The ECETOC Seven Steps for the Identification of Endocrine Disrupting Properties (ECETOC 7SI-ED)

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EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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European Centre for Ecotoxicology and Toxicology of Chemicals
2 Avenue E. Van Nieuwenhuyse (Bte. 8), B-1160 Brussels, Belgium.

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CONTENTS

SUMMARY

In December 2016, the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) published an *Outline of draft Guidance Document for the implementation of the hazard-based criteria to identify endocrine disruptors* (ECHA and EFSA, 2016) that was compiled with support from the Joint Research Centre (JRC). The Guidance Document that is outlined in ECHA and EFSA (2016) shall be applicable in the context of *Regulation (EC) No 1107/2009 on the placing of plant protection products on the market* (EP and Council of the EU, 2009) and *Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products* (EP and Council of the EU, 2012). Section V of ECHA and EFSA (2016) presents seven steps of a *Hazard identification strategy for endocrine disrupting properties* that follow a weight-of-evidence (WoE) approach. As highlighted in ECHA and EFSA (2016), the identification of endocrine disruptors *will be based exclusively on the evaluation of the relevant hazardous properties of a substance,* and *the Guidance is intended [to] be suitable for both applicants and regulatory authorities.*

Against this background, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) convened an Endocrine Disruptors Task Force (ED TF) to provide guidance on how the ECHA and EFSA (2016) draft outline can be put into practice. The ECETOC ED TF has focused its expertise on providing input for consideration under the seven steps of the hazard identification strategy presented in Section V of ECHA and EFSA (2016). This has resulted in the development of the *ECETOC Seven Steps for the Identification of Endocrine Disrupting properties* (ECETOC 7SI-ED). The ECETOC 7SI-ED, that covers both human and environmental health, aligns with the steps presented in ECHA and EFSA (2016). In this ECETOC Report, the ECETOC 7SI-ED is presented as text and as a flow-chart with detailed explanatory notes on how to address each of the seven steps:

Step I: Gathering of relevant data with regard to adverse effects and endocrine modes-of-action (MoA);

Step II: Evaluation of quality, reliability, reproducibility and consistency of the data;

Step III: Evaluation and summary of the evidence for an adverse effect;

Step IV: Evaluation and summary of evidence for endocrine activity;

Step V: Integration of the evidence and evaluation of biological plausibility that adverse effect and endocrine activity are linked by specific endocrine MoA;

Step VI: Identification of uncertainties;

Step VII: Conclusions on endocrine disrupting properties.

While the ECETOC ED TF believes that a more comprehensive approach to the determination of endocrine disruptors, considering aspects beyond hazard data only, would best inform the process of assessment of endocrine disruptors, the ECETOC 7SI-ED has remained focused on the hazard aspects only as specific in the ECHA and EFSA (2016) outline. The ECETOC 7SI-ED builds on the World Health Organisation / International Programme on Chemical Safety (WHO/IPCS, 2002) definition for an endocrine disruptor and its three components. It offers guidance on how to evaluate and integrate information on each of the three

components of the definition, i.e. how to consider (1) available apical studies to identify adverse effects that may be indicative of endocrine MoA; (2) *in vitro* and *in vivo* data on endocrine (or non-endocrine) activity; and (3) the biological plausibility that the adverse effect and endocrine activity are linked by a specific endocrine MoA. The ECETOC 7SI-ED describes how data and information that may be indicative of endocrine disrupting properties can be collected and evaluated, but it does not prescribe any specific testing.

The ECETOC 7SI-ED applies existing relevant scientific concepts and established best practice frameworks and methodologies e.g. the *Joint Research Centre Toxicological data Reliability Assessment Tool (ToxR Tool)*; the *OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters* (OECD, 2012a); the *OECD Guidance Document No. 150* (OECD, 2012b); and the most recent version of the WHO/IPCS MoA/species concordance framework (Meek *et al.,* 2014a, b). By following the ECETOC 7SI-ED, the data for any regulated substance can be transparently organised and evaluated to reveal the WoE available, its strengths and uncertainties, to compare with the WHO/IPCS (2002) definition for an endocrine disruptor and the scientific criteria set out in the context of the EU plant protection products and biocidal products legislation. This enables a conclusion to be drawn on whether, or not, a substance meets this regulatory definition.

While the ECETOC 7SI-ED is conceived as providing general guidance to fulfil the EU legislative remits on plant protection products and biocidal products (Commission, 2016), it does not constitute a hazard assessment strategy. Consistent with the seven steps outlined in Section V of ECHA and EFSA (2016) and the EU plant protection products and biocidal products legislation, the guidance proposed in the ECETOC 7SI-ED is restricted to the identification of endocrine disrupting properties. Notwithstanding, it is the opinion of the ECETOC ED TF that substances that are identified as possessing endocrine disrupting properties should undergo a comprehensive hazard and risk assessment. This entails the determination of safety thresholds, exposure assessment, potency assessment and the determination whether acceptable risk can be demonstrated. Such an approach has already been implemented internationally, e.g., in the USA and Japan. The hazard characterisation and risk assessment of substances that are identified as possessing endocrine disrupting properties should also establish the human health or environmental population-specific relevance of observed effects. ECETOC Reports are available that provide guidance on how to conduct the hazard and risk assessment of endocrine disrupting substances and on how to establish the human health or environmental population-specific relevance of observed effects.

In conclusion, the ECETOC 7SI-ED is recommended as a tool to assess whether or not a substance possesses endocrine disrupting properties. It is based on robust scientific principles and has been designed to be practical and transparent in its utility with the framework being presented as a series of discrete logical steps, each of which is supported by a clear set of questions and considerations to guide decision-making.

1. INTRODUCTION

In December 2016, the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) published an *Outline of draft Guidance Document for the implementation of the hazard-based criteria to identify endocrine disruptors* (ECHA and EFSA, 2016) that was compiled with support from the Joint Research Centre (JRC). The Guidance Document that is outlined in ECHA and EFSA (2016) shall be applicable in the context of *Regulation (EC) No 1107/2009 on the placing of plant protection products on the market* (EP and Council of the EU, 2009) and *Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products* (EP and Council of the EU, 2012). Section V of ECHA and EFSA (2016) presents seven steps of a *Hazard identification strategy for endocrine disrupting properties* that follow a weight-of-evidence (WoE) approach. As highlighted in ECHA and EFSA (2016), the identification of endocrine disruptors *will be based exclusively on the evaluation of the relevant hazardous properties of a substance, and the Guidance is intended [to] be suitable for both applicants and regulatory authorities.*

Against this background, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) convened an Endocrine Disruptors Task Force (ED TF) to provide guidance on how the ECHA and EFSA (2016) draft outline can be put into practice. The ECETOC ED TF has focused its expertise on providing input for consideration under the seven steps of the hazard identification strategy presented in Section V of ECHA and EFSA (2016). This has resulted in the development of the *ECETOC Seven Steps for the Identification of Endocrine Disrupting properties* (ECETOC 7SI-ED). The ECETOC 7SI-ED, that covers both human and environmental health, aligns with the steps presented in ECHA and EFSA (2016). In this ECETOC Report, the ECETOC 7SI-ED is presented as text and as a flow-chart with detailed explanatory notes on how to address each of the seven steps (*cf.* also Figure 1):

Step I: Gathering of relevant information with regard to adverse effects and endocrine modes-of-action (MoA; *cf.* Section 2: Definitions);

Step II: Evaluation of quality, reliability, reproducibility and consistency of the individual studies;

Step III: Evaluation and summary of the evidence for an adverse effect;

Step IV: Evaluation and summary of evidence for endocrine activity (and non-endocrine activity, if available);

Step V: Integration of the evidence and evaluation of biological plausibility that adverse effect and endocrine activity are linked by specific endocrine MoA;

Step VI: Identification of uncertainties;

Step VII: Conclusions on endocrine disrupting properties.

The three elements of the definition for an endocrine disruptor published by the World Health Organisation / International Programme on Chemical Safety (WHO/IPCS, 2002) are reflected in Steps III-V. This definition is equally acknowledged in the EU plant protection products and biocidal products legislations:

1. *An exogenous substance or mixture that alters function(s) of the endocrine system* (Step IV);

2. *And consequently causes* (Step V);
3. *Adverse health effects in an intact organism, or its progeny, or (sub)populations* (Step III).

As described in the *Commission Communication on endocrine disruptors and the draft Commission acts setting out scientific criteria for their determination in the context of the EU legislation on plant protection products and biocidal products* (Commission, 2016), the legal framework underlying the ECHA and EFSA (2016) outline is driven by the ongoing revision of the EU plant protection products and biocidal products legislation. Notwithstanding, the legal framework is likely to eventually apply within further substance-specific legislations.

Consistent with the seven steps outlined in Section V of ECHA and EFSA (2016) and the EU plant protection products and biocidal products legislation, the guidance proposed in the ECETOC 7SI-ED is restricted to the identification of endocrine disrupting properties. Notwithstanding, it is the opinion of the ECETOC ED TF that substances that are identified as possessing endocrine disrupting properties should undergo a comprehensive hazard and risk assessment. This entails the determination of safety thresholds, exposure assessment, potency assessment and the determination whether acceptable risk can be demonstrated. Such an approach has already been implemented internationally, e.g., in the USA and Japan. The hazard characterisation and risk assessment of substances that are identified as possessing endocrine disrupting properties should also establish the human health or environmental population-specific relevance of observed effects (*cf.* Appendix A: Background Information for further details on the rationale to draw up the ECETOC 7SI-ED) .

While the ECETOC 7SI-ED has been structured in line with the seven steps described in Section V of the ECHA and EFSA (2016) outline, in practice, Steps I-VII of the ECETOC 7SI-ED will not necessarily always be followed in a consecutive order. Depending on the substance under investigation, it may be more appropriate to conduct, e.g., Steps III and IV in parallel or to conduct Step IV before Step III, etc. The most appropriate sequence of steps should be determined on a case-by-case basis. This requires expert judgement, and a scientific justification for the selected sequence should be provided.

In the ECETOC 7SI-ED, the framework presented in Section V of ECHA and EFSA (2016) (*cf.* also Appendix A: Background Information) has been elaborated applying existing best practice frameworks and methodologies. The ECETOC 7SI-ED uses the *JRC Toxicological data Reliability Assessment Tool (ToxR Tool)* to assess the quality of individual studies. The *OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters* (OECD, 2012a) is applied to frame available data for the identification of potential endocrine disrupting properties. The *OECD Guidance Document (GD) No. 150 on standardised test guidelines for evaluating chemicals for endocrine disruption* (OECD, 2012b) is referred to for detailed guidance on the types of assays and studies that may serve to identify endocrine activity or adverse effects that may be indicative of endocrine disrupting properties. OECD Test Guidelines (TGs) are considered preferred test methods. Nevertheless, sufficiently relevant and reliable assays and studies that can be used within the ECETOC 7SI-ED are not limited to OECD TGs so long as justification for their consideration is provided.

Currently, OECD GD 150 (OECD, 2012b) only covers the (o)estrogen, androgen, thyroid and steroidogenesis (EATS) pathways, which are also those for which targeted mechanistic and *in vivo* screening test methods are available. The revision of OECD GD 150, underway in 2017, may broaden its scope particularly with respect to the consideration of the recently validated ecotoxicological TGs.

The scope of the draft guidance document outlined in ECHA and EFSA (2016) is also restricted to the EATS pathways, which is further consistent with the United States Environmental Protection Agency (US EPA) Endocrine Disruptor Screening Program (https://www.epa.gov/endocrine-disruption). In regard to environmental hazard identification, ECHA and EFSA (2016) limit the scope to vertebrates (aquatic and terrestrial). Therefore, invertebrates are excluded from the ECHA and EFSA (2016) proposal. For many invertebrate taxa, the understanding of endocrine pathways and diagnostic endpoints for test methods using invertebrates are limited (deFur *et al.*, 1999; deFur, 2004; OECD, 2012b). Consistent with the ECHA and EFSA (2016) outline, the ECETOC 7SI-ED has also been written *with the intention to apply to both human health and the environment, however, where differentiation with regard to humans and non-target vertebrates is required, these aspects will be considered separately* (ECHA and EFSA, 2016).

