response relationship and threshold

- A document summarizing the literature on low-dose studies should be prepared, and if warranted, a workshop on low-dose/concentration related testing and assessment held, or a set of specific research recommendations defined. The rationale for this recommendation is that the low dose issue continues to be an area of uncertainty both with respect to how to conduct tests and how to assess the potential modes of action and hazard posed by endocrine disruptors. Questions involve the shape of the dose/concentration-response curve in the low dose/concentration region and whether or not there is always a threshold. Resolving this issue or defining specific research needs to fill gaps is an important and timely topic.
- Further work is recommended to define dose-response relationships for endocrine disrupter modes of action (receptor based, enzyme, etc) in particular to test the hypothesis that endocrine disrupters may exhibit inverted U shaped dose-response curves, and that threshold of effect can be predicted based on affinity of substance to target.

Metabolic processes

- A survey of the existing literature should be conducted regarding the potential role of chemicals, in the modulation of signalling pathways that may contribute, along with poor nutrition, limited exercise and other underlying medical conditions, to obesity, diabetes and other metabolic disorders that have become a global problem.

Effects in aging animals

- Research is needed into the development of biomarkers, e.g. of underlying epigenetic changes, such that these effects can be reliably detected in younger animals.

Central nervous system

- The development of new endpoints to detect interaction of chemicals with an endocrine action on the central nervous system development and behavior should be a high priority. This research should compare the sensitivity of these endpoints with that of endpoints in existing TGs. The reason for this recommendation is that the central nervous system may be the most sensitive target organ for some endocrine effects.

Predictivity of in vivo mechanistic assay

- There is a research need to continue to probe the relationship between dose giving positive/negative result in lower level tests and results in higher level tests (perhaps from the US Endocrine Disruptor Screening Program evaluation). How effective are positive/negative results in *in vivo* mechanistic assays to predict adverse outcomes?

Concordance of various in vitro assays

- Research is needed to determine concordance of various *in vitro* assays, i.e. that they give similar results across species: are there differences in sensitivities between species? For example, how many different types of estrogen receptor transactivation assays from different species are necessary to get meaningful results for all vertebrates?

Ecotoxicity

- Research is needed to address the following issues/questions:
 - Better knowledge of endocrine systems in invertebrates (e.g. molluscs) to allow the possible development of mechanistic tests should be developed. However, it may be questionable whether we need to understand invertebrate hormone systems to develop adequate invertebrate testing strategies, i.e. are apical tests with invertebrates sufficient in an EDC-testing programme?
 - What is the relationship between receptor binding or gene activation and effects of concern in apical tests e.g. the relationship between vitellogenin (VTG) gene expression, VTG protein induction, and changes in fish reproductive success?
 - Are *in vitro* screens for fish needed in addition to mammalian *in vitro* screens?, i.e. are all the important ED mechanisms conserved across all of the vertebrates? This subject should be investigated further.
 - A future consideration of possible new test species to select under varying circumstances is recommended. For example, should species other than the current standard species be considered for use in future TGs?
 - There are some recently-developed methods involving: 1) metabolism and toxicokinetics in *in vitro* methods (see the OECD document *Detailed Review Paper on the use of metabolising systems for in vitro testing of endocrine disruptors*³); 2) quicker (3-4 days) *in vivo* methods e.g. measuring gene expression for targeted pathways. These merit further investigation because their implementation in Test Guidelines may lead to the use of fewer test animals.
 - A continuing effort should be supported to compare the sensitivity of one-generation and multi-generation ecotoxicity tests. With some fish, for example, it appears likely that such tests are of similar sensitivity to each other when considering the majority of substances, but multi-generation tests may be more sensitive when testing strongly bioaccumulative chemicals. This issue needs to be resolved in order that test costs and the use of animals can be minimised.
 - Are the consequences of possible endocrine effects in plants captured by existing apical guidelines?

Exposure models

- There is a need for better exposure (screening) concepts/scenarios/models in relation to endocrine disrupters.

³ Series on Testing and Assessment N° 97

Annex 1: Test Guidelines and other documents related to endocrine disrupter testing and assessment (adopted or under development)

Adopted Test Guidelines
Stably Transfected Human Estrogen Receptor- α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals (TG 455), 2009
Uterotrophic Bioassay in Rodents: a Short-term Screening Test for Oestrogenic Properties (TG 440), 2007
Hershberger Bioassay in Rats: a Short-Term Screening Assay for (Anti)Androgenic Properties (TG 441), 2009
Fish Short Term Reproduction Assay (TG 229), 2009
21-Days Fish Assay: A Short-term Screening for Oestrogenic and Androgenic Activity, and Aromatase inhibition (TG 230), 2009
28-days repeated dose toxicity study in Rodents (TG 407), last update in 2008
One-Generation Reproduction Toxicity Study (TG 415), 1983
Two-Generation Reproduction Toxicity (TG 416), last update in 2001
Amphibian Metamorphosis Assay (TG 231), 2009
<i>Daphnia magna</i> Reproduction Test (TG 211), last update in 2008
<i>Reproduction/Developmental Toxicity Screening Test (TG 421), 1995</i>
<i>Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (TG 422)1996</i>
Guidance Documents published in the OECD Series on Testing and Assessment
N° 106: Guidance Document for Histologic Evaluation of Endocrine and Reproductive Tests in Rodents
No 82: Guidance Document on Amphibian Thyroid Histology, 2007
No 71: Guidance Document on the Uterotrophic Bioassay - Procedure to Test for Antioestrogenicity, 2007
Detailed Review Papers, Background Review Documents, Validation Reports, and Peer Review Reports published in the OECD Series on Testing and Assessment
No 111: Report of the Expert Consultation to Evaluate an Estrogen Receptor Binding Affinity Model for Hazard Identification
No 110 : Report of the Validation Peer Review for the Hershberger Bioassay (Weanling Model)
No 109 : Literature review on the 21-Day Fish Screening Assay and Fish Short-term Reproduction Assay
No 108 : Report of the Validation of the Hershberger Bioassay (Weanling Model)
No 97: Detailed Review Paper on the Use of Metabolising Systems for In Vitro Testing of Endocrine Disruptors, 2008
No 95: Detailed Review Paper on Fish Life-Cycle Tests, 2008
No 94: Report of the Validation Peer Review for the 21-Day Fish Endocrine Screening Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No 93: Report of the Validation of an Enhancement of OECD TG 211: <i>Daphnia Magna</i> Reproduction Test
No 92: Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of

this Report
No 91: Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3)
No 90: Background Review Document on the Rodent Hershberger Bioassay
No 86: Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α -Reductase Inhibitor in Dose Response Studies by Multiple Laboratories
No 85: Report of the Validation Peer Review for the Hershberger Bioassay, and the Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No 83: Summary Report of the Peer Review Panel on the Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-Up of this Report
No.81: Summary Report of the Validation Peer Review for the Updated Test Guideline 407, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No. 78: Final report of the Validation of the 21-day Fish Screening Assay for the Detection of Endocrine Active Substances. Phase 2: Testing Negative Substances
No. 77: Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study
No. 76: Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol
No. 73: Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol
No. 68: Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report
No. 67: Report of the Uterotrophic Bioassay: Additional Data Supporting the Test Guideline on the Uterotrophic Bioassay in Rodents
No. 66: OECD Report of the Validation of the Rodent Uterotrophic Bioassay: Phase 2 - Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories
No. 65: OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay - Phase 1
No. 62: Final OECD Report of the Initial Work Towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide
No. 61: Report of the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1B)
No. 60: Report of the Initial Work Towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine active Substances (Phase 1A)
No.59: Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats
No. 57: Detailed Review Paper on Thyroid Hormone Disruption Assays
No. 55: Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects

No. 47: Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances
No. 46: Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances
No. 38: Detailed Background Review of the Uterotrophic Bioassay
No. 21: Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals
Projects included in the work plan of the Test Guidelines Programme
Test Guideline on Copepod Reproduction and Development
Test Guideline for Mysid Life Cycle Toxicity Test
Test Guideline for Fish Sexual Development Test
21-Day Female Stickleback Endocrine Screening Assay
Test Guideline for a Chironomid Life-Cycle Toxicity Test
Test Guideline for the Medaka Life-Cycle (MLC)/ Multi generation Test (MMT)
Test Guideline on Amphibian Growth, Development and Reproductive Assay
Test Guideline for Stably Transfected Transcriptional Activation (STTA) Assay for the Detection of Estrogen Receptors Agonists and Antagonists (LUMI-CELL® ER Assay)
Test Guideline for Human Recombinant Estrogen Receptor Alpha Binding Assays (hrERA, 2 protocols)
Test Guideline for H295R Cell-Based Steroidogenesis Assay
Test Guideline for Stably Transfected Transcriptional Activation (STTA) Assay for the detection of androgenic and anti-androgenic activity of chemicals
Stably Transfected Transcriptional Activation (STTA) Assay for the detection of anti-estrogenic activity of chemicals
Guidance Document on the Weanling Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties
Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads
Detailed Review Paper on Molluscs Life-Cycle Toxicity Testing

Annex 2: Summary Case Study

1. Background

1. The OECD Members Countries were asked to submit contributions for the report comprising the description of current and potential assessments of endocrine disrupters (including regulations, guidance, and governmental programmes), strategies under development and prospective strategies, as well as description of relevant case studies.

2. The following countries and stakeholders sent contributions: Denmark, European Commission, France, Germany, Japan, Korea, United Kingdom, United States and Business and Industry Advisory Committee (BIAC). The information received can be grouped into three categories: 1) Regulatory issues and risk assessment policies; 2) Research activities, validation of test methodologies and development of Test Guidelines; 3) Case studies describing how the different TGs and other tools are used in practice.

3. The present report is summarising the received contributions. It has been structured into two main sections:

1. Current activities on endocrine disrupters in different OECD member countries (including regulations, ongoing governmental programmes, advisory activities, test methods validation, information to the public).

2. Current and proposed use of TGs and other tools in decision making across different OECD member countries and selected case studies.

4. The content of this report is based solely on the information included in the contributions received by the Secretariat from countries and BIAC. The respective contributions are included as *Appendices 1-8*:

Appendix 1: Contribution from Denmark

Appendix 2: Contribution from the European Commission

Appendix 3: Contribution from France

Appendix 4: Contribution from Germany

Appendix 5: Contribution from Japan

Appendix 6: Contribution from Korea

Appendix 7: Contribution from United Kingdom

Appendix 8: Contribution from the United States

Appendix 9: Contribution from the Business and Industry Advisory Committee (BIAC)

2. Current activities on endocrine disrupters in different OECD countries

Regulatory requirements for the assessment of endocrine disrupters (ED) in the European Union

5. At the level of the European Union (EU) the regulatory requirements for the assessment of industrial chemicals are laid down by the REACH legislation. One of the key elements of REACH is the authorisation procedure for substances of very high concern, which include those that are carcinogenic, mutagenic or toxic to reproduction (CMR), persistent bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB). In addition, substances not covered by the CMR, PBT, vPvB criteria can be identified on a case-by-case basis when there is scientific evidence of probable serious effects to human health or the environment. These substances are considered of “equivalent level of concern” and can be also subject to authorisation, as specified in the Article 57 (f) of EU Regulation 1907/2006 (REACH). They include substances having endocrine disrupting properties. However, the criteria and data necessary for proof of endocrine disrupting properties so far are not specified in detail and the decision has to be taken on a case-by-case basis.

6. With regard to aquatic toxicity testing, the technical guidance document to REACH (chapter R.7B, Appendix 7.8-5) provides some instructions in form of a risk oriented 3-step procedure that involves: 1) preliminary indication, 2) indication of specific modes of action in intact aquatic organisms, 3) characterisation of long-term effects. Confirmation of endocrine disrupting properties in step 3 forms the basis for bringing the substance for authorisation.

Plant Protection Products (PPP)

7. The assessments of endocrine disrupting activity of PPP are conducted within the EU under the Directive 91/414/EEC concerning the placing of PPP on the market. This directive does not make a direct reference to endocrine disruption but tackles it indirectly via requirements of chronic studies, such as requests to conduct a fish full life cycle test in certain cases. The information requirements are based the EU aquatic and terrestrial guidance documents (SANCO/3268/2001 (rev 4) and SANCO/10329/2002 (rev 2), respectively) in which endocrine disruption is addressed but without specifying a testing strategy

8. In January 2009 the European Parliament adopted a Regulation that replaces the current legislation (Directive 91/414/EEC) on placing plant protection products on the market. The new legislation has to be formally adopted by the Council, and it will enter into force later this year. The new Regulation includes provisions in relation to human health and ecotoxicology that prohibit the use of active substances, safeners and synergists which have endocrine disrupting properties that may cause adverse effects in humans or on non-target animals, unless the exposure of humans or of non-target organisms, respectively, to the active substance, safener or synergist present in a PPP is negligible under realistic proposed conditions of use. Within 4 years from the entry into force of this new Regulation, the European Commission shall present a draft of the measures concerning specific scientific criteria for the determination of endocrine disrupting properties of active substances. Pending the adoption of these criteria, substances that are or have to be classified, in accordance with the provisions of Directive 67/548/EEC, as carcinogen category 3 and toxic for reproduction category 3, 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 Directive 67/548/EEC, as toxic for reproduction category 3 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.

Biocides

9. At the level of the European Union, the EU Directive 98/8/EC concerning the placing of biocidal products on the market addresses endocrine effects in Article 38. Nevertheless, special data requirements and an evaluation strategy to clarify an endocrine mechanism are not explicitly described in this Directive and decisions for testing are made on a case-by-case basis. Therefore, any request for studies on assessment of endocrine effects of biocidal products, even with justified suspicion, is difficult. The Directive 98/8/EC will benefit from the final acceptance of standardised testing methods at the OECD level.

