Workshop: Guidance on Interpreting Endocrine Disrupting Effects 29-30 June 2009, Barcelona

Workshop Report No. 16
and Addendum

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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Brussels, October 2009

ECETOC WORKSHOP REPORT No. 16

European Centre for Ecotoxicology and Toxicology of Chemicals
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Workshop: Guidance on Interpreting Endocrine Disrupting Effects

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Brussels, 22nd December 2009

ADDENDUM TO ECETOC WORKSHOP REPORT NO. 16

WORKSHOP: GUIDANCE ON INTERPRETING ENDOCRINE DISRUPTING EFFECTS - 29-30 JUNE 2009, BARCELONA

Prof. Skakkebaek indicated that the views he had expressed during the workshop were not reflected in the workshop report and therefore he does not support the ECETOC workshop document.

Dr. Bram Versonnen indicated that the general view, expressed during the workshop, of the ECETOC approach is that it represents a good starting point to further develop a scientifically valid approach. One important remark on the approach is that it is not readily applicable to data poor substances and is certainly more suited for data rich substances. In addition he noted that when "human relevance" of a particular mechanism of action is questioned, it should not preclude the consideration that this mechanism of action may lead to potential adverse effects in eco relevant species. Finally the debate on "how to consider endocrine disruption relative to other modes of action of toxicity" for him was not clear cut since some scientists in the audience argued that the endocrine system, in an organism, is indeed different to other physiological systems with respect to chemical toxicity."

ECETOC apologises for the miswriting of one participant's name in the hard copies that were distributed when this report was first published: Dr. José Manuel Navas should have read **Dr. José Maria Navas** (corrected in the PDF version).

1. SUMMARY

This report documents the outcome of a workshop organised by ECETOC to discuss 'Guidance on Interpreting Endocrine Disrupting Effects'. The workshop was held in Barcelona on the 29th and 30th of June 2009. Fifty-five invited experts (from academia, regulatory bodies and industry) discussed an approach developed by an ECETOC Task Force and distributed to participants in the form of a Task Force report (ECETOC, 2009). This report outlined an approach developed by the Task Force providing guidance in the form of flowcharts that could be used as a decision tree for the identification of endocrine disrupting effects in human health (toxicology) and environmental assessments (ecotoxicology). The aim of the workshop was to assess the suitability of such an approach. It was also intended as an open forum for critical analysis and an opportunity to propose improvements to the scheme.

The workshop consisted of a series of invited presentations. The first outlined the regulatory background to the issue. The second reported on German national initiatives to develop toxicology criteria for endocrine disrupters. This was followed by presentations from the ECETOC Task Force introducing the ECETOC approach, including detailed explanations (with case studies) for its application in the toxicology and ecotoxicology fields.

The presentations were followed by four syndicate discussion sessions, each addressing specific issues:

The first breakout group directly evaluated the suitability of the ECETOC approach (see Section 4.1). This group endorsed the ECETOC approach as a means of identifying endocrine disrupting effects in a structured manner. However, it was acknowledged the approach was best suited for data rich substances (such as plant protection products) and further guidance may be required for substances where less data are available. Considerations of mode of action coverage, identifying what constitutes an adverse effect (particularly in ecotoxicity assessments), weight of evidence approaches and the interplay between toxicology and ecotoxicology studies were also discussed.

The second breakout group discussed the appropriateness of risk assessment for endocrine disrupting substances (see Section 4.2). There was a strong consensus that it is scientifically inappropriate to base decisions on hazard alone. A thorough risk assessment of all the factors, which may be involved in evaluating the potential risk of using a substance with endocrine disrupting properties, is required. However, uncertainties in estimating exposures in target animals at various stages of maturation was discussed, although, the group acknowledged that it was better to assess such issues rather than to reject a substance based on hazard potential alone.

The third breakout group discussed whether there was a basis to treat endocrine disruption differently to any other mode of action (see Section 4.3). There was a consensus that there was no basis to treat endocrine disruption differently. However, the discussions highlighted several

points when additional attention was required specifically in relation to low dose effects, timing of exposure and cumulative exposure.

The fourth breakout group discussed the way forward in developing science-based legislation (see Section 4.4). The group agreed that the framework for evaluating endocrine disrupters should be adapted as a function of the legislation. In the short term, for implementing the revision to the plant protection products directive 91/414 (EC, 2006a), it is important to use risk principles and to develop guidance documents built upon integrating hazard evaluation with exposure assessment. The ECETOC guidance provided a structured, science-based process for assessments. It would be useful if REACH (EC, 2006b), the plant protection products and biocides directives used the same evaluation principles and approaches for determining endocrine disruption. The ECETOC guidance was a very useful starting point for such a consistent approach. However, consideration should be given to further work to improve the ECETOC approach. This may be achieved by more case studies of applications of the ECTOC framework and to have additional venues for more in depth discussions.

In conclusion, there was participant endorsement of the ECETOC approach as a science-based evaluative framework. However, areas for further development and improvement were raised. In particular, it was suggested that the systematic and structured approach of the WHO/IPCS conceptual framework for evaluating the mode of action for cancer and non-cancer endpoints be included. In general the participants supported the view that toxicity resulting from endocrine disruption should be subject to risk assessment as is the case for other forms of toxicity. The ECETOC guidance scheme is an appropriate approach. Greater consideration of data poor substances is needed. The ECETOC Task Force is grateful for the expert input and intends to use the thinking and suggestions proposed at the workshop to refine the guidance.

2. WORKSHOP OVERVIEW

2.1 Introduction

Endocrine disrupting properties require specific evaluation under the REACH, revised plant protection products (91/414) and biocides directives. Consequently there is a need to scientifically define criteria that indicate a substance has endocrine disrupting properties.

The REACH regulation indicates that substances (e.g. intermediates, raw materials and formulation inerts) having endocrine disrupting properties will require further investigation. A number of factors (e.g. substitution, exposure control) will be taken into account before such compounds can be authorised for use. Therefore, continued use of such substances will be restricted.

In the draft revision of the 91/414 plant protection products directive, active substances in products considered to have endocrine disrupting properties that may be of toxicological significance in humans or non-target organisms will not be approved.

For both chemicals and pesticides, a definition of endocrine disruption is not elucidated in either directive. Therefore, there is a significant possibility that different interpretations of what is or is not an endocrine disruption effect might lead to inappropriate classification of certain chemicals/pesticides as endocrine disrupters. This would have a serious impact on the registration, use and movement of such substances and, hence, it is critical that the term 'endocrine disruption' is defined in a scientifically sound way. Moreover, clear guidance is needed on the nature and quality of technical data required to conclude that a substance induces endocrine disruption leading to adverse effects through modes of action relevant to humans and non-target organisms.

Consequently, an ECETOC Task Force was formed in June 2008 to address the issue and in May 2009 their Task Force report was published (ECETOC, 2009). The report addressed the key issues, as viewed by the industry representatives of the Task Force. Further, the report developed guidance in the form of series of flowcharts that could be used as a decision tree for the identification of endocrine disrupting effects in mammalian, fish and amphibian, bird and wild mammal assessments. The report was circulated in advance to the participants of the workshop (see Section 2.2). The aim of the workshop was to challenge and improve the thinking presented in the Task Force report.

2.2 Workshop structure

Fifty-five scientific experts from academia, governmental agencies and industry participated in a workshop held in Barcelona on the 29th and 30th of June 2009. It opened with plenary sessions, followed by syndicate sessions, where four themes were discussed:

- Evaluate the ECETOC approach as a concept for identifying endocrine disrupting effects.
- Evaluate the appropriateness of risk assessment in managing endocrine disruption.
- Evaluate whether there is any basis to treat endocrine disruption differently than other mode of action (MoA).
- The way forward in developing science-based legislation.

The discussions from the breakout groups were shared with all participants in a final plenary session where several recommendations were made and conclusions drawn. A list of workshop participants is given in Appendix A, and the programme is detailed in Appendix B.

2.3 Workshop objectives

An ECETOC Task Force had been developing 'Guidance on identifying endocrine disrupting effects'. The aim of the workshop was to introduce, evaluate and discuss improvements to this guidance in order to build a framework of when one can conclude whether a chemical does or does not have endocrine disrupting properties.