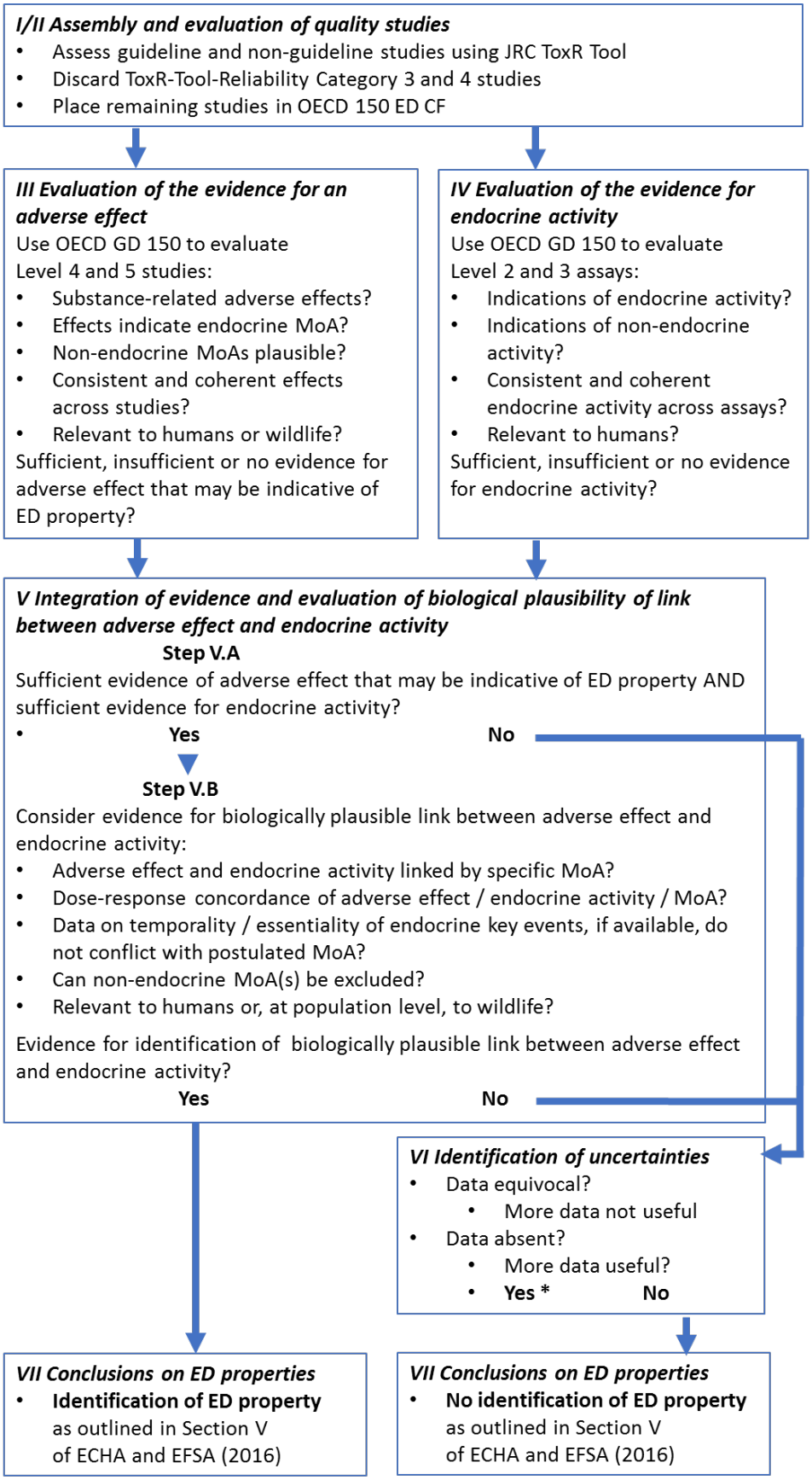
The ECETOC 7SI-ED is intended to provide general guidance to fulfil the EU legislative remits on plant protection products and biocidal products (Commission, 2016). It can be applied to cover those pathways of endocrine disruption and those taxa that are relevant to address a specific scientific question or that are mandatory for regulatory hazard assessment for the substance under investigation. **For this reason, the ECETOC 7SI-ED describes how data and information that may be indicative of endocrine disruption can be collected and evaluated, but it does not identify data gaps or prescribe any specific testing.** In regulatory (eco)toxicology, information requirements will be highly dependent on the specific legislation that is relevant for the substance under investigation (e.g. plant protection product or biocide active substance, personal care product, industrial chemical, etc.). This in turn may dictate the type of evaluation that is possible.

As is also consistent with the ECHA and EFSA (2016) outline, the information collected within the ECETOC 7SI-ED is **evaluated using a WoE approach**. The ECETOC 7SI-ED conforms to the principles of best practice for WoE approaches published by Rhomberg *et al.* (2013) that defined four phases of WoE analyses:

- Phase 1: Define causal question and develop criteria for study selection (covered in Steps I and II of the ECETOC 7SI-ED);
- Phase 2: Develop and apply criteria for review of individual studies (Steps III and IV);
- Phase 3: Integrate and evaluate evidence (Steps V and VI);
- Phase 4: Draw conclusions based on inferences (Step VII).

The ECETOC 7SI-ED uses elements from the template for WoE presented in the most recent version of the WHO/IPCS MoA/species concordance framework (Meek *et al.*, 2014a, b). Importantly, any WoE evaluation should begin with the formulation of a hypothesis or causal question (Rhomberg *et al.*, 2013; Meek *et al.*, 2014a; Dekant and Bridges, 2016a, b). For the identification of endocrine disrupting properties, the hypothesis will most likely state that the substance under investigation does (or does not) cause adverse effects in an intact organism or (sub)populations through an endocrine mechanism.

Figure 1: Simplified flow chart of the ECETOC 7SI-ED that follows the outline presented in Section V of ECHA and EFSA (2016)



Abbreviations: CF: Conceptual Framework; ED: Endocrine disrupting; GD: Guidance Document; MoA: Mode-of-action.
* *cf.* Explanatory note to Step VI on page 24.

2. DEFINITIONS

The following list includes terms, which are generally applicable to (eco)toxicological assays and studies, as well as specific terms used in the identification of endocrine disrupting properties.

Adaptive (non-adverse) effect: A biological effect that does not cause biochemical, behavioural, morphological or physiological changes that affect the general well-being, growth development or life span of an animal (Lewis *et al.*, 2002).

Adverse effect: Change in the morphology / physiology (and pharmacology), growth, development, reproduction, or, life span of an intact organism, or a system or (sub)population, that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO/IPCS, 2009).

Adverse effect with population relevance (environmental assessment): A change in the morphology, physiology, growth, development, reproduction, or life span of an organism that results in an impairment of population stability or recruitment.

Adverse outcome pathway (AOP): A linear sequence of events from the exposure of research animals (or humans) to a potentially toxic substance that results in a molecular initiating event that may lead to early cellular events and, ultimately, an apical effect, e.g., an observable outcome / phenotypic effect (Ankley *et al.,* 2010; OECD, 2013). In contrast to MoA (*cf.* Definition), AOPs are not substance-specific and therefore do not include metabolism considerations. AOPs can help address the biological plausibility of a MoA.

Apical endpoint: An observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant (OECD (2012c), citing Krewski *et al.* (2011)). Further, OECD (2012c) refers to the definition provided within an ecotoxicology testing strategy: Traditional, directly measured whole-organism outcomes of exposure in *in vivo* tests, generally death, reproductive failure, or developmental dysfunction (Villeneuve and Garcia-Reyero, 2011). Alterations in apical endpoints can integrate the effects from multiple MoAs or AOPs. Specifically for the identification of endocrine disrupting properties, this implies that alterations of apical endpoints may or may not result from endocrine changes. For instance, the apical endpoint fecundity may also be affected by perturbations that are unrelated to the endocrine system (OECD, 2012a, b).

Biological plausibility: In accordance with Commission (2016) and ECHA and EFSA (2016), biological plausibility refers to the extent of evidence to support that a specific substance operates via a specific MoA. In Step V of the ECETOC 7SI-ED, specific criteria are presented for the assessment of the extent of evidence for the biological plausibility that adverse effects (Step III) and endocrine activity (Step IV) are linked by a specific endocrine MoA.

Coherence: The extent to which parameters and/or endpoint effects form a logical pattern across different lines of evidence (e.g. *in vivo* mammalian studies, *in vivo* non-mammalian studies, toxicokinetic studies, *in vitro* assays). A coherent pattern is observed when the recorded effects are manifestations of the same primary

effect or MoA (e.g., with respect to endocrine disrupting properties, reduced testicular weight, testicular atrophy, reduced sperm numbers and reduced fertility).

Concordance (biological): The extent to which the pattern of primary events (indicators of MoA and apical effects) stands in accordance with broader biological knowledge (Meek *et al.,* 2014a, b).

Consistency (within study): All parameters recorded in an *in vivo* study / *in vitro* or *in vivo* assay allow concluding on the same adverse effect(s) / type of activity / MoA. (When evaluating MoA in Step V of the ECETOC 7SI-ED, all outcomes match the hypothesised MoA.)

Consistency (amongst different studies): The same adverse effect / type of activity / MoA is recorded in different *in vivo* studies / in different *in vitro* and/or *in vivo* assays, as applicable. (When evaluating MoA in Step V of the ECETOC 7SI-ED, all outcomes match the hypothesised MoA.)

Diagnostic endpoint: A parameter that is determined in *in vivo* studies and that provides an indication for a MoA, but that does not necessarily constitute the observation of an adverse effect.

Endocrine activity: The interaction with one or more elements of the endocrine system (EFSA, 2013).

Endocrine disruptor: An exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations (WHO/IPCS, 2002).

Endocrine MoA: A MoA leading to an observable adverse effect that can be attributed to an alteration in the endocrine system.

Mode-of-action (MoA): The biologically plausible sequence of substance-specific key events, starting with exposure and proceeding through the interaction of the substance or its metabolites with a cell, through functional and anatomical changes leading to an observed effect supported by robust experimental observations and mechanistic data (Sonich-Mullin *et al.,* 2001; Boobis *et al.,* 2009; Fenner-Crisp and Dellarco, 2016).

Relevance: The extent to which a study and its results can be generalised and applied to either humans or ecological species for a given purpose (e.g. the identification of an endocrine disrupting property).

Reproducibility: The extent to which a study can be reconstructed by the information (documentation) provided (e.g. in the publication or study report).

Systematic review: A review that involves a systematic search of the literature by using a detailed and comprehensive plan and search strategy agreed *a priori*, with the goal of reducing bias by identifying, appraising, and synthesizing all relevant studies on a particular topic (Uman, 2011).

Weight of evidence (WoE): An evidence-based approach, in which different pieces of available information are examined and weighted objectively (including strengths and weaknesses) and used collectively to reach a conclusion concerning a property of a substance that may not be evident on the individual data alone. While the WoE approach relies on all the available information, it is recognised that some studies have greater

diagnostic precision than others in determining whether adverse effects will actually occur in living animals, populations, or humans. During WoE evaluations, the completeness and the consistency of the data, which support that a substance causes a specific adverse effect / activity / MoA, is assessed (in contrast to a collection of unrelated observations). A clear picture can only be obtained by considering all data (positive and negative) and by weighting the more relevant and higher quality pieces of information more heavily. For example, direct observation of an adverse effect in an intact animal has greater weight than the response of an *in vitro* assay in isolation.

3. THE STEPS I-VII OF THE ECETOC 7SI-ED

Step I: Gathering of relevant information with regard to adverse effects and endocrine modes-of-action (MoA)

Gather all the available information that may be relevant for the identification of:

1. The substance;
2. Adverse effects that may be indicative of endocrine disrupting properties;
3. Evidence on endocrine (or non-endocrine) MoA that may be relevant for the adverse effects described in bullet point 2;
4. Evidence for other endocrine activity.

Step II: Evaluation of quality, reliability, reproducibility and consistency of the individual studies

Use the JRC ToxR Tool to assess the reliability of the data presented in each relevant study:

- Category 1: reliable without restrictions;
- Category 2: reliable with restrictions;
- Category 3: not reliable;
- Category 4: not assignable.

Studies assigned to Category 3 (not reliable) or Category 4 (not assignable) are summarised and documented, justifying the Category assignment, taking great care to determine how much weight can justifiably be given to such low-quality data. Studies that are not interpretable should be excluded from analysis.

After the reliability assessment, the remaining studies (and other information) are placed into the appropriate level of the OECD CF.

Step III: Evaluation and summary of the evidence for an adverse effect

Use OECD GD 150 to examine OECD CF Level 4 and 5 studies for the presence or absence of adverse effects. Relevant *in vivo* studies using test methods or apical endpoints not mentioned in the OECD CF may also be used, if a justification for their relevance is provided:

1. Are there substance-related adverse effects which may be indicative of endocrine disrupting properties?
2. Are there substance-related adverse effects which may not be indicative of endocrine disrupting properties?

For bullet points 1 or 2, a WoE approach is applied to consider sufficiency of evidence to support a substance-induced adverse effect. First, conclusions on the strength of evidence are made for individual studies and then WoE collectively across studies, with attention given to the quality of the study, as evidence may strengthen in support of an adverse effect (or lack thereof) with higher quality data.

When evaluating the strength of evidence in support of a substance-induced effect in a single study, important considerations include dose-responsiveness (i.e. toxic effects increase in incidence magnitude and/or severity as the dose increases), statistical significance, difference from concurrent controls, and historical control range, and consistency of effects across apical endpoints within one study.

Provided several studies are available, consistency across studies and coherence amongst different lines of evidence are also key considerations (*cf.* Definitions):

- Is the same adverse effect seen in more than one study of the same type?
- Is a consistent pattern of observations emerging across endpoints within one study in support of an adverse effect? Or across similar study types within the same line of evidence (i.e. *in vivo* rodent assays)?
- Is a coherent pattern of observations emerging from other lines of evidence in support of an adverse effect (e.g. *in vivo* mammalian studies, *in vivo* non-mammalian studies)?

Concluding how much to weight a given study with limitations is an important part of the WoE evaluation:

- Low-quality studies are carefully considered, particularly if those studies conflict with results observed in comparable, higher quality studies. The limitations of low-quality studies should not be overlooked.
- Negative or null data are also weighted.