Pharmaceuticals

10. Special assessment of endocrine effects of medicinal products is not foreseen under the EU Directive 2001/83/EC on the Community Code Relating to Medicinal Products for **Human Use**. The ‘Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use’ (EMA/CHMP/SWP/4447/00) includes only a text passage which says that certain substances, such as lipophilic compounds and potential endocrine disruptors, may need to be addressed irrespective of the quantity released into the environment.

11. In the EU Directive 2001/82/EC on the Community Code Relating to **Veterinary Medicinal Products** hormonal effects are directly addressed only in Article 68 which states that Member States shall ensure that only persons empowered under their national legislation in force possess or have under their control veterinary medicinal products, or substances which may be used as veterinary medicinal products that have anabolic, anti-infectious, anti-parasitic, anti-inflammatory, hormonal or psychotropic properties.

Substances used in stock farming

12. Within the European Union, the Directive 96/22/EC concerning the prohibition of the use in stock-farming of certain substances having a hormonal or thyrostatic action, as well as of beta agonists, restricts the use of substances having estrogenic, gestagenic and androgenic effects. This includes substances having a hormonal action for growth promotion and 17-beta estradiol or its ester derivatives used for estrous cycle induction in cattle, horses, sheep or goats.

Control of surface waters

13. The Water Framework Directive (Directive 2000/60/EC) sets environmental objectives of good chemicals status for surface waters and the prevention of pollution of groundwater in the European Union. According to this Directive, the Members States are obliged to take action in order to prevent human exposure to endocrine disrupting substances. They are required to identify chemical pollutants of significance, set quality standards and establish emission control measures to achieve these standards. A specific category includes substances that have proved to be carcinogenic or mutagenic, or to have properties which may affect steroidogenic, thyroid, reproduction or other endocrine related functions in or via the aquatic environment. At the Community level, the Directive sets out a strategy against pollution of surface waters by chemical pollutants (Article 16). The strategy includes the identification of substances of particular concern and the adoption of quality standards and emission control for such substances. The first list of 33 substances (including 21 candidate endocrine disrupting substances for which an evidence or potential evidence or endocrine disrupting effects was found) was adopted in 2001. More details can be found in *Appendix 2*.

Governmental Programmes

Denmark

14. Several governmental programmes related to endocrine disrupting activities of chemicals have been launched in Denmark since 1995. The governmental support resulted in:

- Publication of reports summarising the current knowledge on male reproductive disorders, as well as environmental endocrine disrupting effects caused by chemicals
- Funding of research programmes in the area of endocrine disrupters (e.g. Strategic Research 2003-2005, Pesticides Research Programme; for more details see *Appendix 1*) International cooperation (e.g. with E.C., with the Nordic Countries [Nord-Utte])

15. The Danish government published in 2003 the “Strategy for Human Health and the Environment”, which sets up aims and initiatives to prevent and limit negative environmental effects on human health focused, among others, on reinforcing the efforts towards endocrine disrupting substances.

16. A national strategy for the work related to endocrine disrupting chemicals was presented in 2002. The 3 focus areas are: 1) Knowledge building and development of test methods; 2) Investigations of cause and effect, and preventive efforts, and 3) Regulation. A special grant from the state budget is dedicated to research aimed at strengthening the scientific basis for managing the endocrine disrupter problem.

17. A Centre for Endocrine Disrupters was established in 2008 and is funded by the Danish Government. It functions as a network of scientists and relevant institutions working with knowledge building focusing on authorities’ preventive work. The Centre management is placed at the Department of Growth and Reproduction at the Copenhagen University Hospital.

European Commission (EC)

18. The European Commission adopted in 1999 the ‘Community Strategy for Endocrine Disrupters’, which contains short, medium and long-term activities. The short and medium term activities focus on gathering scientific data on “candidate substances” with the view to prioritize testing, to guide research and monitoring, and to identify specific cases of consumer use and ecosystem exposure. The long term actions focus on the review and possible adaptation of policies and Community legislation.

19. The key short-term action has been the establishment of a preliminary priority list of substances, meant to provide a basis for gathering additional data on the endocrine disrupting effects of those substances, and their further evaluation. The list, created in a step-wise approach, now is comprised of 428 substances. Of the 428 substances, 194 showed a clear evidence of endocrine disrupting effects (category 1), 125 a potential evidence of endocrine disrupting effects (category 2) and 109 showed either no scientific evidence or insufficient data for final inclusion in the list (category 3a and 3b). A database comprising all scientific information was established which provides in a transparent manner the scientific data and references on human health effects and wildlife effects of these substances, as well as categories in terms of priority⁴. Assessment of the legal status of these substances showed that 269 of the category 1 and 2 substances are already subject to a ban or restriction, or are addressed under the existing Community legislations, while 51 substances are neither assessed nor restricted (details in *Appendix 2* of this report). This preliminary list will be used to establish a dynamic working list of substances. For the future, the European Commission plans to develop a methodology to include or remove substances from the list. Where necessary, substances will be fed into relevant legislation in order to manage their risk properly.

⁴ http://ec.europa.eu/environment/endocrine/strategy/short_en.htm

20. In support to the Community Strategy for Endocrine Disrupters, the European Commission under the Research Framework Programmes (FP5, FP6, FP7) sponsored several research projects dealing with endocrine disrupters. These projects were focused on improving the knowledge on endocrine disrupters' hazard and risk characterisation, epidemiological approaches, development of new test methods, investigation of mechanisms of disease development in various organs, endocrine-related reproductive effects in animal models and human studies. All the projects funded by FP6 related to endocrine disrupters are listed in the Annex of the mid-term review report of the Environment and Health Action Plan⁵. More details can be found also in *Appendix 2* to the present document.

France

21. The French National Research Program for Endocrine Disrupters (PNRPE) was launched in 2004. This program aims to answer calls from public authorities and to support fundamental and applied multidisciplinary research into: screening methodologies, biomarkers, mechanisms of action, biokinetics of endocrine disrupters in the organism and their fate in the environment, hazard identification and risk assessment methodologies, monitoring, and related socioeconomic aspects. Two calls for research proposals were launched in 2005 and 2008. The PNRPE programme is an important tool in the evaluation of the endocrine disrupting properties of several chemical pollutants, in particular in the framework of the REACH legislation.

22. France has two other major governmental programmes aimed to assess the impact of several factors (including endocrine disrupters) on the general population and on workers:

- the PNSE (National Environment and Health Action Plan), where one of the major goals is to prevent diseases associated with environmental exposure. Two actions in this plan concern endocrine disrupters: (i) limitation of water and soil pollution; and (ii) call for research projects on monitoring and studying effects of compounds (including endocrine disrupting effects).

- the PST (Occupational and Health Action Plan) is dedicated to workers and aims to improve professional risk prevention. The Objective 1 of PST is (i) to develop knowledge in professional domain, (ii) to manage the use of the resources assigned for research in support to public policies and (iii) to coordinate calls for research projects aimed to improve knowledge on the effects of different types of compounds (including endocrine disrupters compounds) to facilitate public decisions. The Objective 4 of PST promotes the principle of substitution of the most dangerous chemical substances: CMR, PBT, vPvB and endocrine disrupters, in accordance to European Chemical Regulation REACH.

More information on these programmes is available in *Appendix 3* to this report.

Japan

23. The Ministry of Health, Labour and Welfare (MHLW) established a Committee on Health Effects of Endocrine Disrupters, which addresses the evaluation of risk of endocrine disrupters on human health, the necessity to prompt action to protect human health, and the risk communication to the general public. The Committee has developed a framework for the testing of possible endocrine disrupter chemicals, which consists of two tiers: 1) screening assays (including *in silico*, *in vitro* and *in vivo* assays) and 2) definitive tests. The MHLW has carried out screening tests (i.e. including the Hershberger Bioassay and the rodent Uterotrophic Bioassay) on a number of chemicals and a priority list for future definitive testing was established based on the results from these screening assays.

⁵ http://eur-lex.europa.eu/LexUriServ/site/en/com/2007/com2007_0314en01.pdf

24. The Ministry of Economy Trade and Industry (METI) established an advisory body - the Endocrine Disruptive Effect Subcommittee. So far, METI funded studies on hazard assessment of 15 chemicals of potential concern as endocrine disrupters (no significant risk to human health was identified). Moreover, METI has been involved in the OECD Test Guideline Programme and conducted non-animal testing (receptor binding assay, reporter gene assay, steroidogenesis assay, (Q)SAR) and animal testing (Uterotrophic assay, Hershberger assay, enhanced 407 Test Guideline, in utero and lactational exposure study, two-generation reproductive toxicity study) of a number of substances.

25. The Ministry of Environment (MOE) established the “Strategic Program on Environmental Endocrine Disrupters 98” (SPEED 98). In addition the ExTEND 2005 programme was established by MOE in 2005 and it involves basic research on the mechanisms of endocrine disruption, environmental monitoring (observation of wildlife and measurement environmental concentrations and exposure levels), development of test methods, hazard and risk assessment, risk management, promotion of information sharing and risk communication, organization of annual international symposia.

Nordic co-operation, Nord-Utte

26. In 1994 the Nordic Group for the Development for Test Methods (Nord-Utte) was established as part of the Nordic Chemicals Group, under the auspices of the Nordic Council of Ministers. Swedish, Norwegian, Finnish and Danish representatives from the environmental protection agencies participate in the work. Nord-Utte aims at integrating the scientific and regulatory work on testing of chemicals in the Nordic countries. Beyond contributions to the OECD Test Guideline programme, Nord-Utte organizes network meetings and supports projects involved in the development of test methods. One of the main priorities of the group has been the development of test methods for detection of endocrine disrupting substances.

Republic of Korea

27. The relevant ministries of the Government of Korea have established a Mid-Long Term Research Plan for Endocrine Disrupters. The resulting research projects, conducted in the years 1999-2005, dealt mainly with environmental monitoring of endocrine disrupters and the assessment of ecological effects. They included monitoring of terrestrial and marine environment, livestock, food, agricultural products, farmland and drugs. The ministries have recently revised and produced a new five-years research plan (2007-2011). It is focused on reviewing the results of the previous research projects and on preparing appropriate plans for safety management of endocrine disrupters in each ministry. A summary of the results of the research projects and the outline and details of the 5-years research plan are available in *Appendix 6*.

United States

28. The US EPA’s Office of Research and Development (ORD) in 1996 identified endocrine disrupters as one of the top six research priorities. In addition, the passage of the Food Quality Protection Act (FQPA) in 1996 and subsequent amendments to the Safe Drinking Water Act (SDWA) and Federal Food, Drug, and Cosmetic Act (FFDCA) required EPA to: “*develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate [21 U.S.C. 346a(p)].*”

29. In response to this mandate, the US EPA established a multi-stakeholder federal advisory committee, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), under the Federal Advisory Committee Act (FACA). This committee was asked to provide advice to the US EPA on how to design a screening and testing program for endocrine disrupting chemicals (including pesticide and

non-pesticide chemicals). The US EPA considered the recommendations from EDSTAC (details in *Appendix 8*) and adopted a two-tiered testing strategy, which includes estrogen, androgen and thyroid hormonal systems, as well as animal wildlife. The details of the strategy are presented in *Appendix 8* to this report and reviewed in detail on the Endocrine Disruptor Screening Program (EDSP) website⁶.

30. US EPA's Office of Research and Development also developed a Multi Year Research Plan for Endocrine Disruptors that identifies science-specific questions which will be addressed by the Endocrine Disruptor Research Program (EDRP) over the next 5 -10 years. The document is updated every few years to take into account the current state of the science and the updated strategic directions of the program⁷.

31. The ORD has identified several long-term goals in the Multi Year Research Plan. The highest priority has been the development of protocols for the assays critical to the EDSP. The program has conducted much of the underlying research, developed and standardized the protocols, prepared background materials for transfer, briefed US EPA advisory committees, participated on international committees on harmonization of protocols, and/or participated in validation of approximately 19 different *in vitro* and *in vivo* assays for the development and implementation of the two-tiered EDSP. The focus of these activities has been on estrogenic-, androgenic-, and thyroid-mediated mechanisms using mammalian, fish, amphibian and invertebrate models.

32. In addition to test development, there are a number of scientific questions for which research is still needed and that are being addressed by the EDRP (details in *Appendix 8*). These include:

- 1) Understanding of how endocrine disrupting chemicals elicit toxicity through receptor-based interactions, membrane receptors, enzyme alterations, and other non-nuclear receptor-based pathways, particularly at the low end of the dose-response curve;
- 2) Determining the degree to which the effects of endocrine disrupting compounds with defined mechanisms/modes of action (MOAs) can be extrapolated across classes of vertebrates;
- 3) Developing approaches to assess exposure to mixtures of endocrine disrupting compounds, as well as characterizing the occurrence and effects of endocrine active compounds in complex environmental media and developing management approaches to mitigate unreasonable risks;
- 4) Determining the extent to which human development/reproduction is being adversely affected by exposure to endocrine disrupting compounds, as well as determining the critical factors that account for exposures occurring during development resulting in toxicities later in life (e.g., windows of vulnerability, developmental tissue dosimetry, modes of action);
- 5) Developing biomarkers and the next generation of assays for screening chemicals for their potential endocrine disruption;
- 6) Understanding what are the major sources and environmental fates of endocrine disrupting compounds and how can unreasonable risks be managed.

