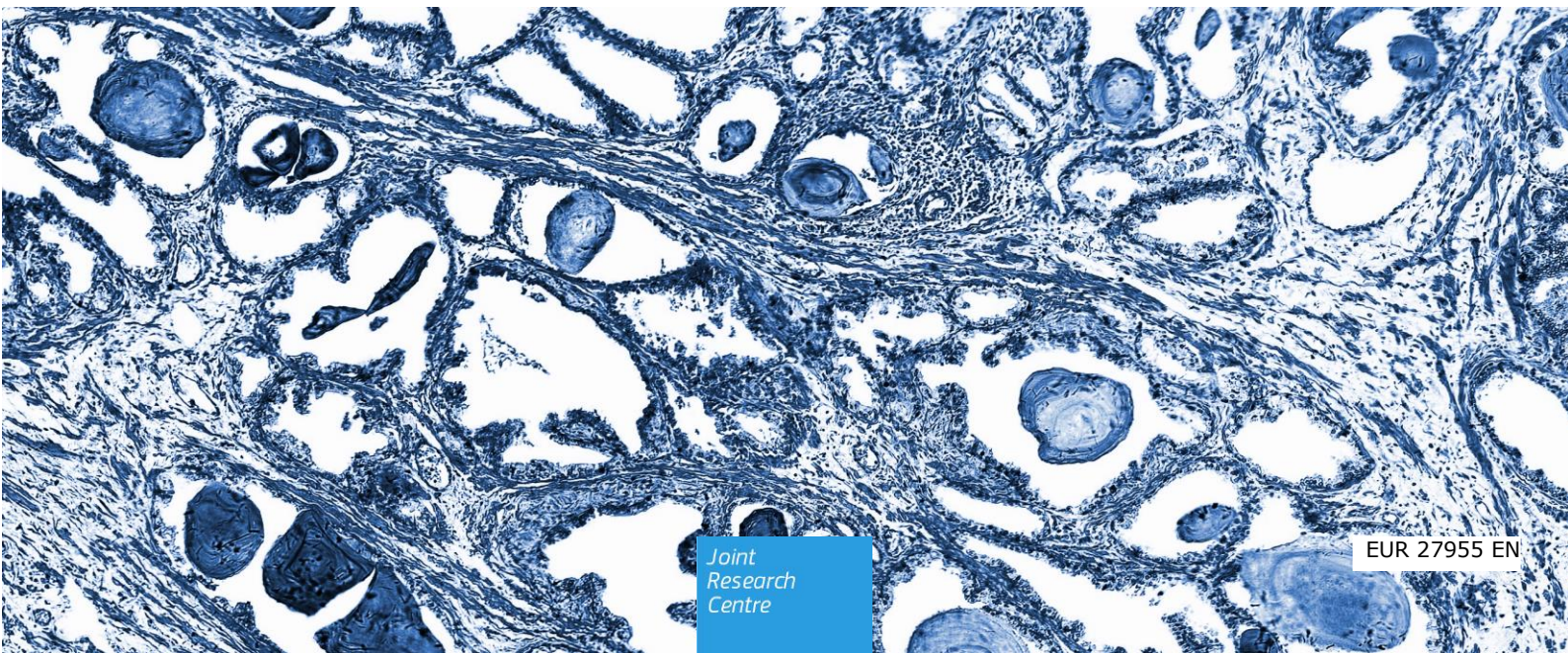
European Commission

JRC SCIENCE FOR POLICY REPORT

Screening methodology to identify potential endocrine disruptors according to different options in the context of an impact assessment

Screening methodology for identification of potential endocrine disruptors according to different options in the context of an impact assessment of proposed criteria for identification of endocrine disruptors and their implementation in EU legislation

2016



Joint Research Centre

EUR 27955 EN

JRC Science Hub
https://ec.europa.eu/jrc

JRC101950

EUR 27955 EN

PDF	ISBN 978-92-79-58906-5	ISSN 1831-9424	doi:10.2788/73203
Print	ISBN 978-92-79-58907-2	ISSN 1018-5593	doi:10.2788/288320