3. PRESENTATION SUMMARIES

3.1 European regulatory perspective on endocrine disruption (REACH and the new Regulation on PPPs, repealing 91/414/EEC)

Bram Versonnen[1], Wim de Coen[1], Rémi Lefèvre[1], Kirsi Sihvonen[1], Jörg Lebsanft[1], Jukka Malm[1], Wolfgang Reinert[2]

[1] European Chemical Agency – ECHA, Directorate B, Assessment

[2] European Commission, Directorate General Health and Consumers, Unit Chemicals, Contaminants and Pesticides, SANCO E3

3.1.1 The new upcoming Regulation on plant production products, repealing 91/414/EEC

The main objectives of the new proposal are:

- To protect human health and environment.
- To safeguard competitiveness of agriculture.
- To improve the functioning of the internal market.
- To speed up decision making.

The key issues (impact assessment) are:

- National provisional authorisations that are introduced.
- The zonal mutual recognition.
- The comparative assessment and substitution principle.
- Data protection and data sharing for renewal of approvals.

The Regulation has been approved by the Parliament, is expected to be adopted by the Council in the third quarter of 2010 and enter into force in 2011.

Importantly, the criteria for approval in the draft Regulation (Annex II.3) state that the following substances shall normally not be authorised: Carcinogens, mutagens and reproductive toxicants class 1 and 2, persistent organic pollutants, persistent-bioaccumulation-toxic substances, very persistent-very bioaccumulation substances, endocrine disrupters. However, carcinogens and reproductive toxicants class 1 and 2 and endocrine disrupters can be exempted from this if negligible exposure can be shown (approval possible for 5 years).

Further, the European Commission has to provide specific scientific criteria within four years for defining endocrine disrupters. In the meanwhile, there is a transitional regime, where

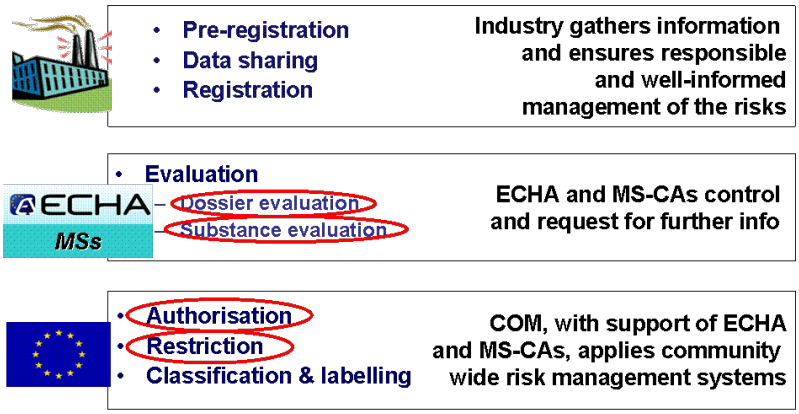
carcinogens and class 3 / reproductive toxicants class 3 + toxic substances shall / may be considered as endocrine disrupters.

3.1.2 REACH Regulation

The aims of the Regulation as stated in Article 1 of the legal text are:

- '…to ensure a high level of protection of human health and the environment… as well as the free circulation of substances on the internal market…';
- '…to ensure that they manufacture, place on the market or use such substances that do not adversely affect human health or the environment…' (→ burden of proof to industry).

Endocrine disruption and 'equivalent concern' are mentioned a number of times in the REACH legal text (e.g. Article 49, 57, 138, Annex I, II, XV). Although tests aiming specifically at endocrine disruption (e.g. vitellogenin assays for the environment) are not part of the standard information requirements outlined in annexes VII to IX, *any other relevant physicochemical, toxicological and ecotoxicological information that is available* has to be submitted by the registrant. The main processes of REACH are shown in the Figure below. The processes where endocrine disruption is likely to be dealt with are circled:



Under Authorisation, *substances – such as those having endocrine disrupting properties* can be included in Annex XIV (the list of substances subject to authorisation), and hence industry is not allowed to place on the market or use these substances unless industry has an authorisation granted by the Commission.

Under Restrictions, manufacture, use and/or placing on the market of a substance on its own, in preparation or in an article can be restricted when an unacceptable risk to human health or the environment and this risk needs to be addressed on a Community-wide basis. Industry has to comply with the conditions of the restriction in Annex XVII for the substance, but no specific dossier has to be submitted.

Substance Evaluation aims at the clarification of a concern for human health or environment and provides a mechanism for Member State Competent Authorities to require registrants to obtain and submit additional information to address the initial concern. This information can go beyond the information requirements mentioned in Annex VII – IX of the legal text.

Existing data on the endocrine disrupting potential of substances can be assessed under Dossier Evaluation. Specific testing to investigate the endocrine disruptive potential of substances is not part of the REACH standard requirements, but some tests cover endocrine related endpoints and all existing data (including on the endocrine disrupting potential) need to be submitted.

An appendix to the Guidance document 7b[1] of the 'Information requirements and chemical safety assessment' guidance, provides a framework to evaluate existing information on endocrine disruption. The emphasis of this appendix is on the aquatic environment.

In conclusion:

- The endocrine disruptive potential of substances is covered in the new draft PPP regulation.
- Specific testing on the endocrine disruptive potential of substances is beyond the standard REACH information requirements, although all *existing* information has to be submitted and can be evaluated.
- The endocrine disruptive potential of substances is further covered under other processes in REACH beyond registration and Dossier Evaluation, namely in Authorisation, Restrictions and Substance Evaluation.

[1] Guidance on information requirements and chemical safety assessment, Volume 5: Endpoint specific guidance for environment, chapter R7b: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm?time=1249469425

3.2 BfR activities for establishment of assessment and decision criteria for substances with endocrine disrupting properties under the new EU legislation for pesticides and biocides

Karen I. Hirsch-Ernst and Roland Solecki
Federal Institute for Risk Assessment (BfR), Berlin, Germany

The draft of the new EU plant protection regulation from January 2009 names endocrine disrupting properties as one of the cut-off criteria for the approval of active substances which are aimed to be used in plant protection products. Furthermore, according to the draft of the proposed new biocide regulation from June 2009, the use of active substances exhibiting endocrine disrupting properties is to be restricted.

While the new plant protection regulation clearly states that an active substance, safener or synergist shall only be approved if it is not considered to have endocrine disrupting properties that may cause adverse effects in humans, unless the exposure of humans under realistic proposed conditions of use is negligible, it fails to provide conclusive scientific criteria for risk assessment. Measures concerning specific scientific criteria for the determination of endocrine disrupting properties are to be presented by the Commission within four years.

To address this need, the BfR will be suggesting scientific assessment and decision criteria for the approval of active substances with potential adverse effects on the endocrine system. To establish a scientifically based position paper, a framework for evaluating the likely relevance of potentially adverse endocrine effects for humans under realistically assumed exposure conditions will be proposed. Central aspects considered within the framework may include dose-dependency, severity and selectivity of effects on the endocrine system, establishment of a mode of action in animals, and qualitative and quantitative comparison of key mechanistic events between experimental animals and humans. The scientific background for such a position paper will be discussed with experts from academia, industry and other authorities at a workshop in November 2009.

Proposed time schedule

May 6, 2009:	Presentation of BfR framework draft at meeting of BfR Committee for Plant Protection Products (CPPP).
June 2009:	Dispatch of the workshop concept, the timetable and the invitations for participation regarding the BfR workshop.
July 2009:	Response of the BfR CPPP to the framework draft.

September 2009: Distribution of a first draft of a concept paper for the assessment of substances with potential adverse effects on the endocrine system to the participants of the international workshop at the BfR.

Nov. 11-13, 2009: International Workshop "Substances with endocrine disrupting properties under new EU PPP regulation - Establishment of assessment and decision criteria" at the BfR, Berlin.

January 2010: BfR-Forum: Discussion of a position paper on establishment of assessment and decision criteria for substances with endocrine disrupting properties in PPP that may cause adverse effects in humans at the BfR, Berlin.

April 2010: Submission to EFSA: A consolidated draft of a position paper for further discussion and development of cut-off criteria for decision on approval or disapproval of substances with potential adverse effects on the endocrine system for their use in pesticides under the new pesticide regulation in Europe.