33. ToxCast⁸ is part of a research collaboration between the US EPA, the National Toxicology Program, the National Institute of Health (NIH) Chemical Genomic Center, and a number of other organizations, with the aim to screen thousands of environmental contaminants and pharmaceutical compounds using a high throughput screening (HTS) *in vitro* approach, in order to identify mechanisms and pathways leading to human toxicity, and based on these pathways to prioritize chemicals for further testing. The Phase I of ToxCast is testing 309 unique chemicals (mostly pesticidal active ingredients, plus a number of high production volume industrial chemicals) in 470 separate assays in order to develop initial predictive approaches that can be used in later large-scale screening phases. The assays include cell-free

⁶ <http://www.epa.gov/scipoly/oscpendo/>

⁷ www.epa.gov/ord/npd/pdfs/Draft-EDCs-MYP-091407.pdf

⁸ www.epa.gov/ncct/toxcast

biochemical assays of receptor binding and enzyme inhibition, gene expression assays, transcription factor activity assays, cell-imaging assays and real-time cellular impedance measurements. Among the assays, 4 evaluate the androgen signalling pathway, 5 relate to estrogen signalling, 4 to thyroid signalling, and 1 one to aromatase activity. Comparisons between ToxCast endocrine profiling and the results from Tier 1 EDSP battery will be made, as there are approximately 55 of the EDSP priority chemicals contained with the ToxCast™ Phase 1 chemical library. More details on the ToxCast programme can be found in [Appendix 8](#) to this report and on the ToxCast website⁵.

Information to the public

Denmark

34. A booklet “Stof til eftertanke – fakta om hormonforstyrrende stoffer” (in English: “Food for thought - facts about endocrine disrupting substances”, only available in Danish) was published in 2002 in a co-operation between the Danish Food Directorate, the Danish Environment Protection Agency (EPA) and the National Board of Health. The booklet informs about endocrine disrupters, describes the effects they might cause and illustrates how and where one can be exposed to them. It aims at all Danish consumers, but has a special focus on pregnant women and parents with small children.

35. The rising public awareness about endocrine disrupters, as well as the indications from animal studies regarding effects on the unborn child after combined exposures to these substances led to the Danish campaign: “Good chemistry to pregnant and nursing mothers – 9 good habits” launched by the Ministry of the Environment. The campaign pointed out 9 easy ways to reduce the exposure of the mother and the child to chemicals (including endocrine disrupters) in cosmetics, toys and baby products. It was a network campaign with midwives, doctors and nurses who distributed the material and used it during dialogues with the pregnant women and nursing parents. The campaign was very successful: 2/3 of the respondents in the target group were aware of the campaign, 2/3 of these had obtained new knowledge. Fifty percent of the respondents who were aware about the campaign followed the advise beforehand, while 30-35% had changed their behaviour as a result of the campaign.

36. Because of the indications and the awareness, Denmark is also promoting the inclusion of combination effects of endocrine disrupters as an area of priority at the 5th WHO Ministerial Conference on Environment and Health to be held in 2010.

37. In 1998 the Danish EPA published a list of undesirable substances, including 26 priority substances that the authorities have considered to restrict or completely ban in the future. The list has been updated three times, most recently in 2004. In 2008 it was announced that all category 1 substances from the EU priority list will be included in the revised list of undesirable substances (expected in 2009). The list serves as guidance to companies that wish to engage proactively in voluntary phasing out undesired substances.

European Commission (EC)

38. The numerous research activities sponsored by the European Commission’s Directorate General for Research will considerably increase the visibility of the phenomenon of endocrine disruption, as project results are made available to the public through leaflets, press releases, websites and workshops.

39. The European Commission has created two websites dedicated to the issue of endocrine disrupters. The website of the European Commission’s Directorate General of Environment⁹ contains a

⁹ http://ec.europa.eu/environment/endocrine/index_en.htm

section, where basic principles of endocrine disruption are explained and the central issues at hand are introduced. The Commission's strategy and a number of reports are also presented in detail. The public website of the European Commission's Directorate General for Research¹⁰ gives an overview of the European Union's activities in the field of endocrine disrupters and includes extensive background information, overview of the EU activities and a list of EU funded research projects in this area, as well as a list of related links.

Korea

40. The Korean Ministry of Environment has established a public website which contains some basic information about endocrine disrupters¹¹.

Test development and validation

Denmark

41. Several activities are undergoing in Denmark in support to the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. They include, among others, the development and validation of test methods to be used at all levels of the conceptual framework.

42. For level 2 (*in vitro* assays providing data on endocrine mechanisms) the QSAR group of the National Food Institute has developed three computer models for predicting endocrine disrupting effects in *in vitro* assays (i.e. estrogen alpha-receptor binding, estrogen receptor gene expression and androgen receptor antagonism). The National Food Institute participated in the OECD validation of an *in vitro* steroid synthesis assay (H295R). In addition, several methods are under development, including an assay to screen for effects on the thyroid receptor (T-screen assay), an androgen receptor reporter gene assay (AR-assay), as well as a method to include metabolism in *in vitro* assays.

43. With regard to methods included in level 3 (*in vivo* assays providing data about single endocrine mechanisms and effects), Denmark participated in the development and validation of the Uterotrophic assay, the Hershberger assay and the Fish Screening Assay. Moreover the National Food Institute is working on the development of an alternative to the frog metamorphosis assay to detect effects on the thyroid. It is an *in vivo* thyroid assay in pregnant rats with the main endpoints being measurement of T4 levels in dams during pregnancy and lactation, and in pups on post-natal day 13 (PND13), as well as growth and development of pups before weaning.

44. For the assays under level 4 (*in vivo* assays providing data about multiple endocrine mechanisms and effects), the National Food Institute participated in the work on the update of the TG 407 and Centre for Maritime and Regional Studies (University of Southern Denmark) together with the DHI - Water & Environment (SDU/DHI) participated in the development and validation of the Fish Sexual Development test, as lead country. Furthermore, the National Food Institute performed a study to investigate whether additional assessment of testosterone and anogenital distance in male fetuses would be useful for investigating the endocrine disrupting effects under the OECD TG 414 (Prenatal Development Toxicity Study). The Danish EPA sponsored the pre-validation, validation and development of a draft OECD TG on reproduction of springtails performed by the Danish Environmental Research Institute.

45. For the level 5 assays (*in vivo* assays providing adverse effects data for endocrine and other mechanisms) the National Food Institute presented several proposals for enhancing generation studies with respect to detection of endocrine disrupting effects, and is involved in the ongoing development of the draft

¹⁰ http://ec.europa.eu/research/endocrine/index_en.html

¹¹ http://eng.me.go.kr/docs/sub2/policy_view.html?id=63&class=14&topmenu=B&cat=250

OECD TG for the Extended One-Generation Study. Finally, Denmark participated in the development of the OECD TG 426 (Development Neurotoxicity Study) and the Fish Full Life Cycle Test. Detailed descriptions of all the above mentioned assays (levels 2-5) can be found in [Appendix 1](#) to this report.

European Commission (EC)

46. The E.C. is supporting the development and validation of test methods by working closely with Members States to coordinate input to OECD at the European Union level, and participated in the OECD Endocrine Disrupters Testing and Assessment Task Force (EDTA). Apart from contributing to the development of new and revision of existing Test Guidelines, the E.C. Joint Research Centre (JRC) is collaborating with the U.S. and Japan on the validation of estrogen receptor (ER) binding assays, androgen receptor (AR) binding assays and steroidogenesis assay. A detailed list of assays currently in (pre)validation can be found in [Appendix 2](#) to this report.

47. Several research projects dealing with the development and validation of new methodologies for hazard identification of endocrine disrupters were funded by the European Commission under the Research Framework Programmes (FP5, FP6, FP7). In particular, the FP6 Integrated Project “REPROTECT”, led by the E.C. Joint Research Centre, has the objective to validate a conceptual framework in the area of reproductive toxicity and to develop substantial number of alternative methods making use of advanced technologies. Within this project six tests for assessing (anti-)estrogenic and (anti-)androgenic compounds have been optimised and are now being analysed for predictive capacity. Two of these tests are continuing validation under the auspices of OECD. A complete list of FP projects related to endocrine disrupters can be found at the website of E.C. DG for Research¹².

Japan

48. The Japanese Ministry of Health, Labour and Welfare provided research grants to study endocrine disrupters on human health, including the development of test methods for detection of endocrine disrupters.

Korea

49. The Korean Government has sponsored and/or carried out the following research projects related to development of screening tools for detecting endocrine disrupters: 1) measuring vitellogenin in bullfrog, 2) development of transgenic yeast for screening sex hormone (estrogen, androgen, progesterone), 3) development of transgenic cells for screening thyroid hormone disruption, 4) development of screening technique for dioxin-like substances using transgenic cells, and 5) development of biomarkers for the screening of exposure level of PAH compounds, polybrominated compounds and heavy metals.

50. Moreover, the following tests which are part of the OECD Conceptual Framework have been established: E-screen assay, enzyme activation assay, estrogen/androgen receptor binding assay, and several *in vivo* assays (Uterotrophic assay, Hershberger assay, one-generation reproductive toxicity study, multi-generation reproductive toxicity study, andrological assay).

51. The development of new screening assays (with high-throughput, high sensitivity), new biomarkers, as well as kits for screening tests and bioassays is foreseen in the new five-years research plan (2007-2011) (see also [Appendix 6](#) to this report).

¹² http://ec.europa.eu/research/endocrine/index_en.html.

United States

52. The assays incorporated the EDSP Tier 1 screening battery (*see also paragraphs 29 and 65-69*) have undergone a five-stage assay validation process by US EPA (*see also paragraphs 88-93*), in collaboration with the OECD. The validation included: 1) test development, 2) pre-validation, 3) inter-laboratory validation, 4) peer-review and 5) regulatory acceptance. For Tier 1 screening methods the relevance was considered to be the ability of an assay or endpoints within an assay to detect chemicals with the potential to interact with one or more of the estrogen, androgen and thyroid hormonal systems, whereas reliability was the reproducibility of those results within and between laboratories. Throughout the validation process of individual assays, the EDSP sought guidance (e.g., on protocol development, selection of known positive and negative test chemicals, and interpretation of results) from within the EPA (Office of Research and Development, ORD) and federal advisory committees such as the Endocrine Disrupter Methods Validation Sub-committee (EDMVS), Endocrine Disrupter Methods Validation Advisory Committee (EDMVAC) and FIFRA SAP.

3. Current and proposed use of TEST GUIDELINES in decision making on testing, assessment and management of endocrine disrupters across different OECD member countries

Denmark

53. Denmark, supported by the Nord-Utte, prepared a case study report, investigating if and how the OECD Conceptual Framework can be used for the assessment of endocrine disrupting substances. The results were presented at EDTA8 meeting in January 2005. Specific proposals for the use of the test results from level 2-5 of the OECD Conceptual Framework can be found in the report from this meeting¹³. The proposed conclusions for regulatory use of test results from each level of the conceptual framework are presented below. It is noted that the report exclusively addressed endocrine disrupter properties in relation to human health and was focused on the EU regulatory framework.

54. The conclusion on the use of level 2 tests for regulatory purposes was that “positive *in vitro* test results indicate potential ED activity and a potential for ED effects *in vivo*. *In vitro* data can provide valuable mechanistic data that is useful for the design of further *in vivo* studies. The *in vitro* tests are relevant for effects in humans because are based on human hormone receptors. Chemicals that bind to these receptors are therefore likely to cause effects in *in vivo* studies and on reproductive function in humans. Negative *in vitro* test results cannot be used to exclude potential ED activity because of limitations such as inability or unknown capacity to metabolically activate toxicants and because ED activity can occur through mechanism other than those tested in the *in vitro* test system. (Q)SAR models for ED activity and reproductive toxicity effects are under development but at present the use for priority setting and risk assessment is undecided”.

55. The conclusion for the use of level 3 tests for regulatory purposes was that: “the assays at level 3 provide an *in vivo* screening of potential endocrine disrupting activity of a substance. Except for the frog metamorphosis assay, the assays at level 3 provide information about the potency of the compound *in vivo*. Furthermore, the outcome of the assays indicates potential for adverse effects in the reproductive developmental studies at level 5. At present, it is uncertain to what extent the frog metamorphosis assay can be used for screening in relation to effects on humans”.

56. The conclusion for the use of level 4 tests for regulatory purposes was that: “the assays at level 4 provide a thorough assessment of the potential endocrine disrupting effects of a substance in pubertal and young adults. Furthermore, level 4 provides information about the potency of a compound to be investigated at level 5. Effects on various endpoints included in the assays can either be considered as adverse or represent an effect on a mechanism relevant for human e.g. changes in hormone levels. Therefore, these assays can be used to provide NO(A)ELs/LO(A)ELs to be used in human risk assessment until further studies are available. The intact male assay and the TG 407 may be more capable for detecting aromatase inhibitors and compounds affecting the steroid synthesis compared to the pubertal male assay. On the other hand, the two assays in intact young males may be less sensitive compared to the Uterotrophic and the Hershberger assay as well as the male and female pubertal assay”.

57. The conclusion for the use of level 5 tests for regulatory purposes was that: “The reproductive toxicity studies provide adverse effect data and are especially useful for risk assessment as they indicate potential for effects in humans. The effects observed in reproductive toxicity studies may be due to other mechanisms than endocrine effects, but the pattern of effects, e.g. decreased anogenital distance and malformations of reproductive organs in males may indicate that endocrine effects are involved. Among

¹³ EDREG (OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters as a Basis for Regulation of Substances with Endocrine Disrupting Properties), <http://www.nord-utte.org/index.php?nyhed=20>

the OECD Test Guidelines for reproductive toxicity, exposure during all vulnerable periods of development is only performed in the two-generation study design. Late effects becoming manifest after weaning of the animals are partly covered in young adults, especially in relation to reproductive function and developmental neurotoxicity, but potentially important late effects are not assessed. Effects becoming manifest during ageing are not included in any guidelines for reproductive toxicity. A number of enhancements of the OECD Test Guidelines for reproductive toxicity for the detection of effects of ED chemicals seem relevant and lack of effects in reproductive toxicity studies can therefore at present not fully exclude the possibility for ED effects caused by chemicals tested negative”.