As this framework is explicit to the identification of substances with endocrine disrupting properties as defined in WHO/IPCS (2002) and by the criteria set out in the ongoing revision of the EU plant protection products and biocidal products legislations, the following considerations are also warranted:

- Is it plausible that any of the adverse effects are caused by an endocrine MoA?
- If more than one adverse effect is observed, is it plausible that they are caused by different endocrine MoAs?
- Are the adverse effects relevant to humans and/or are they relevant at the population level for wildlife?

The evidence for the presence of an adverse effect which may be indicative of endocrine disrupting properties is categorised as sufficient, insufficient or none:

Sufficient evidence: Clear evidence of a substance-related effect(s) that may be indicative of endocrine disrupting properties and that is not related to general systemic toxicity or other potentially confounding (specific) toxicities across a range of study types. Sufficient evidence may include a dose-response for an effect with statistically significant difference from concurrent controls and which is outside the historical control range; and consistently observed in high-quality studies. The evidence is strengthened by being seen in a ToxR Tool Reliability-Category 1 study. Evidence from ToxR Tool Reliability-Category 2 studies is considered to have less strength and, dependent on the nature and the extent of the limitations, may result in a consideration that evidence is insufficient.

Insufficient evidence: A single effect that may be indicative of endocrine disrupting properties, which was not seen in similar studies, or an array of different effects, which do not form a coherent/consistent pattern.

Insufficient evidence includes an effect which was only observed at the highest dose tested, but which was only marginally increased over concurrent control or historical background control levels.

No evidence: No adverse effects identified that may be indicative of endocrine disrupting properties (evidence of absence or absence of evidence). Absence of evidence includes a statistically significant effect which does not form part of a dose-response curve and which is not seen in other studies which might provide evidence of this effect. If there is absence of evidence or evidence of absence of an adverse effect indicative of endocrine disrupting properties, the definition for an endocrine disruptor is not fulfilled and an endocrine disrupting property cannot be identified.

Step IV: Evaluation and summary of the evidence for endocrine activity (and non-endocrine activity, if available)

Use OECD GD 150 to examine OECD CF Level 2 and 3 assays and other reliable assays and studies providing relevant data; further include relevant assays to investigate non-endocrine activities, where available:

1. Are there indications of endocrine activity?
2. Are there indications of non-endocrine activity?

For bullet points 1 or 2, a WoE approach is applied to consider sufficiency of evidence to support substance-induced endocrine activity. First, conclusions on the strength of evidence are made for individual assays and then WoE collectively across assays, with attention given to the quality of the assay, as evidence may strengthen in support of endocrine activity (or lack thereof) with higher quality data.

When evaluating the strength of evidence in support of substance-induced activity in a single assay, important considerations include dose-responsiveness (i.e. activity increases in incidence, magnitude and/or severity as the dose increases), statistical significance, difference from concurrent controls.

Provided several assays (and studies) are available, consistency across assays (and studies) and coherence amongst different lines of evidence are also key considerations (*cf.* Definitions):

- Is the same endocrine activity seen in more than one assay and/ or study of the same type?
- Are indications consistent within similar assay and/or study types?
- Is a consistent pattern of observations emerging across studies in support of a specific endocrine activity and the directional change in this activity (e.g. agonistic versus antagonistic).
- Is a coherent pattern of observations emerging from other lines of evidence in support of a specific endocrine activity (e.g. *in vitro* assays, *in vivo* assays)?

Concluding how much to weight a given assay and/or study with limitations is an important part of the WoE evaluation:

- Low-quality assays and/or studies need to be carefully considered, particularly if they conflict with results observed in comparable, higher quality assays and/or studies.
- Negative or null data should also be weighted.
- The limitations of *in vitro* models must be addressed and weighted, particularly in the presence of incoherent data (e.g. endocrine activity in *in vitro* assays but not in *in vivo* assays).

- Are the indications consistent within similar assay and/or study types?
- For human health: Is the endocrine activity relevant to humans?

The evidence for a substance to be endocrine active is categorised as sufficient, insufficient or none.

Sufficient evidence: Clear evidence of a substance-related effect(s) indicative of endocrine activity from Level 3 *in vivo* assays (or comparable assays and/or studies), which is consistent with indications from Level 2 *in vitro* assays (or comparable assays) of the same type of endocrine activity.

Insufficient evidence: Some endocrine activity in one *in vivo* assay which is not part of a consistent pattern in other *in vivo* assays is assessed as insufficient evidence. Incoherent evidence for endocrine activity, e.g., *in vitro* assays are positive in the absence of any *in vivo* data on endocrine activity (from either Level 3 *in vivo* assays or from diagnostic endpoints, etc., from Level 4 and 5 studies)) is also interpreted as insufficient evidence (i.e. *in vitro* assays positive – no *in vivo* data on endocrine activity). This scenario cannot be taken as conclusive evidence of absence of endocrine activity because endocrine activity could still be seen *in vivo.*

No evidence for endocrine activity: Endocrine activity was not seen in the Level 2 and 3 assays or comparable assays and studies. No evidence for endocrine activity includes activity which is restricted to *in vitro* assays, but which was not seen in the corresponding *in vivo* assay(s) or studies, which should cover both human health and ecotoxicological aspects (i.e. *in vitro* assays positive – comprehensive *in vivo* data on endocrine activity negative).

Step V: Integration of the evidence and evaluation of biological plausibility that adverse effect and endocrine activity are linked by specific endocrine MoA

The evidence from Step III and Step IV is considered together (i.e. integrated) in two phases using the following questions as a guide. If the evidence from Steps III and IV support more than one MoA, the respective data need to be evaluated separately:

Step V.A: Is there sufficient evidence from Step III assessing adverse effect(s) that may be indicative of endocrine disrupting properties and sufficient evidence from Step IV assessing endocrine activity?

- If Yes, proceed to Step V.B.
- If insufficient evidence in Step III and/or Step IV, proceed to *Step VI: Identification of uncertainties* as the scientific criteria set out in the context of the plant protection products and biocidal products Regulations (EC) No 1107/2009 and (EU) No 528/2012 have not been met.
- If there is evidence of absence of adverse effect(s) that may be indicative of endocrine disrupting properties (Step III) or evidence of absence of endocrine activity (Step IV), the WHO/IPCS (2002) definition for endocrine disruptor is not fulfilled and an endocrine disrupting property has not been identified in accordance with the scientific criteria set out in the context of Regulations (EC) No 1107/2009 and (EU) No 528/2012.

Step V.B: The following questions about the evidence from Step III and Step IV are considered:

1. Based upon the WoE from Steps III and IV, is a specified endocrine MoA postulated to be activated / to operate (i.e. can the endocrine activity identified in Step IV plausibly explain the adverse effect(s) observed in Step III)?
2. Is there evidence for the activation of a key event(s) of the postulated endocrine MoA?
3. Is there dose/concentration-response concordance between the evidence for adverse effect(s) and the evidence for endocrine activity for the postulated endocrine MoA?
4. Are there data providing evidence on the temporality or the essentiality of the endocrine key events (i.e. is the sequence of events reversible if a key event is stopped or prevented)?
5. Are there data supporting that a non-endocrine MoA is more likely for the adverse effect(s) than an endocrine MoA?
6. Is the postulated endocrine MoA relevant to human health, and are adverse effect(s) relevant at population level for wildlife?

For each postulated MoA, the answers to these questions are used to determine whether a biologically plausible link has been established. This demands that:

- A specified endocrine MoA is postulated to be activated.
- There is sufficient evidence for the activation of a key event in the postulated endocrine MoA, for example the stimulation or blocking of a receptor that is associated with the MoA. This evidence is most likely to come from Level 2 *in vitro* assays.
- There is sufficient evidence of one or more *in vivo* downstream measurable and relevant consequences which are diagnostic of the postulated endocrine MoA. This evidence is most likely to come from Level 3 *in vivo* assays (or from diagnostic endpoints or other relevant parameters from Level 4 or 5 *in vivo* studies).
- There is sufficient evidence of one or more *in vivo* downstream relevant adverse effects which would be expected from activation of the postulated endocrine MoA. This evidence is most likely to come from Level 4 or 5 *in vivo* studies.
- The dose/concentration-response in each of the three sets of evidence form a coherent pattern. Reference can be made to model substances (e.g., positive control substances) which are known to operate by the postulated endocrine MoA to estimate the relationship which may be expected amongst the three sets of evidence. Differences from the expected relationship should be explained.
- If evidence on the temporality or the essentiality of the endocrine key events is available, the evidence does not conflict with the postulated endocrine MoA.
- Non-endocrine MoA(s), which could also have a biologically plausible link to the adverse effect(s), are considered less likely be activated than the endocrine MoA (based upon the comparison of the available information on biological concordance, dose/concentration-response concordance or temporal association).
- The postulated endocrine MoA is considered to be relevant to human health, or the adverse effect(s) is/are considered to be relevant at population level for wildlife.

A biologically plausible link between an adverse effect and an endocrine MoA can only be established if all these conditions can be met. Proceed to *Step VII: Conclusion on endocrine disrupting properties*.

If a biologically plausible link is not established, then proceed to *Step VI: Identification of Uncertainties*.

Step VI: Identification of uncertainties

Uncertainties are most likely to arise in the assessment of data for endocrine disrupting properties when there is insufficient evidence from Steps III or IV, or when the evidence is sufficient to go to Step V.B, but a biologically plausible link between adverse effect and endocrine MoA cannot be established.

There are 3 major sources of uncertainty:

- The quality of data: Poor quality data (from ToxR Tool Reliability-Category 3 (or 4) studies and assays) from the data quality evaluation in Step II.
- The equivocality of data: Examine conclusions of insufficient evidence from Step III and Step IV to determine if data are equivocal (or absent, see next bullet point). If data are equivocal then consider potentially confounding factors in individual studies (e.g. exceeding of the Maximum Tolerated Dose (MTD) or Maximum Tolerated Concentration (MTC); presence of specific non-endocrine toxicity). If data across ToxR Tool Reliability-Category 1 and 2 studies consistently show equivocal effects, then it is unlikely that more data would reduce uncertainty. Also the inconsistency of effects observed in ToxR Tool Reliability-Category 1 and 2 studies may be a source of uncertainty and not allow a firm conclusion.
- The lack of data: There may be evidence of endocrine activity in one set of data but no data available from one of the other necessary sets of data (e.g. evidence from Level 2 *in vitro* assays, but no Level 3 *in vivo* assays or Level 4 and 5 *in vivo* studies). In this situation, it would be reasonable to generate further complementary data.

Step VII: Conclusions on endocrine disrupting properties

- If a biologically plausible link is established in Step V, then an endocrine disrupting property in accordance with the WHO/IPCS (2002) definition has been identified.
- If there is insufficient evidence for a biologically plausible link in Step V, the WHO/IPCS (2002) definition for endocrine disruptor is not fulfilled and an endocrine disrupting property has not been identified.

4. EXPLANATORY NOTES TO THE STEPS I-VII OF THE ECETOC 7SI-ED

Step I: Gathering of relevant information with regard to adverse effects and endocrine modes-of-action (MoA)

Information that is relevant for the identification of a substance includes its Chemical Abstract Service (CAS) number (where available), International Union of Pure and Applied Chemistry (IUPAC) name, any synonyms, common names, and molecular structure.

Further relevant information includes specific physico-chemical data, as appropriate. For some substances, structural alerts may provide an indication for endocrine activity.

Relevant (eco)toxicological data includes data from (a) OECD TGs; (b) other validated guidelines (e.g. the guidelines from the US EPA Office of Prevention, Pesticides and Toxic Substance (OPPTS) / Office of Chemical Safety and Pollution Prevention (OCSPP); and (c) reliable (i.e. ToxR-Reliability-Categories 1 and 2; *cf.* Step II) research papers (peer reviewed publications or publications that have not been submitted to scientific or regulatory peer review).

(Eco)toxicological and mechanistic information that may be indicative of endocrine disrupting properties can be retrieved from (eco)toxicological study reports that have been conducted for regulatory purposes (applying either standardised or non-standardised test methods) and by conducting open literature searches using defined terms. The endpoints investigated in the assays and studies of the OECD CF can be used as a guide to identify potentially relevant effects in publications from the open literature. However, this does not preclude the inclusion of studies that use different study designs or investigate endpoints for which validated TGs are not yet available or listed in the OECD CF. When such studies are utilised, full details of the procedures followed together with a clear description of strengths and limitations of the methodologies should be provided.

Further, reliable databases should be searched for relevant information (e.g. the US EPA ToxCast Dashboard; available at: https://www.epa.gov/chemical-research/toxcast-dashboard).

Of note, the application of (quantitative) structure activity relationships ((Q)SAR) and other *in silico* tools may become relevant to help identify potential MoAs, e.g., molecular initiating events within AOPs, which in turn may inform on the potential or lack of endocrine activity (Rybacka *et al.,* 2015). However, the application of *in silico* tools is not further addressed in the ECETOC 7SI-ED.

Level 1 of the OECD CF provides guidance on the gathering of existing information.