3.3 Overview of the ECETOC Task Force work

Rémi Bars
Bayer CropScience, France

Although the endocrine system is not fundamentally different to other systems, such as the immune system or the nervous system, the toxicity of this system is referred to as endocrine disruption rather than endocrine toxicity as is the case for the other systems (immunotoxicity and neurotoxicity). However, all these systems function in a similar way with signalling molecules (hormones, cytokines or neuromediators), cellular receptors for these molecules and enzymes that synthesise or catabolise these signalling molecules. Inappropriate interaction of exogenous substances with receptors, enzymes or transporters of any of these systems can lead to adverse effects in the organisms exposed at sufficient dose levels to these exogenous substances. Since the terms 'endocrine disrupter' or 'endocrine disrupting properties' are now entering the legal field with the recent revision of the EU directive on plant protection products and the new regulations on chemicals it becomes critical to have clear guidance for identifying such substances and properties.

Despite the numerous national and international definitions of an endocrine disrupter, there is no set of specific scientific criteria currently available to determine whether a chemical is or is not an endocrine disrupter. To address this deficit an ECETOC Task Force was set-up in June 2008, which comprised of a number of (eco)toxicologists from various sectors of the chemical industry. The objective of this Task Force was to define a set of scientific criteria and develop guidance for the identification of endocrine disrupting effects based on the measurements of endocrine specific endpoints that are assessed in screening/mechanistic and standard regulatory test methods.

The terms of reference for this Task Force were defined by the ECETOC Scientific Committee as follows:

1. Critically review all available definitions of endocrine disruption, which apply to both human health and other organisms in the environment.
2. Identify key and common themes from all definitions, as well as the relevance of these to chemical classification and risk assessment.
3. Provide guidance on the nature and quality of data required to conclude the induction of endocrine disruption and causation of any adverse effects. This should include the ability to evaluate the potency of any endocrine disruption observed.

In the first instance publicly available definitions (five in total) were reviewed and, with the exception of the US EPA definition, they all had a number of themes in common i.e. '*exogenous substance'*, '*adverse effects resulting from changes (alterations) in the endocrine function*',

'*intact organism or progeny*'. Therefore the Task Force worked according to these common themes using the Weybridge definition as a basis.

The first aspect of the definition refers to an '*exogenous substance*' as opposed to an endogenous substance such as hormones which are therefore excluded from the definition. Although the main focus of the ECETOC Task Force was man-made chemicals it was recognised that environmental conditions (e.g. calorie intake, food restriction, temperature, light, salinity, pH or parasites) or natural chemicals (e.g. genistein, daidzein, coumestrol, resveratrol, lignans, forskolin, glycyrrhizin, zearalenone) also have the ability to cause endocrine disruption in laboratory animals, following appropriate exposure. Consequently it was acknowledged by the Task Force that endocrine disruption was not a new form of toxicity and that it was not restricted to man-made chemicals. It was therefore recommended that guidance on how to handle these natural chemicals needed to be considered.

Another aspect of the definition '*adverse effects resulting from changes (alterations) in the endocrine function*' implies that mechanistic information is also required to demonstrate that the adverse effects are due to perturbation of the endocrine system. This need for supporting mechanistic information indicates that the detection of adverse effects is not sufficient *per se* to conclude if a chemical is (or not) an endocrine disrupter.

The last aspect of the definition '*intact organism or progeny*' indicates that care should be paid to the parental and the subsequent generations. In addition, it also implies that *in vitro* test methods are not appropriate to identify an endocrine disrupter as they do not contain an integrated endocrine system.

The ECETOC proposed guidance relies on a general concept, which is based on the understanding of the mode of action of toxicity and subsequent adverse effects that are induced by an exogenous chemical. This concept is described as follows: an exogenous chemical usually interacts with a molecular target present in a target cells/tissue; this interaction can induce a physiological response which may ultimately result in an adverse effect if the dose level is high enough and/or the duration of exposure is long enough. Mode of action investigations are best addressed using the *in vitro* and *in vivo* targeted endpoint studies (OECD level 2-4 assays or US EPA Tier 1 battery) whereas the identification and characterization of the subsequent adverse effects are best addressed using the apical *in vivo* multi-endpoint studies such as the regulatory reproduction studies (in rodents, fish, birds and invertebrates) and chronic/cancer bioassays in rodents. Only when the adverse effects data that indicate a concern for endocrine toxicity, from apical *in vivo* multi-endpoint studies are supported by mechanistic evidence from targeted endpoint studies can it be concluded that there is evidence that the chemical acts as an endocrine disrupter.

An additional requirement of the ECETOC Task Force was to incorporate a potency consideration into the guidance document since the hazard characteristics of chemicals may be very different from one to another. Consequently, the Task Force recommended that a number of factors be taken into account to allow the discrimination of chemicals of high concern from those of lower concern for humans and the environment. These factors include the specificity of the effects (endocrine adverse effects observed at lower doses than other types of toxicity) and the relevance of the mechanism of action to human or the environmental species. If the adverse effects are not specific or not relevant it is recommended that the risk assessment should be based on non-endocrine endpoints. However if the effects are specific and/or relevant, then factors such as dose level, exposure duration, nature/severity/ incidence of adverse effects and number of species affected from regulatory studies should be considered in order to discriminate chemicals of high concern from those of lower concern.

In conclusion, the Task Force focussed mainly (as prescribed by its remit) on the identification of the hazard potential of endocrine disrupting chemicals and paid little attention to exposure consideration. However the risk that chemicals pose to human health and the environment cannot be based simply on an evaluation of hazard but instead should take into account all available scientific information in order to adequately characterise risk based on hazard characteristics, dose response considerations and exposure data.

Questions/comments

- Are you advocating conducting a battery of tests for plant protection products as per US EPA?

Not advocating this in general, however, if there is concern in the database this may be appropriate.

- Would you regulate based on OECD 407?

Depends on the dataset endpoints and effects seen.

- Adverse versus non-adverse is dependent on the endpoints. For example birds incubating eggs / behaviour not addressed?

Such considerations are important details that need to be considered within the scheme and the test guidelines themselves.

3.4 ECETOC approach – Toxicology (flowchart, generic and examples) Case studies: Genistein, glyphosate, 1,3-dinitrobenzene

Ivana Fegert
BASF

The need for specific endocrine disruption criteria for human health under the revised 91/414 and REACH legislations was summarised. The principles of the ECETOC approach were introduced. This approach is based on the Weybridge definition of endocrine disruption and takes into account mode of action, specificity, human relevance, potency and risk assessment. The test methods considered by the Task Force were briefly introduced. They can be divided into three categories, including targeted *in vitro* assays, targeted *in vivo* assays as well as apical and supporting *in vivo* assays. Targeted *in vitro* assays include oestrogen receptor binding, oestrogen receptor transcriptional activation, androgen receptor binding, steroidogenesis and aromatase recombinant assays. Targeted *in vivo* assays being considered are Uterotrophic, Hershberger, pubertal male and pubertal female. Finally, *in vivo* assays are subdivided into supporting studies e.g. OECD TG 407 and the apical studies which are the mammalian two-generation reproduction study and the chronic/oncogenicity bioassays.

The ECETOC approach to identifying endocrine disruption was presented as a flowchart. The flowchart consists of two parts the first illustrating a five-step approach to identify an ED from a mammalian database. A substance would be labelled an ED only if it fulfils the criteria of showing an adverse effect related to ED in an apical study and the corresponding mechanistic evidence of that ED effect. The second part provides guidance on how to discriminate between substances that have been identified as ED in the first part of the flowchart. Case studies for glyphosate, genistein and 1,3-dinitrobenzene were presented to illustrate the appropriateness of the proposed scheme.

In conclusion, there is an array of standard and specific endocrine test methods available to address potential endocrine disruption. The ECETOC approach is based on the Weybridge (1996) definition for ED concluding that ED is only given if adverse health effects in an apical study are supported by mechanistic evidence of the ED mediated effect. Further considerations should be given to the mode of action, specificity, human relevance and potency.

Questions/comments

- Scenario E is rather common under REACH. Is there a recommended study battery?

The Task Force did not suggest a battery since this was beyond the scope of their assignment. A case study for scenario E was therefore not added to the report. However, it is clear that an exhaustive battery has to be presented in order to exclude ED.

- It should be considered that the **in vitro** *targeted studies could give false negative results which would suggest a green light.*

Again, only an exhaustive test battery being negative would suggest no concern for ED. It is clear that this scenario would trigger some discussions when it is complete; the possibility of performing the corresponding *in vivo* test is always given.

- If there are positive results in targeted endpoint studies (positive scenario E) what are the options?

In that case further testing in apical studies can be performed to get a more complete picture.

- The classification of **in vivo** *assays into apical and supporting studies was questioned.*

That is debatable.

- In the genistein example human data have not been incorporated or considered including the EFSA report on isoflavones.