Luxembourg: Publications Office of the European Union, 2016

How to cite: JRC; Screening methodology to identify potential endocrine disruptors according to different options in the context of an impact assessment; EUR 27955 EN; doi:10.2788/73203

Title Screening methodology to identify potential endocrine disruptors according to different options in the context of an impact assessment

Abstract
Several pieces of EU legislation regulate the marketing and use of chemical substances. While several regulations, including the regulations on Plant Protection Products (PPPR), Biocidal Products (BPR) and Chemicals (REACH), include provisions for endocrine disrupting substances (EDs), objective scientific criteria are lacking. In order to evaluate the potential health, socio-economic and environmental impacts of applying four different options for criteria defining EDs across these pieces of legislation, the Commission initiated an Impact Assessment (IA). This IA has been supported by two studies, focusing on (a) selection of substances for the IA and the screening of their potential for identification as EDs according to different options for defining criteria for identification of endocrine disruptors and (b) the potential impacts of various policy options on health, environment, trade, agriculture and socio-economy. This report describes a screening methodology that has been developed by the JRC to support the first study which has assessed almost all pesticide and biocide active ingredients and a selection of substances falling under REACH, the Cosmetic Products Regulation and the Water Framework Directive. This screening methodology is not intended to replace an in-depth risk assessment process, and the results obtained are not intended to pre-empt regulatory conclusions that may eventually be made under different pieces of EU legislation.

Screening methodology to identify potential endocrine disruptors according to different options in the context of an impact assessment

Disclaimer

The present screening methodology was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The methodology was developed for the sole purpose of the screening exercise, which needed to be carried out in a limited time using available evidence (since no additional testing was performed).

The results obtained by applying the screening methodology, which are published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), therefore do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these Regulations.

Table of contents

Acknowledgements

We would like to acknowledge the following authors (in alphabetical order) for their contributions to this report: Raffaella Corvi, Elise Grignard, Marlies Halder, Aude Kienzler, Sander van der Linden, Alfonso Lostia, Federica Madia, Sharon Munn, Malgorzata Nepelska, Alexandra Rolaki and Andrew Worth.

The screening methodology described in this report was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The results obtained by applying the screening methodology, published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these two Regulations.

Executive summary

Several pieces of EU legislation that regulate the marketing and use of substances contain specific provisions for endocrine disrupting substances. The regulations on Plant Protection Products (PPPR)[1] and Biocidal Products (BPR)[2] further require the European Commission to establish scientific criteria to identify substances with endocrine disrupting properties. The European Commission initiated an impact assessment and in 2014 outlined four policy options for identifying endocrine disruptors (EDs) in the corresponding Roadmap (EC, 2014, *Defining criteria for identifying Endocrine Disruptors in the context of the implementation of the Plant Protection Product Regulation and Biocidal Products Regulation)*.

The screening methodology described within this report was developed by DG JRC to be used in the context of an impact assessment to collect and assess, in a limited amount of time, the available evidence regarding endocrine disrupting effects of selected substances. It provides guidance for (a) the identification of data sources, (b) the selection of relevant data and (c) the data analysis procedure to categorise each substance as potential endocrine disruptor or not, including application of a limited weight of evidence. The methodology was used to determine which of approximately 600 substances would be potentially categorised as ED under the following four policy options set out in the EC Roadmap.

- Option 1: Interim criteria, no policy change. Assessment based on the CLP classification (as carcinogen category 2 or toxic for reproduction category 2, harmonised or proposed) and toxicity to endocrine organs. Outcome of the screening is ED or Unclassified.

- Option 2: Assessment based on the IPCS/WHO definition of an ED (*i.e.* "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"). Outcome of the screening is ED or Unclassified.

- Option 3: Assessment based on the IPCS/WHO definition of an ED, while acknowledging different levels of uncertainty in the data. Outcome is of the screening is ED (Cat I), Suspected ED (Cat II), Endocrine active (Cat III) or Unclassified. ED under option 2 is equivalent to ED (Cat I) under option 3.