To ensure that all available relevant scientific data are retrieved, the principles of systematic review should be applied (Uman, 2011), which provide transparency, rigour and reproducibility to the task of data collation and selection. For this purpose, the ECETOC 7SI-ED refers to the EFSA guidance for *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009* (EFSA, 2011). This EFSA guidance provides instructions on how to identify and select scientific peer-reviewed open literature for inclusion in plant protection product dossiers and how these searches should be reported.

For example, recommendations are made on how to document literature search methods, databases, inclusion and exclusion criteria for study selection. Whilst the EFSA guidance requires clear *a priori* documentation of the relevance criteria, it does not provide specific advice on how to define relevance criteria, which will be very specific in WoE approaches to evaluate potential endocrine disrupting properties. The assessment of relevance concerns the ability of the selected study to address the specific property being investigated. Rudén *et al.* (2016) provide guiding questions to determine the relevance of ecotoxicological studies for regulatory decision-making by the alignment to the protection goal(s). The general considerations of this guidance with respect to biological relevance are equally applicable to the assessment of toxicological studies.

Step II: Evaluation of quality, reliability, reproducibility and consistency of the individual studies

Consistent with the title of the second step presented in Section V of ECHA and EFSA (2016), Step II of the ECETOC 7SI-ED describes how the *quality (reliability), reproducibility and consistency of individual studies* may be assessed. The main purpose of this step of the ECETOC 7SI-ED is to select those studies that are of sufficient quality (reliability) to be used within the WoE evaluation, and to exclude those studies that are of insufficient quality. The criteria that are evaluated in determining study quality also allow a determination of the reproducibility of studies. Further, Step II provides information that may support the determination of within-study consistency. Notwithstanding, within-study consistency and consistency amongst different studies are factors that are applied in the WoE evaluations conducted in Steps III and IV.

The JRC ToxR Tool (available at: https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/toxrtool accessed March 2017) is used to assess the reliability of the data presented in each study. This Excel-based tool provides comprehensive criteria and guidance for evaluations of the inherent quality of toxicological data, making the decision process of assigning reliability categories transparent. It comprises a list of 21 criteria for *in vivo* studies and 18 criteria for *in vitro* studies. Each criterion can be assigned either a '1' (one point; i.e. 'criterion met') or a '0' (no point; i.e. 'criterion not met'). The JRC ToxR Tool specifies indispensable criteria (e.g. substance identification, specification of test species and of route of administration). Only if all indispensable criteria are rated as '1', the tool will assign a study to the Reliability-Categories 1 or 2, irrespective of the total score obtained.

The ToxR Tool spreadsheet includes explanations and guidance for most of the criteria. Further, the ToxR Tool contains free text fields to justify individual scores. Comprehensive use of these free text fields can aid in ensuring transparency of the assigned Reliability-Categories.

Criteria for evaluating *in vivo* or *in vitro* study reliability were established congruently to the largest extent possible and are grouped into five groups of criteria for either *in vivo* or *in vitro* studies:

1. Test substance identification;
2. Test system characterisation;
3. Study design description;
4. Study results documentation;
5. Plausibility of study design and results.

Groups 1-4 contain criteria that are mainly related to documentation, and they are relevant to determine the reliability of studies. Further, test system characterisation and study design description are relevant to assess the reproducibility of studies. Group 5 goes beyond documentation and asks for an assessment of the internal plausibility of the experimental approach used in the study. Such information is relevant to determine the power of a study to inform causality between substance exposure and outcomes, or toxicological significance. By comparison, study consistency is a factor applied during the Step III and IV WoE evaluation.

Whilst the JRC ToxR Tool criteria and guiding explanations for *in vivo* studies were primarily designed for the assessment of toxicological studies, the general principles of the criteria are also applicable to the evaluation of ecotoxicological studies (Roberts and Leopold, 2016). Specific guidance for the evaluation of ecotoxicological tests is being developed and should be consulted where additional justification for the individual criteria is required (e.g., Moermond *et al.*, 2016a). Alternatively, for an assessment of reliability and relevance of ecotoxicology studies, the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) evaluation method might be used (Moermond *et al.*, 2016b).

Each study is assigned into one of the categories of the JRC ToxR Tool as described above, taking into account specific guidance for the evaluation of ecotoxicological tests as appropriate. Thereafter, all assays and studies that shall be used in the WoE evaluation are placed into the appropriate Level of the OECD CF:

- Level 2: *In vitro* assays providing data about selected endocrine mechanism(s) / pathway(s);
- Level 3: *In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s);
- Level 4: *In vivo* studies providing data on adverse effects on endocrine-relevant endpoints;
- Level 5: *In vivo* studies providing more comprehensive data on adverse effects on endocrine-relevant endpoints over more extensive parts of the life cycle of the organism.

By analogy to the OECD CF Levels 2 and 3, *in vitro* and *in vivo* assays providing data about non-endocrine mechanism(s) / pathway(s) are also collected and sorted for subsequent use in Step IV.

Data from Level 2 and 3 assays and diagnostic endpoints included in Level 4 and 5 studies (e.g. hormone levels, vitellogenin, etc.), provide mechanistic information that is used for the evaluation of endocrine activity in Step IV. Further Level 4 and 5 study parameters (e.g. organ weight and histopathological parameters) may also provide information that is relevant to determine the consistency and coherence of endocrine activity.

Apical endpoints from the *in vivo* studies in Levels 4 and 5 provide information on adverse effects that are evaluated in Step III. Apical effects may also be identified in specific Level 3 *in vivo* assays (e.g. fecundity from the fish reproductive screening assay; OECD TG 229). However, screening assays are designed to inform whether further testing is needed, and therefore only include, e.g., limited portions of life cycles and fewer concentrations than the extensive Level 4 and 5 studies. For this reason, decisions on adverse effects should not be made based on apical effects from Level 3 *in vivo* assays alone (Wheeler *et al.,* 2014). They should only be used in a WoE approach to support apical data derived from Level 4 and 5 studies.

While the ECHA and EFSA (2016) outline has excluded studies with invertebrates from its scope and OECD GD 150 does not provide specific guidance on invertebrate tests, the OECD CF (OECD, 2012a) does include Level 4 and Level 5 invertebrate partial and life cycle tests, which measure apical endpoints that are population-relevant. However, the majority of invertebrate test designs do not provide mechanistic insight, and no specific

TGs exist yet to characterise endocrine activity in invertebrates at Levels 2 or 3 of the OECD CF (Coady *et al.,* 2017). This has been identified as a research need and new test methods are in development (OECD, 2016).

Step III: Evaluation and summary of the evidence for an adverse effect

The purpose of Step III of the ECETOC 7SI-ED is to examine the available evidence for adverse effects which may be indicative of endocrine disrupting properties and to determine the sufficiency of those data to conclude on adversity. There is nothing different about the way adverse effects are assessed for endocrine disrupting endpoints compared to established procedures for non-endocrine disrupting endpoints. When identifying endocrine disrupting properties, a specific next step after the determination that an adverse effect is present is its characterisation to possibly help direct which specific endocrine activity may merit follow-up in Step IV. The recognition of adverse effects and the differentiation between adverse and non-adverse effects is made as described by Lewis *et al.* (2002). For environmental assessments, adverse effects with population relevance are identified. For this purpose, it is important to determine which toxicity test endpoints are population-relevant and also what levels of effect on the endpoints may impact a population (Weltje *et al.*, 2013).

OECD CF Level 4 and 5 studies (or other relevant studies that use different study designs or investigate endpoints, for which validated TGs are not yet available or listed in the OECD CF) provide information on whether a substance causes adverse effects on apical endpoints that may be indicative of endocrine disrupting properties. As compared to the Level 3 *in vivo* assays (that are not designed to assess adversity, but to assess a specific endocrine activity (Wheeler *et al.,* 2014)), Level 4 and 5 studies are sensitive to a variety of toxicological MoAs, including one or more endocrine MoA, and they cover numerous apical endpoints across different life stages and larger portions or all of the life cycle of the test species. For this reason, the Level 4 and 5 studies can provide a more thorough assessment of the possible or actual endocrine disrupting effects of a substance in developing or adult organisms than the Level 3 assays. While apical effects identified in Level 3 *in vivo* assays should also be taken into consideration for a comprehensive WoE evaluation in Step III, such findings are not decisive in the absence of Level 4 or 5 data to identify adverse effects that may be indicative of endocrine disrupting properties.

Generally, in Step III a treatment-related, significant and biologically relevant effect that may be indicative of endocrine disrupting properties flags the need for further consideration (in Steps IV and V) to elucidate the MoA involved in the causation of the adverse effect. Effects which may be indicative of an endocrine MoA can also be caused by other (non-endocrine) MoAs. Many of the Level 4 and 5 studies (and similar study types) are designed to identify a range of adverse effects, and not only those which may be indicative of endocrine disrupting properties. These considerations notwithstanding, scenarios are conceivable in which diagnostic endpoint data from a Level 4 and 5 study could be used in Steps IV and V as part of the WoE approach, which is used to establish the biologically plausible link between an adverse effect elicited in this same study and the endocrine MoA. A substance that does not cause adverse effects on apical endpoints that are indicative of endocrine disrupting properties in a suite of Level 4 or 5 studies (or other relevant study designs) does not fulfil the criterion for adverse effects in the WHO/IPCS (2002) definition of an endocrine disruptor.

For human health evaluations of endocrine disrupting properties, adverse effects may constitute changes in the morphology, physiology, growth, development, reproduction, or life span of the organism that result in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO/IPCS, 2009). The Level 4 and 5 chronic / carcinogenicity and developmental / reproductive toxicity studies provide data on adverse effects and add to the WoE concerning the potential for impacts in humans, while at the same time providing data on dose/concentration-response.

For human hazard identification, it is essential to assess whether the adverse effect(s) observed in *in vivo* animal studies are relevant to humans. The current default assumption is to assume human relevance. However, this assumption can be re-evaluated if there are scientifically valid data to demonstrate non-relevance to humans. The most well-known example for non-human relevance of adverse endocrine-mediated effects in animals is the susceptibility of the rat to disruption of thyroid function, and it can be explained by species-specific differences in synthesis, binding, metabolism/clearance and transport of the thyroid hormones (Lewis, 2013). These considerations should also consider toxicodynamic and biological differences in species responses such as already accepted for the human versus rodent relevance of receptor-mediated toxicity via peroxisome proliferator-activated receptor alpha for certain liver tumours (Corton *et al.,* 2014).

For wildlife evaluations of endocrine disrupting properties, the assessment of adverse effects needs consideration of the population relevance of effects in order to reflect the protection goal of environmental assessments. The WHO/IPCS (2009) definition of adverse effects includes consideration of population effects. Therefore, it is crucial to determine which apical endpoints are population-relevant, as well as what level of effect on these endpoints may impact a population, bearing in mind that the magnitude of change will depend on the dose or concentration tested. Population-relevant endpoints are generally assessed by survival, growth, development (including sexual) and reproduction in Level 4 and 5 studies. Whilst it is important to determine what level of effect on these endpoints may impact populations, guidance on this aspect is still lacking. However, these endpoints can also be affected by generalised and specific non-endocrine toxicities, which highlights the need for mechanistic evaluation (covered in Step IV). A number of mechanistic endpoints that are diagnostic for endocrine disruption are included in more recently adopted OECD TGs; e.g. OECD TG 234 and 241 (Level 4) and OECD TG 240 (Level 5), whereas others do not provide any mechanistic insight; e.g. OECD TG 206 (Level 5).

For human health and wildlife evaluations of endocrine disrupting properties alike, effects seen above the recommended limit dose or concentration for the studies should not be considered, and those observed at the highest tested dose/concentration require careful consideration (Wheeler *et al.,* 2013; Mihaich *et al.,* 2017). Systemic toxicity at these doses/concentrations can lead to non-specific adversity which can result in apical changes that could be associated with endocrine disruption. However, such changes may be related to the overload of the organism, resulting in stress, or occur concomitant with generalised, systemic toxicity (Wheeler and Coady, 2016). If the MTD or MTC is reached or exceeded in a study, and generalised, systemic toxicity is observed, then any endocrine relevant-effects observed at these doses/concentrations should generally be excluded from considerations on endocrine activity/disruption (Wheeler *et al.*, 2013). The relevance of effects occurring at doses that exceed the inflection point of nonlinear toxicokinetic behaviour, i.e. a 'kinetically derived maximum dose', should also be carefully considered, particularly, if there is an

adequate margin with human exposures and the relevance of the toxicokinetic processes in the test species to humans is reasonably established (Creton *et al.,* 2012).

In determining the consistency and coherence of effects amongst different studies, it should be taken into consideration that the route of exposure may not be representative of normal conditions of use making direct extrapolations difficult (e.g. intraperitoneal exposure in a study when human exposure is dermal or oral). Similarly, species-specific differences in substance adsorption, distribution, metabolism, and elimination (ADME) should be taken into account in assessing the consistency and coherence of effects amongst different studies. This is particularly important when considering the consistency and coherence of mammalian and non-mammalian toxicity data. Expert judgement is required on a case-by-case basis to evaluate whether data are conflicting and to determine the implications of conflicting data.

OECD GD 150 provides guidance on the specific apical endpoints that are covered by the individual Level 4 and Level 5 studies and on the relevant endocrine MoA(s) that are indicative of these apical endpoints.

