Risk Assessment of Endocrine Disrupting Chemicals 9-10 May 2011, Florence

Workshop Report No. 21

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Risk Assessment of Endocrine Disrupting Chemicals

CONTENTS

1. SUMMARY

This report documents the outcome of a workshop organised by ECETOC to discuss the 'Risk Assessment of Endocrine Disrupting Chemicals'. The workshop was held in Florence on the 9th and 10th of May 2011. Thirty-eight invited experts (from academia, regulatory bodies and industry) discussed approaches for the risk assessment of endocrine disrupting chemicals. The aims of the workshop were to evaluate emerging guidance produced by regulatory authorities, and academic and industry scientists, identify areas of concordance and difference, consolidate the common scientific themes, provide a platform for constructive debate on areas of difference, and invite a wider critique of the proposed approaches.

The workshop consisted of a series of invited presentations. The first set of presentations dealt with human safety, whilst the second set covered environmental safety. National initiatives and developments to define and test criteria for the identification of endocrine disrupting chemicals were presented. This was followed by presentations from the ECETOC Task Force on the ECETOC approach, which included refinements and further development of their original proposal 'Guidance on Identifying Endocrine Disrupting Effects (TR106)' (ECETOC, 2009a).

The presentations were followed by four syndicate discussion sessions, which addressed four specific themes. Each theme was considered from both toxicological and ecotoxicological perspectives.

Theme I was concerned with the use of weight of evidence (WoE) for decision making. The participants concluded that a consistent approach for the WoE of endocrine disrupting chemicals is required, which would be applicable under various regulatory regimes. There was general support for a requirement to demonstrate both an adverse effect in an intact organism (extended to population level impacts for the ecotoxicological assessment) and a plausible endocrine mode of action. For human health assessment there was general support for using the WHO/IPCS mode of action framework (WHO/IPCS, 2007). For ecotoxicological assessment it was acknowledged that no direct equivalent to this WHO/IPCS framework exists, but several specific WoE frameworks for the evaluation of endocrine disrupting effects have been published. These should be evaluated and combined for the requirements under current legislation.

Theme II covered discussions on the human and population relevance of endocrine related endpoints. It was noted that there were some rodent cases for which non-relevance to humans has been demonstrated, but that the number of such cases is low. The default position is to assume human relevance.

Specific guidance was considered necessary to aid in the identification of endpoints in ecotoxicological studies that are of population relevance. Some endpoints are clearly directly

population related, whereas others are more diagnostic in nature, and in order to infer their population relevance, they need to be used as part of a cluster of endpoints.

Theme III dealt with the evaluation of lead toxic effects and the specificity of endocrine effects when identifying endocrine disrupting chemicals. While it was seen as scientifically sound, most participants thought that the application of this criterion would depend on the degree of separation between a non-endocrine mediated lead effect and the endocrine-mediated effect, as well as the relative severity and seriousness of the lead *versus* ED-mediated effect. The acceptable degree of separation should be assessed on a case by case basis, and for EDs of very high concern a factor of 10 was suggested as a conservative starting point. This could be a useful approach for the REACH legislation, which requires that individual exposure scenarios need to be addressed to guarantee safety for different uses of the same chemical. For ecotoxicological assessments the participants felt that further work was required before a value for the degree of separation could be recommended.

Theme IV was concerned with using potency to differentiate between endocrine disrupting chemicals. It was highlighted that the concept of potency assessment could be introduced as a surrogate for risk assessment following the legislative introduction of a hazard based cut-off criterion for endocrine disrupting chemicals. Equivalent categories already exist for repeated dose toxicity. The potency assessments (cut-off criterion) proposed by the German and British authorities (BfR and CRD respectively) and ECETOC would only apply to identify substances of high regulatory concern which would be refused marketing authorisation. All other (less potent) endocrine disrupting chemicals which are part of PPPs and biocides would still undergo standard risk assessment.

2. WORKSHOP OVERVIEW

2.1 Introduction

Recent European legislation (Plant Protection Products Regulation 1107/2009 [EC, 2009a]; proposed new Biocidal Products Regulation COM(2009)267 [EC, 2009b]) has created a hazard based cut-off criterion that only allows the marketing and use of chemicals on the basis that they do not induce endocrine disruption which may lead to an adverse outcome in humans and/or wildlife species. Substances with endocrine properties are also subject to authorisation under the REACH Regulation (1907/2006) (EC, 2006). However, there is currently no agreed guidance on how to identify and evaluate endocrine activity and disruption. Consequently, an ECETOC Task Force was formed in June 2008 to address the issue and in May 2009 a Task Force report 'Guidance on Identifying Endocrine Disrupting Effects (TR106)' was published (ECETOC, 2009a). The report developed guidance in the form of a 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.

In June 2009 ECETOC held a workshop to discuss their proposed guidance. This provided a stimulating discussion on the scientific basis for identifying endocrine disrupting chemicals (ECETOC, 2009b). The outcomes from this workshop were used to refine the guidance and this was published by Bars *et al* (2011). However, it was recognised that certain elements of the guidance still needed further development. For human health these included the relevance to man of the endocrine mechanism of toxicity, the specificity of the endocrine effects with respect to other potential toxic effects, and the potency of the endocrine effect. For ecotoxicological assessment consideration of specificity and potency, population relevance of the observed endocrine related effects, and definition of negligible exposure were the areas chosen for refinement.

A considerable amount of work has also been undertaken by individual member states, which has generated approaches that have significantly progressed the thinking in this area. The aim of this workshop was to debate, combine and consolidate these rapidly evolving approaches.

2.2 Workshop structure

- A review of evolving schemes provided by the German BfR and UBA, UK CRD, Denmark and ECETOC.
- A series of breakout groups to tackle areas for further development.
- A plenary session to clearly identify areas of consensus and future debate.

2.3 Workshop objectives

Specifically to:

- Evaluate emerging guidance produced by regulatory authorities, academic and industry scientists.
- Identify both areas of agreement and differences of opinion.
- Consolidate the common scientific themes.
- Provide a platform for constructive debate on areas of difference.
- Invite a wider critique of the proposed approaches.

3. PRESENTATION SUMMARIES

3.1 Report on criteria for endocrine disrupters from the Danish Centre on Endocrine Disrupters

Ulla Hass
Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark

The Report on Criteria for Endocrine Disrupters was carried out by the Danish Centre on Endocrine Disrupters (CEHOS) as a project contracted by the Danish Environmental Protection Agency. CEHOS is an interdisciplinary scientific network without walls and the main purpose of the Centre is to build and gather new knowledge on endocrine disrupters (EDs) with focus on information needed for the preventive work of the regulatory authorities. The overall aim of the report is to propose scientific criteria for the identification of ED substances of concern for human health and the environment.

The widely used definitions of EDs and potential EDs according to WHO/IPCS (2002) were used as a starting point. However, these two definitions seem to represent the two 'ends' of the spectrum of knowledge on ED properties and effects and consequently a definition of suspected ED was inserted in between. A number of other issues relevant for the development of criteria for EDs were also considered such as potency, lead effects, specificity and relevance for humans and the environment. Based on these considerations the proposed scientific criteria can be summarised as:

Group 1 - Endocrine disrupter:

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

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

Group 2a - Suspected endocrine disrupter:

Substances are placed in group 2 when there is some evidence from humans or experimental animals, and where the evidence is not sufficiently convincing to place the substance in group 1.

If for example limitations in the study (or studies) make the quality of evidence less convincing, group 2a could be more appropriate. Such effects should be observed in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effect should be considered not to be a secondary non-specific consequence of other toxic effects.

Group 2b - Potential ED:

Substances are placed in group 2b when there is some *in vitro* / *in silico* evidence indicating potential for endocrine disruption in intact organisms. The evidence could also be observed effects *in vivo* that may, or may not, be ED-mediated.

It has been proposed that in cases where ED-induced effects are not the lead toxic effects but are seen at dose levels significantly higher than those causing other toxic effects, the substance is not an ED of regulatory concern. Also, potency has been proposed as part of the criteria for identifying EDs, i.e. defining an effect level below which a substance can be identified as an ED substance (and consequently above which it would not be identified as an ED). These approaches are not considered relevant for ED identification. They are clearly in contrast to the CLP criteria for classification of (CM)Rs, where only specificity of effects is required and such substances are considered of regulatory concern. Also, the WHO definition of EDs does not include considerations of this. Furthermore, dismissing the ED properties in a regulatory context, if there are other more sensitive toxic effects for the individual chemical, or based on potency, would invalidate the evaluation of mixtures of chemicals with similar types of ED effects, but differences in lead toxic effect or potency.

The report describes the scientific evidence needed for fulfilling these criteria based on the OECD Conceptual Framework for endocrine testing and assessment and considers non-test methods, test methods, epidemiology and field studies. This can be summarised as:

Evidence for ED (group 1)

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

Evidence for suspected ED (group 2a)

- *In vivo* assays providing data on adverse effects linked to endocrine or other mechanisms (OECD, level 5), but where ED mode is suspected.

- Good quality epidemiological studies showing associations between exposure and adverse human health effects related to endocrine systems.
- *In vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, level 3).
- In some cases, read across, chemical categorisation and/or QSAR approaches may provide the necessary data in combination with ADME information and *in vitro* data.

Evidence for indicated ED (group 2b)

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

The regulatory use of these ED criteria in relation to REACH article 57(f) and the new PPP regulation is also considered. It is proposed that EDs in group 1 should be identified as SVHC in REACH article 57(f) and as ED substances under PPP. For suspected and potential EDs (group 2a and 2b), further data may be necessary to evaluate whether the substances is an ED (group 1).

As such, the overall purpose of the report is to provide scientific background for Danish input to the ongoing EU work within this field.

3.2 Joint UK-DE proposal for a regulatory definition of an endocrine disrupter in relation to human health[1]

Susy Brescia
Chemicals Regulation Directorate, Health & Safety Executive, UK

This is a proposal for a definition of an endocrine disrupter (ED) in relation to human health that can be applied in a regulatory context. The stimulus for this work was the introduction into the new European Union Plant Protection Products (PPP) Regulation (1107/2009 [EC, 2009a]) of an exclusion criterion for non-approval *(IF: Exclusion for non-approval: double negative makes a positive???)*, which explicitly indicates that any active substance, safener and synergist with endocrine disrupting properties cannot be approved for marketing and use, unless exposure is negligible. A similar non-approval exclusion criterion has been introduced in the proposed new EU Biocidal Products Regulation (COM(2009)267 [EC, 2009b]). Substances with endocrine disrupting properties are also targeted within the REACH Regulation (1907/2006 [EC, 2006]). Identification of substances as EDs may lead to their inclusion in the list of substances subject to the Authorisation requirements of REACH. Hence, the regulatory consequences of identifying a substance as an ED are severe – these provisions are stringent, hazard-based criteria that should be reserved only for those substances genuinely posing a potential real threat to human health and/or the environment. With such considerations in mind, it is problematic that at the present time there is no definition and/or set of criteria within regulations, by which to identify EDs.

The widely accepted scientific definition of ED by WHO/IPCS is proposed as a starting point for characterising an ED for regulatory purposes. This is a well-established and widely recognised definition produced by a global, authoritative organisation through a world-wide initiative of high scientific rigour (WHO/IPCS, 2002):

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

The WHO/IPCS definition is still a very broad description, which does not have the power to discriminate between substances meriting regulatory action for their ability to harm health because of disruption to the endocrine system, and substances that justify lesser concern in relation to any endocrine-disrupting ability and for which such severe regulatory action is not justified. Therefore, the aim is to use the WHO definition as the starting point to arrive at a regulatory definition of an ED by adding a number of criteria that need to be satisfied before an ED requiring regulatory action can be identified. These are as follows:

[1] The joint DE-UK proposal is available on the CRD website at: [http://www.pesticides.gov.uk/approvals.asp?id=3034] and on the BfR website at: [http://www.bfr.bund.de/cm/349/regulatory_definition_of_an_endocrine_disrupter_in_relation_to_potential_threat_to_human_health.pdf].