- Option 4: Assessment based on the IPCS/WHO definition of an ED, but incorporating the dose at which the effect occurs (giving a measure of the substance potency). Outcome of the screening is ED (if dose is below a certain cut-off) or Unclassified.

All selected substances are currently subject to at least one of the following Regulations on Plant Protection Products (PPPR), Biocidal Products (BPR), Chemicals (REACH) or Cosmetic Products (CPR) and a few are also listed as priority substances under the Water Framework Directive (WFD). Substances were selected according to the criteria described in the document "Selection of chemical substances to be screened in the context of the IA on criteria to identify endocrine disruptors" published on the DG SANTE website[3] and in Annex 4 of the impact assessment report.

[1] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market

[2] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products

[3] http://ec.europa.eu/health/endocrine_disruptors/impact_assessment/index_en.htm

The screening methodology focuses on humans and wildlife and unless specifically stated otherwise, all mammalian toxicity data were regarded as being relevant for both humans and mammals in the environment (wildlife). As the understanding regarding the disturbance of the endocrine system of many invertebrate species is limited, the effects on wildlife were confined to the effects observed in mammals, fish, amphibians and to a very limited extent in birds and reptiles.

In addition, this methodology relies solely on existing data (no additional experimental work was performed) and is limited to the effects on the estrogenic, androgenic, thyroid and steroidogenesis (EATS) pathways. These pathways are relatively well understood and consensus guidance on the interpretation of effects observed in OECD Test Guidelines is available from OECD Guidance Document 150[4]. Perturbations of other non-EATS pathways - although potentially relevant for ED - were largely beyond the scope of this methodology. Human epidemiological data, whilst potentially informative, were not included unless already part of a regulatory assessment. *In silico* data (such as (Q)SAR predictions) were also not considered.

The data used were primarily data already evaluated from existing regulatory assessment reports. As the data in these documents have been assessed independently by the Member State Competent Authorities, they are assumed to be of high quality and relevant by default. This information was supplemented by information gathered from specific databases (or the references they provide) and from targeted scientific literature searches which focussed on endocrine effects and included non-regulatory studies.

It is important to emphasise that this screening methodology was not intended to result in a full assessment of the selected substances. Existing data on the EATS pathways may be scarce for many substances and the available test guidelines do not consider all relevant species, pathways, or timeframes of exposure. Moreover, within the time constraints of the project it was neither possible to assess in detail the quality of individual studies nor to carry out an in-depth weight of evidence assessment across all available data for each substance. Due to these limitations, this screening methodology is neither equivalent to nor intended to replace an in-depth assessment process as usually carried out for regulatory purposes. Hence, the outcome of the screening does not pre-empt in any way the formal regulatory conclusions that may eventually be made under different pieces of EU legislation.

[4] OECD, *Guidance Document on standardised test guidelines for evaluating chemicals for endocrine disruption, Series on Testing and Assessment No. 150,* 2012.

The screening methodology described in this report was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The results obtained by applying the screening methodology, published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these two Regulations.

1 Background

Several pieces of EU legislation that regulate the marketing and use of substances contain specific provisions for substances that can (potentially) cause adverse effects by disrupting the endocrine system, so called endocrine disruptors (EDs). The regulations on Plant Protection Products (PPPR)[5] and Biocidal Products (BPR)[6] further require the European Commission (EC) to establish scientific criteria to identify substances with endocrine disrupting properties. In addition the 7th Environment Action Programme[7] states that harmonised hazard-based criteria for the identification of EDs should be developed.

Endocrine disruption encompasses a range of mechanisms incorporating the many hormones secreted directly into the circulatory system by the glands of the endocrine system and their specific receptors, transport proteins and associated enzymes (Kortenkamp *et al*., 2011). With hormones orchestrating virtually all physiological processes, the identification of relevant (*in vivo*) endpoints that indicate a specific perturbation of endocrine processes is challenging. This makes it difficult to identify and hence regulate substances that can (potentially) cause adverse effects by disrupting the endocrine system, so called endocrine disruptors (EDs).