Step IV: Evaluation and summary of the evidence for endocrine activity (and non-endocrine activity, if available)

The purpose of Step IV of the ECETOC 7SI-ED is to determine whether a substance elicits endocrine activity in Level 2 and 3 assays. Evidence for endocrine activity may also be available from specific diagnostic endpoints included in some Level 4 and Level 5 studies or from assays and studies not mentioned in the OECD CF that provide comparable data (if justification of their comparability is provided). Further parameters from Level 4 and 5 studies (e.g. organ weight and histopathological parameters) may also be relevant to indicate the presence or absence of endocrine activity.

As compared to the corresponding step presented in Section V of ECHA and EFSA (2016), the focus of Step IV of the ECETOC 7SI-ED has been slightly modified to specify that the assays evaluated in this step allow identifying endocrine activity, and not endocrine MoA. MoAs extend to the manifestation of apical effects that are addressed in the studies evaluated in Step III, but not necessarily in the assays evaluated in Step IV. Additionally, Step IV has been expanded to cover not only the evaluation of *in vitro* and *in vivo* mechanistic evidence that is relevant to identify endocrine activity, but also available mechanistic evidence that is relevant to identify possible non-endocrine mechanisms that could explain the adverse effects identified in Step III. In Step V, such information may be relevant to identify the most plausible (endocrine or non-endocrine) MoA.

Level 2 assays are *in vitro* screening assays used for the identification of possible endocrine activity. Positive *in vitro* test results indicate the possibility of endocrine effects *in vivo.* However, *in vitro* assays do not include consideration of toxicokinetics (ADME). Therefore, their ability to predict effects in humans or in environmentally relevant species may be limited (OECD, 2012b). Consideration of toxicokinetics is particularly critical for the evaluation of taxonomic differences in effects. Current *in vitro* tests covered by the OECD CF are largely based on mammalian systems, but they are considered relevant for vertebrate wildlife species, because many of the assays are based on hormone receptors and molecular mechanisms that are highly conserved across vertebrates (OECD, 2012b). Mechanistic insight into endocrine activity is presently not available for all invertebrate groups (deFur *et al.,* 1999; deFur, 2004; Weltje *et al.,* 2013). Generally, an

evaluation of the ecotoxicological relevance of endocrine activity identified in Level 2 for non-mammalian wildlife requires case-by-case expert judgement.

Level 3 assays are *in vivo* assays that allow screening for possible endocrine activity. While they may encompass apical endpoints, their study design as screening assays does not allow drawing conclusions on the presence of adverse effects that may be indicative of endocrine disrupting properties. The currently available Level 3 *in vivo* assays are designed to provide a yes/no (qualitative) answer about a substance's ability to interact with the EATS pathways. These assays are designed to detect alterations in endocrine-sensitive tissues thereby providing alerts to substances with possible endocrine activity (Borgert *et al.,* 2011, 2014; de Peyster and Mihaich, 2014). Therefore, they favour false positives rather than negatives and are in some cases highly sensitised models, e.g. neutered animals without a functional hypothalamic-pituitary-gonadal axis, which are therefore unable to compensate fully for endocrine perturbations.

In evaluating the consistency and coherence of types of activity amongst different assays, it should be taken into account that the routes of exposure applied in different assays may not be representative of normal conditions of use making direct extrapolation for relevance difficult (e.g. intraperitoneal exposure in a study when human exposure is dermal or oral).

OECD GD 150 provides guidance on the interpretation of Level 2 and 3 assays. Each Level 2 *in vitro* assay is primarily designed to detect a specific endocrine activity (e.g., oestrogen agonism, androgen antagonism, etc.), whilst some Level 3 *in vivo* ecotoxicological assays may provide information on more than one form of endocrine activity. For instance, the fish screening assays (OECD TG 230 and 229) can provide information on *in vivo* activity via the EAS pathways, whilst the amphibian metamorphosis assay (OECD TG 231) specifically detects *in vivo* activity via the T pathway.

Step V: Integration of the evidence and evaluation of biological plausibility that adverse effect and endocrine activity are linked by specific endocrine MoA

The purpose of Step V of the ECETOC 7SI-ED is to determine if adverse effects identified in Step III and endocrine activity identified in Step IV are linked by a specific endocrine MoA. The ECHA and EFSA (2016) outline calls for a *reasonable* degree of proof that adverse effects identified in Step III and the endocrine activity identified in Step IV are linked by a specific endocrine MoA.

As compared to the corresponding step presented in Section V of ECHA and EFSA (2016), the title of Step V of the ECETOC 7SI-ED has been reformulated to highlight that the adverse effects identified in Step III and the endocrine activity identified in Step IV are (or are not) linked by a specific endocrine MoA. Therefore, evidence supporting different endocrine MoAs needs to be evaluated separately in Step V. In accordance with ECHA and EFSA (2016), the biological plausibility for this link is established, i.e. the extent of evidence to support that a specific substance operates via a specific endocrine MoA (Commission, 2016). In the following, specific criteria are presented for the assessment of the extent of evidence for a biologically plausible link.

The ECETOC 7SI-ED uses aspects of the modified Bradford Hill Considerations presented in the updated WHO/IPCS MoA/species concordance framework (Meek *et al.,* 2014a, b) to determine if there is reasonable

degree of proof for a biologically plausible link as defined in ECHA and EFSA (2016). The WoE evaluation conducted in Step V (just as in Steps III and IV) should never be based upon a mere weighing up of positive versus negative outcomes.

Biological concordance: There is evidence of an adverse effect(s) that may be indicative of endocrine disrupting properties (Step III) and evidence for endocrine activity (Step IV), and the endocrine activity is known to be associated with the endocrine MoA that is relevant for the adverse effect(s). The pattern of adverse effects identified in Step III and the evidence for endocrine activity identified in Step IV should be indicative of the same (hypothesised) endocrine MoA.

Dose/concentration-response concordance: The endocrine activity (key event(s)) observed in the Level 2 and 3 assays is observed at similar or lower doses/concentrations than the adverse effects that are indicative of endocrine disrupting properties seen in the Level 4 and 5 apical studies. The principles used within the *in vitro-in vivo* extrapolation model presented by Wetmore *et al.* (2012) are recommended to address the extrapolation of *in vitro* to *in vivo* data.

Plausible key events of the endocrine MoA and temporal association: Plausible key events of the endocrine MoA that are affected by the substance have been identified. If applicable, depending on the design of the available assays and studies, temporal association of the key events and adverse effects can be determined. For instance, if adverse effects only occur in juvenile stages, whereas endocrine activity is only observed in the adult, the link between the two is not biologically plausible.

Essentiality of key events: To establish a MoA, the essentiality of the key events can be demonstrated by showing that the downstream events are absent or reversed if the key event is stopped or prevented. To demonstrate this conclusively, relevant inhibitors, antagonists or agonists can be used.

AOPs, i.e. the evaluation and integration of many different types of chemical and biological information following an MoA-based approach to understanding adverse effects (OECD, 2012d; Munn and Goumenou, 2013), have the potential to aid in the determination of the biological concordance of an endocrine MoA for adverse effects observed in *in vivo* (eco)toxicological studies. As such, they can help identify if an observed adverse effect can be plausibly linked to an endocrine mechanism. While the term AOP originated in ecotoxicology (Ankley *et al.,* 2010), the AOP approach is equally applicable in toxicology, just as the WHO/IPCS MoA concept that was originally developed for toxicology is also applicable in ecotoxicology (Munn and Goumenou, 2013). The *ECETOC Guidance on assessment and application of AOPs relevant to the endocrine system* (ECETOC, 2016) presents a detailed scheme of additional considerations for assessing the biological plausibility of a link between adverse effect and endocrine MoA.

Step VI: Identification of uncertainties

The major uncertainties will have been addressed in the evaluation of the evidence in Step III and Step IV. Therefore, the specific guidance presented in Step VI refers back to these Steps. Uncertainties are likely to arise when the conclusion has been for insufficient evidence in either Step III or Step IV. For Level 4 and 5 studies (Step III), uncertainty mostly arises from the complexity of the studies and because of the interference of potentially endocrine and non-endocrine mechanisms. There are only few apical endpoints which are

unambiguously indicative of an endocrine MoA. This highlights the importance of exploring the entire toxicity profile of a substance, and not only data pointing toward a potential endocrine MoA.

There are 3 major sources of uncertainty:

- The quality of data: Poor quality data (from ToxR Tool Reliability-Category 3 (and 4) studies and assays) from the data quality evaluation in Step II.
- The equivocality of data: On occasion, it is difficult to draw conclusions from the data e.g. because the dose-response curve does not follow a consistent pattern of increasing effects with dose. Usually, equivocality of data can be resolved by reference to other studies. If no other studies are available, then it would be reasonable to generate more data. If other studies are available and they also show equivocal data, then there is no reason to generate more data.
- The lack of data: There may be evidence of endocrine activity in one set of data but no data available from one of the other necessary sets of data (e.g. evidence from Level 2 *in vitro* assays, but no Level 3 *in vivo* assays or Level 4 and 5 *in vivo* studies). In this situation, it would be reasonable to generate further complementary data.

Step VII: Conclusions on endocrine disrupting properties

The conclusions reached in Step V of the ECETOC 7SI-ED should be explained. If a biologically plausible link between an adverse effect in an intact organism and endocrine activity has been identified in Step V, then it is reasonable to conclude that an endocrine disrupting property in accordance with the WHO/IPCS (2002) definition has been identified.

If there is evidence for endocrine activity, which however does not meet the criteria in Step V, then an endocrine disruptor has not been identified. Although such substances may be considered by some to be ‘suspected endocrine disruptors’, the Commission considers that establishing categories of what *may be* an endocrine disruptor does not help to define what *is* an endocrine disruptor for the purposes of regulatory decision-making (Commission, 2016; (Italics are by the Commission). Furthermore, such categorisation would decrease legal certainty for regulators and stakeholders, without established benefits in terms of protection of health and the environment.

5. CONCLUSION

In response to the publication of the ECHA and EFSA (2016) *Outline of draft Guidance Document for the implementation of the hazard-based criteria to identify endocrine disruptors*, ECETOC provides guidance on how the strategy described in ECHA and EFSA (2016) can be put into practice. This has resulted in the development of the ECETOC 7SI-ED. The ECETOC 7SI-ED focuses on how to use a step-wise WoE approach to gather and assess available information, to integrate it and determine its sufficiency to identify the presence or absence of endocrine disrupting properties of a substance in accordance with the three components of the WHO/IPCS (2002) definition of an endocrine disruptor and the criteria set out in the ongoing revisions of the EU plant protection products and biocidal products legislations.

The ECETOC 7SI-ED is envisioned to cover human and environmental health, and it applies existing relevant scientific concepts and established best practice frameworks and methodologies. By following the ECETOC 7SI-ED, the data for any regulated substance can be transparently organised and evaluated to reveal the WoE available, its strengths and uncertainties, to compare with the regulatory definition for an endocrine disruptor. This enables a conclusion to be drawn on whether, or not, a substance meets this definition. However, the seven-step tool to identify the endocrine disrupting properties of a substance forms only the first step of the actual hazard and risk assessment of endocrine disrupting substances. The next step for any substance that meets the definition criteria is to undergo a comprehensive hazard and risk assessment. ECETOC Reports are available that provide guidance on how to conduct the hazard and risk assessment of endocrine disrupting substances (ECETOC, 2009a, b, 2011; Bars *et al.,* 2011, 2012; Weltje *et al.,* 2013). Substance hazard and risk assessment entails the determination of safety thresholds, exposure assessment, potency assessment and the determination whether acceptable risk can be demonstrated. By comparison, the seven steps outlined in Section V of ECHA and EFSA (2016) are restricted to the identification of endocrine disrupting properties. Boobis *et al.* (2016, 2017) provide an in-depth discussion of the limitations of hazard identification-based classification schemes.

The ECETOC 7SI-ED is recommended as a tool to assess whether, or not, a substance meets the regulatory definition of an endocrine disruptor. It is based on robust scientific principles and has been designed to be practical and transparent in its utility with the framework being presented as a series of discrete logical steps, each of which is supported by a clear set of questions and considerations to guide decision-making.

ABBREVIATIONS

7SI-ED	Seven steps for the identification of endocrine disrupting properties
ADME	Adsorption, distribution, metabolism, and elimination
AOP	Adverse outcome pathway
BP	Biocidal product
CAS	Chemical abstract service
CF	Conceptual framework
CRED	Criteria for Reporting and Evaluating Ecotoxicity Data
EATS pathways	(O)estrogen, androgen, thyroid and steroidogenesis pathways
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ED	Endocrine disrupting
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
GD	Guidance document
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre
MoA	Mode-of-action
MTD / MTC	Maximum tolerated dose / concentration
OPPTS	Office of Prevention, Pesticides and Toxic Substance
OCSPP	Office of Chemical Safety and Pollution Prevention
PPP	Plant protection product
(Q)SAR	(Quantitative) structure activity relationship
TG	Test guideline
ToxR Tool	Toxicological data reliability assessment tool
US	United States
WHO	World Health Organisation
WoE	Weight-of-evidence

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APPENDIX A: BACKGROUND INFORMATION

Introduction

ECETOC is an industry-funded, scientific, not-for-profit think tank that strives to enhance the quality and reliability of science-based chemical risk assessment. To assist in the development of practicable guidance to move regulatory application of criteria to identify endocrine disrupting properties forward in the EU, it is proposing the *ECETOC Seven Steps for the Identification of Endocrine Disrupting Properties (ECETOC 7SI-ED)*.