Adverse consequences during testing: Adverse effects potentially related to endocrine disruption to have been seen in one or more toxicity studies conducted in intact animals and to be of acceptable quality, in which the substance was administered by a route relevant for human exposure.

ED mode of action: A mode of action link between the toxic effects of concern and endocrine disruption to have been established.

Test results relevant to humans: The effects seen in experimental animals to be judged to be of potential relevance to human health.

Potency of the effect: The adverse effect(s) related to endocrine disruption to have been produced at a dose at or below the relevant guidance value for the application of Category 1 'Specific Target Organ Toxicity - Repeated Exposure, STOT-RE' classification & labelling (CLP Regulation).

These proposed criteria were tested in a number of case studies and showed that they are able to discriminate between genuine EDs of regulatory concern and substances for which regulatory action is not justified.

3.3 Impact project on proposed decision criteria for substances with endocrine disrupting properties

Philip Marx-Stölting and Roland Solecki
Federal Institute for Risk assessment, Berlin, Germany

The new plant protection products (PPP) regulation, Reg. (EC) 1107/2009 (EC, 2009a), introduces endocrine disrupting properties as one of a number of new cut-off criteria for the approval of PPP. Since no specific science-based measures for the assessment of substances with endocrine disrupting properties have been agreed upon and a draft of specific measures is to be presented by the European Commission only at the end of 2013, the development of assessment and decision criteria represents a key challenge concerning the implementation of this new legislation. These criteria should also be applicable for other substances with endocrine disrupting properties within the European legislation, such as REACH-chemicals and biocides.

A science-based proposal for specific decision criteria for substances with potential endocrine disrupting properties in human health risk assessment has been developed by the German Federal Institute for Risk Assessment (BfR). The proposed conceptual framework includes assessment of adversity of effects, establishment of a mode / mechanism of action in animals, considerations concerning the relevance of effects to humans and regulatory decision criteria which are exposure-based (option 1) or categorisation-based (option 2).

Option 1 and option 2 of these decision criteria were tested upon 36 active substances in PPP for their applicability and also to analyse the potential impact of the new PPP regulation on active substances currently approved for use. The collection of the substances evaluated in this impact study was based on their classification (i.e. carcinogenicity, reproductive toxicity) and on random selection, respectively. Furthermore, the outcome of the conceptual framework was compared to the interim criteria of Regulation (EC) 1107/2009 (option 3). The first results of this impact study are presented here and illustrate the application of the BfR framework.

3.4 ECETOC proposal to identify endocrine disrupting chemicals of regulatory concern for human health

Rémi Bars[1], Ivana Fegert[2] and Dick Lewis[3]
[1] *Bayer CropScience, Sophia Antipolis, France*
[2] *BASF SE, Limburgerhof, Germany*
[3] *Syngenta Ltd, Jealott's Hill, Bracknell, Berkshire, United Kingdom*

In June 2009 an ECETOC workshop was organised in Barcelona, Spain, to discuss a proposal of scientific criteria to be used to identify endocrine disrupting chemicals.

The criteria proposed by the ECETOC Endocrine Task Force were based on the two requisite elements contained in the endocrine disrupting chemical definitions from WHO/IPCS, EC, Weybridge and Japan i.e. the necessity to observe adverse effects and the demonstration that these effects are indeed caused by an endocrine mechanism of action. It was proposed that while the detection of adverse effects is best addressed by the regulatory toxicity studies (apical and supporting studies), the demonstration of the mechanism of action is best addressed by the recently validated *in vitro* / *in vivo* screening mechanistic studies illustrated in the US EPA Tier 1 endocrine test battery or the OECD level 2-4 of the conceptual framework for the testing and assessment of endocrine disrupting chemical chemicals.

Evidence for endocrine disrupting properties was considered to be met when the adverse effects that raised concern from regulatory toxicity studies could eventually be explained by the screening / mechanistic studies or vice versa i.e. when the indication of endocrine activity from the screening / mechanistic studies could eventually be confirmed through the manifestation of adverse effects in the supporting and/or apical regulatory toxicity studies. However, since all endocrine disrupting chemicals may not represent the same hazard for humans a number of criteria were proposed to discriminate chemicals of high concern from those of lower concern.

An illustration of the fact that not all endocrine disrupting chemicals represent the same hazard to humans is given by the evaluation of a number of common chemicals that humans are exposed to on a routine basis. This evaluation was performed in the *in vitro* screening assay for steroidogenesis using the H295R adrenal cells. The results of these evaluations indicate that six chemicals (caffeine, gingerol, paracetamol, vitamin C, vitamin B6, vitamin B3) out of ten tested are capable of interfering with steroidogenesis[2]. For caffeine this endocrine activity, which is manifested by an activation of steroidogenesis (increased oestradiol formation) may be the cause for the adverse effects observed in endocrine tissues in apical toxicity studies (increased incidence of pituitary and mammary gland tumours in rat and mouse chronic studies respectively

[2] Tinwell H, Colombel S, Bars R. 2011. Evaluation of substances routinely taken in everyday life in the H295R steroidogenesis assay. Poster (abstract no. 2351). Society of Toxicology, Washington, USA, March 2011.

and ovarian and sperm changes in rat and mouse reproduction studies respectively)[3,4,5,6]. Consequently, a set of clear criteria is needed to discriminate chemicals of high concern from those of lower concern. If these criteria are not put in place the possibility exists that, by using specific and sensitive endocrine screens in combination with apical studies, numerous chemicals (both natural, like caffeine, and man-made) will eventually be considered as endocrine disrupting chemicals and could therefore be subjected to exclusion criteria whilst posing no harm to humans.

When considering the criteria first developed by ECETOC as well as those elaborated by national regulatory authorities (HSE-CRD and BfR), it appears that some criteria are essential for discrimination of chemicals. Such key criteria include the relevance of the mode of action of toxicity to humans, the specificity/lead effect, the potency of the chemicals and some exposure considerations whereby a margin of exposure greater than 1000 should be sufficient to protect humans from any potential adverse effects.

For the relevance of the mode of action (MoA) to humans, it is reasonable to assume that, in the absence of appropriate data, the MoA is relevant to humans. There are not many examples where an MoA of endocrine toxicity detected in animals has been demonstrated not to be relevant to humans; however, rodent thyroid toxicity mediated by liver enzyme induction is one of them and has been extensively investigated.

The criterion of specificity/lead effect applies to chemicals that have multiple target tissues (e.g. liver, kidney, blood as well as endocrine tissues) and for which serious adverse effects on non-endocrine tissues are found at much lower dose level than the endocrine effects. However there is a need to agree within the scientific and regulatory community on an acceptable degree of separation between the non-endocrine lead effect and the endocrine effect.

The potency criterion is also very important, since a chemical such as zearalenone that has endocrine activity and induces adverse endocrine effects at 200 µg/kg/day should be considered differently to caffeine, which has also endocrine activity but only produces adverse endocrine effects from 50 mg/kg/day. Although the difference between zearalenone and caffeine in terms of potential harm to humans is obvious it is difficult to establish, at a scientific level, a clear set of cut-off values. A pragmatic approach could, however, be as recommended by HSE-CRD and BfR, to use the guidance values of the CLP regulation for serious adverse effects (Specific Target Organ Toxicity following Repeated Exposure [STOT-RE]).

[3] Yamagami T, Handa H, Juji T, Munemitsu H, Aoki M, Kato Y. 1983. Rat pituitary adenoma and hyperplasia induced by caffeine administration. Surg Neurol 20:323-331.

[4] Welsch CW, DeHoog JV, O'Connor DH. 1988. Influence of caffeine consumption on carcinomatous and normal mammary gland development in mice. Cancer Res 48:2078-2082.

[5] Bradford JC, Caldwell JA, Barbolt TA, Drobeck HP. 1983. Chronic administration of caffeine to two generations of rats. Teratology 27:32A.

[6] Gulati *et al*. 1984. NTP-85-097.

Finally, for chemicals which have endocrine toxicity but for which the exposure level in humans is very limited (margin of exposure more than a factor of 1000), it should be considered that the risk for humans is so low that these chemicals are not of regulatory concern and should not therefore be affected by exclusion criteria.

Overall, these different criteria need to be reviewed and discussed in the light of concrete examples in order to move the debate from concept to pragmatism so that a fair, scientific and realistic regulation can be implemented.

3.5 Endocrine Disruption - German Approach to pesticide assessment

Christoph Schäfers
Fraunhofer IME, Schmallenberg, Germany

In the Regulation (EC) No 1107/2009, plant protection products (PPP) and their active ingredients that cause endocrine disruption in man and wildlife receive specific attention (EC, 2009a).

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

The most common definition for Endocrine Disruptors (ED) applies for the human hazard assessment, as the protection goal is the individual organism (Weybridge, 1996).

> *"An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary (consequent) to changes in endocrine function. A potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism."*

However, for environmental hazard assessment, the protection goal is the (sub)population of a potentially affected species. Thus, the scale of adverse health effects was extended (WHO/IPCS, 2002).

> *"… adverse health effects in an intact organism, or its progeny, or (sub-)populations"*

Population-relevance and focus of environmental hazard assessment of ED

Population-relevant effects influence the intrinsic rate of population increase by affecting growth and development time, sex ratio, fecundity, fertility or stage specific survival. These endpoints are decisive for environmental hazard assessment, but not necessarily related to endocrine disruption: Sex ratio and fertility are clearly better indicators of endocrine effects than mortality or growth. However, even mortality may be a hint of highly specific effects on regulatory systems such as the sexual-endocrine system, if it occurs at very high acute-chronic ratios (e.g. $EC50_{96h}/EC50_{28d} > 10000$ for ethinyloestradiol).

More specific indicators of endocrine effects such as biomarkers (e.g. concentrations of hormones, hormone synthesis enzymes or indicator proteins like vitellogenin) do not affect population growth or maintenance by themselves, but may be linked to effects on population-

relevant parameters. Consequently, they can be used for causal analysis of population-relevant effects. Strong indicators directly affecting population relevant endpoints are secondary sex characteristics, mating behaviour and gonad histopathological findings. However, for quantitative risk assessment, the apical population-relevant parameters are relevant.

To date, the main focus of environmental hazard assessment is on aquatic vertebrates. In the scope of priority setting this is justified. As life processes depend on physiological aqueous solutions, properties of endocrine active substances have to enable transport in aqueous solutions supported by lipoproteins. Beside via ingestion, aquatic organisms are exposed via the respiration media. This especially applies for high metabolic performers such as fish. Thus, exposure to endocrine disrupting chemicals is most problematic in the aquatic compartment.

As ecologically adverse effects occur at limited recovery and recolonisation potential, organisms with long generation time and relatively low fecundity are at particular risk, especially when accessibility of freshwater habitats is limited, which again applies for aquatic vertebrates (fish, amphibians).

For these taxonomic groups, several testing protocols are available (see OECD-EDTA). The main focus is on disruption of sex hormone production and signalling in fish with the testing and risk assessment strategy being available (Knacker *et al*, 2010; OECD, 2011), followed by thyroid hormone interactions in amphibians. There are still assessment gaps for birds and molluscs that are shown to be sensitive to sexual ED. To date there is a severe shortcoming concerning the ways to come to the initial suspicion for ED in wildlife. Moreover, most EU substance regulations mostly lack ED-specific data requirements as well as clear guidance for ED-specific assessment and decision making.

Specific Situation for Pesticides

Whereas the development of harmonised approaches for all groups of substances is advantageous and required, specific conditions for PPPs should be considered:

- For PPPs, large data packages are available, containing extended toxicological and ecotoxicological information due to the testing requirements and information on mode of action (MoA) due to intended use.
- For PPPs, authorisation is a condition for use. Before authorisation, the benefits of use have to be proven and the risk has to be assessed. Risk management measures are part of the authorisation.
- For several groups of active substances, the endocrine system is target organ and basis for very specific effects on target organisms. Thus, the ED activity is intended, especially in invertebrates and plants. The high selectivity of ED mechanisms is usually more appropriate

for the environment than other MoA, provided a thorough and comprehensive environmental risk assessment is conducted.