Germany

58. In Germany, except for some guidance provided in the technical guidance document to REACH (chapter R.7B, Appendix 7.8-5) related to aquatic toxicity testing (see also *Paragraph 4 and 5*), no testing strategy for **environmental assessment** of endocrine disruption exist. General screening for endocrine disruption is not intended in any legal framework and case-by-case decisions on preliminary indication are necessary. Depending on the legislative background it is possible to require the respective studies from applicants to assess endocrine effects.

59. To address this problem a workshop took place recently in Germany on the ‘Characterization of endocrine mediated effects in fish’. The workshop discussed besides others the issues of:

- 1) What constitutes for environmental relevant substances a suspicion for endocrine disruption,
- 2) According to which rules (criteria for weight-of-evidence) a decision for performing a fish screening assay (FSA) is made,
- 3) What is the function of the FSA (clarifying the mode of action),
- 5) How the results from FSA are used in regulatory decision making,
- 6) Which test is more suitable: full life cycle test (FLCT) or a two-generation-test?

Experts from Germany representing industry, regulatory authorities and academia agreed on the basic design of a (national) testing and assessment strategy for endocrine effects in fish (the report from the workshop is available only in German).

60. A project was funded by the German Environmental Research programme UFOPLAN, aimed to develop and pre-validate a full life cycle test (FLCT) with zebra fish, with the aim of identifying relevant endpoints and defining a conceptual approach on the use of this FLCT as a definite test for endocrine disrupting activity in fish (details in *Appendix 4* to the present report).

United Kingdom

61. In case that there are strong suspicions about the endocrine disrupting activities of specific chemicals, the basic approach in UK is to rely on the full risk assessment of a given chemical at the European, rather than national, level. The UK has led such risk assessments for several chemicals that have featured prominently in discussions about endocrine disruption.

62. For the assessment of the potential risks of **new chemicals** to human health, the UK regulatory authorities require the chemicals to be tested using standard toxicology test requirements and do not generally recommend to carry out specific tests for substances known or suspected to have endocrine disrupting potential. If standard toxicity tests reveal effects on an endocrine system, then the significance for human health would be assessed before a decision on approval or risk reduction measures is taken. The most likely use of specific tests for endocrine disruption is within industry, during screening of new candidate chemicals for endocrine testing effects, which is done prior to making a decision on whether to take the compounds forward for standard toxicological testing.

63. With regard to **plant protection products** UK does not have a set procedure for testing chemicals in this category for endocrine disrupting activity. Assessments are conducted under EU Directive 91/414/EEC, based on information provided in the EU aquatic and terrestrial guidance documents (see also *Paragraph 12*).

64. When considering **environmental assessments**, the UK tends to take a pragmatic approach to the testing of potential endocrine disrupting chemicals, so that non-standard but scientifically reliable data on endocrine effects are taken into account. For the use of biomarkers of endocrine disrupting activity (e.g. vitellogenin, ovotestis in fish) this is considered an area of work of high priority, as they might be used in a supportive role, as well as to guide test concentrations election and aid efficient test design. The use of biomarkers in definitive apical tests (e.g. fish lifecycle tests) is likely to be desirable rather than compulsory, e.g. for use in the rare cases when a substance is being tested for ED-related hazards without previous knowledge of its mode of action from mammalian or *in vitro* studies. However, where data are obtained from tests that have not been validated and internationally standardised, especially where there are difficulties in replicating the results, then disputes over interpretation can easily arise. Thus, the UK fully supports the OECD in attempting to speed up the test validation process and sees it as a high priority activity.

United States

65. The US EPA Endocrine Disruptor Screening Program (EDSP) has developed a two-tiered system for screening and testing of the potential interactions of pesticide and non-pesticide chemicals on the estrogen, androgen and thyroid (EAT) hormonal systems with application to human health, animal wildlife and the environment.

66. The Tier 1 of the EDSP consists of *in vitro* and mammalian and non-mammalian *in vivo* screening assays designed to cover agonistic and antagonistic effects involving the estrogen and androgen modes of action and respective steroidogenic pathways, as well as the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroidal (HPT) axis. It is foreseen that complimentary and corroborating evidence among assays within the Tier 1 battery shall be used with a weight-of-evidence approach to determine whether or not a test chemical interacts with the endocrine system.

67. The **assays selected for Tier 1** are listed below:

In vitro assays

- Estrogen receptor (ER) binding – Rat uterus or recombinant
- Estrogen receptor α (hER α) transcriptional activation - Human cell line (HeLa-9903)
- Androgen receptor (AR) binding – Rat prostate
- Steroidogenesis – Human cell line (H295R)
- Aromatase – Human recombinant

In vivo assays

- Uterotrophic (rat)
- Hershberger (rat)
- Pubertal female (rat)
- Pubertal male (rat)
- Amphibian metamorphosis (frog)
- Fish short-term reproduction

68. The basis for selecting a candidate assay to be included in the battery involved: 1) the capacity of that assay to detect estrogenic- and androgenic-mediated effects by various modes of action including receptor binding (agonist and antagonist) and activation/transcription, reproductive steroidogenesis, and

hypothalamic-pituitary-gonadal (HPG) feedback, and 2) the degree to which *in vitro* and *in vivo* assays complemented one another in the battery. In addition, rodent and amphibian *in vivo* assays were selected for the proposed battery based on their capacity to detect direct and indirect effects on thyroid function (hypothalamic-pituitary-thyroidal, HPT, feedback). A detailed description of the endocrine modes of action and degree of complementarity among the EDSP Tier 1 assays can be found in [Appendix 8](#) to this report

69. A science-based approach to **interpretation of the results** of the battery will generally follow the principles:

1. Interpretation of the battery will be considered in light of the results of all assays in the battery, using a weight-of-evidence approach, taking into consideration *in vitro/in vivo* discrepancies (if any), metabolism, and route of exposure.
2. When all screening assays are performed and all assays are *negative*, it may be concluded that the chemical will not likely interact with EAT hormonal processes included in the battery.
3. If results from Tier 1 are *positive*, indicating a substance does exhibit the potential to interact with the E, A or T hormonal pathways, then more complex and definitive dose-response testing would likely be done in Tier 2 to further identify the potential hazard and to assess adversity and risk to the public and the environment.

Business and Industry Advisory Committee to OECD (BIAC)

70. The chemical industry supports the development of internationally harmonised procedures to prioritise substances efficiently, to screen for endocrine activity, to test for adverse effects and to evaluate substances in the framework of a coherent chemicals policy. These procedures should provide for a globally harmonized, tiered, hierarchical scientific framework in which OECD validated *in vivo* and *in vitro* screening assays are used to identify substances with endocrine activity and prioritise substances for further, more definitive testing where exposures will be evaluated in the complete and intact endocrine system encompassing critical life stages and processes. The definite tests that use validated, harmonized protocols will provide data on adverse effects and dose response necessary for hazard and risk characterization.

71. For hazard characterization, the chemical industry calls for the development of a "weight-of-evidence" evaluation process that consists of a comprehensive, objective, transparent and balanced interpretation of the totality of scientific evidence regarding hormonal activity and adverse effects that might result from an endocrine mechanism. A detailed description of the proposal towards the establishment of a weight-of-evidence approach to prioritizing action in relation to endocrine disruption can be found in *Appendix 9* to this report.

72. As the OECD finalizes validation of test methods and adopts new Test Guidelines, BIAC stresses that there is a pressing need for a globally harmonized evaluative processes and framework because such assays will soon be deployed in the U.S. as part of the EPA's Endocrine Disruptor Screening Program and in Europe as part of REACH. Therefore, BIAC proposes an **Endocrine Screening and Testing Hierarchical Framework** which consists of three stages (for mammalian and ecotoxicity, respectively) (details in *Appendix 9*).

Stage 1: Initial assessment to set priorities for further evaluation

73. This step consists of the evaluation of all available data (including production volume and patterns of use, exposure information, predicted environmental properties (e.g. fate, persistence, bioaccumulation), toxicological data from existing studies), quantitative structure activity relationships ((Q)SARs) and molecular screening results. The evaluation of these data should be done using a weight-of-

evidence approach. This should enable to promptly rapidly recognise substances of concern and permit more a rapid prioritisation and assessment.

Stage 2: Screening assay

74. This step allows to efficiently and effectively develop information as to whether a substance has the potential to interact with one or more components of the endocrine system. It includes *in vitro* assays and *in vivo* assays providing mechanistic information/data on single mechanisms, and *in vivo* assays providing information on multiple endocrine mechanisms. For flexibility, the option to proceed directly to *in vivo* assays should not be precluded, nor should *in vitro* assay results be required if *in vivo* results are available. The *in vivo* assays (i) incorporate substance-specific complexities that cannot be obtained from *in vitro* assays, including absorption, distribution, metabolism and excretion, and (ii) reflect the complex and dynamic homeostasis and operation of the intact endocrine system. Therefore, *in vivo* results would supersede *in vitro* results.

The assays in Stage 2 include:

For mammalian toxicity:

- Estrogen and androgen receptor binding assays
- Transfected mammalian cell assays (ER, AR and TR)
- *In vitro* aromatase
- *In vitro* steroidogenesis

For ecotoxicity:

- Receptor binding (presumably will be in principle applicable across vertebrate classes and to any invertebrate expressing similar receptors)
- Fish screening assay (vitellogenin and secondary sex characteristics)
- Frog metamorphosis assay
- OECD Fish Screening assay (VTG and secondary sex characteristics as mandatory endpoints, other endpoints are optional)

As the results from assays comprising the Stage 2 provide only mechanistic information and not complete evidence for adverse effects, these screening results do not indicate that a compound is an ‘endocrine disrupter’.

75. A weight-of-evidence process needs to be implemented in order to integrate results across the complement of assays. Substances which are positive based on overall consideration of the weight-of-evidence in Stage 1 and 2 are considered to be high priority candidates for further evaluation in definitive tests (Stage 3). However, prior to initiating additional work, it is appropriate to consider the potential for human exposure and potential for entrance into the environment. The term ‘potential endocrine disrupter’ could be easily misinterpreted, and generally the use of this term should be avoided. From a scientific perspective, it is important to determine the overall weight-of-evidence of the performance of a substance in the screening assays/battery, as described above.

Stage 3: Definite testing

76. This step aims at evaluation of apical endpoints, adverse effects and dose response to accurately and effectively identify and characterize the hazard(s) from chemicals.

Stage 3 includes reproduction and developmental tests, and in particular:

For the mammalian toxicity:

- Repeated dose 28-days oral toxicity study in rodents (TG 407)
- Reproductive/developmental; screening test (TG 421)
- Combined repeated dose with reproduction/developmental screening (TG 422)
- One-generation reproductive toxicity (TG 415)
- Two-generation reproductive toxicity (TG 416)
- Extended one-generation reproductive toxicity (TG under development at OECD)

For the ecotoxicity:

- Partial and full cycle assays in fish, birds, amphibians and invertebrates (developmental and Reproduction tests)
- Fish full life cycle

77. The array of assays in Stage 3 should be viewed as a matrix of available options, and not as a sequential list of assays and tests. It would not be necessary to conduct all tests, but the appropriate tests should be selected. For example, in the interests of flexibility and to minimize animal use and resources, the enhanced Repeated 28-Days Oral Dose Toxicity Study (TG 407) and the shorter-scope reproduction/developmental tests would not be required in cases where a longer scope test is already available or has been planned.

78. Overall, hazard characterization for hormonally active chemicals requires an objective evaluation of whether the effects produced are adverse, and/or whether adverse effects are due to the hormonal activity of the chemical. This includes consideration of the proposed Stage 2 screening tests and Stage 3 definitive tests, and results from standard toxicity studies.

79. Risk characterization requires integration of scientific data and knowledge of hazard, dose-response and exposure, as well as an evaluation of the foundations of the hazard data and inherent uncertainties. In cases of low potency and low or negligible actual and potential exposures, test methods such as the Repeated Dose 28-days Oral Toxicity Study (TG 407) or the Reproduction/Developmental Toxicity Screening Tests (TG 421/422) could be used to provide dose-response data of effects on apical endpoints. This would serve to focus the more extensive testing only on substances that have high production volume and the highest potential for human and ecological exposures. In all cases, results from definitive testing outweigh or supersedes results from screening.

80. It is important to stress that all stages should make use of standardized, validated and internationally harmonized test methods. In case that new methods and studies with non-standard species which provide important scientific information are used, BIAC suggests doing a thorough review of the study report, if possible replicating the study in another laboratory. Then, if the findings are shown to be reproducible, then two courses of action would be advised:

- subject the test method to standardization and validation within the OECD TG program (EDTA) or within a similar formal program sponsored by a national government or recognized scientific organization (e.g., ISO, ASTM),

or

- evaluate the substance of concern in one of the wide variety of existing validated test method using standardized OECD TG methods and species (or similarly validated scientific methods, for example those promulgated by ISO, ASTM, US EPA). Results of this study would then be evaluated within the tiered hierarchical OECD EDTA Framework. In general, this would be the preferred course of action.