Appendix A to this report provides relevant background information to substantiate the rationale and justification for the structure and contents of the ECETOC 7SI-ED:

- Activities of the OECD related to endocrine disruptors;
- Activities of the European Commission (in the following: the Commission) related to the establishment of legislative provisions on endocrine disruptors;
- Summary of prior ECETOC work related to the establishment of a science-based framework for the identification and hazard and risk assessment of endocrine disruptors.

Generally, the ECETOC 7SI-ED follows the framework and the provisions outlined by the Commission in 2016. Specifically, provisions have been outlined in the *Commission Communication on endocrine disruptors and the draft Commission acts setting out scientific criteria for the determination of endocrine disruptors in the context of the EU legislation on plant protection products [PPP] and biocidal products [BP]* (Commission, 2016a) and the ECHA and EFSA *Outline of draft guidance document for the implementation of the hazard-based criteria to identify endocrine disruptors* (ECHA and EFSA, 2016). The Commission plans to implement scientific criteria concerning the hazard-based identification of endocrine disruptors in the context of *Regulation (EU) No 528/2012 concerning the making available on the market and use of BP* (EP and Council of the EU, 2012) and *Regulation (EC) No 1107/2009 on the placing of* PPP *on the market* (EP and Council of the EU, 2009).

As described in Commission (2016a) and ECHA and EFSA (2016), a conclusion on the identification of endocrine disrupting properties is based upon an evaluation of all available relevant data and information, and it encompasses three pillars, i.e. (1) an assessment of the evidence for endocrine MoA; (2) an assessment of the evidence for adverse effects in an intact organism, or its progeny, or (sub)populations; (3) an assessment of whether identified endocrine MoAs are causally related to the adverse effects.

Activities of the OECD related to endocrine disruptors

Information on OECD activities with respect to endocrine disruptors is available at:
http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm

All OECD Test Guidelines (TG) are available at:
http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

(Websites were accessed March 2017.)

Within its Testing of Chemicals Programme, the OECD has been and continues to be dedicated to the topic of endocrine disrupting chemicals. In 2010, it convened an advisory group on testing and assessment of endocrine disrupters (EDTA AG). One of the outcomes of the work of the EDTA AG was the publication of a *Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters* (OECD, 2012a) and a *Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption* (OECD, 2012b). A revision of this Guidance Document is underway in 2017. Further, the OECD published *Guidance document No 150 on standardised test guidelines for evaluating chemicals for endocrine disruption: Case studies using example chemicals* (OECD, 2012c). As presented in further detail in Table 1**,** the OECD CF consists of the following five levels, and for each level, relevant non-standardised and standardised test methods (the latter: OECD Test Guidelines (TGs)) are listed (OECD, 2012a). The OECD CF is intended to be used as a guide to the available tests that may be helpful for identifying potential endocrine disruptors but it does not constitute a 'pick list' of test methods or a testing strategy. It is possible to enter and exit the OECD CF at any test level depending on the outcome of evaluations:

Level 1: Collection of existing data and non-test information.

Level 2: *In vitro* assays providing data about selected endocrine mechanism(s) / pathway(s); i.e. screening assays used for hazard detection, identification of possible MoAs, prediction of AOPs, priority-setting, and WoE-based judgements leading to a conclusion.

Level 3: *In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s): i.e. *in vivo* screening for possible endocrine disrupting activity; designed to provide a yes/no (qualitative) answer about the ability to interact with the (o)estrogen, androgen, thyroid and steroidogenesis (EATS) pathways.

Level 4: *In vivo* studies providing data on adverse effects on endocrine-relevant endpoints. This entails a more thorough assessment of the possible or actual endocrine disrupting effects of a chemical in developing or adult organisms because they are sensitive to more than one mechanism that may or may not be endocrine-related.

Level 5: *In vivo* studies providing more comprehensive data on adverse effects on endocrine-relevant endpoints over more extensive parts of the life cycle of the organism. The developmental and reproductive toxicity studies provide data on adverse effects and are especially useful for risk assessment as they add to the WoE concerning the potential for impacts in humans and vertebrate wildlife, and provide data on dose/concentration-response. Effects in these studies can be sensitive to more than one mechanism that may or may not be endocrine-related.

Table 1: Overview of Levels 2-5 of OECD CF for testing and assessment of endocrine disruptors (adapted from: OECD, 2012a)

Level	Description	Mammalian toxicology	Non-mammalian toxicology
2	*In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) (Mammalian and non mammalian methods)	(O)estrogen or androgen receptor binding affinity (O)estrogen receptor transactivation (OECD TG 455 – OECD TG 457) Androgen or thyroid transactivation (OECD TG 458 *) Steroidogenesis *in vitro* (OECD TG 456) MCF-7 cell proliferation assays (ER ant/agonist) Other assays as appropriate	
3	*In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s) [1]	Uterotrophic assay (OECD TG 440) Hershberger assay (OECD TG 441)	Xenopus embryo thyroid signalling assay (when/if TG is available) Amphibian metamorphosis assay (OECD TG 231) Fish reproductive screening assay (OECD TG 229) Fish screening assay (OECD TG 230) Androgenized female stickleback screen(OECD GD 140)
4	*In vivo* studies providing data on adverse effects on endocrine relevant endpoints [2]	Repeated dose 28-day study (OECD TG 407) Repeated dose 90-day study (OECD TG 408) 1-generation reproduction toxicity study (OECD TG 415) Male pubertal assay (*cf.* OECD (2012b); Chapter C4.3) [3] Female pubertal assay (*cf.* OECD (2012b); Chapter C4.4) [3] Intact adult male endocrine screening assay (*cf.* OECD (2012b); Annex 2.5) Prenatal developmental toxicity study (OECD TG 414) Chronic toxicity and carcinogenicity studies (OECD TG 451-453) Reproductive screening test (OECD TG 421; if enhanced) Combined 28-day/reproductive screening assay (OECD TG 422; if enhanced) Developmental neurotoxicity (OECD TG 426)	Fish sexual development test (OECD TG 234) Fish reproduction partial life cycle test (when/if TG is available) Larval amphibian growth & development assay (OECD TG 241*) Avian reproduction assay (OECD TG 206)
5	*In vivo* studies providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism	Extended one-generation reproductive toxicity study (EOGRTS) (OECD TG 443) [4] 2-Generation reproduction toxicity study (OECD TG 416; most recent update)	FLCTT (Fish life cycle toxicity test) (when TG is available) Medaka multi-generation test (MMGT) (when TG is available) Medaka One Generation Reproduction Test (MEOGRT; TG 240) ** Avian 2 generation reproductive toxicity assay (OECD, 2007 *)

Footnotes as provided in the OECD CF (OECD, 2012a):

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

2: Effects can be sensitive to more than one mechanism and may be due to non-endocrine disrupting mechanisms.

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

4: The EOGRTS (OECD TG 443) is preferable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints in the juvenile and adult F1, which are not included in the 2-generation study (OECD TG 416) adopted in 2001.

Further footnotes to Table 1:

* TG or Detailed Review Paper not (yet) included in the OECD CF (OECD, 2012a).

** Method not (yet) included in the OECD CF (OECD, 2012a).

The OECD CF (OECD, 2012a) also lists the following non-mammalian toxicology tests on invertebrates, and it notes: At present, the available invertebrate assays solely involve apical endpoints which are able to respond to some endocrine disrupters and some non-endocrine disruptors. Those in Level 4 are partial life cycle tests, while those in Level 5 are full- or multiple life cycle tests.

In Level 4, the following tests on invertebrates are listed: Mollusc partial life cycle assays (when TG is available); Chironomid toxicity test (TG 218-219); Daphnia reproduction test (with male induction) (OECD TG 211); Earthworm reproduction test (OECD TG 222); Enchytraeid reproduction test (OECD TG 220); Sediment water lumbriculus toxicity test using spiked sediment (OECD TG 225); Predatory mite reproduction test in soil (OECD TG 226); Collembolan reproduction test in soil (OECD TG 232).

In Level 5, the following tests on invertebrates are listed: Mysid life cycle toxicity test (when TG is available); Copepod reproduction and development test (when TG is available); Sediment water chironomid life cycle toxicity test (OECD TG 233); Mollusc full life cycle assays (when TG is available); Daphnia multi-generation assay (if TG is available).

Activities of the Commission related to endocrine disruptors

Information on the Commission procedure to define criteria for endocrine disruptors is available at:
http://ec.europa.eu/health/endocrine_disruptors/next_steps_en

The EFSA work (that is focused on PPP) is described at:
http://www.efsa.europa.eu/en/press/news/161202

The ECHA work (that is focused on BP) is described at:
https://echa.europa.eu/-/endocrine-disruptors-echa-and-efsa-start-work-on-guidance

(Websites were accessed March 2017.)

In 1999, the Commission adopted the *Community strategy for endocrine disruptors COM(1999)706* (Commission, 1999) that set out the actions the Commission would undertake to address the potential environmental and human health impacts of endocrine disruption. Since, specific regulatory provisions for endocrine disruptors have been laid down in the areas of BPs and PPPs, covered by *Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products* (EP and Council of the EU, 2012) and *Regulation (EC) No 1107/2009 on the placing of plant protection products on the market* (EP and Council of the EU, 2009). Appendix B of this ECETOC Report provides an overview of the respective provisions that refer to endocrine disruptors. Article 5(3) of the BP Regulation (EP and Council of the EU, 2012) requires the Commission to determine how the criteria for endocrine disruptors should be defined, by drawing up acts *specifying scientific criteria for the determination of endocrine-disrupting properties*.

Accordingly, in 2016, the Commission published the *Communication COM(2016)350 final on endocrine disruptors and the draft Commission acts setting out scientific criteria for their determination in the context of the EU legislation on PPP and BP* (Commission, 2016a); *cf.* Commission (2016b) for executive summary of the related impact assessment. Amendments to the respective relevant Annexes of the PPP and BP Regulations are being prepared and have been published in:

The Draft Commission Regulation (EU) *setting out scientific criteria for the determination of endocrine disrupting properties and amending Annex II to the PPP Regulation (EC) 1107/2009* (Commission, 2017) – *cf.* Appendix C of this ECETOC Report for details on the (most recent) respective provisions.

The Draft Commission delegated Regulation (EC) *setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to the BP Regulation (EU) No 528/2012* (Commission, 2016c) – *cf.* Appendix C of this ECETOC Report for details on the (most recent) respective provisions.

The Commission Communication on endocrine disruptors

The Commission Communication COM(2016)350 final on endocrine disruptors and the draft Commission acts setting out scientific criteria for their determination in the context of the EU legislation on PPP and BP (Commission, 2016a) provides important information on issues that the Commission considers relevant for the identification of endocrine disruptors.

Specifically, the Commission states that it will follow the WHO/IPCS (2002) **definition of endocrine disruptor**, *i.e. an exogenous substance or mixture that*

1. alters function(s) of the endocrine system
2. and consequently causes
3. adverse health effects in an intact organism, or its progeny, or (sub)populations (*cf.* also WHO/UNEP, 2012).

In further specifying the three essential components of this definition, the Commission (2016a) refers to the following definitions:

Endocrine MoA (referring to bullet point 1 of the definition of endocrine disruptor, i.e. the way in which the function of the endocrine system is altered): *The inherent ability of a substance to interact or interfere with one or more components of an endocrine system* (EFSA, 2013). Consistent with the opinion of the EFSA, the Commission highlights that *an endocrine MoA is not a (eco)toxicological hazard in itself* (Commission, 2016a).

Adverse effect (referring to bullet point 3 of the definition of endocrine disruptor): *Change in the morphology / physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences* (WHO/IPCS, 2009).

For the determination if an effect is adverse or adaptive, Commission (2016a) highlights that *expert judgement will […] be required to assess on a case-by-case basis the toxicological relevance of [...] changes. In general, transient, inconsistent and minor fluctuations at the biochemical and molecular level may be considered adaptive (i.e. non-adverse), whilst sustained, consistent and permanent changes at the cell, organ- or organism-level, resulting in pathology or functional impairment in vivo, as well as altered timing of development, may be considered adverse* (EFSA, 2013).

Commission (2016a) further explains that endocrine-related adverse effects which are only indirectly triggered by a non-endocrine-related toxicity are not adverse effects that are relevant for the identification of a substance as an endocrine disruptor. In such cases, the reactions of the endocrine system would be the consequence of any given generalised toxicity, rather than the cause of the specific adverse effect observed.

The EFSA (2013) cites the US National Research Council (NRC, 2007) that explore the different implications of adverse and adaptive effects: *The consequences of a biologic perturbation depend on its magnitude, which is related to the dose, the timing and duration of the perturbation, and the susceptibility of the host. Accordingly, at low doses, many biologic systems may function normally within their homeostatic limits. At somewhat higher doses, clear biologic responses occur. They may be successfully handled by adaptation, although some susceptible people may respond. More intense or persistent perturbations may overwhelm the capacity of the system to adapt and lead to tissue injury and possible adverse health effects* (NRC, 2007).