Endocrine acting pesticides that adversely affect non-target organisms are to be labelled as such by hazard-based cut-off-criteria and excluded from use, regardless of their exposure-related risk. This view is transferred from the REACH process, where however, endocrine disrupting properties result in the need for product authorisation, which includes risk estimation. As pesticides aim to specifically affect target organisms at low environmental concentrations, 'low-dose-effects' are intended and common. Consequently, the established risk assessment concepts are also valid for endocrine disrupting PPPs, as long as the test strategy is able to detect threshold concentrations for endocrine-mediated adverse effects in non-target organisms potentially at risk. There is a need for differentiation to account for the specific situation in pesticides as well as for the political intention to ban EDs in the EU. The presented German approach differentiates endocrine disrupting pesticides by grouping them in 1) PPPs with intended endocrine effects in target organisms (only plants and invertebrates), 2) PPPs with relevant endocrine side-effect potential (effects on non-target organisms), and 3) PPPs with non-relevant endocrine side-effect potential.

When ED properties are the basis of the biological activity in target organisms (only invertebrates and plants), PPPs should be regulated based on environmental risk assessment (ERA) and risk management.

The risk-related properties of active substances with such MoA are characterised by high risk for taxonomically and physiologically related non-target organisms, which has to be assessed and managed properly, but very low hazard and risk potential for vertebrates including humans. Consequently, selectivity has to be clearly demonstrated to exclude not intended endocrine side-effects. The pesticide effect classes with intended ED-effects consist of plant growth stimulators (synthetic auxins) and inhibitors, and of insecticides / acaricides interfering with growth and moulting regulation or acting as pheromones.

When comparison of appropriate ecotoxicological effect data reveals that not intended ED-effects of PPPs are most sensitive and thus drive the authorisation process they should not be approved.

The legal requirement to replace ED is based on political rather than scientific reasons. Specific and potent endocrine disrupting properties causing side-effects are generally undesirable and the use and development of less critical pesticides will be promoted.

When comparison of appropriate ecotoxicological effect data reveals that non-endocrine endpoints are clearly more sensitive than endocrine endpoints and thus relevant for risk assessment and risk mitigation, ED-relevant exposure is unlikely (is negligible).

This means, ED-effects are not specific and potent according to the definition and the established risk assessment and management should be applied.

Discussed PPP effect classes

Neuronal effects. Pyrethroids, pyrethrins, carbamates, organophosphates and neonicotinoids dominantly act trough their neuronal toxicity especially in arthropods. The regulatory decisive lethal endpoints should be by far more sensitive than the endocrine disrupting ones in unrelated species. For pyrethroids, due to their physico-chemical properties several chronic fish tests have been performed. So far there are no hints of endocrine disruption in fish full life cycle tests according to the extended Weybridge definition.

Modulation of signal transduction, e.g. MAP/histidine-kinase (dicarboximides), quinolines. The dicarboximide Vinclozolin exhibits anti-androgenic side-effects and is regarded as a potential endocrine disruptor in mammals, birds and fish, e.g. by US EPA (2000). Anti-androgenic effects in zebrafish full life cycle tests with flutamide were shown on reproduction (mating behaviour) (Knacker *et al*, 2010). Different to other endocrine mechanisms of action this effect seems to be reversible. Regarding pesticide use and exposure patterns, population relevance and relation to non-endocrine endpoints should be discussed.

DMI Fungicides (triazoles, imidazoles or pyrimidines) inhibit ergosterol synthesis in fungi cells. The target enzyme is partly analogous to the aromatase and demethylases in steroid synthesis pathway of e.g. vertebrates. Endocrine effects by aromatase inhibition were shown in chronic fish studies providing a comparably data rich situation. Aromatase inhibition exhibits a clear concentration-response relationship. The testing strategy to identify relevant ED effects is available (Knacker *et al*, 2010) and there is high experience in performance, evaluation and interpretation of appropriate studies. Aromatase inhibition is particularly adverse when causing irreversible effects during sexual maturation. Other endocrine-mediated endpoints (egg quality) may recover and can be discussed regarding population relevance.

The approach is summarised by the wildlife-related presentation by Tobias Frische (UBA) at the DG ENV ad hoc meeting on ED activities on 26th November 2010 in Brussels (6 figures follow).