Selected case studies

Dimethylation inhibitor (DMI)-fungicides (case study provided by Germany)

81. Active ingredients of plant protection products (PPP) of the DMI-fungicide group show a potential for endocrine disruption. These assumptions, based on the intended mechanism of action, have been confirmed in long-term studies on mammals, birds and fish. For this reason the German Registration Authorities regard all representatives of the DMI-fungicide group as potential endocrine disrupters. Registrations of this group are now allowed until appropriate data have been submitted and reviewed (e.g. the fish life cycle test (FLCT)). Thus, in the last 2-3 years the endocrine effects of DMI-fungicides were assessed in detail in Germany in the framework of the PPP authorization.

82. One project dealt with "Characterization of endocrine mediated impacts in fish – relevant parameters for the development of new OECD test methods and the application in environmental risk assessment". This study aimed at the collection and comparison of existing data from fish screening assays, full-fish cycle studies and two-generation studies. This allowed to derive general conclusion about endpoint sensitivity and predictivity, and to propose a tiered testing strategy for assessment of potential ED activity of chemicals. A short report containing the main conclusions of the study is available in [Appendix 4](#).

83. A second study on the "assessment of the safety of an extrapolation from growth data of Early Life Stage and Juvenile Growth tests (OECD 210, 204, 215) to the No-Observed Effect Concentration (NOEC) of Fish Full Life Cycle Tests in the risk assessment of DMI fungicides" was commissioned in Germany by the Agricultural Industry Association (Industrieverband Agrar, IVA) to determine a criterion to allow registration of DMI-fungicides. The study aimed to identify a suitable factor that would allow extrapolating an FLCT NOEC from existing fish chronic studies, and thus allowing for a preliminary risk assessment. Data on acute and chronic toxicity (OECD TG 203, 204, 210, 215), Fish Screening Assay, Fish Sexual Development Test and bioconcentration potential (OECD 305) were the basis of this analysis. The study concluded that for the effects caused by DMI-fungicides it is possible to apply an extrapolation factor of 1/5 to the NOECs from Early Life Stage (ELS) and Juvenile Growth (JG) studies, which allows to estimate a NOEC in FLCT useful for a preliminary risk assessment. However, for definite risk assessment it is still necessary to perform a FLCT as a definitive method. A detailed description of the methodologies, the study results and the recommendations are available in [Appendix 1](#).

Azol Fungicides (case study provided by Denmark)

84. The Danish National Food Institute has investigated the effects of several azole fungicides in various *in vitro* assays, the Hershberger assay and the pre- and postnatal development toxicity studies. The overall results showed that in general azole fungicides have a similar profile of action *in vitro*, but the profile of action *in vivo* may differ, which indicates that the main mechanism responsible for the ED activity lies in the interaction with key enzymes responsible for steroid hormones synthesis. Epidemiological studies in greenhouse workers showed elevated estrogen activity in females exposed to this group of chemicals; preliminary studies have also shown increased incidence of impaired reproductive development in sons of female workers. Thus, although some of the azole fungicides are still approved for use, the Danish EPA is now establishing stricter guidelines for handling azole fungicides in greenhouses. Full case study description is available in [Appendix 1](#).

Mixture effects/combined exposure (case study provided by Denmark)

85. For single compounds the current risk assessment is based on the No-Observed Adverse Effect Levels (NOAELs). Single chemicals alone may be present in human tissues at levels too low to cause concerns for adverse reproductive effects. However, several anti-androgenic chemicals have been found in humans as mixtures. Thus, the Danish National Food Institute investigated the ability of mixtures of anti-androgens to induce disruption of male sexual differentiation after perinatal exposure in extensive dose-response studies.

The results of these *in vivo* studies indicate that the joint effects of combined exposure to more androgens in many cases can be predicted by dose-addition models (a method to estimate cumulative risk¹⁴) and that marked effects can occur at doses below NOAELs for the single chemicals. In conclusion, risk assessment based on NOAELs for single anti-androgens alone might severely underestimate the risk for antiandrogenic adverse effects in humans. A more detailed description of this case study can be found in [Appendix 1](#).

Endocrine disrupters in freshwater fish (case study provided by Denmark)

86. Endocrine disruption in fish was already investigated in the late 1990'ies. The causal mechanisms were in contrast to other rather well understood endocrine related effects. This provided the basis for establishing in 1998 the Nordic work on developing an OECD Test Guideline for detection of endocrine disrupting effects of chemicals in Zebra fish. The work, supported by the Danish EPA and the Nordic co-operation Nord-Utte, resulted in the development of the OECD Test Guideline proposal "Fish Sexual Development Test" (FSDT). In the FSDT, the endpoints are vitellogenin in male fish and change of the sex-ratio. These endocrine related effects have been observed in exposed wild fish populations in Danish watercourses and the extent of these findings were investigated in a series of projects (details in [Appendix 1](#)). It was concluded that the observed endocrine disruption in freshwater fish by far and most probably was caused by exposure to natural estrogens (excreted to sewage by the female part of the population and to a minor extent by farm animals to manure and via agricultural runoff to water courses) and not by exposure to synthetic estrogens or other chemicals with estrogenic activity. Furthermore, it was concluded that the majority of Danish wastewater is treated sufficiently to reduce the amount of estrogens released to the environment to a level which is considered of low significance.

Phthalates (case study provided by Denmark)

87. The use and consumer exposure to phthalates and the effects of these substances in the environment were investigated by the Danish EPA for the first time in 1984. Over the last decades several studies have been conducted in Denmark to assess the potential of phthalates for being carcinogenic and/or toxic to reproduction. Also, phthalates had been measured in the environment and had been shown to accumulate in aquatic organisms and sediments. The detailed review of the studies is available in [Appendix 1](#). The Danish EPA has used these studies as documentation for the reproductive effects of phthalates both within the EU programme for risk assessment of existing substances and in the subsequent restrictions of use of phthalates in consumer products. These studies have also contributed with important information in relation to the use of anogenital distance and nipple retention as new end-points for detection of endocrine disrupting effects and input for improvement of the TG 414 and the one-generation study.

The validation of EDSP Tier 1 screening battery (case study provided by the US)

88. Examples of test chemicals known to interact with the estrogen, androgen and thyroid hormonal pathways have been selected to illustrate the mode of action and complementarity of the Tier 1 screening assays. The results presented here are part of the validation process that was conducted by the EPA and OECD (see also [Appendix 8](#)). A detailed account of the EPA validation process and the results for each assay are reviewed at the EDSP website¹⁵.

89. Methoxychlor is a pesticide that binds weakly to the estrogen receptor, as compared to its metabolites. The results from the *in vitro* ER assays and *in vivo* uterotrophic assay showed a relatively

¹⁴ Chen et al, (2001) Using Dose Addition to Estimate Cumulative Risks from Exposures to Multiple Chemicals, Reg. Tox Pharm, 34: 35-41.

¹⁵ <http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/status.htm>

weak but positive response to methoxychlor. This response was confirmed by the relatively strong positive response of apparent methoxychlor metabolite(s) in the *in vivo* female pubertal and fish reproduction assays. Hence, the combined positive responses among all assays provided evidence that methoxychlor interacts with the estrogen hormonal pathway in an agonistic way. Consequently, further testing would be indicated in the EDSP Tier 2.

90. Bisphenol A is an industrial compound primarily used in the production of polycarbonate plastics. The compound gives positive responses in the *in vitro* ER binding assay and the estrogen receptor α (hER α) transcriptional activation (HeLa-9903). Also, the *in vivo* uterotrophic and fish reproduction assays with the subcutaneous and aquatic routes of exposure, respectively, were positive. However, the oral route of exposure and the first-pass metabolism of Bisphenol A in the pubertal female assay led to a negative response. These combined results demonstrate the strength of the EDSP Tier 1 screening battery by incorporating both *in vitro* and *in vivo* assays with different routes of exposure and taxa to minimize false negatives. Thus, a positive response in a majority of the assays provides sufficient evidence to indicate Bisphenol A interacts with the estrogen hormonal pathway in an agonistic way. Consequently, further testing would be indicated in the EDSP Tier 2.

91. Vinclozolin is a pesticide that has anti-androgenic effects. In the AR binding assay using rat prostate cytosol vinclozolin gave ambiguous results (a partial binding curve that did not cross the 50% binding threshold). Nevertheless, in the Hershberger assay, pubertal male assay and fish short term reproduction assay, vinclozolin gave positive results, despite the corroborative results in the *in vitro* AR assay. Unlike the *in vivo* assays, the AR *in vitro* assay does not have the capacity for metabolism, which apparently led to the equivocal response. In this case, positive responses among the *in vivo* assays provided greater evidence over the equivocal response in the *in vitro* assay to indicate vinclozolin interacts with the androgen hormonal pathway in an antagonistic way. Consequently, further testing would be indicated in the EDSP Tier 2.

92. Ketoconazole is a pesticide and a pharmaceutical that alters steroidogenic enzymes resulting in enhanced progesterone and reduced estrogen and androgen production. In the *in vitro* steroidogenesis assay (H295R cell line assay) ketoconazole inhibited the production of both testosterone and estradiol. It also provided a full inhibition curve in the recombinant aromatase assay. This is consistent with a chemical that is a non-specific P450 inhibitor as it inhibits enzymes upstream and downstream of testosterone. The results of the pubertal female and male assays (alterations in ovarian and testicular morphology, delayed puberty in male) and the fish short term reproduction assay were corroborated by results in the *in vitro* steroidogenesis and aromatase assays. Hence, the positive responses among all *in vitro* and *in vivo* assays provide a strong evidence to indicate ketoconazole likely interferes with the steroidogenic pathway. Consequently, further testing would be indicated in the EDSP Tier 2.

93. Perchlorate is a pesticide and pharmaceutical that alters steroidogenic enzymes resulting in enhanced progesterone and reduced estrogen and androgen production. In the pubertal male and female assays perchlorate induced histopathological changes of the thyroid gland and respective alterations of T4, T3 (in female) and TSH. In the amphibian metamorphosis assay perchlorate increased follicular cell height and reduced colloid area in the thyroid gland, as well as delayed the developmental stage progression at higher doses. Summing up, alterations in thyroid gland weight, changes in histomorphology and associated alteration of hormone levels in the *in vivo* female and male pubertal and amphibian metamorphosis assays provided a strong evidence that perchlorate interferes with thyroid development and function. Consequently, further testing would be indicated in the EDSP Tier 2.

Atrazine (case study provided by the US)

94. An understanding of atrazine's neuroendocrine mode of action (MoA) in laboratory animals (rats) was used to address issues about the relevance of the data to human risk assessment. Studies showed

that atrazine inhibits the pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus, which in turn suppresses the release of luteinizing hormone (LH) from the pituitary. From this it was concluded that the MoA leading to the formation of rat mammary gland tumors was not relevant to humans because the LH suppression was affecting a reproductive aging process unique to the rat.

95. However, atrazine's effect on LH and consequent effects on development and reproduction in the rat was assumed to be relevant to humans given the homology of the hypothalamic-pituitary control on normal reproductive development and function. Thus, LH suppression was used as the basis of the dose-response assessment (NOAEL = 1.8 mg/kg b.w. per day; LOAEL 3.65 mg/kg b.w. per day from a 6 month dietary rat study). Basing the point of departure and derivation of the reference dose (RfD) on LH suppression would provide protection from LH-dependent effects on reproduction and development. In addition, understanding atrazine's neuroendocrine MoA served as the basis for grouping certain chloro-s-triazine pesticides by a common mechanism of toxicity and served as the basis for a cumulative risk assessment, i.e., an assessment considering the risk due to co exposure to all chloro-s-triazine pesticides.

96. Potential endocrine effects were considered but were determined inconclusive on aquatic organisms and wildlife.

97. Results observed in the EDSP Tier 1 studies are consistent with the EPA risk assessment and the understanding of atrazine's neuroendocrine mode of action. Negative findings were observed in the *in vitro* ER and AR binding assays and the uterotrophic assay, but a delay in female and male puberty was found in rat pubertal assays. This would be expected, given atrazine's ability to suppress the pituitary LH surge, which plays a role in sexual maturation. The overall results from the EDSP Tier 1 fish assay indicated that atrazine exposure had no statistically significant effects on assay endpoints.

Mancozeb (case study provided by the US)

98. Mancozeb is a thyroid toxicant. Toxicity was manifested as alterations in thyroid hormones, increased thyroid weight, and microscopic thyroid lesions (mainly thyroid follicular cell hyperplasia), and thyroid tumors. Mancozeb is metabolized and environmentally degraded to ethylene thiourea (ETU); thus, some of the thyroid toxicity of Mancozeb may due to ETU since it is also known to disrupt thyroid hormone homeostasis. The oral exposure assessment is based on decreased thyroxine (LOAEL = 17.82 mg/kg/day) from a subchronic rat toxicity study. The inhalation exposure assessment is based on thyroid hyperplasia and decreased thyroxine (females) (LOAEL = 0.326 mg/L) from a subchronic rat inhalation study. The thyroid effects found in the mammalian (rat) studies indicate a potential endocrine effect for both human and ecological systems.

99. Three avian reproduction studies are available on mancozeb. Effects include a reduction in the weight of 14-day old surviving birds, hatchling weight, egg production, early and late embryo viability, hatchability, offspring weight at hatch, and a decline in the number of 14-day old survivors. These results can be due to a number of factors and cannot be clearly concluded as endocrine related.