Consequently causes (bullet point 2 of the definition of endocrine disruptor): The Commission (2016a) explains that this link between the MoA and the adverse effect is the *heart* of the definition. In accordance with Commission (2016a), the question remains about the extent to which this link should be clearly established, i.e. the degree to which a strict causality should be required. The Commission follows the conclusion from the EFSA (2013) that there *must be a reasonable evidence base for a biologically plausible*

causal relationship between the [endocrine MoA] and the adverse effects seen in intact organism studies, i.e. a *reasonable evidence base* to determine causality.

Commission (2016a) explains that the alternative would have been a more rigid approach to causality, asking for example for *conclusive* evidence of the connection and that the Commission considers that in practice, it will be very difficult to demonstrate *conclusive evidence* of causality. Therefore, the Commission intends to follow a concept of a reasonable evidence (*biological plausibility*) to determine causality (Commission, 2016a).

The following definitions for the terms conclusive and reasonable are provided in the online version of Black's Law Dictionary (available at: http://thelawdictionary.org):

Conclusive: Shutting up a matter; shutting out all further evidence; not admitting of explanation or contradiction; putting an end to inquiry; final; decisive.

Reasonable: Agreeable to reason; just; proper. Ordinary or usual.

The explanations from the Commission point to the importance of determining whether a link between endocrine disrupting activity and adverse effect is **biologically plausible**. In the *JRC Report of the Endocrine Disrupters Expert Advisory Group: Key scientific issues relevant to the identification and characterisation of endocrine disrupting substances* (Munn and Goumenou, 2013), a biologically plausible linkage would constitute a *causal chain of events from initial interaction of a substance with its target site in the organism through to the adverse outcome … biologically plausible linkage… depends on MoA and type of effect observed*.

With respect to biologically plausible linkage, the EFSA (2013) states: *Demonstration of all the key events of an endocrine MoA leading to the adverse outcome is not necessary, as this requires a very high burden of proof. However, it is important that there is logical and plausible reasoning to explain any (potential) causal relationship between the observed endocrine activity and the endocrine-mediated adverse effects. This concept of „plausibility" implies expert judgement. A minimum set of criteria was described by Bradford Hill (Bradford Hill, 1965) to provide adequate evidence of a causal relationship between an incidence and a consequence (e.g. exposure and ill health), including biological plausibility, consistency of findings, specificity, predictivity, coherence, concordance of dose response relationships and temporal associations and characterisation of uncertainties.*

Since different scientific issues have to be addressed for the identification of endocrine properties, different types of data have to be combined in **WoE approaches** to come to a conclusion on endocrine disruption, as also the EFSA (2013) highlights: *In principle, no single assay is likely to provide all the information needed to decide whether a substance is an ED (according to the WHO/IPCS definition endorsed by the Scientific Committee) because of the need to provide both mechanistic information showing how the substance interacts with the endocrine system, and apical information describing the adverse effects this interaction may cause. The results from a combination of tests increase the WoE and further elucidate the AOP* (EFSA, 2013).

ECHA and EFSA outline of draft Guidance Document for the implementation of the hazard-based criteria to identify endocrine disruptors

On 20 December 2016, the ECHA and the EFSA published an *Outline of draft guidance document for the identification of the hazard-based criteria to identify endocrine disruptors* (ECHA and EFSA, 2016) that was prepared with support from the Commission's Joint Research Centre (JRC). ECHA and EFSA (2016) describes that although the (identical) criteria for endocrine disruptors will formally apply only in the context of the BP and PPP legislation, the scientific approach(es) described in the Guidance could be relevant for other substances as well, since the endocrine disruptor identification step will be based exclusively on the evaluation of the relevant hazardous properties of a substance.

The Guidance outlined in ECHA and EFSA (2016) will focus on the data and information needed for endocrine disruption hazard identification. It will also provide an indication on which information may be considered sufficient to conclude on the endocrine disrupting properties of a substance in accordance with the criteria. The evaluation approach will take toxicological and ecotoxicological information into account in an integrated manner and provide guidance for identifying data gaps that would trigger the need for additional data across the human health and environment domains. The scope of the Guidance will be restricted to the EATS pathways, which are the best characterized pathways. Regarding the groups of (non-target) organisms to be considered in the Guidance, its coverage will be limited to vertebrates, including mammals, fish, birds, amphibians and reptiles. This is also justified on the basis of the available test methods.

In accordance with ECHA and EFSA (2016), the Guidance will describe **information sources for the identification of endocrine disruptors** (Section IV of the Guidance). This Section will distinguish between:

(i) *In vivo* tests for the identification of endocrine disruptor-relevant adverse effects;
(ii) Mechanistic information from *in vitro* and *in vivo* tests;
(iii) (Quantitative) structure activity relationships ((Q)SAR), read across and category approaches;
(iv) Epidemiological data, field studies and population models.

This section on information sources will be built, to a large extent, on the *in vitro* and *in vivo* laboratory studies identified in OECD GD 150 (OECD, 2012b), updated with additional information sources and considering the JRC methodology. Accordingly, *in vivo* studies for the identification of adverse effects will cover the OECD CF Levels 4 and 5; the mechanistic *in vitro* assays will cover OECD Level 2; and the mechanistic *in vivo* assays will cover OECD Level 3.

Regarding the test methods that are relevant for the identification of adverse effects, two sub-sections are envisioned to cover human health and ecotoxicology, and the Guidance will include an indication of which endpoint is informative on which of the EATS modalities. In order to facilitate the evaluation of adverse effects, it will be described which apical adverse effects could be considered as

(i) Diagnostic for endocrine MoA;
(ii) Indicative for an endocrine MoA (i.e. possibly, but not exclusively linked to an endocrine MoA);
(iii) and those adverse effects that in principle are not related to an endocrine MoA.

Similarly, test methods informative on the endocrine MoA of a substance will be identified, indicating the endocrine modalities (axes, pathways) that each specific test is aimed at or informative on. As some TGs include optional endpoints that are informative on alterations in the endocrine system, e.g. the level of hormones in the blood, the overview will also indicate whether the endpoint is standard or optional (at least according to the TGs).

As described in EFSA and ECHA (2016), Section V of the Guidance will present a **hazard identification strategy for endocrine disrupting properties**. The Guidance will describe endocrine disruptor hazard identification from two different starting points to ensure applicability of the proposed methodology for substances for which the available information differs in type. One approach will consider starting the evaluation with apical studies indicative of endocrine-mediated adverse effects and will set out how to evaluate if, indeed, an endocrine mechanism would be the cause of the adverse effect observed. The second approach will consider starting the evaluation with endocrine disruptor-relevant mechanistic information and set out how to investigate whether the observed endocrine activity would result in adverse effects in intact organisms.

The ECETOC 7SI-ED follows the structure of Section V of the ECHA and EFSA (2016) outline for a draft guidance document. As described in EFSA and ECHA (2016), the Commission hazard identification strategy for endocrine disrupting properties will be built to allow evaluating the information in a WoE approach. This WoE approach will cover the following seven steps:

1. Gathering of relevant information with regard to adverse effects and endocrine disrupting MoAs as described in Section IV of the ECHA and EFSA (2016) Guidance.

2. Evaluation of quality, reliability, reproducibility and consistency of the individual studies: Guidance on the evaluation of the relevant studies with regard to quality, reliability, reproducibility and consistency. Reference to documents describing such evaluation approaches, as appropriate.

3. Evaluation and summary of the evidence for an adverse effect: Guidance on how to assess and conclude on the strength of evidence in terms of adversity relevant to humans and non-target vertebrates. Where differentiation with regard to humans and non-target vertebrates is required, these aspects will be considered separately.

4. Evaluation and summary of the evidence for MoAs: Guidance on how to assess and conclude on strength of evidence in terms of MoA(s) for the EATS modalities with specific sub-sections on how to evaluate MoAs for (anti)oestrogenicity; (anti)androgenicity; thyroid effects; and steroidogenesis.

5. Integration of the evidence and evaluation of biological plausibility of a link between endocrine MoA and adverse effect: Guidance on how to integrate the available evidence on adverse effects for humans and non-target vertebrates with the evidence on (endocrine) MoA(s) in order to enable a conclusion on the existence of a biologically plausible link between the observed adverse effect(s) and the endocrine MoA(s). Approaches will be described on how the available information on adversity and endocrine activity should be considered together in a WoE approach, in order to conclude on the biological plausibility. The evaluation will cover human health and non-target vertebrates.

6. Identification of uncertainties: Guidance on how to assess uncertainties. Reference will also be made to existing documents addressing this issue.

7. Conclusions on endocrine disrupting properties: Guidance on how to conclude on the endocrine disrupting properties of a substance in accordance with the criteria for endocrine disruptors both with regard to human health and non-target vertebrates, considering WoE and identified uncertainties.

For the third step, i.e. the evaluation and summary of the evidence for an adverse effect, ECHA and EFSA (2016) suggest the following contents:

- How to evaluate conflicting results, e.g. both positive and negative results;
- Consistency of the data;
- Consideration of the pattern and coherence of the results between studies of a similar design and across different species;
- Route of exposure, toxicokinetic and metabolism studies;
- Concept of the limit dose, and international guidelines on maximum recommended doses and for evaluating confounding effects of excessive toxicity;
- Evaluation of the relevance of adverse effects for humans and at the population-level for non-target vertebrates.

For the seventh and final step, i.e. the conclusion on endocrine disrupting properties, ECHA and EFSA (2016) announces that indication will be provided on which information may be considered sufficient to conclude on the endocrine disrupting properties of a substance in accordance with the criteria. For some substances the information identified as relevant may not be sufficient to reach a firm conclusion. For such cases, guidance will be provided for different scenarios on what missing information should be generated in order to enable a conclusion on the endocrine disrupting properties of the substance in question.

Prior ECETOC work related to endocrine disruptors

In recognition of the Commission's activities for the establishment of a legislative-based strategy for endocrine disruption, the ECETOC has been proactively engaged in developing guidance to implement regulatory actions for endocrine disrupting substances. The first European industry task force (named Environmental Oestrogens) to address endocrine disruption was formed by ECETOC in 1995. In 2009, ECETOC published a Technical Report (TR) Guidance on identifying endocrine disrupting effects (ECETOC, 2009a) that addressed comments provided at a Workshop Guidance on interpreting endocrine disrupting effects that took place on 29-30 June 2009, in Barcelona, Spain (ECETOC, 2009b). Specific scientific criteria for the determination of endocrine disrupting properties that integrate information from both regulatory toxicity and ecotoxicity studies and mechanistic/screening studies were proposed. These scientific criteria rely on the nature of the adverse effects detected in the studies that give concern for endocrine toxicity and the description/understanding of the MoA of toxicity which scientifically support and explain the adverse effects. Importantly, the TR (ECETOC, 2009a) and the follow up publications Bars *et al.* (2011, 2012) provided guidance, in the form of flow charts that could be used as decision trees for the identification of endocrine disrupting effects in human health and environmental hazard assessments.

As further explored in ECETOC (2009b), ecotoxicity test methods typically focus on measuring impacts on development, growth and reproduction, which often give limited insight into the toxicological MoA that leads to the adverse effect. Environmental hazard assessment also differs from human health hazard assessment in that the protection goal is at the population rather than the individual (human) level. Beyond this, environmental hazard assessment has to consider many species over different taxonomic groups. Oftentimes, the data that are available from mammalian toxicity studies drive the cause for concern for endocrine disruption and trigger further investigation in environmental species.

Since all substances having endocrine disrupting properties may not represent the same hazard, ECETOC (2009a) and Bars *et al.* (2011, 2012) propose specific criteria for potency to assess the level of hazard resulting from endocrine toxicity and to discriminate substances of high concern from those of lower concern. These criteria should be considered collectively, using a WoE approach, to determine the potency of the substance as an endocrine disruptor:

- The dose level at which adverse effects on endocrine endpoints occur.
- The duration of exposure that is required for an adverse effect to be induced.
- The nature, incidence and severity of the adverse effects.
- The number of animal species from regulatory toxicity studies showing adverse endocrine effects.

Another option to quantify the potency of a substance is to compare its effect thresholds with those of a reference substance. The reference substance would be assigned a potency of 1 and the test substance is normalized by dividing the (no) effect concentration of the reference substance by the (no) effect concentration of the test substance (Weltje *et al.,* 2013).