Figure 1

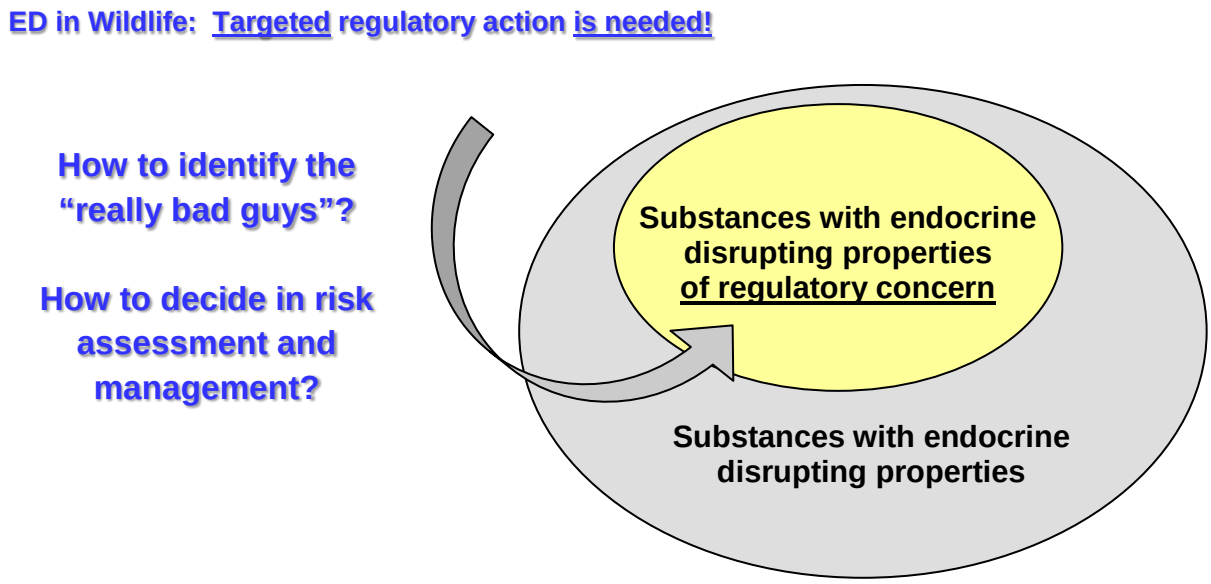


Figure 2

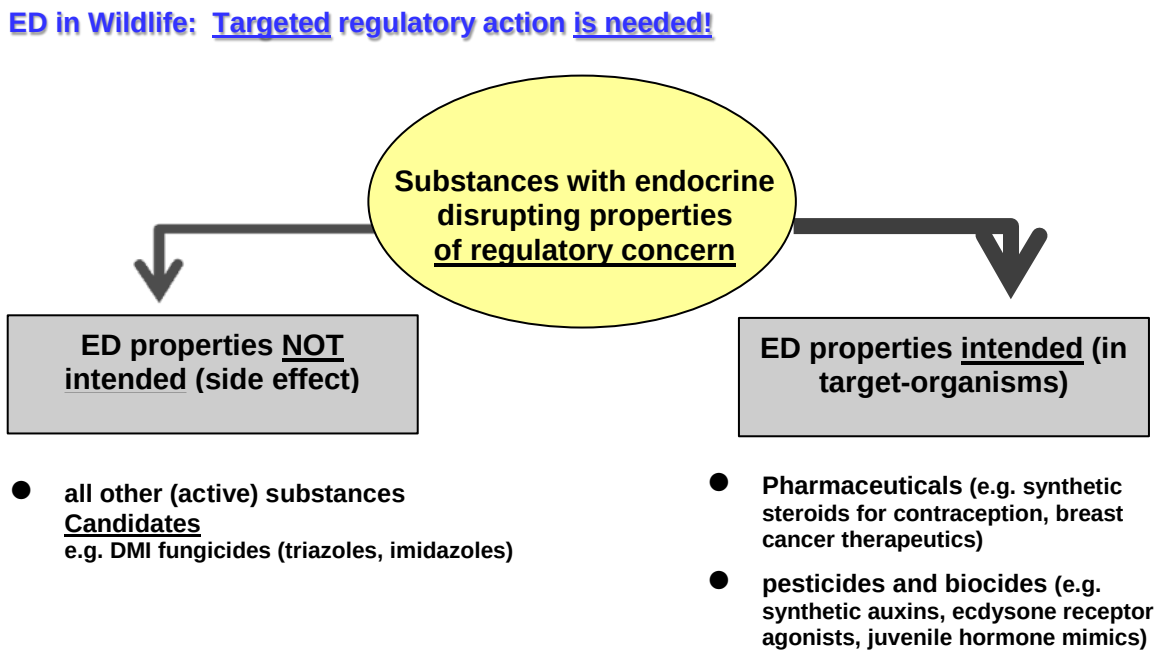


Figure 3

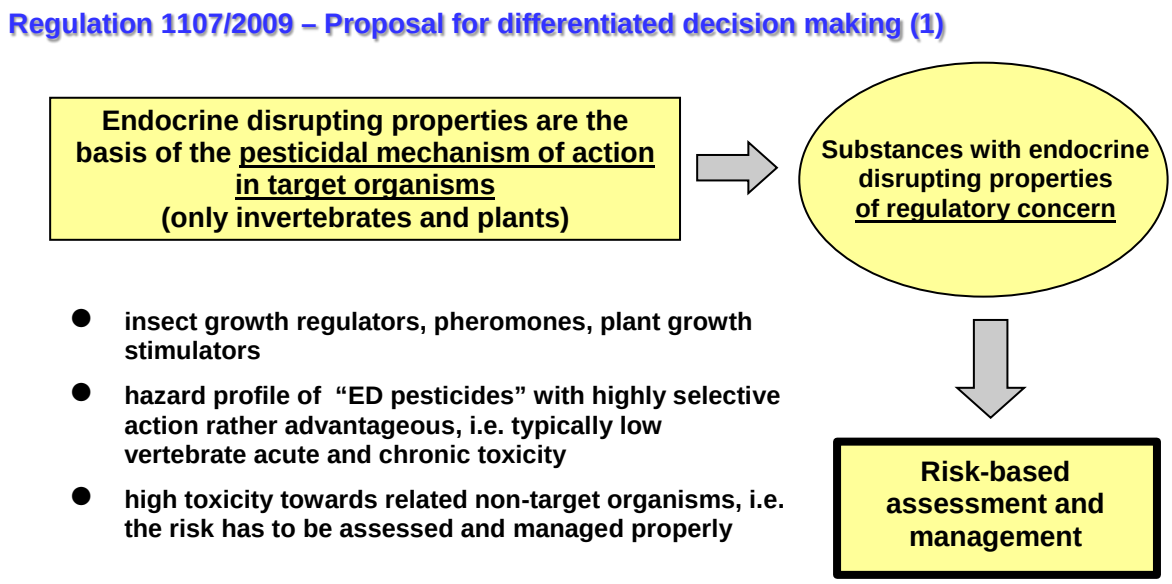


Figure 4

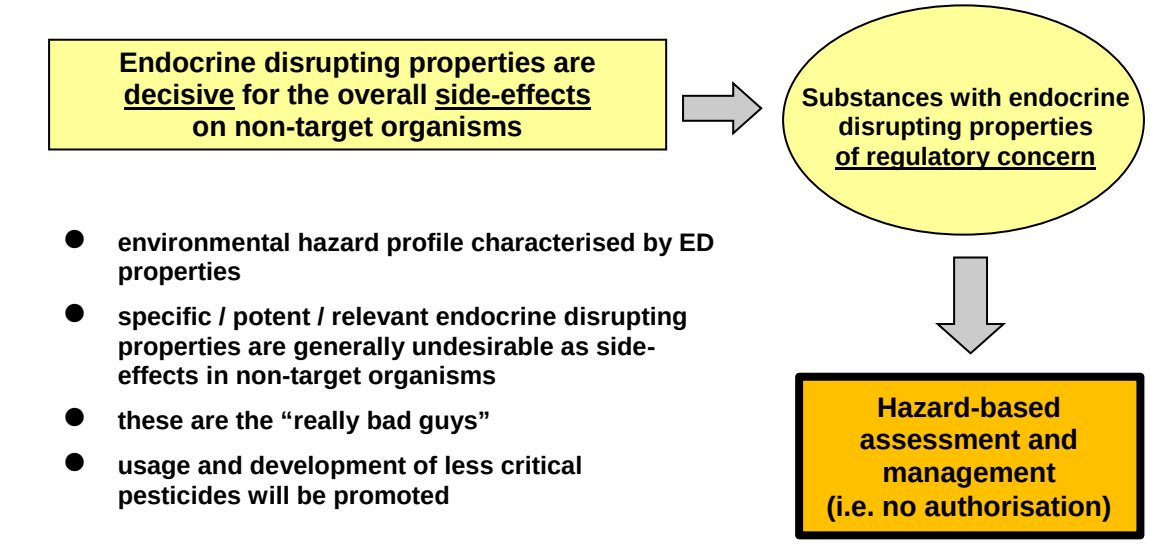


Figure 5

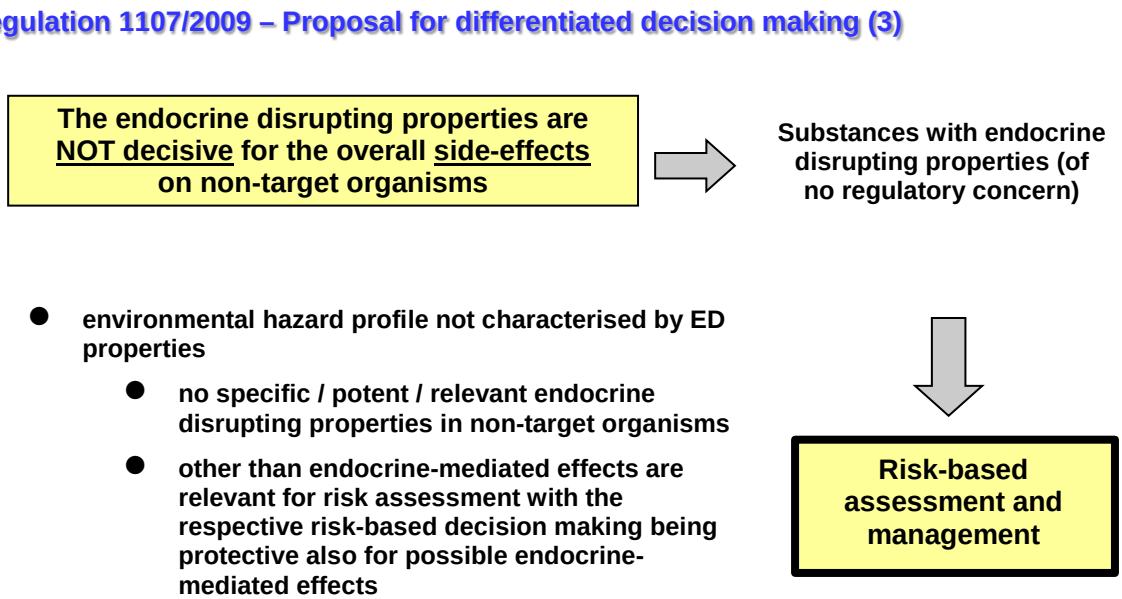
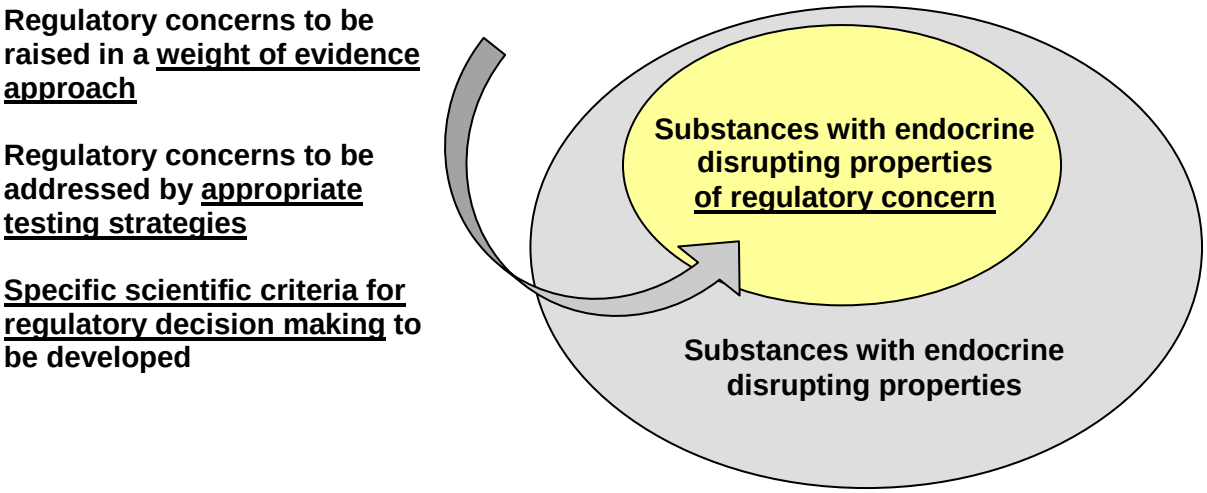


Figure 6



3.6 UK CRD proposal for a regulatory definition of an ecotoxicological endocrine disrupter

Catherine Pepper
Chemicals Regulation Directorate, Health & Safety Executive, UK

Under new and draft regulations, chemicals with endocrine disrupting properties are to be subject to approval exclusion criteria. Despite these stipulations, at the present time there is no definition and/or set of criteria within these pieces of legislation, by which to identify substances that are endocrine disrupters (EDs), in relation to potential effects on human health and/or other species in the environment.

The aim of this work is to propose a definition and associated interpretative criteria that can be applied to identify EDs, specifically focusing on ecotoxicological EDs. The proposal aims to identify EDs of concern for which regulatory action can be taken within the provisions of the current legislative framework and has been developed in the context of the needs and characteristics of EC Plant Protection Products (pesticides) legislation, in terms of availability of data and regulatory consequences. As such, the proposal stipulates that in addition to the internationally recognised definition of an endocrine disrupter (as adopted by the WHO/IPCS in 2004) the following additional criteria should be observed:

a) Evidence of an endocrine mode of action and an adverse effect on population stability or recruitment in an intact organism;
b) prominence of the endocrine effect with respect to the lead toxic effect on the target organism.

These proposals could also be relevant to the way in which endocrine disruption is intended to be a focus of attention under forthcoming EC biocides legislation; and to the requirements of identifying industrial chemicals as EDs and thereby potentially subject to authorisation under REACH. In these cases it might be that some adjustment in the criteria by which EDs are identified is necessary to accommodate the characteristics of these pieces of legislation and the substances and situations they cover.

3.7 Refinement of the ECETOC approach to identify endocrine disrupting properties of chemicals in ecotoxicology

Lennart Weltje[1], James R. Wheeler[2], Arnd Weyers[3] and Malyka Galay Burgos[4]
[1] *BASF, Limburgerhof, Germany*
[2] *Syngenta, Bracknell, Berkshire, United Kingdom*
[3] *Bayer CropScience, Monheim, Germany*
[4] *ECETOC, Brussels, Belgium*

The first ECETOC technical report (ECETOC, 2009a) and associated workshop (ECETOC, 2009b) presented a science-based proposal on how to identify endocrine disrupting properties of chemicals for both human health and the environment. The synthesis of the technical report and the workshop report was published by Bars *et al* (2011). However, to be able to discriminate chemicals with endocrine disrupting properties of low concern from those of higher concern (for regulatory purposes), it was recognised that the concept needed further refinement.

This paper elaborates various aspects that are deemed critical for the ecotoxicological assessment. The following aspects were discussed: Adversity, population relevance, specificity, potency and negligible exposure. While some aspects are common also to toxicology, i.e. adversity, specificity and potency, other aspects are specific to ecotoxicology. One of the main differences is the aim to protect populations instead of individuals. This allows ecotoxicologists to accept some individual level effects as long as the population is not impacted. Common definitions for what constitutes an adverse effect (e.g. ECETOC, 2002), but also the definition for what constitutes an endocrine disruptor (e.g. Weybridge, 1996), are at present defined for toxicology (i.e. for individuals) and need to be adapted to populations to reflect the protection goal of ecotoxicological risk assessments. This implies that an endocrine disruptor in toxicology is not necessarily an endocrine disruptor in ecotoxicology. It is therefore crucial to determine which endpoints are population relevant and also what level of effect on these endpoints may impact a population. Thus, both statistical and biological relevance should be considered in this assessment. A detailed inspection of endpoints from chronic studies with fish, amphibians, birds and mammals has started to determine their relevance for populations and also their relation with endocrine mechanisms.

Another significant difference is the consideration of all species (except humans) in an ecotoxicological assessment. This obviously increases the complexity of the assessment, and it should also be recognised that mechanistic insight in the toxic mode of action (including a possible endocrine-mediated one) for each taxonomic group deemed relevant in the risk assessment is presently not available. However, when considering specificity (i.e. is the endocrine-mediated effect occurring at the lowest concentration and thus driving the risk assessment?), other species may come into play as they could have more sensitive (lower) non-endocrine-mediated endpoints. Therefore, the endocrine-mediated NOEC/NOEL is first

compared with other NOECs/NOELs within the same species group to determine if it is indeed the lowest endpoint. If it does constitute the lowest endpoint, then the endocrine NOEC/NOEL is compared with those of other species within the same compartment (e.g. aquatic) to determine if there are lower endpoints. For example, an endocrine-mediated effect in fish with a NOEC, which is higher than the NOEC for *Daphnia*, is considered of less regulatory concern as the endocrine-mediated NOEC is not driving the aquatic risk assessment. The concern is obviously lower as there is an extra margin of safety. The size of the margin is likely to differ between aquatic organisms and terrestrial vertebrates and may also depend on the specific regulation by which the chemical is regulated.

Another aspect is the consideration of 'negligible exposure' that is part of the regulatory context for plant protection products and biocides. As negligibility is not defined, it is implied to lie somewhere between zero and a regulatory acceptable concentration as determined through an appropriate risk assessment. The introduction of the words 'negligible exposure' into a paragraph describing an otherwise hazard-based cut-off criterion implies that exposure does play a role. After all, negligibility can only be determined when both hazard and exposure are known.

Finally, the potency of a chemical should be taken into account by using various measures such as the acute-to-chronic (endocrine) ratio, the exposure duration necessary to cause an effect and a comparison of no-effect concentrations between the test chemical and a reference chemical. The most practical aspect is the acute-to-chronic (endocrine) ratio for aquatic organisms for which a trigger of 10 can be set below which effects are not considered specific and thus of no special concern. Knacker *et al* (2010) suggested a trigger of 20 appropriate. For terrestrial organisms the acute-to-chronic ratio concept needs to be developed. Another aspect of potency is to consider if endocrine-mediated effects already occur after a short exposure duration (in contrast to sustained exposure in worst-case laboratory studies). As short or pulsed exposures are realistic exposure scenarios it is important to determine if there is reversibility or even a lack of endocrine effects after such exposures. If that is the case, then the chemical is of lower concern.

Further, as some chemicals are made to interfere with hormone systems of their target pests (e.g. insect growth regulators such as juvenile hormone analogues and ecdysone receptor agonists or plant growth regulators such as synthetic auxins) special consideration should be given to such chemistries. It is proposed that chemicals targeting non-vertebrates undergo a normal risk assessment, but that chemicals targeting vertebrate endocrine systems should undergo an assessment such as the ECETOC approach.