The EC carried out an Impact Assessment (IA) to evaluate the health, socio-economic and environmental impacts of various options for the criteria and their implementation in the legislation as described in an EC roadmap[8] (hereafter, the Roadmap). The IA has been supported by two studies, focusing on (1) selection of substances and screening of their potential to be identified as EDs according to the various options for criteria and (2) assessing the potential impacts on health, environment, trade, agriculture, and socio-economy based on the different criteria and policy options in the Roadmap.

The Roadmap outlines four policy options for identifying EDs. These options include the application of so-called interim criteria as specified in the BPR and the PPPR, as well as three options based on the International Programme on Chemical Safety/World Health Organisation (IPCS/WHO, 2002) definition (with varying degrees of strength of evidence or additional inclusion of elements of hazard characterisation).

To guide the screening of the substances, the methodology described in this report was developed by the Directorate General (DG) JRC under the terms of an Administrative Arrangement with DG SANTE. The methodology draws on the JRC Report of the Endocrine Disruptors Expert Advisory Group (JRC, 2013), which specifies the key scientific issues relevant to the identification and characterisation of endocrine disrupting substances. The method was consulted with DGs SANTE, ENV, GROW, SG, and the European Food Safety Authority (EFSA) and with the European Chemicals Agency (ECHA). The methodology has been applied by an external contractor to DG SANTE to all approved pesticide and biocide active ingredients (with a few exceptions as listed in Appendix A) and to a selection of substances falling under Registration, Evaluation,

[5] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market

[6] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products

[7] Decision No 1386/2013/EU of the European Parliament and of the Council of 20 November 2013
on a General Union Environment Action Programme to 2020 "Living well, within the limits of our planet"

[8] European Commission (2014) Roadmap: Defining criteria for identifying Endocrine Disruptors in the context of the implementation of the Plant Protection Product Regulation and Biocidal Products Regulation

Authorisation and Restriction of Chemicals (REACH) Regulation[9], the Cosmetic Products Regulation (CPR)[10] and the Water Framework Directive (WFD)[11]. The selection of the substances to be screened was made by the DGs SANTE, ENV and GROW and the criteria used for the selection are reported in Appendix A. The screening results have served as input to assessment and comparison of the impacts of the different policy options on substances falling under the PPPR and the BPR.

A draft methodology was supplied to the contractor at the beginning of the study. The methodology was further modified, in consultation with the contractor, during the course of the study, particularly with respect to the development of the data summary template and on the application of weight of evidence (WoE) analysis considerations. The development of this methodology has been an iterative process balancing the need for a sound scientific strategy against the need to screen many substances in a limited amount of time. The assessment of whether a substance has endocrine disrupting properties in humans or wildlife populations was based only on existing data. For the purposes of this screening methodology, the endocrine relevant effects were limited to effects on the estrogen, androgen, thyroid and steroidogenesis pathways.

As the screening was conducted in the context of an impact assessment, the results do not substitute evaluations of individual substances to be carried out under the respective legislations. The screening methodology is neither equivalent to nor intended to replace the usual in-depth assessment process carried out for regulatory purposes. The results obtained are not intended to pre-empt and they do not pre-empt the formal regulatory conclusions that may eventually be made under different pieces of EU legislation.

[9] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

[10] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products

[11] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

2 Scope

The scope of this document is to describe the screening methodology used in the context of an IA to collect and assess the available evidence regarding endocrine disrupting effects of selected substances. The aim was to assess in a limited amount of time the potential endocrine disrupting properties, based solely on already existing data, of approximately 600 selected substances that are subject to one or more of the following EU regulations/directives: PPPR, BPR, REACH, CPR and the WFD. For all selected substances, based on the evidence collected, it was determined whether they would be categorised as ED under four different policy options that are set out in the Roadmap (Appendix B).