This is correct. The examples were not meant to be discussed in detail but used to show the applicability of the proposed scheme.

3.5 ECETOC approach – Ecotoxicology (flowchart, generic and examples) Case studies: Genistein, flutamide

James Wheeler
Syngenta

The need for specific endocrine disruption criteria for environmental (non-target) species under the revised 91/414 and REACH legislations was summarised. Specific differences between the fields of ecotoxicology and toxicology were highlighted, as these in part explain the differences in approach taken for toxicology and ecotoxicology ECETOC flowcharts. Ecotoxicology test methods typically focus on measuring impacts on development, growth and reproduction, which often give a limited insight into the toxicological mode of action that leads to the adverse effect. Conversely toxicology studies, in addition to measuring these apical effects, assess mechanistic endpoints that can be used to elaborate modes of action including endocrine disruption. Environmental assessments also differ from human health assessments in that the protection goal is at the population rather than the individual (human) level. Beyond this, environmental assessments have to consider many species over different taxonomic groups. Therefore, the environmental flowchart differs from the toxicological approach. To determine if a substance is an endocrine disrupter requires a separation of the effects in ecotoxicology studies (apical and endocrine specific) based on mode of action; i.e. it is necessary to demonstrate an endocrine mode of action in the environmental model (species). This explains why, in general, it is the toxicological database that drives the cause for concern for endocrine disruption and 'triggers' further investigation in environmental species.

The test methods considered by the Task Force were briefly summarised. These include non-endocrine specific methods such as standard (without specific endocrine endpoints) fish full lifecycle, avian reproduction and the suite of mammalian toxicology tests, including the rat two-generation study. Endocrine specific screening and definitive methods were also discussed. The OECD fish screen and short-term reproduction assays for the Hypothalamic-Pituitary-Gonad axis and the amphibian metamorphosis assay for the Hypothalamic-Pituitary-Thyroid axis were highlighted. Definitive tests include modified standard methodologies with the inclusion of endocrine specific endpoints (biomarkers and apical). These include the fish sexual development test, fish full lifecycle tests and amphibian tests (precise designs currently unclear), whilst the mammalian toxicology package could be used for a wild mammal assessment. For birds, specifically designed partial or critical life stage tests (addressing endpoints not currently included in the reproduction study) may be performed. Additionally, the US EPA is currently developing a two-generation study with Japanese quail.

The ECETOC approach to identifying endocrine disruption was presented with case studies for flutamide and genistein. This can be found in the original report (ECETOC, 2009) and so will not be further discussed here.

In conclusion, there is an array of standard and endocrine specific methodologies available to address potential endocrine disruption. The ECETOC approach relies on a weight of evidence evaluation of *in vivo* toxicology (and *in vitro*) data to trigger concern for specific evaluation.

Questions/comments

Why were invertebrate studies not included in the scheme?

This was considered by the Task Force. It was acknowledged that our understanding of invertebrate endocrinology is limited. However, there are a number of lifecycle and partial tests with invertebrates currently in existing regulatory frameworks e.g. *Daphnia* reproduction, mysid chronic and chironomid development tests. Additionally, several invertebrate lifecycle methodologies are currently in development or validation by the OECD (e.g. copepod and chironomid lifecycle tests and a mysid two-generation test). Although these do not necessarily offer mechanistic power to identify endocrine disruption, they do offer exposure over a whole lifecycle (practically, ethically and financially feasible with invertebrates). Therefore, it is possible to measure adverse apical effects at all life stages so that any relevant adverse effects from an endocrine disrupting mode of action are likely to be captured and so considered in the risk assessment.

In this context, insect growth regulators (juvenile hormone analogues and ecdysone (ant)agonists) were discussed. These are effectively designed endocrine disrupting chemicals and would fulfil the environmental cut-off criterion. Removal of such products from the market may be an unintended consequence of the regulation.

Need to consider false negative results in the **in vitro** *screens*

This is a potential issue. However, the combination of *in vitro* information with an analysis of the *in vivo* toxicology studies should overcome this potential for data rich substances. However, for data poor substances this may be of greater concern. This general issue will be considered further by the ECETOC Task Force following the workshop.

If there are effects on the thyroid system you may not detect these in fish **in vivo** *studies*

The ECETOC scheme relies on the fact that thyroid activity is flagged in the *in vivo* toxicology studies. This would direct the investigator to conduct an amphibian metamorphosis assay that is specifically directed to assess impacts on the Hypothalamic-Pituitary-Thyroid axis.

If no mammalian concern is identified you would exit the scheme. Issues of mammals being dosed orally, whereas fish and amphibians exposed via the gills and skin. Issues of metabolism differences?

The issue of metabolism and differing exposure routes was raised elsewhere during the workshop. Therefore, the ECETOC Task Force will consider this when revising the scheme in light of the workshop recommendations.

Fish screening assay is not particularly sensitive / consistent to detect anti-androgens

It was discussed that during the validation studies for the fish screening assay large differences were noted amongst laboratories (and species?) in their ability to detect anti-androgenic activity with flutamide. However, the study has successfully detected anti-androgenicity for vinclozolin. It was discussed that it is possible to add the measurement of 11-ketotesterone to the fish screening assay to address this mode of action. It was further noted that the OECD is currently considering a Standard Project Submission Form submitted by the United Kingdom for a stickleback assay specifically designed to assess anti-androgenic activity in 'androgenised' females.

4. SYNDICATE SESSIONS

4.1 Theme I: Evaluate the ECETOC approach as a concept for identifying endocrine disrupting effects

Moderators:	Dick Lewis	Christoph Schäfers
Rapporteurs:	Alberto Mantovani	Tobias Frische
	Carole Besret	Mohamed Benahmed
	Melanie Gross	Willie Owens
	Karen Hirsch-Ernst	Masanori Seki
	Taisen Iguchi	Lennart Weltje
	Arnd Weyers	

General Concept

The approach was endorsed as a valuable concept that can be useful for identifying endocrine disrupting effects in a structured manner. Several points of clarification and emphasis were agreed.

- The approach is particularly suited to data rich situations such as plant protection products and certain high production volume chemicals.
- The approach is most useful when used as a scheme for evaluating existing data; it is not intended or well suited as a framework laying out what data should be generated. For example a positive finding in an apical study should be investigated in the most scientifically appropriate manner using approaches matched to the finding and should not be confined to a range of predetermined experimental approaches.
- Care should be used when arriving at a weight of the evidence judgement that key or apical studies cover endocrine sensitive endpoints. For example, prior to 1998 the guidelines governing the two-generation study did not include the range of endocrine sensitive endpoints mandated after this time.
- A greater clarity is needed in how to deal with data poor situations, and in particular the entrance criteria need defining. Similarly, more thought should be given within the concept as to how best to follow up on situations where the only data available indicate a positive outcome from *in vitro* or *in vivo* screening studies and where data from apical studies are not available.

Modes of action coverage

When considering the coverage and selectivity / specificity of the modes of endocrine action considered in the proposed scheme the following aspects were discussed and highlighted.

- Thyroid mode of action and the thyroid as a target. In addition to direct measurements of thyroid toxicity such as histopathology, other findings that may be a consequence of thyroid effects (reduced growth, neurobehavioral differences, resorptions and difficult parturition) should be evaluated in the context of the thyroid as a potential mode of action. Mechanistic studies conducted in order to establish the MoA should cover all potential relevant mechanisms to include enzyme inducers altering metabolism, protein transport as well as iodination and receptor mediated effects.
- For ecotoxicity definition and identification of a thyroid MoA are more difficult although the Xenopus metamorphosis assay is viewed as an effective *in vivo* screen, but *in vitro* and QSARs are not well developed. For ecotoxicity sex steroid effects, methods are in place but are only partly specific. The precise definition of concern is needed in order to address with the most appropriate test model.
- Attention should be focussed on MoAs other than those targeting sex steroids or thyroid. Apical studies when expanded to include 90-day and chronic studies (which should be able to identify for example diabetes-like lesions, mammary neoplasms and neoplastic and preneoplastic conditions in other endocrine organs) can identify endpoints relevant to endocrine activities other than those which disrupt oestrogen, androgen and thyroid systems. However fewer methods are available to investigate the MoA for these potential activities.
- As for ecotoxicology the main population-relevant effects are reproduction and development (so sex steroids and thyroid effects are main MoA targets).