4. REPORTS FROM THE SYNDICATE SESSIONS

4.1 Syndicate A (Toxicology)

Moderator: Paul Foster (*Theme I*)
Rapporteur: Sharon Munn (*Theme I*)

Moderator: Roland Solecki (*Theme II*)
Rapporteur: Nina Hallmark (*Theme II*)

Rémi Bars
Mohamed Benahmed
Bernard Bottex
Ivana Fegert
Aldert Piersma
Petra Winkler

Theme I: Use of weight of evidence (WoE) for decision making

What constitutes a sufficient WoE to identify a chemical as an endocrine disrupting chemical?

It was the view of this group that the WoE required to identify a chemical as an endocrine disrupting chemical should be evaluated on a case-by-case basis, and that the application of expert judgment was required. A WoE should not consist simply of the number of positive studies versus the number of negative studies. WoE evaluations require that consideration is given to the full spectrum of available data and not just isolated incidences of a change in a single parameter (such as ano-genital distance). For the identification of an endocrine disrupting chemical an adverse health effect needs to be established in *intact* organisms (or their progeny) and then the biological plausibility of an ED-related mode of action needs to be established.

Effects normally measured in standard toxicology studies should be evaluated to determine if ED-related endpoints are affected. Where this is the case, additional information may be required to decide whether the substance is an endocrine disrupting chemical. (This is central to the concept of WoE – our clear position is that adverse effects in apical studies in the absence of clear compelling MoA evidence are insufficient to regard a chemical as an endocrine disrupting chemical.) The submitter should then be asked for additional mechanistic information to fulfil this need, and the assays required should be selected based on the potentially ED-related endpoints observed in the apical studies.

How do we deal with data poor situations in a WoE approach?

EDs can be identified in standard toxicology tests that are routinely performed to fulfil the requirements of various regulatory programmes. In particular, ED-mediated toxicity can be detected in repeated-dose, reproductive and developmental toxicity, and carcinogenicity studies required by pesticides and biocides regulatory programmes. However for lower tonnage substances under REACH endocrine screening data are currently not part of regulatory packages for chemicals, biocides or pesticides. In case of data poor substances, the starting point for endocrine disrupting chemical assessment would be alerts from any available information, including SAR or QSAR data, as well as (at 10 tonnes) data from 28-day toxicity studies. The function of mechanistic studies is to give pointers to the potential significance of *in vivo* effects and not to discount those findings, as well as providing the link to endocrine disrupting mode of action and/or relevance to humans. However, the group discussion did not resolve whether the next step would normally be the generation of *in vitro* mechanistic data or whether to go directly to further investigations *in vivo*. In case of data rich substance endocrine-mediated toxicity is usually suspected by analysing the data from *in vivo* apical studies (reproductive / developmental, chronic and carcinogenicity studies).

For chemicals regulated under REACH, the group discussed what the next steps should be in the case of positive Herschberger or uterotrophic assays, i.e. could the results from such screening assays be used directly in risk assessment or would further *in vivo* investigations be required? The group agreed that the results from such screening assays were not sufficient by themselves, since they would not fulfil the agreed definition for endocrine disrupting chemicals (causing *adverse effects* in *intact* organisms). In such cases, further *in vivo* investigations would usually be required. However, in the plenary session the Herschberger protocol with the non-castrated male was referred to as one that may be useful in the risk assessment as this would be an intact organism.

The group debated on the existence of a threshold for endocrine disrupting effects. Any substance that enters the body will have a physiological effect and homeostatic mechanisms exist to deal with this. The question is at what level of response does this become *adverse*? The threshold would be at the point where the effect becomes adverse. The consensus conclusion from this group was that endocrine disrupting effects do have a threshold although it was acknowledged that thresholds are not easy to identify and may require specific study designs that are different from current testing approaches.

The sensitivity of *in vivo* studies to detect ED-related effects was discussed. Some *in vivo* regulatory protocols have been updated to encompass enhancements which include ED-sensitive parameters. However, the question arose as to whether further enhancements are

needed, e.g. by starting dosing earlier *in utero* or juvenile stages in cancer bioassays, to cover potentially sensitive life stages.

Group A also gave consideration to the following issues in relation to WoE assessments:

- How many endocrine disrupting chemicals-related mechanisms should be included? Endocrine disruption is not just limited to oestrogen, androgen and thyroid (EAT) targets. However, since most assays currently available are limited to these modalities, focus could be on these first.
- How should reversibility of effects be dealt with in relation to determining whether an effect is adverse or not? For example, are effects on ano-genital distance transient? If a transient effect is not considered adverse, would a substance that caused a transient effect on ano-genital distance not be determined as an endocrine disrupting chemical? Conversely, an agent may cause a delay in puberty. Puberty still occurs (i.e. this is not a permanent effect), but such an effect is still considered adverse and indicative of endocrine disruption. This issue is also related to exposure duration, e.g. does continuous exposure lead to a 'continuous reversible / transient' effect, which by nature of the exposure duration becomes effectively constant and 'irreversible'.
- One mode of action may be manifest in different ways across species (e.g. interference in progesterone production is manifest in the rat in the form of dystocia, but could manifest in different adverse outcomes in other species).

How do we implement a consistent approach to WoE assessment?

It is important and desirable to have a consistent approach, particularly across different regulatory regimes. The aim should be for consistent tools, which will increase the likelihood that the same conclusions will be reached. This can be achieved by developing guidance which is shared across the various regulatory programmes. The IPCS MoA framework (WHO/IPCS, 2007) was supported as a way of presenting the evidence in a WoE assessment. Examples of using this approach for endocrine disrupting chemicals are available in the literature (e.g. Boobis *et al*, 2008; WHO/IPCS, 2002[7]).

[7] Chapter 7: Causal criteria for assessing endocrine disruptors – A proposed framework. [http://www.who.int/ipcs/publications/en/ch7.pdf].

Theme II: Human and population relevance

What are the general accepted MoAs for non-relevance to Humans?

It was considered that there are a few cases where adverse effects observed in rodent studies have been concluded not to be relevant to humans (e.g. some thyroid tumours in rodents). New tools may expand the knowledge base for non-relevance to humans, but these are not ready yet.

Both IPCS MoA approaches for the assessment of human relevance of cancer and non-cancer endpoints, provide a framework to address this issue.

It was suggested that case studies should be used to investigate non-relevance cases for endocrine disruption, because the situation is different to the normal assessment undertaken for reproductive and developmental toxicity. For example, progestin-mediated dystocia in rodents may be an alert for progestin effects in humans although the endpoint itself may differ. In other words, the same mechanism may be of relevance but it may translate into a different health effect in humans. The assessment should begin with the weight of evidence for MoA in the test species from the available test data and then the human relevance should be considered. In doing so, the applicability of the test species would be taken into consideration.

A number of open questions were presented:

- Is the rat the best default model for reproductive toxicology? If not, what are the alternative options?
- Are we expecting or hoping for programmes like ToxCast to support a move away from default models to more human relevant models? However it is difficult to see how, in the near future, isolated cell systems can replace the integrated biology of the endocrine system.
- What about alternative species and *in vitro* tools? How will these tools be used within predictive approaches to risk assessment or are they only useful for prioritisation?
- What will be the role of a QSAR approach with class-specific testing paradigms?
- It was considered that there was still a long way to go before such approaches were commonly used in risk assessments rather than as tools for prioritisation of chemicals for further assessment.

What evidence is needed to support non-relevance cases?

Whilst respecting that the participants agreed that we would need (in many cases) more than just findings from (routine) apical studies, as exemplified in the thyroid case above, to support non-relevance cases for endocrine disrupting chemical, the extent and nature of additional

mechanistic data required was unclear. In any case the demonstration of a non-relevance case is the responsibility of the submitter (Industry).

The assessment of comparative metabolism was raised as a question. Would an evaluation of differences in comparative metabolism be sufficient to decide on a non-relevant case? Should or could such an assessment be based on toxicokinetics? Would this have to be on a qualitative (e.g. an active metabolite is not formed in the human compared to the rodent) versus quantitative (the level of the active metabolite in humans is only 20% of the rodent levels) basis? What would the role of assessment factors be? (WHO/IPCS, 2005)

The participants respected that in the absence of convincing evidence, in accordance with the IPCS mode of action and human relevancy framework, the default was to assume human relevancy.

Overarching issues:

In addition to the specific questions set by the organising committee, the group also discussed some overarching issues, which are bulleted below.

- The importance of understanding differences in susceptibilities during key life stages.
- The need for international harmonisation of test guidelines, definitions, identification criteria and WoE approaches.
- Is the situation more difficult or different for endocrine disrupting chemicals due to high public concern level?
- What is the role of targeted research and education? For example, one task could be to apply the IPCS framework to endocrine disrupting chemicals in a broader context and then analyse for human relevance.
- BfR (German Federal Institute for Risk Assessment) and CRD (Chemicals Regulation Directorate) have plans for an evaluation of chemicals to test the criteria for endocrine disrupting chemical properties according to the DE/UK proposal for practicability.
- It should be considered to include all interested stakeholders e.g. COM, other EU members states, NGOs and Industry in such a project to work together through selected case studies. Several government authorities have proposals that could adopt such a way forward, but it would be improved by having multiple stakeholders involved (i.e. government, academia, industry, etc.) in the task of working through specific case studies exploring the utility of the approach.

4.2 Syndicate B (Toxicology)

Moderator: Susy Brescia (*Theme III*)
Rapporteur: Chris Willoughby (*Theme III*)

Moderator: Helen Håkansson (*Theme IV*)
Rapporteur: Jenny Odum (*Theme IV*)

Neil Carmichael
Pierre Crettaz
Ellen Dhein
Philippa Edwards
Ulla Hass
Dick Lewis
Philipp Marx-Stölting
Ben van Ravenzwaay
Maurice Whelan

Theme III: Lead toxic effect / specificity

In identifying endocrine disrupting chemicals should specificity be taken into consideration?

Group B modified this question to assess sensitivity (i.e. the lead effect) rather than specificity. The syndicate agreed that a specificity criterion was redundant because when MoA information is available showing that the underlying mechanism of the observed adverse effect(s) is endocrine, the adverse effect, by default, is deemed a specific endocrine effect and cannot be regarded as the secondary consequence of other toxic effects. The majority of participants in this group agreed that sensitivity should be taken into account, especially with reference to EDs of 'Very High Regulatory Concern', but no consensus could be reached on how to do this. As a generalisation, if a substance is regulated based on the most sensitive endpoint, then this should provide a safety margin for less sensitive effects. The main issues surrounding lead effects were:

1. Although the consideration of a lead effect is an important criterion for many compounds, for EDs the severity of lead and secondary responses need to be taken into account. A spectrum of effects may be observed at different doses, with less severe (or adverse) effects occurring at lower doses. However, these can be associated or possibly be indicative of more severe effects at higher doses. Which specific effect within the spectrum should be chosen as the lead effect? Some participants felt that to address this potential problem, there should be a factor of at least 10-fold between the lead effect (the effect occurring at the

lowest dose) and the endocrine effect. This would provide further reassurance that the endocrine disrupting effect is not disregarded.

2. The irreversibility of effects at critical time windows of effects needs to be given due consideration when assessing lead effects. Substances may exert irreversible effects during critical time windows of development and are therefore more serious than potentially reversible effects in the mature animal.
3. If there is exposure to a mixture of EDs acting with a similar MoA, the lead effect criterion might lead to insufficient human health protection. However, some participants noted that even if some substances were not to be identified as EDs on the basis of these hazard-based criteria, these substances would still undergo the standard risk assessment and risk management methodologies, including combined risk assessment if at the present time this is mainly applicable to PPPs and biocides.
4. In practice, the lead effect has no huge impact when potency is considered in the evaluation of endocrine disrupting substances of 'Very High Regulatory Concern'.

What degree of separation between the lead effect and an endocrine effect is required?

A factor of 10-fold degree of separation between the lead effect and an endocrine effect was mentioned in the group discussion as a conservative starting point. However, each substance would have to be assessed on a case-by-case basis, taking into account the nature and severity of both the primary lead effect and the endocrine effects.

Theme IV: Using potency to differentiate endocrine disrupting properties

What is the basis for the proposed potency concept?