100. In the California Red Legged Frog (CRLF) Risk Assessment for mancozeb, it was assumed that chronic exposure EECs and toxicity are mainly related to ETU. The endpoint determined from a chronic freshwater invertebrate toxicity test conducted with ETU was used to assess potential indirect effects to the CRLF via reduction of prey items (freshwater invertebrates). ETU adversely affected growth and reproduction of *Daphnia magna* at 4.1 ppm. Survival and lack of growth effects were observed on fathead minnow (*Pimephales promelas*) at a LOAEC of 2.19 ppb; however, this value was not used in the risk assessment.

101. ETU, which is a thyroid synthesis inhibitor, was found to alter metamorphic development and thyroid gland histology in the amphibian metamorphosis screening assay.

Vinclozolin (case study provided by the US)

102. The principal toxic effects induced by vinclozolin are related to its anti-androgenic activity: vinclozolin and two of its metabolites bind and inhibit the function of the androgen receptor. Chronic exposure of adult animals leads to Leydig cell tumors and short term exposures during critical developmental periods could potentially lead to male reproductive tract malformations. The acute dietary pesticide risk assessment was based on the most sensitive developmental effects noted following *in utero* exposure: decreased prostate weight, weight reduction in other sex organs, nipple/areolas development, and decreased ano-genital distance in male rats.

103. Results observed in the EDSP Tier 1 studies confirm vinclozolin's anti-androgen activity. The EPA risk assessment described above is consistent with the findings from the EDSP Tier 1 assays. In the EDSP Tier 1 fish study, male secondary sex characteristics, testicular degeneration, and male gonad weight and GSI were the most robust and sensitive endpoints which would be consistent with vinclozolin's activity as an AR antagonist. Vinclozolin was also positive in the rat Hershberger assay which is designed to detect androgen antagonists and agonists. In the rat male pubertal assay, vinclozolin showed a profile expected for an anti-androgen (*e.g.*, delayed puberty, testicular histopathology, and epididymis weight as well as increases in testosterone at higher doses).

104. In a modified fish life-cycle toxicity test, fathead minnows were exposed for 112 days under flow-through conditions to 0.12 mg total vinclozolin residues/L [vinclozolin, metabolite B (acid), and metabolite E (amide)]. In the F₀-generation the number of spawns/female was statistically-reduced and the number of eggs/spawn was notably higher in the vinclozolin treatment group. In the F₁-generation hatch survival was statistically-reduced both at the start and end of hatch compared to controls. However, because only one concentration was tested, the study could not be used to define a NOAEC and/or LOAEC.

The following part of this annex summarises the approaches to testing and assessment of endocrine disrupting substances proposed by the United States, Denmark and BIAC, and compares these approaches with the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. The document is based on contributions received from countries and BIAC, which are included in the appendices to this document.

US EPA Endocrine Disruptor Screening Program (EDSP)

105. The US EPA Endocrine Disruptor Screening Program (EDSP) is a two-tiered system for screening and testing the potential interactions of pesticide and non-pesticide chemicals on the estrogen, androgen and thyroid (EAT) hormonal systems with application to human health, animal wildlife and the environment.

Tier 1

106. The Tier 1 consist of a screening battery that includes *in vitro* and mammalian and non-mammalian *in vivo* screening assays, and was designed to cover multiple modes of action and provide corroborating information among assays within the battery that will support a weight-of-evidence approach.

107. The screening battery was designed to work as a whole. The basis for selecting a candidate assay to include in the battery involved: 1) the capacity of that assay to detect estrogenic- and androgenic-mediated effects by various modes of action including receptor binding (agonist and antagonist) and activation/transcription, reproductive steroidogenesis, and hypothalamic-pituitary-gonadal (HPG) feedback, and 2) the degree that *in vitro* and *in vivo* assays complemented one another in the battery. In addition, rodent and amphibian *in vivo* assays were selected for the proposed battery based on their capacity to detect direct and indirect effects on thyroid function (hypothalamic-pituitary-thyroidal, HPT, feedback). Thus, the robustness of the proposed Tier-1 Screening Battery is based on the strengths of each individual assay and their complementary nature within the battery to detect effects on EAT hormonal function. The Tier 1 battery is presented in the table below (compared with the levels of the OECD conceptual framework).

108. The complimentary and corroborating evidence among assays within the Tier-1 battery can be used with a weight-of-evidence approach to determine whether or not a test chemical interacts with the endocrine system. When all Tier-1 assays are performed and all assays are negative within the battery, it may be concluded that the test substance will not likely interact with EAT hormonal processes. If results from Tier 1 indicate that a substance does exhibit the potential to interact with E, A or T function, then more complex and definitive dose-response testing would be done in Tier 2 to further identify the potential hazard and to assess adversity and risk to the public and the environment.

109. The Tier 1 screening battery largely corresponds to the levels 2 and 3, and partly level 4 of the OECD Conceptual Framework. It is important, however, to note that the screening battery is recommended to be used as a whole.

Tier 2

110. The purpose of Tier 2 testing is to identify the effects and characterize dose-response relationship of EAT disruption in humans, fish and wildlife. Tier 2 is comprised of multigenerational tests in five taxa: mammals, fish, birds, amphibians and invertebrates. Unless a rationale exists to limit the test to 1 generation, tests for endocrine disruption will usually encompass 2 generations including effects on

fertility and mating, embryonic development, sensitive neonatal growth and development, and transformation from the juvenile life stage to sexual maturity.

111. The outcome of Tier 2 is designed to be conclusive in relation to the outcome of Tier 1 and any other prior information. Thus, a negative outcome in Tier 2 will supersede a positive outcome in Tier 1. Furthermore, each full test in Tier 2 has been designed to include those endpoints that will allow a definitive conclusion as to whether or not the tested chemical substance is disruptor for EAT in that species/taxa. Conducting all five tests in the Tier 2 testing battery would provide a more comprehensive profile of the effects a chemical substance or mixture could induce via EAT disruption mode(s)/mechanism(s) of action than would be the case if only a subset of tests or less comprehensive tests were performed. Considerations for determining whether the full battery of comprehensive tests should be implemented include an understanding of mechanisms of action, environmental fate and transport, persistence, potential for bioaccumulation, and potential exposure.

Table 1. Comparison of the US EPA EDSP approach with the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals

OECD Conceptual Framework			Approach in the U.S. EPA EDSP				
Level	Assays for mammalian toxicity	Assays for ecotoxicity	Stage	Description	Assays for mammalian toxicity	Assays for ecotoxicity	
Level 1: Sorting and prioritization based upon existing information	<ul style="list-style-type: none"> - Physical & chemical properties, e.g. MW, reactivity, volatility, biodegradability - Human & environmental exposure, e.g. production volume, release, use patterns - Hazard, e.g. available toxicological data 		Not described				
Level 2: <i>In vitro</i> assays providing mechanistic data	<ul style="list-style-type: none"> - ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis <i>in vitro</i> - Aryl hydrocarbon receptor recognition/binding - QSAR - High-throughput pre-screens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate) 		Tier 1 Screening Assays (mode of action)	<i>In vitro</i> assays providing mechanistic information / data	<ul style="list-style-type: none"> - Estrogen receptor (ER) binding (rat uterus or recombinant) - Estrogen receptor α (hERα) transcriptional activation (human cell line (HeLa-9903)) - Androgen receptor (AR) binding (rat prostate) - Steroidogenesis (human cell line (H295R)) - Aromatase (human recombinant) 		
Level 3: <i>In vivo</i> assays providing data about single mechanisms and effects	<ul style="list-style-type: none"> - Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor binding mediated hormone function - Others (e.g. thyroid) 	<ul style="list-style-type: none"> - Fish vitellogenin (VTG) assay (estrogenic related) 		<i>In vivo</i> assays providing mechanistic information / data on single or multiple endocrine mechanisms	<ul style="list-style-type: none"> - Uterotrophic (rat) - Hershberger (rat) - Pubertal female (rat) - Pubertal male (rat) - Amphibian metamorphosis (frog) 	<ul style="list-style-type: none"> - Fish short-term reproduction - Amphibian metamorphosis (frog) 	
Level 4: <i>In vivo</i> assays providing data about multiple mechanisms and effects	<ul style="list-style-type: none"> - Enhanced OECD TG 407 (endpoint based endocrine effects) - Male and female pubertal assays - Adult intact male assay 	<ul style="list-style-type: none"> - Fish gonadal histopathology assay - Frog metamorphosis assay 					

<p>Level 5: <i>In vivo</i> assays providing data on effects on endocrine & other mechanisms</p>	<ul style="list-style-type: none"> - One-generation assay (TG 415 enhanced) - Two-generation assay (TG 416 enhanced) - Reproductive screening test (TG 421 enhanced) - Combined 28-day/reproduction screening test (TG 422 enhanced) 	<p>Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)</p>	<p>Tier 2 definitive (dose-response) testing</p>		<ul style="list-style-type: none"> - Two generation mammalian assay (TG 416 enhanced) or - Extended one-generation assay (<i>currently being drafted</i>) 	<ul style="list-style-type: none"> - Avian two-generation reproductive effects* - Fish multigeneration reproductive effects* - Amphibian growth and reproduction test* - Invertebrate multigen (daphnia or copepod)* <p><i>* currently being validated</i></p>
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Denmark (Nordic countries)

112. Denmark supports the view that the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters may be used differently with regard to testing for toxicological effects than for testing for ecotoxicological effects. In principle, toxicological and ecotoxicological test methods are designed with particular reference to different protection purposes, as the use of ecotoxicological tests is aimed at protection on an ecosystem level (i.e. protection of populations) whereas toxicological tests are aimed at protection of a single species (humans) and furthermore single individuals.

113. Since criteria for assessment of endocrine disrupters have not yet been established within the chemicals regulation amongst other reasons due to the lack of proper test method, one of the focus areas in the Danish national strategy is development of test methods. Currently endocrine disrupters are identified case-by-case and in this work decisions based on all available and reliable information including preliminary indications are necessary. The above mentioned strategy and activities have resulted in a number of initiatives and projects related to the identification of endocrine disrupters and preventive measures.

114. The Nordic Coordination Group for the Development of Test Methods in Toxicology and Ecotoxicology (Nord-UTTE) in 2004 finalized a project called EDREG (OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters as a Basis for Regulation of Substances with Endocrine Disrupting Properties) which focused on how to interpret and use test results for regulatory purposes. The aim of this project was to assess and draw up a model for the use of the Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (confirmed in OECD in June 2002). The model should be a basis for regulatory initiatives in relation to substances with endocrine disrupting properties, in order to ensure that they are covered by the chemicals legislation.

115. The project focused on the health aspects, especially effects on estrogenic and androgenic activity with regard to effects on human reproduction (effects on thyroid system were included to a limited extent).

116. The first part of the report from this project includes a thorough assessment of each type of test at each level (only levels 2-5 of the Conceptual Framework are discussed, not level 1), specification of the endpoint for the test, and the reliability and relevance for effects in humans. In the second part of the report criteria were proposed for making conclusions as regards endocrine disrupting properties based on data according to the types of information described at the different levels of the Conceptual Framework. It aims at clarifying how to interpret and use test results when endocrine disrupters are assessed in a case by case approach, and in general how to interpret and use test results for regulatory purposes. The report has been published at the website of the Nordic Council of Ministers: (<http://www.norden.org/pub/miljo/miljo/sk/TN2004555.pdf>).

117. The outcome of this project served as a Nordic contribution to the discussion in EU about how to integrate endocrine disrupters in the new EU chemicals regulation for industrial chemicals, REACH. It also served as an input for discussions at the OECD level on the regulatory use of the OECD Conceptual Framework for endocrine disrupting chemicals. The main conclusions from the project are presented in the Table below.

Table 2. Conclusions based on the Nord-Utte EDREG project on the proposed regulatory use of the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals

OECD Conceptual Framework		Conclusions based on the Nord-Utte EDREG project
Level	Assays for mammalian toxicity	
Level 1: Sorting and prioritization based upon existing information	<ul style="list-style-type: none"> - Physical & chemical properties, e.g. MW, reactivity, volatility, biodegradability - Human & environmental exposure, e.g. production volume, release, use patterns - Hazard, e.g. available toxicological data 	Not discussed in the report of the Nord-Utte EDREG project
Level 2: in vitro assays providing mechanistic data	<ul style="list-style-type: none"> - ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis <i>in vitro</i> - Aryl hydrocarbon receptor recognition/binding - QSAR - High-throughput pre-screens - Thyroid function - Others (as appropriate) 	<p>Positive <i>in vitro</i> test results indicate potential ED activity and a potential for ED effects <i>in vivo</i>.</p> <p><i>In vitro</i> data can provide valuable mechanistic data that is useful for the design of further <i>in vivo</i> studies.</p> <p>The <i>in vitro</i> tests are relevant for effects in humans because many of these tests are based on hormone receptors or interaction with key enzymes or other key molecules involved in the regulation of the hormone levels. Chemicals that bind to these receptors or otherwise interfere with key processes of hormone regulation are therefore likely to cause effects in <i>in vivo</i> studies and on reproductive function in humans.</p> <p>Negative <i>in vitro</i> test results cannot be used to exclude potential EDC activity because of limitations such as inability or unknown capacity to metabolically activate toxicants and because EDC activity can occur through mechanism other than those tested in the <i>in vitro</i> test system.</p> <p>(Q)SAR models for ED activity and reproductive toxicity effects are under development but at present the use for priority setting and risk assessment is undecided.</p>
Level 3: in vivo assays providing data about single mechanisms and effects	<ul style="list-style-type: none"> - Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor binding mediated hormone function 	<p>Assays at level 3 provide an <i>in vivo</i> screening of potential endocrine disrupting activity of a substance. At present, it is uncertain to what extent the frog metamorphosis assay can be used for screening in relation to effects on humans</p> <p>Except for the frog metamorphosis assay, the assays at level 3 provide information about the potency of the compound <i>in vivo</i>.</p> <p>The positive outcome of the assays indicates potential for adverse effects in the reproductive developmental studies at level 5.</p> <p>However, a compound found negative in these assays may still have endocrine</p>