Identifying adverse effects

There are established schemes available that can help judgments on adversity. Most concerns in this area are around ecotoxicity assessments. Behavioural effects in birds and fish are population relevant and not sufficiently covered currently. Similarly sex ratio and time to first reproduction in fish will be addressed in future but not currently well covered experimentally.

What constitutes sufficient evidence of no endocrine disrupting activity?

ED effects should be *specific* with the lowest NOAEL represented by endocrine adverse effects. It was considered that apical studies will not miss the signals of endocrine activity if well conducted and if we are able to identify and interpret the outcome. Targeted studies should not be confined to OECD guidelines, but should be expanded to cover the most scientifically

appropriate approach. It is recognised that in the ecotoxicity area the data-poor nature of many situations makes assurance of lack of effect more difficult.

Weight of the evidence assessment

The weight of the evidence approach should follow the principles of evidence based toxicology. There are a number of existing frameworks (such as the Cefic approach), which can give a robust and transparent approach to assigning levels of weight to individual pieces of evidence (e.g. a well conducted and complete two-generation study versus a receptor assay *in vitro*). Industry and regulators are to be encouraged to show in a transparent way the evidence used in coming to a judgment, the weight assigned to the individual elements of the evidence and which data may have been discarded in arriving at an overall position.

Understanding the models used to generate data

Some key themes were recognised including the need to generate data on hormone levels in a scientifically appropriate manner; profiles are preferable to single point estimates which can be variable and misleading. Certain experimental animal strains (for example SD rats) have high percentage of background thyroid alterations which can confound interpretation. Confounding factors for ecotoxicity studies should be identified for each study type.

Triggering ecotoxicity studies for toxicity data and vice versa

If there is a positive signal in the area of ecotoxicity, this should trigger more data on mammalian toxicity and *vice versa*. This holds true only for triggering not for extrapolation: e.g. o'p'DDT has much higher binding affinity with fish ER than mammalian ER. Oestrogenicity triggering further work may be possible but triggering for androgenicity may be different. Other insights and watch-outs included the observation that fish are directly exposed via gills, avoiding liver metabolism. Reproduction is different between mammals and birds (the egg concentrates lipophylic substances).

4.2 Theme II: Evaluate the appropriateness of risk assessment in managing endocrine disruption

Moderators:	Angelo Moretto	Beat Lang
Rapporteurs:	Chris Willoughby	José María Navas
	ZhiChao Dang	Dean Leverett
	Ellen Dhein	Niels Skakkebaek
	Ivana Fegert	Ben van Ravenzwaay
	Katherine Flynn	James Wheeler
	Helen Håkansson	

Introduction

The initial reaction of the breakout groups to the theme and questions posed, focused on concern that substances under the new 91/414 guideline would, if classified as endocrine disruptors, find their use blocked on the basis of hazard and the value of the material would not be subjected to objective risk assessment to put findings into perspective.

Endocrine disruption can be a mechanism to explain some causes of reproductive toxicity and was on a par with other complex multi-causal toxicity patterns as involved in carcinogenesis and immunotoxicity. The groups considered that there was no justification for creating another class of toxicity specifically for endocrine disruptors and that they should be treated like any other chemicals causing toxic effects.

Q1: Should all endocrine toxicants be treated equally? Or should potency also be considered to discriminate the potent from the weak ones?

The groups believe that all endocrine disruptors (ED) should be treated equally; but that it is essential that exposure is taken into account in considering how the ED will be dealt with under the various regulations.

In the current climate it is likely that once a test material is classified as an ED in some parts of the world, use would be restricted by this classification and no risk assessment would be undertaken. For this reason it is essential to be clear whether the ED activity occurs at the lowest NOAEL and is pivotal in setting the NOAEL or whether ED effects are only seen at dose levels higher than those at which other target organ toxicity has been detected. It was not possible, in such a brief debate, to attempt to define the relationship between the minimal NOAEL (the critical one for risk assessment) and the ED NOAEL and at which ratio we could consider the

material should be labelled as an endocrine disruptor with respect to future hazard and risk assessment. The development of this concept was considered to be a potentially valuable step in the categorisation of EDs.

The effect of an ED will depend on its chemical potency – the ability of the molecule to interact with receptors at which concentration and what is the maximal effect – and the ability to reach the target receptor(s). In turn, local exposure will depend on dose level (in terms of mg/kg), transfer of the molecule to the active site, metabolism and excretion of the molecule so that effective concentrations are maintained at the active site.

Consideration should also be given with respect to the duration of exposure that is required for the substance to cause endocrine disruption effects: is the effect seen within a few hours or days of exposure or does it take extended period of exposure before the effect can be detected? Is the effect reversible, and how?

There are a wide range of ED effects that can be detected by the variety of testing paradigms. Whilst binding activity and *in vitro* effect may be demonstrable, an effect *in vivo* may be limited because of the natural background of hormone levels and the ability of the intact animal to compensate for varying levels of exogenous ED. Concern would be greater for an ED which generated an effect at low dosage over a short period of time and resulted in a substantial change in performance or structural abnormality rather than a temporary change in the rate of maturation with no ultimate detectable effect upon fertility. Effects detectable in many species would be regarded as more important for interspecies extrapolation on hazard and risk than where effects were limited to one species or *phylum*.

Q2: In what situation would hazard alone be considered as the only criteria to authorise or not authorise chemicals?

It is not appropriate to use hazard as the sole criteria for the restriction of EDs. This approach throws enormous, unwarranted weight on the decision whether the material is or is not a primary ED and does not allow for thorough assessment of all the factors which may be involved in the evaluating the potential risk of using an ED. The trend, with the latest planned regulation revisions that will replace the EC Directive 91/414 and the REACH regulations, is to impose severe usage restrictions on materials identified as EDs or even block usage without risk assessment. However in the US, risk assessment based authorisation will continue to be used to establish appropriate restrictions on use.

It is biologically plausible that there will be functional threshold levels for EDs as the exogenous material interacts with the natural feedback systems within the animal. Despite potential ED activity and hazard, it is important that all aspects of exposure and severity of potential reaction

are weighed in the evaluation of the ED and that risk assessment should be applied to all potential EDs where there is a justified use for the material.

Risk with EDs will vary with the timing of exposure relative to maturity so that effects at early stages of development may be damaging and permanent, whilst in the mature animal there might be no discernable effect.

There are also concerns with respect to the interactions with other materials with ED activity within the environment. Effects could be additive at the same time of exposure because of similar mechanism or because of complementary mechanism or additive because of different temporal exposure. With current standard testing methods, it seems unlikely that the interactions of molecules within the apical *in vivo* tests will be practical and evaluation will have to be made by extrapolation from effects in simpler tests and on MoA consideration.

Risk assessment can be complicated by many uncertainty factors in estimating exposures in target animals at various stages of maturation, but it is considered always more worthwhile to assess these points rather than to reject a material on hazard potential alone.

Q3: Should regulatory decisions on chemicals which are based on the same set of data be very different (hazard based versus risk assessment) across the globe?

The situation in which regulatory decisions on chemicals should be based on hazard in one part of the world, but on risk in another part of the world is clearly untenable and every effort should be made by the scientific community to guide the political overlords towards making decisions based on risk – a well assessed compilation of all factors – rather than a nervous twitch response to a perceived hazard where confounding factors have not been assessed in setting limits as to how a material might be handled and used safely. The US approach of applying risk assessment to all materials where there is ED concern is considered to be the most satisfactory approach from the scientific perspective.

Q4: Is hazard based classification which leads to exclusion of a chemical (for which safe uses can be demonstrated) ever justified?

We do not believe that hazard based classification can be justified. The whole process of development of a test material requires identification of a market for use. Once the market is defined then necessary precautions can be investigated and put in place to limit exposure (and hence risk) to operators and the general population who may come into contact with the material. The ultimate definition of risk will depend on dose level, exposure duration, type and

severity of ED effects, and, for extrapolation amongst species, the number of species observed to be affected in the test systems.

As long as there is no separate labelling classification for EDs then they can only be categorised within the scale of reproductive hazard and risk.

Discussion and conclusion

In considering hazard rating and risk assessment it is essential that the criteria for labelling a material as an endocrine disruptor are clearly defined to relate ED activity relative to other toxicities. Once a material is labelled as an ED hazard then a risk assessment should be completed to define appropriate limitations of use.