Two opinions were voiced in the group discussions:

- **Option 1:** EDs that require severe regulatory action (prohibition under PPPR and draft BPR and authorisation under REACH) should be defined by a series of criteria that include potency considerations. The basis for this is two-fold: 1) Toxic endocrine effects that occur at excessively high dose levels tend to represent the unspecific and generalised response of the body to the chemical insult; 2) Stringent regulatory measures such as prohibition under PPPR and draft BPR and authorisation under REACH should be reserved to the more potent EDs. These same stringent regulatory measures apply to CMR cat 1A or 1B substances (but not to CMR cat 2) under these regulations. CMR cat 1A or 1B substances possess serious, well-established and specific hazard properties. Therefore, only the more potent EDs can be considered of equivalent concern to CMR 1A or 1B substances. It was also noted that the less potent EDs are not completely disregarded as these substances would

still undergo standard risk assessment and risk management (risk evaluation under PPPR and BPR and Chemical Safety Assessment under REACH) for chemicals of more than 10 tonnes CSA if classified.

- **Option 2:** EDs that result in clear adverse effects should be considered to be of 'Very High Regulatory Concern' regardless of the dose at which this occurred. This is because there are no dose cut-off values in the criteria for CMR substances. An additional category of 'E' was proposed for EDs.

The majority of the group considered that the use of the CLP STOT-RE guidance values to take potency into account is a pragmatic way of making the new legislation not only more workable and more proportional but also more in line with scientific principles of risk assessment (i.e. option 1). They considered potency to be important to distinguish between chemicals of very high concern and those of lesser concern, although some concern was expressed that potency had no place in identifying endocrine disrupting chemicals.

How should the studies and their exposure durations be integrated into the potency concept?

To start the evaluation, sufficient data are needed to assess whether a chemical is an endocrine disrupting chemical. The studies included in the evaluation have to be reproducible and robust; the data quality needs to be assessed. Non-guideline studies or studies not performed according to GLP should only be included in the assessment if the data are of sufficient quality. The point was also made that although guideline studies conducted under GLP would be desirable to ensure confidence in the data produced, it was yet to be fully established that the design of current guidelines is sufficient to detect endocrine disrupting specific effects at the relevant life-stages and in the more susceptible populations.

The STOT-RE guidance values could be used to grade specific effects on endocrine organs. However, the group noted that the STOT-RE guidance values (normally used to grade toxic effects in adult animals) may not be appropriate when grading toxic effects observed in developing animals (i.e. NOAELs established in adults may be too high for young animals). In addition, the nature of specific endpoints observed and their sensitivity need to be considered within the potency concept, e.g. foetal genital malformations versus behavioural effects. However, some participants felt that this concern was not supported by the available scientific evidence and that effect and no effect levels derived in studies conducted in relevant sensitive life stages (such as foetal effects in developmental toxicity studies or post natal effects in reproduction studies) could be appropriate endpoints on which to base an assessment of potency.

Should the dose (NOAEL/LOAEL) be compared to exposure in order to assess risk?

The group agreed that the answer to this question was 'yes', and that the level of exposure in animal studies should be compared with the expected or known human exposure.

It was highlighted that the concept of potency has been introduced in some proposals because EU legislation had introduced hazard-based cut off-criterion for endocrine disrupting chemicals. The concept of potency could make these hazard-based criteria more in line with the scientific principles of risk assessment so that more potent EDs would be regulated more stringently than less potent EDs.

4.3 Syndicate C (Ecotoxicology)

Moderator: Daniel Pickford
Rapporteur: Melanie Gross

Malyka Galay Burgos
Raimund Grau
Christoph Schäfers
Chris Turner
James Wheeler
Jochen Matthes (observer)

Theme I: Use of weight of evidence (WoE) for decision making

What constitutes a sufficient WoE to identify a chemical as an endocrine disrupting chemical?

There was general acceptance and support of the UK proposal presented before the breakout sessions. The key points were that a) an adverse effect of population relevance for ecotoxicology needs to be observed in an intact organism in apical studies, and b) coherent evidence from screening and mechanistic studies are required to identify an endocrine mode of action. Both lines of evidence are required to determine whether a substance is defined as an endocrine disrupting chemical for regulatory purposes.

How do we deal with data poor situations in a WoE approach?

The group agreed that a WoE assessment will highlight any data gaps and/or concerns. Identification of data gaps or potential concerns will then inform the development of an appropriate testing strategy.

The group also considered the situation where no information at all is available for a substance. In this instance the first steps would be to read across from other similar chemicals and initiate *in silico* and *in vitro* investigations.

How do we implement a consistent approach to WoE assessment?

In order to implement a consistent approach to WoE assessment, an agreed methodology is required as a first step. Various guidances exist for WoE approaches, both general e.g. the recent

ECHA guidance on reporting weight of evidence[8], and specific to endocrine disruption e.g. Brown *et al* (2001), Cefic EMSG (1999) and Borgert *et al* (2011). These existing methodologies need to be evaluated and useful elements combined for the current requirements, and this may benefit from further evaluation of other potential WoE approaches e.g. driven by Multi-Criteria Decision Analysis (MCDA). Once the Commission issues draft criteria for the identification of endocrine disrupting properties, this activity should become a priority.

Common components of the existing WoE methodologies are:

a) Assessment of data quality, e.g. Klimisch assessment – however, the Klimisch codes need to be modified and expanded to be more relevant for ecotoxicological assessments.
b) A form of weighting based on the relevance of individual studies for the assessment of endocrine disrupting chemicals.

The group agreed that it is mainly the transparency of the WoE method that was of key importance. This could likely take the form of a logical decision tree; quantitative methods for weighting studies may not ultimately be required.

Theme II: Human and population relevance

What evidence is needed to support non-relevance cases?

Although this question was a human health-related question, the group discussed the point that non-relevance cases did not extend to ecotoxicological assessment. In other words, any mammalian data related to mode of action are relevant for the ecotoxicological assessment of endocrine disruption in non-human mammalian and wildlife species. For example, there might be a chemical which exhibits some thyroid-activity in a rodent model, that while not relevant to human health risk assessment owing to physiological differences between the rat and human thyroid axes, would nevertheless suggest a mode of action which could be relevant to and indicative of potential for thyroid disruption in wildlife species that might have population relevance (e.g. amphibians).

Which endpoints are considered population relevant?

The group agreed that specific guidance to cover this aspect was required. Some endpoints are clearly population-relevant in their own right, whereas others may need to be considered as part of clusters of supporting endpoints. Therefore endpoints from ecotoxicological studies would ideally be weighted, in order to distinguish between endpoints with population relevance in their own right, and those providing additional supporting evidence when co-occurring in a cluster.

[8] 2010 Practical guide 2: How to report weight of evidence (available in 22 languages). European Chemicals Agency, 2010: http://echa.europa.eu/doc/publications/practical_guides/pg_report_weight_of_evidence.pdf

For example, in fish tests there are some endpoints that are clearly population-relevant endpoints in their own right (e.g. fecundity, fertility, time to maturity and sex ratio), while others are more diagnostic in nature (e.g. secondary sexual characteristics) but that when taken together with other related endpoints (e.g. sexual behaviour) might be considered to have population relevance as a cluster.

Whether a specific endpoint is population relevant can depend on the life history and reproductive strategy of the species in question. For example, time to maturity can matter for certain fish species with defined / limited spawning periods. As we work with a limited number of species in ecotoxicology studies, there needs to be consideration of extrapolation for these endpoints to life history strategies relevant for species in the wild.

It was also highlighted that some clearly population-relevant endpoints, such as sex ratio, can be affected for reasons other than endocrine disruption (e.g. sex specific mortality) and that such other reasons should be considered.

The amphibian assay was discussed. Limitations of a higher-tier, apical frog test with respect to reproductive endpoints (inability for Xenopus / Silurana to breed spontaneously in standard laboratory test conditions) means that it may be hard to definitely confirm population relevance of changes in reproduction-related endpoints in supporting *in vivo* studies (e.g. the amphibian metamorphosis assay - AMA) or in mechanistically diagnosing *in vivo* or *in vitro* studies. Equally, it is not clear what additional population-relevant endpoints concerning development would be incorporated in a higher tier definitive amphibian test, that are not already embedded in the AMA (i.e. developmental stage progression through larval development).

What levels of change in population relevant endpoints are considered significant?

The level of change in a population relevant endpoint that should be considered significant will be different for the various endpoints (and life history strategies of the species in question). The group agreed, however, that this assessment should be based on the *biological* significance and not only on the statistical significance of a specific effect. This is particularly crucial in the case of data coming from toxicology used for assessing effects on terrestrial mammals. Because of the excessive homogeneity of the animals used in these studies extremely small changes often become statistically significant. Current ongoing work on modelling population-relevant endpoints was touched upon in this discussion group, i.e. studies in which endpoints are fed into models together with life history information to determine whether population stability is affected. The group agreed that modelling approaches may help to tease out what level of change is biologically significant. Eco-epidemiology may be beneficial in supporting the extrapolation of modelling approaches to wild species with various life histories; however, causal analysis will be difficult in nearly any case.

4.4 Syndicate D (Ecotoxicology)

Moderator: Peter Day
Rapporteur: Mike Roberts

Larry Frey
Ionna Katsiadaki
Catherine Pepper
Lennart Weltje
Arnd Weyers

Theme III: Lead toxic effect / specificity

In identifying endocrine disrupting chemicals should specificity be taken into consideration?

The group agreed that specificity of endocrine effects should be taken into consideration, but that the details of how this should be done in practice still needed further discussion. When evaluating a data set for a specific compound, specificity should be considered within species first, and then between species.

How is a real difference in specificity within a study and a database recognised?

The group reviewed and discussed the Weyers *et al* (2011) poster presented during the poster session of the workshop (Guidance in identifying endocrine disrupting effects: Specificity of environmental species) (see Appendix A). This was considered to be a valid approach. In the poster, a hypothetical example of a data set for a plant protection product was presented. The lowest non endocrine endpoint (aquatic plants) was 30 fold lower than the endocrine-mediated endpoint in fish and there was general consensus that this margin was protective to ensure that no endocrine effects would occur in the field under conditions of safe use[9].

What degree of separation between the lead effect and an endocrine effect is required?

The group felt that an absolute value could not be recommended. However, there was agreement that a higher degree of separation between the lead and an endocrine effect was needed for aquatic species than terrestrial species. This is due to several factors: (a) The greater diversity of species (vertebrates and invertebrates) considered in the aquatic risk assessment compared to the

[9] Please note that the draft CRD paper "Definition of an ecotoxicological endocrine disruptor for regulatory purposes" (2011) refers to "orders of magnitude" for such cases.

taxonomically less diverse group of birds and mammals in the terrestrial risk assessment, and (b) for mammals (and birds) there are usually reproduction data available. The difference in test designs (fewer dose groups with a larger spacing factor for birds and mammals) needs to be taken into account in deciding on the required degree of separation. The above argument is also reflected in the currently used assessment factors in acute and chronic PPP risk assessment for aquatic organisms (100 and 10) and terrestrial vertebrates (10 and 5) respectively.

Theme IV: Using potency to differentiate endocrine disrupting properties

What is the basis for the proposed potency concept?

There was agreement amongst the group that potency should be used to differentiate between substances of high and low endocrine disrupting concern, but that potency should not be used to *define* whether a substance was an endocrine disrupting chemical. It was stressed that the discussion of using a potency assessment in this way was only in relation to the endocrine disruption approval criterion for plant protection products under Regulation 1107/2009 (EC, 2009a). It was also highlighted that less potent substances passing this approval criterion would still need to undergo a normal risk assessment.

Should potency be compared with exposure in order to assess risk?

Yes, potency should be compared with exposure. Two examples were briefly discussed to illustrate the importance of comparing potency with exposure to assess risk: a) A low potency compound with high exposure could be of concern, whereas b) a high potency compound with very low exposure could be of no concern. In the plenary feedback session, the majority of the participants expressed their preference for a risk assessment over a hazard-based cut off criterion. There was no ad-hoc proposal how to achieve this, given the current wording of the PPP regulation 1107/2009 (EC, 2009a).