	- Others (e.g. thyroid)	<p>disrupting properties mediated through other mechanisms than those covered by these <i>in vivo</i> screening tests.</p> <p>The results (NOEL/LOEL) from the Uterotrophic and Hershberger assays can be used to perform preliminary risk assessment (until further studies are available), and are useful when considering hazard classification of a chemical for e.g. reproductive and developmental toxicity.</p>
Level 4: <i>in vivo</i> assays providing data about multiple mechanisms and effects	<ul style="list-style-type: none"> - Enhanced OECD TG 407 (endpoint based endocrine effects) - Male and female pubertal assays - Adult intact male assay 	<p>Assays at level 4 provide a thorough assessment of the potential endocrine disrupting effects of a substance in pubertal and young adults.</p> <p>Level 4 assays provide information about the potency of a compound to be investigated at level 5. Effects on various endpoints included in the assays can either be considered as adverse or represent an effect on a mechanism relevant for human e.g. changes in hormone levels. Therefore, these assays can be used to provide NO(A)ELs/LO(A)ELs to be used in human risk assessment until further studies are available.</p> <p>The intact male assay and the TG 407 may be more capable for detecting aromatase inhibitors and compounds affecting the steroid synthesis compared to the pubertal male assay. On the other hand, the two assays in intact young males may be less sensitive compared to the Uterotrophic and the Hershberger assay as well as the male and female pubertal assay.</p>
Level 5: <i>In vivo</i> assays providing data on effects on endocrine & other mechanisms	<ul style="list-style-type: none"> - One-generation assay (TG 415 & the draft TG on the extended one generation study, Cooper et al. 2007) - Two-generation assay (TG 416 enhanced) - Reproductive screening test (TG 421 enhanced) - Combined 28-day/reproduction screening test (TG 422 enhanced) - Developmental Neurotoxicity study (TG 426) 	<p>The reproductive toxicity studies provide adverse effect data and are especially useful for risk assessment as they indicate potential for effects in humans. The effects observed in reproductive toxicity studies may be due to other mechanism than endocrine effects, but the pattern of effects, e.g. decreased anogenital distance and malformations of reproductive organs in males, may indicate that endocrine effects are involved.</p> <p>Among the current OECD Test Guidelines for reproductive toxicity, exposure during all vulnerable periods of development is only performed in the two-generation study design. Late effects becoming manifest after weaning of the animals are partly covered in young adults, especially in relation to reproductive function and developmental neurotoxicity, but potentially important late effects are not assessed. Effects becoming manifest during ageing are not included in any guidelines for reproductive toxicity.</p> <p>A number of enhancements of the OECD Test Guidelines for reproductive toxicity for the detection of effects of EDCs seem relevant and lack of effects in reproductive toxicity studies can therefore at present not fully exclude the possibility for ED effects caused by chemicals tested negative.</p>

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118. BIAC supports the use of a tiered hierarchical scientific framework (comprising of 3 stages) in which validated screening assays are used to identify substances with endocrine activity and prioritize substances for further, more definite testing that provides data on adverse effects and dose response which are necessary for hazard and risk characterization. Definitive testing using validated harmonized protocols is necessary to identify adverse effects caused by alterations to endocrine system functions. Using such a tiered approach the results from definite tests must outweigh or supersede results from screening assays in guiding policy and management in both the public and private sectors.

119. The interpretation of data coming from testing using the tiered approach should be based on a weight-of-evidence evaluation. For hazard characterization the chemical industry supports the development of a weight-of-evidence evaluation process that consists of a comprehensive, objective, transparent and balances interpretation of the totality of scientific evidence regarding hormonal activity and adverse effects that might result in endocrine mechanisms.

120. The proposed 3-stage testing approach is presented below.

Stage 1: Initial Assessment

121. This step consists of the evaluation of all available data (including production volume and patterns of use, exposure information, predicted environmental properties (e.g. fate, persistence, bioaccumulation), toxicological data from existing studies), structure activity relationships ((Q)SARs) and molecular screening results. The evaluation of these data should be done using a weight-of-evidence approach. This should enable to rapidly recognize those substances where scientifically relevant data exist to permit more rapid prioritization and assessment.

This stage corresponds to level 1 of the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals.

Stage 2: Screening.

122. This step allows to efficiently and effectively develop information as to whether a substance has the potential to interact with one or more components of the endocrine system.

It includes *in vitro* assays and *in vivo* assays providing mechanistic information/data on single mechanisms and *in vivo* assays providing information on multiple endocrine mechanisms. For flexibility, the option to proceed directly to *in vivo* assays should not be precluded, nor should *in vitro* assay results be required if *in vivo* results are available (see table below).

The *in vivo* assays incorporate (i) substance-specific complexities that cannot be obtained from *in vitro* assays, including absorption, distribution, metabolism and excretion, and (ii) reflect the complex and dynamic homeostasis and operation of the intact endocrine system. Therefore, *in vivo* results would supersede *in vitro* results.

123. As the results from assays comprising the Stage 2 provide only mechanistic information and not evidence for adverse effects, these screening results do not indicate that a compound is an 'endocrine disruptor'.

124. A weight-of-evidence process needs to be implemented in order to integrate results across the complement of assays. Substances which are positive based on overall consideration of the weight-of-evidence in Stage 1 and 2 are considered to be high priority candidates for further evaluation in definitive tests (Stage 3). However, prior to initiating additional work, it is appropriate to consider the potential for

human exposure and potential for entrance into the environment. The term ‘potential endocrine disruptor’ could be easily misinterpreted, and generally use of this term should be avoided. From a scientific perspective, it is important to determine the overall weight-of-evidence of the performance of a substance in the screening assays/battery, as described above.

125. This stage largely corresponds to levels 2, 3 and 4 of the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals

Stage 3: Definitive Testing

126. This step aims at evaluation of apical endpoints, adverse effects and dose response to accurately and effectively identify and characterize the hazard(s) from chemicals.

127. The array of assays in Stage 3 (see Table below) should be viewed as a matrix of available options, and not as a sequential list of assays and tests. It would not be necessary to conduct all tests, but the appropriate tests should be selected. In the interests of flexibility and minimizing animal and resources, for example the enhanced repeat dose study and the shorter-scope reproduction/developmental tests would not be required in cases where a longer scope test is already available or planned.

Overall, hazard characterization for hormonally active chemicals requires an objective evaluation of whether the effects produced are adverse and whether adverse effects are due to a hormonal activity of the chemical. This includes consideration of the proposed Stage 2 Screens and Stage 3 Definitive Tests, and results from standard toxicity studies.

128. Risk characterization requires integration of scientific data and knowledge of hazard, dose-response and exposure, as well as an evaluation of the foundations of the hazard data, inferences drawn from the data, and inherent uncertainties. In cases of low potency and low or negligible actual and potential exposures, test methods such as the Repeat Dose Study (TG 407) or the Reproduction/Developmental Toxicity Screening Tests (TG 421/422) could be used to provide dose-response data of effects on apical endpoints. This would serve to focus the more extensive testing only on substances that have high production volume and the highest potential for human and ecological exposures. In all cases, results from definitive testing outweigh or supersedes results from screening.

129. This stage corresponds to level 5 of the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals.

130. It is important to stress that all stages should make use of standardized, validated and internationally harmonized test methods. In case that new and novel methods, and studies with non-standard species which provide important scientific information, are used, BIAAC suggests doing a thorough review of the study report, if possible replicating the study in another laboratory. Then, if the findings are shown to be reproducible, then two courses of action would be advised:

- subject the test method to standardization and validation within the OECD TG program (EDTA) or within a similar formal program sponsored by a national government or recognized scientific organization (e.g., ISO, ASTM),

or

- evaluate the substance of concern in one of the wide variety of existing validated test method using standardized OECD TG methods and species (or similarly validated scientific methods). Results of this study would then be evaluated within the tiered hierarchical OECD EDTA Framework. In general, this would be the preferred course of action.

Table 3. Comparison of the approach proposed by BIAC with the OECD Conceptual Framework for the Testing and Assessment of ED Chemicals.

OECD Conceptual Framework			Approach proposed by BIAC			
Level	Assays for mammalian toxicity	Assays for ecotoxicity	Stage	Description	Assays for mammalian toxicity	Assays for ecotoxicity
Level 1: Sorting and prioritization based upon existing information	<ul style="list-style-type: none"> - Physical & chemical properties, e.g. MW, reactivity, volatility, biodegradability - Human & environmental exposure, e.g. production volume, release, use patterns - Hazard, e.g. available toxicological data 		Stage 1: initial assessment to set priorities for further evaluation	<ul style="list-style-type: none"> - Predicted environmental properties, e.g., fate - Production volume and pattern of use, available exposure information - Toxicological data, especially endocrine-relevant data (i.e., results of histopathology on reproductive organs from repeat dose studies, developmental or reproductive toxicological information.) - Structure activity relationship - Molecular screening results 	<p>All relevant studies</p> <p>It is presumed that SAR for receptor mediated modes of action will be applicable across mammalian orders.</p> <p>All relevant studies</p>	<p>It is presumed that the data review will incorporate both mammalian and ecotoxicological issues.</p> <p>It is presumed that SAR will be applicable across vertebrate classes. Invertebrates may have unique receptors.</p> <p>All relevant studies</p>

Note: Assays marked in “**bold**” indicate differences between the OECD Conceptual Framework and the BIAC approach (placed at different levels).

OECD Conceptual Framework			Approach proposed by BIAC			
Level	Assays for mammalian toxicity	Assays for ecotoxicity	Stage	Description	Assays for mammalian toxicity	Assays for ecotoxicity
Level 2: <i>In vitro</i> assays providing mechanistic data	- ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis <i>in vitro</i> - Aryl hydrocarbon receptor recognition/binding - QSAR - High-throughput pre-screens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)		Stage 2 Screening Assays (mode of action)	<i>In vitro</i> assays providing mechanistic information / data	- E and R receptor binding assays - Transfected mammalian cell assays (ER, AR, TR) - <i>In vitro</i> Aromatase and steroidogenesis	It is presumed that receptor binding will in principle be applicable across vertebrate classes and to any invertebrates expressing similar receptors.
Level 3: <i>In vivo</i> assays providing data about single mechanisms and effects	- Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor binding mediated hormone function - Others (e.g. thyroid)	- Fish vitellogenin (VTG) assay (estrogenic related)		<i>In vivo</i> assays providing mechanistic information / data on single endocrine mechanisms	-Uterotrophic screening assay (estrogen and anti-oestrogen) -Hershberger screening assay (androgen and anti-androgen)	-Fish screening assay (vitellogenin and secondary sex characteristics) -Frog metamorphosis assay
Level 4: <i>In vivo</i> assays providing data about multiple mechanisms and effects	- Enhanced OECD TG 407 (endpoint based endocrine effects) - Male and female pubertal assays - Adult intact male assay	- Fish gonadal histopathology assay - Frog metamorphosis assay		<i>In vivo</i> assays providing mechanistic information / data on multiple endocrine mechanisms	- Enhanced TG407* - Male Pubertal assay - Female Pubertal assay - Adult Intact Male assay	OECD Fish Screening Assay (VTG and secondary sex characteristics as mandatory endpoints; other endpoints are optional)

Note: Assays marked in “**bold**” indicate differences between the OECD Conceptual Framework and the BIAC approach (placed at different levels).

* Remark: Depending on the situation a TG 407 would suffice to establish a NOAEL in selected instances. Such instances could include, for example, substances with low potency, minimal human exposure likely intermediates or substances manufactured in closed system, and limited potential for environmental release. This would serve to focus the more extensive testing only on substances that have high exposure potential.

OECD Conceptual Framework			Approach proposed by BIAC			
Level	Assays for mammalian toxicity	Assays for ecotoxicity	Stage	Description	Assays for mammalian toxicity	Assays for ecotoxicity
Level 5: in vivo assays providing data on effects on endocrine & other mechanisms	<ul style="list-style-type: none"> - One-generation assay (TG 415 enhanced) - Two-generation assay (TG 416 enhanced) - Reproductive screening test (TG 421 enhanced) - Combined 28-day/reproduction screening test (TG 422 enhanced) 	Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)	Stage 3 Definitive Testing: evaluation of apical endpoints, adverse effects and dose response for hazard identification and characterization	Reproduction/developmental tests – shorter scope – includes in utero exposure, developmental, and reproductive capacity endpoints	<ul style="list-style-type: none"> One generation reproductive toxicity (TG 415) Two generation reproductive toxicity (TG 416) Reproductive /developmental screening test (TG 421) Combined repeat dose with reproduction / developmental screening (TG 422) (TG407* (as adopted on October 03.2008) depending on the exposure situation as the method does not include the reproductive phase) [Enhanced one generation reproductive toxicity - if and when a final OECD TG is developed] 	<ul style="list-style-type: none"> - Partial and full life cycle assays in fish, birds, amphibians and invertebrates (developmental and reproduction)

Note: Assays marked in “**bold**” indicate differences between the OECD Conceptual Framework and the BIAC approach (placed at different levels).

* Remark: Depending on the situation a TG 407 would suffice to establish a NOAEL in selected instances. Such instances could include, for example, substances with low potency, minimal human exposure likely intermediates or substances manufactured in closed system, and limited potential for environmental release. This would serve to focus the more extensive testing only on substances that have high exposure potential.