Within the various areas of chemical types where there might be concern over ED activity there are two clear classes of material: The chemicals marketed for plant protection/pesticide use where there is generally a comprehensive assessment of toxicity, usage and exposure, and the broader chemical market covered by REACH – including an enormous range of materials and intermediate molecules used in manufacture.

For this latter group, it is very difficult to estimate population exposure or even individual exposure. It might be assumed that the more material that is produced the greater the burden on the world and the increased risk to potential target species be it man, mammals, fish or insects. However many of these compounds rarely leave the sealed environment of the factory and the risk of general exposure is limited.

For the chemicals designed to be used in plant protection there is a much greater risk that the public will be exposed to the chemicals, if only as residues on treated crops. The chemicals are designed to be reactive and harmful to target species and may be persistent to be effective, but the presence of these residues can be quantified and a valid risk benefit assessment made.

In conclusion, identification of chemicals as endocrine disruptors should be used with caution to set potential hazard labelling and trigger appropriate risk assessment which will permit safe use of the test material.

4.3 Theme III: Evaluate whether there is any basis to treat endocrine disruption differently from other MoA

Moderators:	Paul Foster	Cliff Elcombe
Rapporteurs:	Tamara Galloway	Peter Day
	Gerard Cooke	Susan Jobling
	Malyka Galay Burgos	Annegaike Leopold
	Nina Hallmark	Giuseppe Malinverno
	Keith Houck	Tinka Murk
	Grace Panter	Patricia Pazos

Five specific questions were addressed by the groups. The discussion below combines and summarises the thoughts and ideas of both groups.

Q1: What are the hazards for humans and the environment from endocrine toxicity?

- The groups agreed that different hazards (endpoints) would be identified (toxicity versus ecotoxicity) but that the same evaluation approach could be used. To expand, human toxicology studies will always have as their basis the protection from harm of the individual whereas in environmental risk assessment the focus is on protection of populations of individuals, communities of different populations and ecosystems in which different communities co-exist. The focus is on growth, survival and reproduction.
- The most appropriate endpoints to consider for endocrine disruptors include carcinogenicity, reproduction and development (including metamorphosis) and long-term longevity. The consideration of data from a broad range of invertebrate and wildlife species is considered of benefit for Environmental Risk Assessment (ERA).
- Any assessment should consider individuals when assessing hazards (but should always consider individuals *and populations* when assessing risk).

Q2: Is there any basis to treat endocrine disruption differently than other modes of action?

- Simply, the answer is no, however ….
- Scientifically, the basis for taking a different approach is centred around discussions of the biphasic nature of many hormone-regulated responses and the potential for subtle low dose effects to occur that are not detectable under acute, short term test conditions. Recent legislative focus on endocrine disruption reflects these scientific and societal concerns. Because of its complexity, endocrine disruption should be considered carefully in a similar way to carcinogenicity.

- *"The timing and context of the dose makes the poison".*
- Developmental changes occurring early in the lifecycle may theoretically lead to irreversible effects, potentially to vulnerable sub-populations (consider the very great need to protect children).
- The adult population is vulnerable to cumulative exposures and these long term effects may not easily be identified through simple short-term laboratory tests.
- We consider molecular toxicology / epidemiology studies that combine exposure assessment of human populations and the exploration of associations with health outcomes and identification of mechanisms of impact to be of key importance in identifying population-level effects. These kinds of studies, when properly conducted, must be given greater consideration in reviewing the 'weight of evidence' for conducting risk assessments.

Q3: In which situations should hazard alone be considered to be the only criteria to authorise or not authorise chemicals?

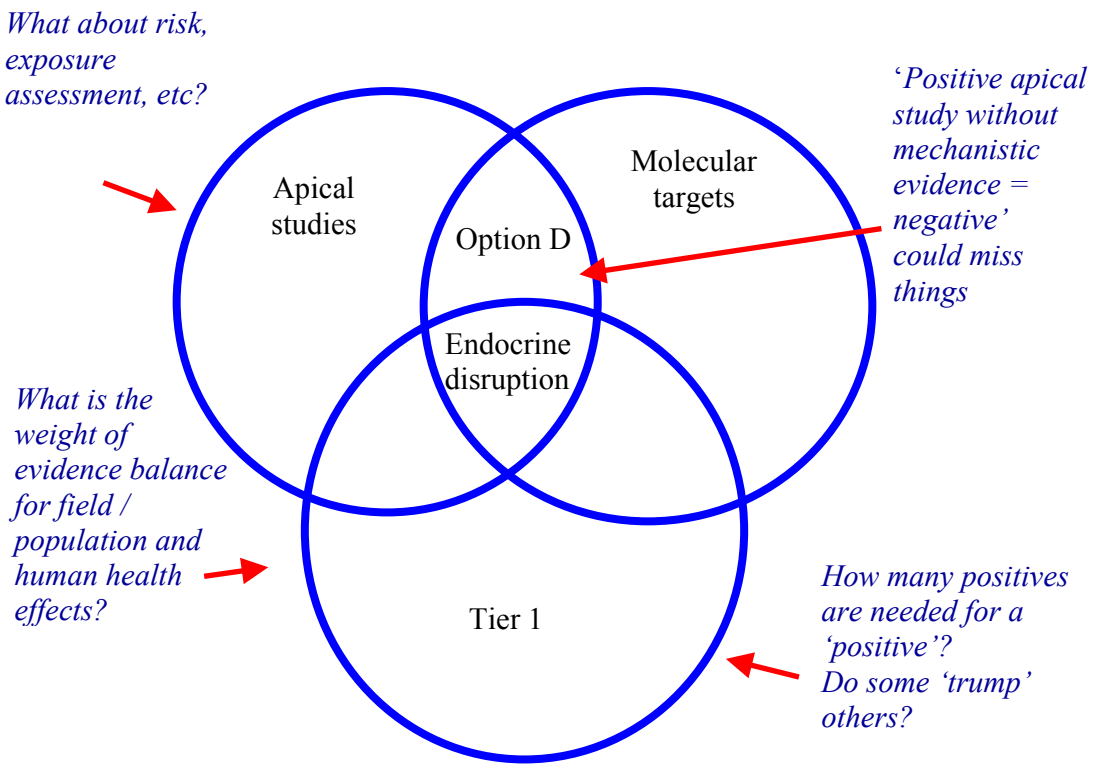
- No, under no circumstances should hazard alone be used as a criterion.

Q4: What regulatory measures should be taken towards natural endocrine disrupters (plant components and mycotoxins) and physical (temperature, light) toxicants?

- Greater regulatory attention should be given to industrial substances.
- In principle natural and industrial substances should be treated similarly, but…
- There is a question of practicability in the regulation of natural substances (no one 'owns' them).
- Lifestyle choice, public protection versus nanny state (e.g. tobacco versus genistein).

Q5: What is the relative hazard of man-made chemicals compared to natural endocrine toxicants? Are the natural ones less hazardous than man-made ones?

- Yes and no – it depends on the natural and man-made substances in question. Assessment should be on a case by case basis.
- This is more of a risk management / perception issue where consent and information are the key. Are there gaps in legislative coverage? Is this actually an issue?
- Concepts to be considered include 'risk' versus 'benefit', 'relevance'.
- Consider the case of zearalenone as an example, a fungal phytooestrogen restricted in foodstuffs, i.e. adopt an advisory tone for phyto-oestrogens.
- Another example of relevance is in the risk assessment of soy infant formula.
- The following diagram provides the group's summarised overview of some of the challenges faced during the construction of adequate risk assessment strategies for endocrine disruptors.


What about risk, exposure assessment, etc?
Apical studies
Molecular targets
Option D
'Positive apical study without mechanistic evidence = negative' could miss things
Endocrine disruption
What is the weight of evidence balance for field / population and human health effects?
Tier 1
How many positives are needed for a 'positive'? Do some 'trump' others?

4.4 Theme IV: The way forward in developing science-based legislation

Moderators:	Neil Carmichael	Ian Dewhurst
Rapporteurs:	Richard Sharpe	Rick Becker
	Fabrice Broeckaert	Susan Jobling
	Christine Fuell	Rémi Bars
	Anne Gourmelon	Miriam Jacobs
	Bruno Hubesch	Peter Korytar
	Bram Versonnen	Roland Solecki

Several overarching themes emerged from the discussions of the two breakout groups focused on the path forward with respect to current and upcoming legislation in Europe and implementation of legislatively mandated evaluation of substances to identify endocrine active substances (EAS).