The methodology comprises the following sequential steps:

1) Identification of data sources and data types to be collected from these sources
2) Collection and storage of data considered relevant to inform on potential endocrine disrupting properties of a substance
3) Analysis of data in order to categorise substances under the four policy options of the Roadmap.

Each step comprises a number of different components. A detailed explanation of each step is provided in the following sections. Figure 1 provides a schematic representation of the methodology.

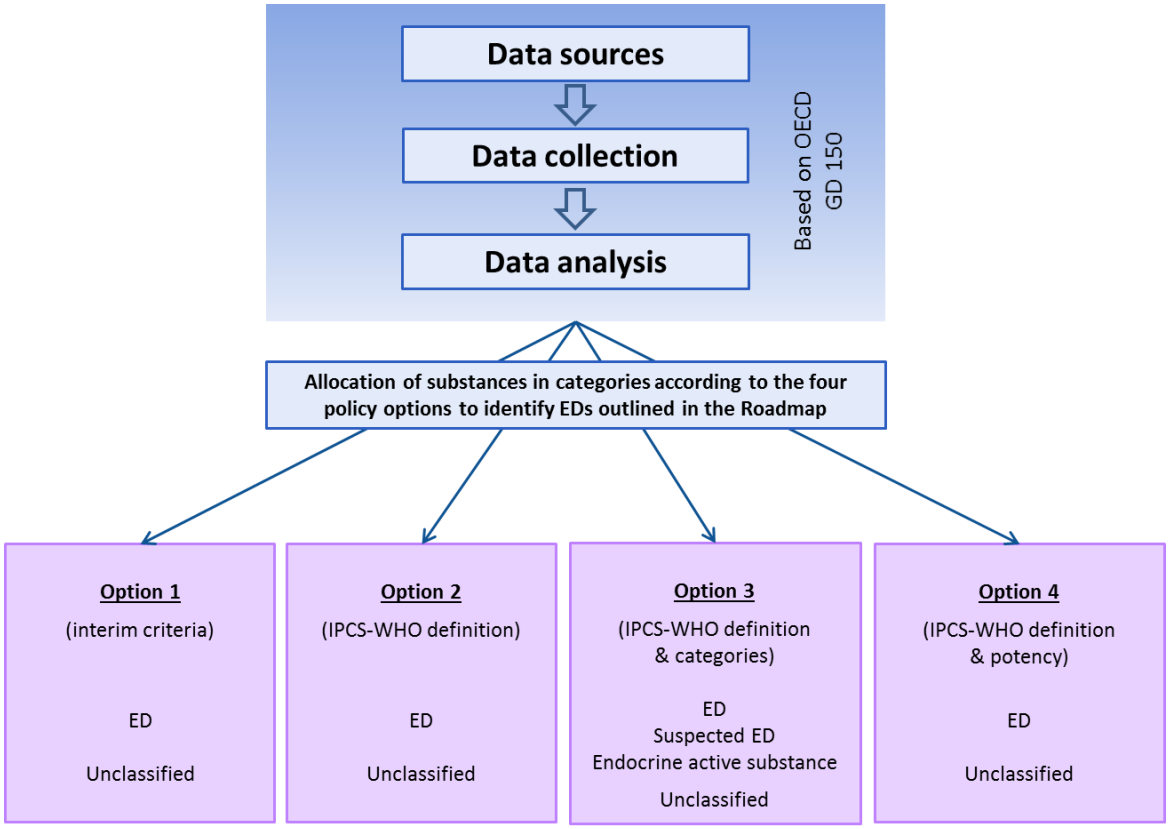


Figure 1. Schematic representation of the screening methodology to potentially identify which substances might be categorised as endocrine disruptors under four policy options.

The screening methodology described in this report was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The results obtained by applying the screening methodology, published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these two Regulations.

The development of this methodology has been an iterative process balancing the need for a sound scientific strategy against the need to screen many substances (about 600) in a limited amount of time (about 10 months). This required some pragmatic and practical decisions to accommodate the limited timeframe whilst still retaining to the extent possible scientific rigour, consistency and transparency.