How should the studies and their exposure durations be integrated into the potency concept? For ecotoxicology how can potency be measured?

These two questions were grouped together for the discussion and feedback sessions. The group discussed the concepts of: a) ACR (acute-to-chronic ratio), b) duration of exposure before effects are observed, c) using a potent reference substance for comparison and d) the number of species affected.

There was agreement in the group that ACR and duration until effects are observed are likely to be better differentiators of potency, while comparisons with a reference compound and the number of species affected were considered less helpful. It was noted that aquatic ACR data were currently available, and that further data for terrestrial species (mammalian and avian) could be collated.

During the plenary feedback session the ACR concept was discussed further. It was commented that generally an ACR of 15 would indicate that a substance is not acting via a specific mode of action (MoA). If a specific MoA (such as an endocrine MoA) is involved, then the ACR for a substance would be larger. Therefore the ACR could be used as an indication of a specific MoA and as a trigger for further evaluation to be conducted. This raised the question in the plenary session as to whether the ACR was actually more related to specificity of a compound rather than potency. For considerations of potency, the dimensionless ACR would need to be combined with information on the concentration or dose level (NOEC/NOEL) for endocrine-mediated effects. It was further suggested that an equivalent of the STOT-RE values proposed for the potency assessment in human health, be developed for ecotoxicology.

5. CONCLUSIONS AND RECOMMENDATIONS

Several key conclusions and recommendations arose from the breakout sessions and these are summarised below under their respective themes.

Theme I: Use of weight of evidence (WoE) for decision making

The participants of the breakout groups recommended that the full spectrum of available data be evaluated in a WoE assessment of endocrine disrupting properties. The WoE should be conducted on a case-by-case basis, and the application of expert judgement for the interpretation of the data set as a whole is required. The joint UK-DE position, as well as the Danish and ECETOC approaches presented during the workshop, provided an excellent platform for the discussions. The key point highlighted was the requirement for more than one line of evidence. The data need to demonstrate:

1. An adverse effect in an intact organism.
2. Evidence from mechanistic studies showing a biologically plausible endocrine mode of action conducted and assessed according to internationally accepted criteria.
3. In the absence of convincing evidence, in accordance with the IPCS mode of action and human relevancy framework, the default was to assume human relevancy.
4. For ecotoxicological assessments, there is a further requirement that the adverse effect also needs to be of population relevance.

In data poor situations the WoE assessment will highlight the data gaps and direct the development of a testing strategy. If no data are available at all, then the first steps would be to consider read-across from other similar chemicals and *in silico* and *in vitro* investigations. For low volume chemicals under REACH the starting point would be (Q)SAR and (at 10 tonnes) 28-day study data to look for possible alerts, as well as read-across. It was stressed that risk assessments should be performed using the results from apical studies. There was also general agreement that endocrine disruption has a threshold of toxicity, i.e. this would be the point at which the effect becomes an adverse effect. However, it was recognised that threshold of endocrine toxicity may require appropriate study designs to be identified.

The participants concluded that a consistent approach for WoE assessments was required, which would be applicable under each regulatory regime. For human health assessments there was general support for using the WHO/IPCS mode of action framework (WHO/IPCS, 2007). A recommendation was made to run a few case studies through this framework. For ecotoxicological assessments it was acknowledged that there was no direct equivalent to the WHO/IPCS mode of action framework. However, specific WoE frameworks for endocrine disruption have been published, and these should be evaluated and combined for the requirements

under current legislation. Key aspects that need to be included are the assessment of data quality and relevance weighting of each study type for the evaluation of endocrine disruption.

Theme II: Human and population relevance

In relation to human relevance the participants concluded that there are only few cases where effects observed in rodent studies are considered not to be relevant to humans. The only example mentioned was the rodent thyroid hyperplasia and tumours caused by increased hepatic metabolism and clearance of thyroid hormones following exposure to some substances that do not occur in humans under similar exposure. It was highlighted that the default position is to assume human relevance unless good data demonstrate otherwise. A recommendation was made that the IPCS framework be applied to endocrine disrupting chemicals specifically to evaluate human relevance as case studies.

The participants agreed that specific guidance was required to aid in the identification of endpoints in ecotoxicological studies that are of population relevance. Some endpoints are clearly population related on their own, whereas others are more diagnostic in nature and are needed as part of clusters of endpoints to infer population relevance. Some endpoints fulfil both these aspects. There was a consensus amongst the participants, that the level of change in population-relevant endpoints that should be considered significant should be based on biological significance, not statistical significance alone. Current and future work in the area of population modelling and eco-epidemiology may help to define the significance levels further.

Theme III: Lead toxic effect / specificity

There was broad support in the discussion groups for the concept of taking into account the lead toxic effect *versus* specificity of endocrine-mediated effects. However, it was noted that this would depend on the degree of separation between the lead effect and the endocrine-mediated effect. The acceptable degree of separation should be assessed on a case-by-case basis, and endocrine disrupting chemical chemicals of very high concern would need a larger safety margin.

For toxicological assessments, a factor of 10 was suggested i.e. if the degree of separation is >10X the substance should not be considered an endocrine disrupting chemical of concern but if the degree of separation is <10X then the substance should be considered as endocrine disrupting chemicals of concern.

For ecotoxicological assessments, it was recommended that specificity should first be evaluated within species, and then between species. The participants felt that further work in ecotoxicology was required before a concrete value for the degree of separation could be recommended.

However, a greater degree of separation between the lowest lead effect and the endocrine-mediated effect would be required for the aquatic environment than the terrestrial environment in order to cover the higher diversity of species, which is in line with current risk assessment approaches (e.g. for pesticides).

There was general agreement that the method proposed in a poster presented at the workshop by the ECETOC Task Force (Weyers *et al*, 2011) was a useful and valid approach to identify a real difference in specificity within a study and an ecotoxicological database.

Theme IV: Using potency to differentiate endocrine disrupting properties

The majority view was that as the relevant regulations were hazard-based, introducing the concept of potency served to discriminate the substances of highest concern from those of lesser concern. This was considered a poor substitute for risk assessment but was, in some measure, a surrogate for it. Potency is a key concept in (eco)toxicology and is used in many areas to determine chemicals according to intrinsic toxicity in classification and labelling systems.

Substances with endocrine disrupting properties which are not caught by the potency assessment and cut-off criterion are still considered as endocrine-active substances, but rather than being prohibited, they should undergo standard risk assessment.

Potency should also be considered in ecotoxicological assessments in order to differentiate between levels of concern for endocrine disrupting chemicals. It was suggested that the acute-to-chronic ratio could provide an indication of whether a specific mode of action was involved, and when combined with the NOEC for endocrine-mediated effects could be used as a measure of potency. Another useful measure of potency to be considered is the duration of exposure until effects are observed.

A view was expressed that potency had no place to define whether a substance was an endocrine disrupting chemical but has its place when taking into consideration exposure levels, since a low potency substance with high exposure level could be of concern whereas a high potency substance with very low exposure level could be of no concern.

The workshop provided a forum for stimulating discussion of the key issues in the risk assessment of endocrine disrupting chemicals. The Task Force is grateful for the input received at the workshop and intends to use this to refine the ECETOC guidance further.

ABBREVIATIONS

ACR	Acute-to-chronic ratio
ADME	Absorption, distribution, metabolism, and excretion
AMA	Amphibian metamorphosis assay
BfR	German Federal Institute for Risk Assessment
BPR	Biocidal products regulation
Cefic	The European Chemical Industry Council
CEHOS	Danish Centre on Endocrine Disrupters
CLP	Classification labelling and packaging of substances and mixtures
CMR	Carcinogenic, mutagenic or reprotoxic
COM	(European) Commission
CRD	Chemicals Regulation Directorate
CSA	Chemical Safety Assessment
DG ENV	Directorate General Environment
DMI	Demethylation inhibitors
EAT	Oestrogen, androgen and thyroid
EC	European Commission
EC_{50}	Effective concentration, 50%
ECHA	European Chemicals Agency
ED	Endocrine disrupter / Endocrine disrupting chemical
EDTA	Endocrine disrupters testing and assessment
EMSG	Endocrine modulators steering group
EPA	Environmental Protection Agency
ERA	Environmental risk assessment
EU	European Union
GLP	Good laboratory practice
HESI	Health and Environmental Sciences Institute
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
LOAEL	Lowest observed adverse effect level
MAP	Mitogen-activated protein
MCDA	Multi-criteria decision analysis
MoA	Mode of action

NGO	Non-Governmental Organisation
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
OECD	Organisation for Economic Co-operation and Development
PPP	Plant protection products
PPPR	Plant protection products regulation
QSAR	Quantitative structure activity relationship
REACH	Registration, evaluation, authorisation and restriction of chemicals
SAR	Structure activity relationship
STOT-RE	Specific target organ toxicity - repeated exposure
SVHC	Substance of very high concern
TR	Technical report
UBA	Umweltbundesamt / German Federal Environment Agency
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation
WoE	Weight of evidence

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[http://www.pesticides.gov.uk/approvals.asp?id=3034];
and on the BfR website at:
[http://www.bfr.bund.de/cm/349/regulatory_definition_of_an_endocrine_disrupter_in_relation_to_potential_threat_to_human_health.pdf].

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APPENDIX A: POSTER

Guidance on identifying endocrine disrupting effects: Specificity for environmental species

Weyers A, Weltje L, Wheeler JR, Galay Burgos M. 2011.

Specificity

1107/2009 EC (PPP) and 1907/2006 (Reach): restrictions for substances with "endocrine disrupting properties that cause adverse effects in non-target species"

Definitions of ED relate to effects in intact organisms (Weybridge, WHO/IPCS)

➔ Specificity within one study /taxon is required (endocrine effect is lower than general toxicity) to differentiate primary endocrine-mediated effects from secondary ones caused by systemic toxicity

Specificity across taxa:

Overview of the compartment assessments allows for a margin of safety preventing endocrine specific effects to occur.

➔ Under conditions of safe use the potential endocrine effects would not occur and under natural conditions the substance is not an endocrine disrupter for the environment, or exposure to the substance is negligible.

The hypothetical example (adapted from a herbicide case) illustrates the concept

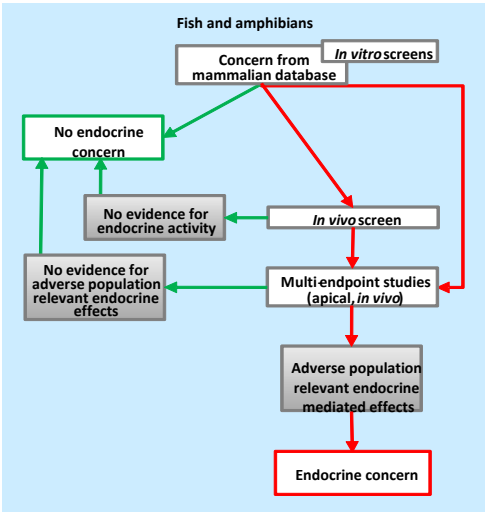

Fig. 1: ECETOC guidance (ECETOC 2009a, b; Bars et al. 2011) applied to a hypothetical substance. Initial concern from database is estrogenic activity, hence sex ratio would be a corresponding relevant endpoint.