1. The dynamic tension between hazard classification and risk-based evaluation

Legislation directing REACH and the plant protection product directive are already in place and cannot be changed in the short term. Both the past and current classification legislations are hazard-based, as is GHS. This raised the questions, "Is Classification being used in a way it was not designed for? Is it really appropriate to base exclusion criteria simply on hazard classifications?" Clearly, deeper discussion on this topic may be useful, and while the legislation could have benefited from additional scientific input, the discussants agreed the frameworks for evaluating EAS should be adapted as a function of the legislation. For implementing the plant protection product directive it is important to use risk principles and to develop guidance documents built upon integrating hazard evaluation with exposure assessment.

Furthermore, as revisions to the Biocides legislation are coming, we believe the legislation would greatly benefit from primary reliance on risk assessment principles for EAS assessment. The basic analytical framework should not be reduced to a black and white decision paradigm based on consideration of inherent hazard properties alone. Even the most hazardous substances can be used safely when exposures are controlled. The ECETOC framework indicates it is not necessary to ban or rule out all uses of some chemicals based on endocrine disruption, but instead a risk-based approach can guide decision making that can be used to control exposures to balance risks and benefits of such substances. Classification is overtaking risk assessment (chemicals in or out); but the best scientific approach for authorisations should consider dose response, exposures and therefore risk.

2. The dichotomy of requiring more in depth hazard evaluation while at the same time discouraging expanded animal testing

For plant protection products, where molecules are designed to be biologically active to exert effects to protect agricultural practices from pests, the registration and licensing provisions require development of extensive hazard testing data, and detailed information on environmental fate, transport and detailed exposure assessments. This provides a rich set of data from which to assess both hazards and risks. The study protocols which comprise the set of data developed for a pesticide active ingredient, typically focus on measuring apical adverse effects. Apical adverse effects are not specific to any one mechanism, and they can arise from many different mechanisms, including endocrine mechanisms. Thus it is expected that use and exposure guidelines developed from up to date risks assessments for pesticide active ingredients will protect against significant health and environmental risks. However, the practical effect of legislation which calls for evaluating the endocrine activity of such molecules is that additional mechanistic studies will likely be necessary, and such studies will in many cases include additional studies using laboratory animal models. While some mechanistic information can be attained by *in vitro* and *in silico* approaches (e.g. QSAR), the laboratory animal models provide the only practical means for evaluating substances which require absorption and biotransformation for interaction with components of the intact (endocrine) system. In the REACH legislation, ED is included as a part of the authorisation, and while no specific additional toxicity testing is currently legislatively required for such authorisation of EAS, information from many of the same apical and mechanistic tests discussed for ED evaluation in plant protection products may be needed to arrive at a risk-based decision for REACH authorisation of EASs. Therefore, discouraging additional animal testing under REACH is counter intuitive to the need for a solid scientific foundation of data for risk-based decision making. In the case of REACH substances, while the criteria for entry into the ECETOC framework clearly needs more consideration, use of all relevant information – QSAR, structural alerts, categories, read across, and available results of toxicity testing, including effects on the environment and ecological receptors, as well as exposure potential, needs to be integrated into a priority setting process to guide further testing. Integrated testing frameworks are encouraged.

3. The ECETOC evaluative framework is the basis of a pragmatic, science-based approach

The ECETOC evaluative framework provides a structured, science-based process to evaluate results from a variety of different toxicity tests and mechanistic studies and to integrate knowledge of adverse effects and mode of action from these studies to reach conclusions regarding endocrine disruption, in accordance with the Weybridge definition. There are three inter-related pieces of legislation in Europe – REACH, PPP and Biocides Directives – and although there are differences in the testing frameworks for each of these different categories of substances, the evaluation principles and approaches for determining ED should be consistent and

harmonised to the greatest extent practical. The ECETOC framework is a very useful starting point for such a consistent approach. It is strongly recommended that opportunities be sought to involve all relevant parties in discussions leading to EAS evaluation frameworks, and that the various government and industry sectors avoid fragmented approaches.

Consideration should be given to further work to improve the ECETOC evaluative framework. Specifically, incorporation of a systematic and structured approach using the WHO/IPCS/ILSI weight of evidence, mode of action and human relevance should be discussed. Such a structured and systematic approach will encourage both consistency and scientific rigor of analyses across compounds and over time and provide the transparency needed for stakeholders from all sectors to understand the analytical procedures, the assumptions made and their justifications, and this will promote greater overall confidence in the assessment procedures. The WHO/IPCS/ILSI weight of evidence, mode of action and human relevance provides a means to evaluate whether the effects measured relate more closely to primary endocrine interactions or to secondary effects resulting as a corollary to toxicity at other, non-endocrine, target tissues. In addition, there is an explicit step in this that specifically focuses on determination of the relevance to humans, including subpopulations, and can include, when approved by appropriate ethical panels, the use of human data.

4. The pressing need for improving the ECETOC frameworks to aid in implementation of the PPP directive for ED

Many agreed that use of classification based solely on hazard properties seems to be overtaking risk assessment, and that if not implemented in a risk-based framework, certain beneficial substances could be removed from commerce in the European Union. As such, it is imperative that the broader impacts of such an implementation approach be considered. If there are no convincing scientific criteria from Europe, then there will be considerable difficulties with global acceptance (e.g. PPP, Codex and Joint FAO/WHO Meeting on Pesticides Residues [JMPR]). Therefore, there was general agreement that the more appropriate scientific approach would be to consider hazard, dose response, exposures, to integrate these to provide a risk characterization, and employ a risk basis for authorisation. Therefore, where there are words without clear definitions – 'negligible exposure' and 'human relevance' for example, there are opportunities to build interpretive approaches, such as the ECETOC ED framework, to bring forward science based definitions and decision analyses that integrates apical tests, mechanistic studies and exposures. There was general agreement that derivation of a margin of exposure (MoE) would provide a risk benchmark to guide decision making. When possible, existing definitions that have been agreed to at OECD and other authoritative bodies should be used as the starting point.

There is a desire to have more case studies of applications of the ECETOC framework developed and to have additional venues for more in depth discussions. Refinement of the ECETOC

framework examples to include consideration of defining 'negligible exposure' in the context of risk-based exposure values would be particularly useful in stimulating discussions of such an approach. Similarly, one or more examples employing the WHO/IPCS/ILSI mode of action human relevance framework, including, where supported by the data, data-derived uncertainty factors to arrive at a MoE should be considered. Opportunities for pooling case studies and making these more broadly available should be encouraged across ECETOC, industry groups and other organisations such as IPCS, WHO and OECD.

Looking ahead, in addition to refining the ECETOC frameworks, science opportunities over next few years include building better understanding of potential biological responses at low, environmentally relevant levels of exposures, and thresholds of biological responses. Significant challenges exist in understanding the dose dependent relationships in biological pathways, including endocrine pathways. Specifically, more scientific research is needed to understand when perturbations vary within homeostatic range, and when these progress by transitioning first to an adaptive state and then to a state reflecting an adverse effect. And understanding what the key events are along such a pathway. In addition, exploration of possible development and application of threshold of toxicological concern (TTC) approaches should be considered. And, to support risk-based approaches, there is a need to have adequate exposure information for EAS substances with thresholds, including exposures of any identifiable susceptible subpopulations.

4.5 Overall summary of the syndicate sessions

The breakout group sessions reached consensus on a number of items and also recommended several actions to be taken.

Concerning the ECETOC approach as a concept for identifying endocrine disrupting effects, there was consensus that this approach was scientifically valid, particularly for data rich compounds. For data poor substances it was recommended that entrance criteria (to start an evaluation) are developed. Evidence of endocrine activity should only be considered 'positive' if the effects are specific. Transparency and documentation of the evaluation leading to a specific conclusion in the ECETOC approach should be part of process.

Concerning the appropriateness of risk assessment in managing endocrine disruption there was consensus that such an effect should be considered as any other mode of action. Consequently the assessment of endocrine disruption should be based on risk and not on hazard and that the assessment process should be harmonized globally. It was recommended that criteria be developed on how to use dose level, exposure duration, specificity and the type of endocrine effect as elements to be considered within the ECETOC approach.

Concerning the question of whether endocrine disruption should be considered as different to other modes of action there was consensus that there was no scientific evidence to treat endocrine disruption differently, a conclusion also reached by breakout group 2. Consequently, identification of endocrine effects alone should not be a reason for non authorisation of substances. The identification of endocrine disruption demands the careful evaluation of all relevant data and it was recommended that particular consideration is given to those cases where adverse effects giving concern for endocrine toxicity are detected from in vivo studies but for which no clear mode of action can be identified.