In 2011, the discussion on best practices for the risk assessment of endocrine disrupting substances was further advanced at an ECETOC workshop *Risk assessment of endocrine disrupting chemicals* (ECETOC, 2011). There was agreement that the hazard and risk assessment of endocrine disruptors would require a consistent WoE approach, which would be applicable under various regulatory regimes. For human health hazard assessment, the systematic and structured approach of the WHO/IPCS framework for evaluating the MoA for cancer and non-cancer endpoints (Sonich-Mullin *et al.,* 2001; Boobis *et al.,* 2006, 2008, 2009; Fenner-Crisp and Dellarco, 2016) was suggested as practicable means to evaluate available mechanistic and apical information (ECETOC, 2011). For ecotoxicological assessment, it was acknowledged that no direct equivalent to this WHO/IPCS framework existed. However, several specific WoE frameworks for the evaluation of endocrine disrupting effects had been published. These should be evaluated and combined for the requirements under current legislation. Specific guidance was considered necessary to aid in the identification of endpoints in ecotoxicological studies that were of population relevance. Some endpoints are clearly directly population related, whereas others are more diagnostic in nature and do not necessarily lead to an adverse population-relevant effect(s). As a follow-up to these ECETOC workshops, the flow charts originally published in ECETOC (2009a) were further refined as described and presented in Bars *et al.* (2011, 2012); Lewis (2013) and Weltje *et al.* (2013).

Finally, in 2016, ECETOC published a TR *Guidance on assessment and application of AOPs relevant to the endocrine system* (ECETOC, 2016). AOPs can help identify if an observed adverse outcome can be plausibly linked to an endocrine mechanism, which is a key requirement for identification of a substance is an endocrine disruptor according to the WHO/IPCS (2002) definition. AOPs could also potentially be used to help predict

the potential for an adverse outcome *in vivo* based on the results of *in vitro* mechanistic data. If AOPs are to be used to identify endocrine disrupting properties, it must be ensured that they are sufficiently robust and fit for purpose. To this end, the TR (ECETOC, 2016) provides guidance on identifying the basic requirements of a defined AOP and how to establish the minimum scientific standards that allow the use of AOPs in different contexts, such as hazard identification, read-across and risk assessment (ECETOC, 2016).

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APPENDIX B: PROVISIONS IN THE CURRENT PPP AND BP REGULATIONS THAT RELATE TO ENDOCRINE DISRUPTORS

Regulation (EC) No 1107/2009 concerning the placing on the market of PPP

Annex II of Regulation (EC) No 1107/2009 concerning the placing on the market of PPP (EP and Council of the EU, 2009), describes *procedures and criteria for the approval of active substances, safeners and synergists pursuant to Chapter II:*

- Section 3: Criteria for the approval of an active substance
  - Section 3.6: Impact on human health; specifically for ED: 3.6.5
  - Section 3.8: Ecotoxicology; specifically for ED: 3.8.2

Section 3.6.5. of Annex II of the PPP Regulation states:

An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a PPP, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005.

By 14 December 2013, the Commission shall present to the Standing Committee on the Food Chain and Animal Health a draft of the measures concerning specific scientific criteria for the determination of endocrine disrupting properties to be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 79(4).

Pending the adoption of these criteria, substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.

In addition, substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.

Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances in accordance with the PPP Regulation

Data requirements for active substances in accordance with the PPP Regulation are set out in Commission Regulation (EU) No 283/2013 (Commission, 2013a). The specific test methods are listed in a Commission Communication (Commission, 2013b).

In Part A of the Annex to Commission Regulation (EU) No 283/2013 (Commission, 2013a), Section 5 describes toxicological and metabolism studies, and Sub-section 5.8 other toxicological studies, with 5.8.3 describing endocrine disrupting properties:

5.8.3. Endocrine disrupting properties

If there is evidence that the active substance may have endocrine disrupting properties, additional information or specific studies shall be required:

- to elucidate the mode/mechanism of action,
- to provide sufficient evidence for relevant adverse effects.

Studies required shall be designed on an individual basis and taking into account Union or internationally agreed guidelines, in the light of the particular parameters to be investigated and the objectives to be achieved.

Regulation (EU) No 528/2012 concerning the making available on the market and use of BP

In Article 5, the BP Regulation refers to EDs as follows (consistent with PPP Regulation):

No later than 13 December 2013, the Commission shall adopt delegated acts in accordance with Article 83 specifying scientific criteria for the determination of endocrine-disrupting properties. Pending the adoption of those criteria, active substances that are classified in accordance with Regulation (EC) No 1272/2008 as, or meet the criteria to be classified as, carcinogen category 2 and toxic for reproduction category 2, shall be considered as having endocrine-disrupting properties. Substances such as those that are classified in accordance with Regulation (EC) No 1272/2008 as, or that meet the criteria to be classified as, toxic for reproduction category 2 and that have toxic effects on the endocrine organs, may be considered as having endocrine-disrupting properties.

APPENDIX C: DRAFT COMMISSION REGULATIONS SETTING OUT SCIENTIFIC CRITERIA FOR THE DETERMINATION OF ENDOCRINE DISRUPTING PROPERTIES AND AMENDING THE PPP AND BP REGULATIONS

The two draft legal acts setting the criteria to identify EDs that the Commission presented on 15 June 2016 (described below) now need to be adopted according to the relevant procedures, which in both cases involve Parliament and Council. Three further rounds of discussions with experts and Member States took place on 21 September 2016, 18 November 2016, and 21 December 2016. The revised criteria clarify the scope of the WHO/IPCS (2002) definition, the burden of proof, the scope of the criteria, and the kind of scientific evidence that can be used to identify EDs. The original text concerning PPP has been split into one text containing the criteria and one text containing the technical amendment to the clause on negligible exposure.

Draft Commission Regulation setting out scientific criteria for the determination of endocrine disrupting properties and amending Annex II to the PPP Regulation

For the identification of EDs, the draft Commission Regulation (Commission, 2017) describes the following amendment to Annex II to the PPP Regulation (EC) No 1107/2009. Nota bene, this version (from the Commission General Directorate Health and Food Safety (SANTE)) does not contain a qualification that is either related to negligible risk or negligible exposure, but only a qualification that is related to the human health relevance of effects.

In Point 3.6.5. the following paragraphs are added after the fourth paragraph:

From [date of application], an active substance, safener or synergist shall be considered as having endocrine disrupting properties that may cause adverse effect in humans if, based on points (1) to (3) of the sixth paragraph it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effects identified are not relevant to humans:

(1) it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;

(2) it has an endocrine MoA, i.e. it alters the function(s) of the endocrine system;

(3) the adverse effect is a consequence of the endocrine MoA.

The identification of an active substance, safener or synergist as having endocrine disrupting properties that may cause adverse effect in humans in accordance with the fifth paragraph shall be based on all of the following:

(1) all available relevant scientific data (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo, in vitro, or, if applicable, in silico studies informing about endocrine MoA):

(a) scientific data generated in accordance with internationally agreed study protocols […];
(b) other scientific data selected applying a systematic review methodology […];

(2) an assessment of the available relevant scientific data based on a WoE approach in order to establish whether the criteria set out in the fifth paragraph are fulfilled; in applying the WoE determination, the assessment of the scientific evidence shall, in particular, consider all of the following factors:

(a) both positive and negative results;
(b) the relevance of the study designs, for the assessment of adverse effects and of the endocrine MoA;
(c) the biological plausibility of the link between the adverse effects and the endocrine MoA;
(d) the quality and consistency of the data, considering the pattern and coherence of the results within and between studies of a similar design and across different species;
(e) the route of exposure, toxicokinetic and metabolism studies;
(f) the concept of the limit dose, and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity;

(3) adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor."

In Point 3.8.2. the following paragraphs are added after the sole paragraph:

"From [date of application], an active substance, safener or synergist shall be considered as having endocrine disrupting properties that may cause adverse effects on non-target organisms if, based on points (1) to (4) of the third paragraph, it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effects identified are not relevant at the (sub)population level for non-target organisms:

(1) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;

(2) it has an endocrine MoA, i.e. it alters the function(s) of the endocrine system;

(3) the adverse effect is a consequence of the endocrine MoA.

The identification of an active substance, safener or synergist as having endocrine disrupting properties that may cause adverse effects on non-target organisms in accordance with the second paragraph shall be based on all of the following:

(1) all available relevant scientific data (*in vivo* studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as *in vivo*, *in vitro*, or, if applicable, *in silico* studies informing about endocrine MoA):

(a) scientific data generated in accordance with internationally agreed study protocols […];
(b) other scientific data selected applying a systematic review methodology […];

(2) an assessment of the available relevant scientific data based on a WoE approach in order to establish whether the criteria set out in the second paragraph are fulfilled; in applying the WoE determination, the assessment of the scientific evidence shall consider all of the following factors:

(a) both positive and negative results, discriminating between taxonomic groups (e.g. mammals, birds, fish, amphibians) where relevant;
(b) the relevance of the study design for the assessment of the adverse effects and its relevance at the (sub)population level, and for the assessment of the endocrine MoA;
(c) the adverse effects on reproduction, growth/development, and other relevant adverse effects which are likely to impact on (sub)populations. Adequate, reliable and representative field or monitoring data and/or results from population models shall as well be considered where available;
(d) the biological plausibility of the link between the adverse effects and the endocrine MoA;
(e) the quality and consistency of the data, considering the pattern and coherence of the results within and between studies of a similar design and across different taxonomic groups;
(f) the concept of the limit dose and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity.

(3) Adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms;

(4) If the intended plant protection mode of action of the active substance being assessed, within the meaning of point 3.6. of Part A of the Annex to Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances, consists of controlling target organisms via their endocrine systems, the effect on organisms being of the same taxonomic phylum as the targeted one, shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms.

Draft Commission Delegated Regulation setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to the BP Regulation

The following excerpts from the Draft Commission delegated Regulation setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 (Commission, 2016c) describe which criteria for the identification of ED properties are currently stipulated (for BPs):

Section A - Endocrine disrupting properties with respect to humans

(1) An active substance shall be identified as having endocrine disrupting properties that may cause adverse effect in humans if, based on points (a) to (d) of point (2), it is a substance that meets all of the following criteria, unless there is information demonstrating that the adverse effects identified are clearly not relevant to humans:

(a) it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences;

(b) it has an endocrine MoA, i.e. it alters the function(s) of the endocrine system;

(c) the adverse effect is a consequence of the endocrine MoA.

(2) The identification of an active substance as having endocrine disrupting properties that may cause adverse effect in humans in accordance with point (1) shall be based on all of the following:

(a) all available relevant scientific data:

(i) scientific data generated in accordance with internationally agreed study protocols (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo or in vitro studies informing about MoA). In particular, ECHA guidance on the implementation of the BP Regulation shall be considered;

(ii) other relevant scientific data selected applying a systematic review methodology.

(b) an assessment of the available relevant scientific data based on a WoE approach in order to establish whether the criteria set out in point (1) are fulfilled.

(c) in applying the WoE determination, the assessment of quality, reliability, reproducibility and consistency of the scientific evidence shall, in particular, consider all of the following factors:

(i) both positive and negative results.

(ii) the relevance of the study designs for the assessment of adverse effects and of the endocrine MoA.

(iii) the biological plausibility of the link between the adverse effects and the endocrine MoA.

(iv) the quality and consistency of the data, considering the pattern and coherence of the results within and between studies of a similar design and across different species.

(v) the route of exposure, toxicokinetic and metabolism studies.

(vi) the concept of the limit dose, and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity.

(d) adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as ED.

Section B - Endocrine disrupting properties with respect to non-target organisms

(1) An active substance shall be considered as having endocrine disrupting properties that may cause adverse effects on non-target organisms if, upon the application of points (a) to (d) of point (2), it is a substance that meets all of following criteria, unless there is information demonstrating that the adverse effects identified are not relevant at the (sub)population level for non-target organisms:

(a) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences, considered relevant at the (sub)population level;

(b) it has an endocrine MoA, i.e. it alters the function(s) of the endocrine system;

(c) the adverse effect is a consequence of the endocrine MoA.

(2) The identification of an active substance as having endocrine disrupting properties that may cause adverse effects on non-target organisms in accordance with point (1) shall be based on all of the following:

(a) all available relevant scientific data:

- *(i) scientific data generated in accordance with internationally agreed study protocols (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo or in vitro studies informing about endocrine MoA). In particular, ECHA guidance on the implementation of the BP Regulation shall be considered.*
- *(ii) other relevant scientific data selected applying a systematic review methodology.*

(b) an assessment of the available relevant scientific data based on a WoE approach in order to establish whether the criteria set out in point 1 are fulfilled.

(c) in applying the WoE determination, the assessment of quality, reliability, reproducibility and consistency of the scientific evidence shall consider all of the following factors:

- *(i) both positive and negative results, discriminating between taxonomic groups (e.g. mammals, birds, fish) where relevant.*
- *(ii) the relevance of the study designs for the assessment of the adverse effects and its relevance at the (sub)population level, and for the assessment of the endocrine MoA.*
- *(iii) the adverse effects on reproduction and growth/development, as these are the effects most likely to impact on (sub)populations. Adequate, reliable and representative field or monitoring data and/or results from population models shall be considered where available.*
- *(iv) the biological plausibility of the link between the adverse effects and the endocrine MoA.*
- *(v) the quality and consistency of the data, considering the pattern and coherence of the results within and between studies of a similar design and across different taxonomic groups.*
- *(vi) the concept of the limit dose and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity.*

(d) adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms.

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Lucy Wilmot	ECETOC, B (Contracted to ECETOC by PFA-Brussels)

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Responsible Editor:
Dr Alan Poole
ECETOC AISBL
Av. E. Van Nieuwenhuyse 2 (box. 8)
B-1160 Brussels, Belgium
VAT: BE 0418344469
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