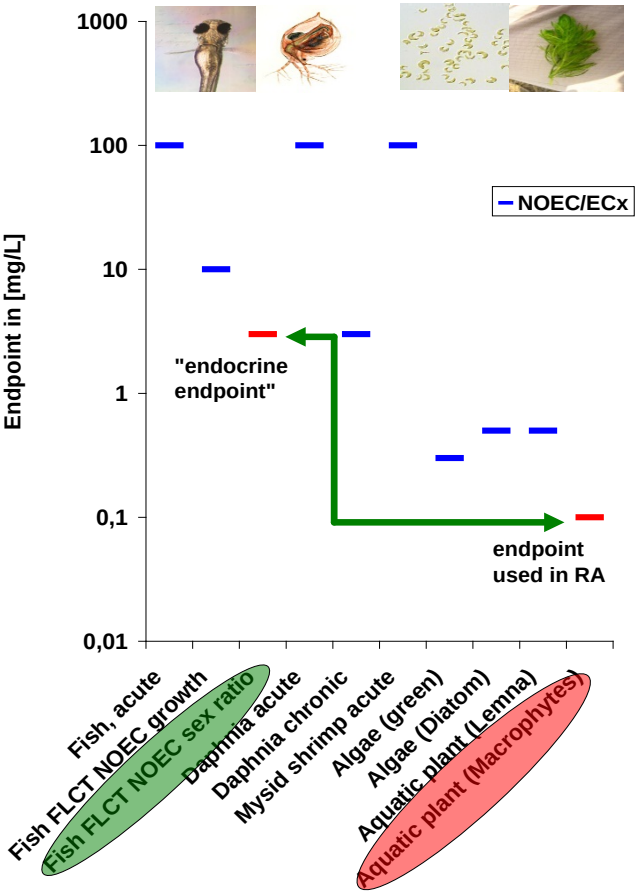

Fig. 2: Endpoints for hypothetical substance (herbicide). Factor 30 between "endocrine" fish endpoint and regulatory endpoint that drives risk assessment.
The endocrine endpoint in fish is non-specific and the substance is not an endocrine disruptor under realistic exposure conditions.

Discussion and Conclusion

Specificity should be considered in the assessment of substances for endocrine activity.

Specificity within one study (or taxon), i.e. the question if the endocrine effect is the lowest effect observed, should be considered early in the endocrine assessment, when linking apical in-vivo effects and mechanistic (*in vitro* and *in vivo*) data (Fig. 1)

When endocrine concern has been confirmed, **specificity across taxa should also be considered**. In the example presented above, there is a factor 30 between the "endocrine" fish endpoint and the regulatory endpoint (macrophytes) that drives risk assessment. Thus under conditions of safe use as detailed in the risk assessment, the potential endocrine effects would not occur and the **substance does not have endocrine disrupting properties that cause adverse effects in non-target species**.

The question remains, **what margin of safety is minimally required** to arrive at this conclusion if the difference between endocrine mediated endpoints and non-endocrine endpoints is smaller.

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APPENDIX B: LIST OF PARTICIPANTS

First Name	*Name*	*Affiliation*	*E-mail*
Rémi	Bars	Bayer CropScience, France	remi.bars@bayer.com
Mohamed	Benahmed	CHU Nice (Inserm), France	benahmed@unice.fr
Bernard	Bottex	EFSA, Italy	bernard.bottex@efsa.europa.eu
Susy	Brescia	HSE CRD, UK	susy.brescia@hse.gsi.gov.uk
Neil	Carmichael	ECETOC, Belgium	neil.carmichael@ecetoc.org
Pierre	Crettaz	Swiss Office of Public Health, Switzerland	pierre.crettaz@bag.admin.ch
Peter	Day	ECPA, Belgium	peter.day@ecpa.eu
Ellen	Dhein	Bayer AG, Germany	ellen.dhein@bayer.com
Philippa	Edwards	Health Protection Agency, UK	philippa.edwards@hpa.org.uk
Ivana	Fegert	BASF, Germany	ivana.fegert@basf.com
Paul	Foster	NIEHS, USA	foster2@niehs.nih.gov
Larry	Frey	Wildlife International Ltd., USA	lfrey@wildlifeinternational.com
Malyka	Galay Burgos	ECETOC, Belgium	malyka.galay-burgos@ecetoc.org
Raimund	Grau	Bayer CropScience, Germany	raimund.grau@bayer.com
Melanie	Gross	WCA-environment, UK	melanie.gross@wca-environment.com
Helen	Håkansson	Karolinska Institutet, Sweden	helen.hakansson@ki.se
Nina	Hallmark	ExxonMobil, Belgium	nina.hallmark@exxonmobil.com
Ulla	Hass	National Food Institute, Technical Univ. of DK, DK	ulha@food.dtu.dk
Ionna	Katsiadaki	CEFAS, UK	ionna.katsiadaki@cefas.co.uk
Dick	Lewis	Syngenta, UK	dick.lewis@syngenta.com
Philip	Marx-Stölting	BfR, Germany	philip.marx-stoelting@bfr.bund.de
Jochen	Matthes	ECHA, Finland	jochen.matthes@echa.europa.eu
Sharon	Munn	European Commission, DG JRC, Italy	sharon.munn@ec.europa.eu
Jenny	Odum	RSA, UK	jenny.odum@regulatoryscience.com
Catherine	Pepper	HSE CRD, UK	catherine.pepper@hse.gsi.gov.uk
Daniel	Pickford	Brunel University, UK	daniel.pickford@brunel.ac.uk
Aldert	Piersma	RIVM, NL	aldert.piersma@rivm.nl
Michael	Roberts	DEFRA, UK	mike.roberts@defra.gsi.gov.uk
Christoph	Schäfers	Fraunhofer Institute, Germany	christoph.schaefers@ime.fraunhofer.de
Roland	Solecki	BfR, Germany	roland.solecki@bfr.bund.de
Chris	Turner	Caleb Management Services, UK	chris@calebgroup.net
Ben	van Ravenzwaay	BASF, Germany	bennard.ravenzwaay@basf.com
Lennart	Weltje	BASF, Germany	lennart.weltje@basf.com
Arnd	Weyers	Bayer CropScience, Germany	arnd.weyers@bayer.com
James	Wheeler	Syngenta, UK	james.wheeler@syngenta.com
Maurice	Whelan	European Commission, DG JRC, Italy	maurice.whelan@ec.europa.eu
Chris	Willoughby	Huntingdon, UK	willougc@ukorg.huntingdon.com
Petra	Winkler	Johnson & Johnson, Germany	pwinkle2@its.jnj.com

APPENDIX C: WORKSHOP PROGRAMME

Monday 9th May 2011

12.00 – 12.45	Registration and lunch	
Chairman:		Remi Bars, Bayer CropScience
12.45 – 13.00	**Introduction and aim**	Neil Carmichael ECETOC
13.00 – 13.15	**Report on criteria for endocrine disrupters from the Danish Centre on Endocrine Disrupters**	Ulla Hass National Food Institute, Technical University of Denmark

HUMAN SAFETY

13.15 – 13.45	**Joint UK-DE proposal for a regulatory definition of an endocrine disrupter in relation to human health**	Susy Brescia Chemical Regulation Directorate, HSE
13.45 – 14.15	**Impact project on proposed decision criteria for substances with endocrine disrupting properties**	Philipp Marx-Stölting and Roland Solecki BfR
14.15 – 14.45	**ECETOC proposal to identify endocrine disrupting chemicals of regulatory concern for human health**	Rémi Bars Bayer CropScience
14.45 – 15.00	Coffee break	

ENVIRONMENTAL SAFETY

15.00 – 15.30	**German approach to pesticide assessment**	Christoph Schäfers Fraunhofer Institute
15.30 – 16.00	**UK CRD proposal for a regulatory definition of an ecotoxicological endocrine disrupter**	Catherine Pepper Chemical Regulation Directorate, HSE
16.00 – 16.30	**Refinement of the ECETOC approach to identify endocrine disrupting properties of chemicals in ecotoxicology**	Lennart Weltje BASF
16.30 – 17.15	Coffee break and **poster session**	
17.15 – 17.30	**Questions and discussion** **Introduction to the breakout group sessions**	Dick Lewis Syngenta

17.30 – 19.30 **Breakout Group Sessions**

Theme I Use of weight of evidence (WoE) for decision making

(i) What constitutes a sufficient WoE to identify a chemical as an endocrine disrupting chemical?
(ii) How do we deal with data poor situations in a WoE approach?
(iii) How do we implement a consistent approach to WoE assessment?

Theme II Human and population relevance

(i) What are the general accepted MoAs for non-relevance to Humans?
(ii) What evidence is needed to support non-relevance cases?
(iii) Which endpoints are considered population relevant?
(iv) What levels of change in population relevant endpoints are considered significant?

Theme III Lead toxic effect / specificity

(i) In identifying endocrine disrupting chemicals should specificity be taken into consideration?
(ii) How is a real difference in specificity within a study and a database recognised?
(iii) What degree of separation between the lead effect and an endocrine effect is required?

Theme IV Using potency to differentiate endocrine disrupting properties

(i) What is the basis for the proposed potency concept?
(ii) How should the studies and their exposure durations be integrated into the potency concept?
(iii) Should potency be compared to exposure in order to assess risk?
(iv) For ecotoxicology how can potency be measured?

20.30 – 22.30 Dinner

Tuesday 10th May 2011

08.30 – 10.45 ***Rapporteurs'* feedback from breakout groups and plenary discussion** Moderator: Aldert Piersma RIVM

10.45 – 11.00 **Conclusions** Ben van Ravenzwaay BASF

12.00 – 13.00 Lunch

Close of workshop

APPENDIX D: ORGANISING COMMITTEE

Rémi Bars (Chairman)
Bayer CropScience
355, rue Dostoïevsky
F-06903 Sophia Antipolis Cedex

Ivana Fegert
BASF
Carl Bosch-Str. 38
D-67056 Ludwigshafen

Malyka Galay Burgos
ECETOC
Avenue Van Nieuwenhuyse, 4
B - 1160 Brussels

Dick Lewis
Syngenta
Jealott's Hill International Research Centre
UK - Bracknell, Berkshire, RG42 6EY

Lennart Weltje
BASF
Speyerer-Strasse 2
D - 67117 Limburgerhof

Arnd Weyers
Bayer CropScience
Alfred-Nobel-Str. 50
D - 40789 Monheim

James Wheeler
Syngenta
Jealott's Hill International Research Centre
UK - Bracknell, Berkshire, RG42 6EY

ECETOC Workshops Reports

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels (Published December 2003)
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 (Published March 2004)
No. 3	Workshop on the Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff (Published November 2004)
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin (Published October 2004)
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Paris (Published December 2004)
No. 6	Workshop on Chemical Pollution, Respiratory Allergy and Asthma. 16-17 June 2005, Leuven (Published December 2005)
No. 7	Workshop on Testing Strategies to Establish the Safety of Nanomaterials. 7-8 November 2005, Barcelona (Published August 2006)
No. 8	Workshop on Societal Aspects of Nanotechnology. 7-8 November 2005, Barcelona (Published October 2006)
No. 9	Workshop on the Refinement of Mutagenicity/Genotoxicity Testing. 23-24 April 2007, Malta (Published September 2007)
No. 10	Workshop on Biodegradation and Persistence. 26-27 June 2007, Holmes Chapel (Published September 2007)
No. 11	Workshop on the Application of 'Omics in Toxicology and Ecotoxicology: Case Studies and Risk Assessment. 6-7 December 2007, Malaga (Published July 2008)
No. 12	Workshop on Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study. 14-15 April 2008, Barza d'Ispra (Published August 2008)
No. 13	Counting the Costs and Benefits of Chemical Controls: Role of Environmental Risk Assessment in Socio-Economic Analysis. 4 June 2008, Brussels (Published September 2008)
No. 14	Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. 24-25 June 2008, Brussels (Published February 2009)
No. 15	The Probabilistic Approaches for Marine Hazard Assessment. 18-19 June 2008, Oslo (Published June 2009)
No. 16	Guidance on interpreting endocrine disrupting effects. 29-30 June 2009, Barcelona (Published October 2009)
No. 17	Significance of Bound Residues in Environmental Risk Assessment. 14-15 October 2009, Brussels (Published December 2009)
No. 18	The Enhancement of the Scientific Process and Transparency of Observational Epidemiology Studies. 24-25 September 2009, London (Published December 2009)
No. 19	'Omics in (Eco)toxicology: Case Studies and Risk Assessment. 22-23 February 2010, Málaga (Published June 2010)
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Responsible Editor:
Dr. Neil Carmichael
ECETOC AISBL
Av. E. Van Nieuwenhuyse 4 (bte. 6)
B-1160 Brussels, Belgium
VAT: BE 0418344469
www.ecetoc.org
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