Concerning the way forward there was consensus that legislation should be science based and that a risk based analysis should be introduced as part of the evaluation process. The ECETOC approach was considered to be pragmatic in bringing science (back) into the evaluation process. It was recommended that, within the current European legislative context, wording such as 'negligible exposure' and 'human relevance' should be explored as possibilities to bring the best science forward. Research on the effects of low doses and mixtures should be advanced and the use of margin of exposure and threshold of toxicological concern should receive more attention.

CONCLUSIONS AND RECOMMENDATIONS

Overall the ECETOC evaluation framework was considered scientifically sound and was viewed as a valuable contribution to the definition of specific scientific criteria that are required for the determination of endocrine disrupting properties. However whilst it was acknowledged that the ECETOC framework was particularly suited for chemicals with comprehensive toxicology and ecotoxicology databases, it was lacking in guidance for those chemicals with poor databases. In particular practical guidance should be given on what data need to be generated for chemicals with partial or significant data gaps (in respect to the ECETOC scheme). To this end, it was recommended that additional case studies should be included in the ECETOC evaluation framework using both data poor and data rich chemicals.

To further refine the ECETOC evaluation framework it was recommended that the systematic and structured approach of the WHO/IPCS conceptual framework for evaluating the mode of action for cancer and non-cancer end points be included, as this conceptual framework is part of a large project on the harmonisation of approaches for the assessment of risk from exposure to chemicals.

It was also recommended that approaches for determining endocrine disrupting properties should be consistent and harmonised for the three inter-related pieces of EU legislation that concerns chemicals under REACH, biocides and plant protection products directives.

Several conclusions also arose from the breakout group sessions, which can be summarised as follows. First, it was considered inappropriate to introduce a new class of chemical toxicity specifically for the endocrine disrupters given that the adverse effects resulting from endocrine disruption can be detected in apical studies (reproductive, development and chronic toxicity studies as well as the cancer bioassays) and are, therefore covered by the existing EU or GHS classifications. It was also recognised that there was no scientific reason to approach the toxicity resulting from an endocrine mode of action differently to other types of toxicity (e.g. neuro-, immuno-) resulting from non-endocrine modes of action. Overall there was a general consensus that European regulatory decisions to authorise or not authorise chemicals purely on the basis of hazard in the absence of proper risk assessment was not scientifically justified and contradicted approaches taken by non-European authorities. However it was recommended that appropriate risk assessment should be performed which take into account a number of issues i.e. threshold of biological responses, potential for ‘low dose’ effects, and mixtures of chemicals acting by similar modes of action.

The ECETOC Task Force appreciated the opportunity to present the approach to a wide audience of experts in the field. Further the Task Force is grateful for the input received at the workshop and intends to use this to revise the ECETOC guidance. It is hoped that this will be valuable in moving the debate and tools available forward to assist in the regulatory challenges faced by the issue of endocrine disruption.

ABBREVIATIONS

CA	Competent Authority
Cefic	European Chemical Industry Council
CPPP	Committee for Plant Protection Products
EAS	Endocrine active substance
ECHA	European Chemicals Agency
ED	Endocrine disrupter
EFSA	European Food Safety Authority
ER	Oestrogen receptor
ERA	Environmental risk assessment
GHS	Global harmonised system
ILSI	International Life Sciences Institute
IPCS	International Programme for Chemical Safety
JMPR	Joint FAO/WHO Meeting on Pesticides Residues
MoA	Mode of action
MoE	Margin of exposure
MS	Member State
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PPP	Plant protection products
QSAR	Quantitative structure activity relationship
REACH	Registration, evaluation, authorisation and restriction of chemicals
TTC	Threshold of toxicological concern
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

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APPENDIX B: WORKSHOP PROGRAMME

Monday 29th June 2009

12.00 - 13.30	Registration and lunch	
13.30 - 13.45	**Introduction to ECETOC and the overall context of the project**	Neil Carmichael ECETOC
13.45 - 14.15	**European Regulatory Perspective on endocrine disruption (Revision of 91/414/EC Directive and REACH)**	Bram Versonnen ECHA
14.15 - 14.30	**BfR activities for establishment of criteria for endocrine disruption under the new EU legislation**	Karen Hirsch-Ernst / Roland Solecki BfR
14.30 - 15.00	**Overview of the ECETOC Task Force work**	Rémi Bars Bayer CropScience
15.00 - 16.00	**ECETOC approach**	
	Toxicology (flowchart, generic and examples) **Case studies: Genistein, glyphosate, 1,3 DNB**	Ivana Fegert BASF
	Ecotoxicology (flowchart, generic and examples) **Case studies: Genistein, flutamide**	James Wheeler Syngenta
16.00 - 16.30	Coffee break and **poster session**	
16.30 - 16.45	**Introduction to the breakout group sessions**	Rémi Bars
16.45 - 19.00	**Breakout Group Sessions**	
	Theme I	**Evaluate the ECETOC approach as a concept for identifying endocrine disrupting effects**
	I a:	Moderator: Dick Lewis; *Rapporteur*: Alberto Mantovani
	I b:	Moderator: Christoph Schäfers; *Rapporteur*: Tobias Frische
	Theme II	**Evaluate the appropriateness of risk assessment in managing endocrine disruption**
	II a:	Moderator: Angelo Moretto; Rapporteur: Chris Willoughby
	II b:	Moderator: Beat Lang; Rapporteur: José María Navas
	Theme III	**Evaluate whether there is any basis to treat endocrine disruption differently than other MoA**
	III a:	Moderator: Paul Foster; Rapporteur: Tamara Galloway
	III b:	Moderator: Cliff Elcombe; Rapporteur: Peter Day
	Theme IV	**The way forward in developing science-based legislation**
	IV a:	Moderator: Neil Carmichael; Rapporteur: Richard Sharpe
	IV b:	Moderator: Ian Dewhurst; Rapporteur: Rick Becker
20.30 - 22.00	Dinner	

Tuesday 30th June 2009

09.00 - 10.45	**Rapporteur feedback from the Breakout Groups**	Chair: Rémi Bars
10.45 - 11.15	Coffee break	
11.15 - 12.15	**Plenary discussion**	Moderator: Neil Carmichael
12.15 - 12.45	**Conclusions and next steps**	Ben van Ravenzwaay BASF
12.45 - 14.00	Lunch	

APPENDIX C: ORGANISING COMMITTEE

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ECETOC WORKSHOP REPORTS

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels
No. 2	Strategy Report on Challenges, Opportunities and Research Needs Arising from the Definition, Assessment and Management of Ecological Quality Status as Required by the EU Water Framework Directive Based on the Workshop EQS and WFD versus PNEC and REACh - Are They Doing the Job? 27-28 November 2003, Budapest
No. 3	Workshop on Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Crécy-la-Chapelle
No. 6	Workshop on Chemical Pollution, Respiratory Allergy and Asthma. 16-17 June 2005, Leuven
No. 7	Workshop on Testing Strategies to Establish the Safety of Nanomaterials. 7-8 November 2005, Barcelona
No. 8	Workshop on Societal Aspects of Nanotechnology. 9 November 2005, Barcelona
No. 9	Workshop on the Refinement of Mutagenicity / Genotoxicity Testing. 23-24 April 2007, Malta
No. 10	Workshop on Biodegradation and Persistence. 26-27 June 2007, Holmes Chapel
No. 11	Workshop on the Application of 'Omics in Toxicology and Ecotoxicology: Case Studies and Risk Assessment. 6-7 December 2007, Malaga
No. 12	Workshop on Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study. 14-15 April 2008, Barza d'Ispra
No. 13	Counting the Costs and Benefits of Chemical Controls: Role of Environmental Risk Assessment in Socio-Economic Analysis. 4 June 2008, Brussels
No. 14	Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. 24-25 June 2008, Brussels
No. 15	Workshop on the Probabilistic Approaches for Marine Hazard Assessment. 18-19 June 2008, Oslo

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ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) was established in 1978 as a scientific, non-profit making, non-commercial association and counts as its members the leading companies with interests in the manufacture and use of chemicals. An independent organisation, ECETOC provides a scientific forum through which the extensive specialist expertise of manufacturers and users can be harnessed to research, evaluate, assess, and publish reviews on the ecotoxicology and toxicology of chemicals, biomaterials and pharmaceuticals.