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Annex 4: Workshop Agenda**Tuesday, 22nd September 2009**

- 9:00 – 9:05** **Opening and welcome** (*Claus Torp, director, Danish EPA*)
- 9:05 – 9:15** **Setting the scene** – presentation of the objectives of the meeting - *Secretariat*
- 9:15 – 10:00** **Status of OECD Work on Endocrine Disrupters** –*Secretariat*
- Test Guidelines: available TGs, TGs under preparation, methods under validation
 - The updated OECD Conceptual Framework
- 10:00 – 12:30** **Activities on testing, assessment and management of ED in the different regions**
- a) Presentation from US (30 min – *Steve Bradbury*)
- Coffee/tea break (30 min)*
- b) Presentation from E.U. (30 min) – *Peter Korytar and Pia Juul Nielsen*
- c) Presentation from Japan (30 min) – *Jun Kanno and Taisen Iguchi*
- d) Questions and discussion (30 min)
- 12:30 – 13:30* *Lunch*
- 13:30 – 13:45** **Introduction to the breakout groups discussions on the use of TGs and other tools for the assessment of ED – including the presentation of the proposed revision of the Conceptual Framework** – *Agnieszka Kinsner-Ovaskainen on behalf of the OECD Secretariat*
- 13:45 – 17:00** **Breakout group discussions: The use of TGs and other tools for the assessment of ED**
(*coffee/tea break when appropriate*)
- The participants will be divided into four groups: *i*) human health, *ii*) ecotoxicity, *iii*) two mixed (health & eco)
- 17:00 – 18:00** **Plenary session: Report from the breakout groups**
(*10 min. presentation from each group*)
- 19:00** **Diner** (*Restaurant Søren K, Søren Kirkegaards Plads 1, Copenhagen*)

Wednesday, 23rd September 2009

9:00 – 9:30 **The use of TG and other tools for the assessment of endocrine disrupters – views from ENV NGOs – Gwynne Lyons**

9:30 – 10:00 **The use of TG and other tools for the assessment of endocrine disrupters – views from BIAC – Rick Becker**

10:00 – 10:30 **Plenary discussion**

10:30 – 11:00 *Coffee/tea break*

11:00 – 12:00 **General wrap-up from Day 1, introduction to breakout group discussion and if relevant presentation of additional questions (to be decided by the steering group on the basis of Day 1 discussions)**

12:00 – 13:30 *Lunch*

13:30 – 16:30 **Breakout group discussions (continued) and formulation of recommendations (coffee/tea break when appropriate)**

16:30 – 18:00 **Plenary session – report from breakout groups and general discussion**

Thursday, 24th September 2009

9:00 – 12:00 **Plenary session**

Wrap-up, conclusions. General recommendations, next steps.
Presentation of the outline of the workshop report. *(coffee/tea break when appropriate)*

12:00 – 13:00 **Lunch**

Annex 5: Questions to the breakout groups**A) Which topics/data/information are essential for different types and levels of decision making?**

How much confidence is needed for different types and levels of decisions for ED assessment?

This includes considerations concerning:

- 1) *Adverse effects* caused by EDC and *the level of evidence*
- 2) The *scientific basis* for linking such endocrine activity causally to “adversity of effects” for man and/ or organisms in the environment
- 3) The *potency* and nature of endocrine activity
- 4) If and how the above mentioned considerations are used together with *other relevant information* (intrinsic properties of the substance as well as emission/exposure potential/considerations)

Adverse effects caused by EDCs:

- Discuss indications that “adverse effects” may be triggered by endocrine active substances. In these discussions, consider:
 1. Understanding/definition of “adverse effects” (see Background document on definitions)
 2. The scientific basis for linking such endocrine activity causally to “adversity of effects” for man and/or the environment

Level of evidence

ED is regarded as a mode of action of particular concern. Identify the different types of adverse effects caused by EDCs. For other substances of particular concern the amount/level of evidence (CMR in relation to GHS) is being used to conclude either:

1. proven status (CMR cat. 1) or
 2. substantiated suspected status (CMR cat 2)
- Discuss whether a similar distinction between *proven and suspected EDCs* may be relevant, warranted or of regulatory usefulness (see Background document on definitions:
 1. “an endocrine disrupter is an exogeneous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function”
 2. “a potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism”

Principles of hazard and risk assessment

- Discuss whether and how information regarding the *potency* of ED active substances can be used. Is it possible to identify a *threshold* for individual ED active substances?
- Discuss the *principles of hazard and risk assessment* of substances with ED activity. In these discussions, consider:
 1. human health and/or ecotoxicity
 2. (anti)oestrogenic chemicals and/or (anti)androgenic chemicals and/or thyroid hormone interfering chemicals

B) How the available TGs and the other tools can/should be used together with weigh of evidence based conclusions?

- Identify and discuss ways forward in relation to *whether all types of effects caused by ED are currently adequately covered* by available and draft OECD TGs (use e.g. the (revised) Conceptual Framework for this)
- Regarding the Conceptual Framework, besides referring to types of information in relation to its nature and comprehensiveness, do the various levels of the framework also include reference to other considerations, such as strength for decision making?
- Discuss how non standard test data on ED may be used and e.g. whether non standard test data should always be superseded by data from adopted OECD TGs.
- Discuss whether information from lower levels of the Conceptual framework should always be superseded by information from higher levels, and whether and if so when there is a need for repeating positive or negative studies
- How to use non test information (Threshold of Toxicological Concern (TTC), (Q)SAR predictions, chemicals categories and read across) concerning ED in making conclusions?
- Should further guidance be developed?
- Are there new species, endpoints and response variables that should be further validated and/ or standardised for inclusion in *new* or revised *OECD TGs*? Is there in particular a need for a fish *in vitro* screen in addition to the mammalian *in vitro* screens?

C) Which are the *data or knowledge gaps* that should be particularly addressed in the near future?

- Is there an urgent need for extensive research in particular areas or endpoints and response variables?

D) How are the *Test Guidelines and other tools* used in the different countries for decision making concerning identification and evaluation of chemicals with endocrine activity/disrupting properties?

- Identify commonalities and differences

In relation to national decision making framework on ED, discuss the following topics:

- How is the OECD *Conceptual framework* understood and possibly reflected in your *decision making framework* concerning ED?
- How are TG data and other types of information being used in the decision of whether *further information or testing* on suspected EDC is warranted? – in particular:
- How and when is *exposure*-related information being used?
- How are other chemicals intrinsic properties being used together with ED activity in your priority setting:
 1. *Non test information*
 2. *In vitro* test data
 3. *In vivo* data indicating endocrine activity but when test data from the highest level of the conceptual framework is not (yet) available:
 - Oestrogenic activity
 - (anti)androgenicity
 - Thyroid hormone activity
 - Other?
 - When further information/testing on a suspected EDC is prioritized/decided to be necessary, discuss how to handle hazard and risk communication and management. (Including: “What to do while waiting for more definitive information?”)
- Discuss the same issues as above in relation to various *other regulatory decision* making issues, such as:
 1. Need for classification and labelling
 2. Need for providing information to the public
 3. Need for giving incentives to industry to consider substitution
 4. Need for setting conditions for production, emission and/or use (Emission norms, Limit values, Restriction of production or certain or all uses, Authorisation (with conditions) for production and certain or all use)
- Discuss pros and cons for different types of decision making framework (*hierarchical/decision tree, weigh of evidence, etc*).
- Do you in your decision making framework take account of the possible *combined effects* of simultaneous exposure to ED active and similarly acting chemicals? If so how?
- Exemplify how *WoE approaches* are being used – and try to make a generalized description of this.

Annex 6: Conceptual Framework for the Testing and Assessment of Endocrine Disrupters Chemicals

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

<p>Level 1 Sorting & prioritization based upon existing information</p>	<ul style="list-style-type: none"> - physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability, - human & environmental exposure, e.g., production volume, release, use patterns - hazard, e.g., available toxicological data 	
<p>Level 2 <i>In vitro</i> assays providing mechanistic data</p>	<ul style="list-style-type: none"> - ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis <i>in vitro</i> - Aryl hydrocarbon receptor recognition/binding - QSARs 	<ul style="list-style-type: none"> - High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
<p>Level 3 <i>In vivo</i> assays providing data about single endocrine Mechanisms and effects</p>	<ul style="list-style-type: none"> - Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non-receptor mediated hormone function - Others (e.g. thyroid) 	<ul style="list-style-type: none"> - Fish VTG (vitellogenin) assay (estrogenic related)
<p>Level 4 <i>In vivo</i> assays providing data about multiple endocrine Mechanisms and effects</p>	<ul style="list-style-type: none"> - enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay 	<ul style="list-style-type: none"> - Fish gonadal histopathology assay - Frog metamorphosis assay
<p>Level 5 <i>In vivo</i> assays providing data on effects from endocrine & other mechanisms</p>	<ul style="list-style-type: none"> - 1-generation assay (TG415 enhanced)¹ - 2-generation assay (TG416 enhanced)¹ - reproductive screening test (TG421 enhanced)¹ - combined 28 day/reproduction screening test (TG 422 enhanced)¹ <p>¹ Potential enhancements will be considered by VMG mamm</p>	<ul style="list-style-type: none"> - Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)

Notes to the Framework

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

Note 2: In level 5, ecotoxicology should include endpoints that indicate mechanisms of adverse effects, and potential population damage

Note 3: When a multimodal model covers several of the single endpoint assays, that model would replace the use of those single endpoint assays

Note 4: The assessment of each chemical should be based on a case by case basis, taking into account all available information, bearing in mind the function of the framework levels.

Note 5: The framework should not be considered as all inclusive at the present time. At levels 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. Once developed and validated, they will be formally added to the framework.

Note 6: Level 5 should not be considered as including definitive tests only. Tests included at that level are considered to contribute to general hazard and risk **assessment**.

Annex 7: Extract from the breakout group contributions (not reviewed in detail by the plenary)

This annex only reports on breakout group contributions that may be useful for further discussions regarding implementation of the Workshop recommendations.

Conceptual Framework:

A revised Conceptual Framework including 4 levels instead of 5 was proposed by the small group that prepared the workshop. From the discussions in the breakout groups, it appeared that more discussions would be necessary to reach agreement on a revised Conceptual Framework. Groups 2 and 4 made specific comments on the proposed revised Conceptual Framework. These comments are not included in this document to avoid confusion; however, they will be used for any further discussion on the conceptual framework.

Group 1

Changing the number of levels of the Conceptual Framework was not thought to be important. Some members of the group recommended not merging levels 3 and 4 and keeping a five level structure. It was pointed out that the uterotrophic and Hershberger are not comparable to an intact animal. In vivo studies are multi-target; the Hershberger and uterotrophic are artificial systems because they use animals without competent HPG axes. The biological meaning of level 3 and 4 are different. It would make more sense to keep the five levels but refine their definitions. Level 3 would consist of screening level in vivo assays. Level 4 would be include apical endpoints but would not be definitive. Level 5 would be the definitive tests. It was felt that the five level system would be better suited to the future when upstream events are used in hazard assessments. TG 421 and 422 should move from level 5 to level 4.

Some proposed defining the five levels as follows: Level 1: same ; Level 2: same; Level 3: *in vivo* screens using artificially manipulated animals; Level 4: *in vivo* assays providing apical endpoints; Level 5: definitive one-generation or multigeneration tests.

The members supporting the four level system believed that grouping all of the *in vivo* screens together was simpler and easier to understand.

Should carcinogenicity assays be included in the top level? Should we list every assay that could provide useful information regarding the endocrine system, or should it be more limited to provide guidance regarding assays that would be most frequently used in an ED testing strategy? It was concluded that the conceptual framework is a toolbox and should only contain studies that would be used in a testing strategies; therefore, such studies as the carcinogenicity bioassay would not be included.

Group 2

Group 2 is content with the proposed change to a four-level CF, as this appears to be in line with the types of currently available tools. In general, higher level endocrine-sensitive tests (e.g. fish lifecycle tests) can supersede the need for some lower level tests (e.g. in vitro tests; screening assays) providing their endpoints take account of the suspected mode of action.

Group 3

It is recommended to add TG 408 (90 day repeat dose toxicity assay) to Level 4 of the existing Conceptual Framework).

Group 4

Group 4 has no strong feelings whether the CF needs being revised now as proposed. There was consensus that a revision could be considered within a few years where more experience and scientific progress has been gained. Consider adding the pubertal assay to the OECD Test Guidelines

Guidance related to the use of the Conceptual Framework

A proposed Structure for this guidance is:

- Part 1: Description of the different levels: scenarios of use of Test Guidelines at each level; what is a positive or negative result? How best to use data? What is logical progression of tests? Presentation of different scenarios for different substances (eg., for data rich chemicals and data poor chemicals)
- Part 2: Description of the Test Guidelines: aim, principle, mandatory endpoints; Interpretation (including limitations) of positive/negative result; logical use of data; logical progression of testing (where do you go to further evaluate implications of result?).
- Part 3: Using actual case studies (as provided by member countries, ECETOC), guidance on interpretation of data from endocrine disrupter Test Guidelines.(Group 3)

Assessment

- It may be prudent to avoid making an important regulatory decision based on one run of a single in vitro test. At the least, it is recommended that conduct of a repeat in vitro test should be considered in support of a critical decision, in addition to considering all other relevant information. The reason for this is that laboratories have sometimes been unable to replicate certain data indicative (or not) of possible endocrine disruption (Group 2).

Exposure

- It is recommended that the OECD Task Force on exposure assessment consider whether some tools may already be available to consider / can be developed in a medium timeframe (Group 4).