Based on the policy options identified in the Roadmap[4] (Appendix B) the first step was to identify the relevant types of data required under each of the options and to identify the sources of these data. Three out of four of the options in the Roadmap (options 2, 3 and 4) are based on the IPCS/WHO definition (IPCS/WHO, 2002) of an endocrine disrupting substance. In option 3 the substances are allocated in one of three categories based on the different weight of evidence for fulfilling the IPCS/WHO definition.

These categories are the following:

- Endocrine Disruptor (Category I)(equivalent to ED under option 2)
- Suspected Endocrine Disruptor (Category II)
- Endocrine active substance (Category III)

Option 4 introduces, in addition, the concept of potency as an element of hazard characterisation.

The IPCS/WHO definition states that:

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

In analysing the elements of the definition, it is clear that evidence of alteration of the endocrine system, evidence of adverse effects and evidence that the one leads to the other are required to draw conclusions on the ED properties of a substance. Consequently, in the context of this methodology, there was a need to operationalise these terms by further defining them and identifying the sources and types of information that are most informative in providing evidence of their occurrence in relation to exposure to a specific substance.

The assessment of whether a substance has endocrine disrupting properties in humans or wildlife populations was based only on existing data and focuses solely on the active substances for biocides and pesticides and not on the formulations. Unless specifically stated otherwise, all mammalian toxicity data was regarded as being relevant for humans. In addition, mammalian effects, if population relevant, were also used to assess potential for endocrine disruption in wildlife populations. As the understanding regarding the disturbance of the endocrine system of invertebrate species is limited, the focus for wildlife effects was limited to the effects observed in mammals, fish, amphibians and to a limited extent in birds and reptiles.

The type of data which relates to how an adverse effect arises is described usually as either mechanistic or mode of action (MoA) data. Hence an "alteration of the endocrine system" leading to an adverse effect could be described as an adverse effect arising from an endocrine disrupting MoA or mechanism. The initial focus for test guideline (TG) development of mechanistic assays to understand endocrine disruption has been in relation to interference with the function of hormones (estrogen and androgen) related to sexual development and fertility as well as production of these hormones (steroidogenesis) and, to a certain extent, disruption of the action of thyroid hormones. Consequently, there is a relatively good mechanistic understanding on how perturbations of the Estrogenic, Androgenic, Thyroid and Steroidogenesis (EATS) pathways may lead to certain adverse effects.

Relevant TGs and standardised test methods have been incorporated into the Organisation for Economic Co-operation and Development's (OECD) Conceptual Framework (OECD, 2012a) for the testing and assessment of endocrine disrupters which organises the tests (including non-test information) in five levels according to the type of information that the tests provide. The OECD Guidance Document (GD) 150 (OECD, 2012b) focuses on interpretation of these (draft) TGs with respect to adverse effects which may be caused by disruption of EATS pathways as well as the available *in vivo* and *in vitro* mechanistic assays which inform on disruption of EATS pathways.

Consequently, for the purposes of this screening methodology, the endocrine relevant effects were limited to effects on the EATS pathways, as these are relatively well understood and consensus guidance on the interpretation of effects observed in OECD TGs is available from the OECD GD 150. Perturbations of other non-EATS pathways – although potentially relevant for ED - were largely beyond the scope of this methodology since data are limited and consensus guidance on their interpretation towards endocrine disrupting MoA is currently lacking. Human epidemiological data, whilst potentially informative, were not included unless already part of a regulatory assessment. *In silico* data (such as (Q)SAR predictions) were also not considered, due to lack of scientific consensus on how to select and apply such data.

It is important to emphasise that this screening methodology was not intended to result in a full assessment of the selected substances. Existing data on the EATS pathway may be scarce for many substances and the available TGs do not consider all relevant species, pathways, or timeframes of exposure. Moreover, within the time constraints of the project it was not possible to assess in detail the quality of individual studies nor to carry out an in depth WoE assessment across all available data for each substance. Due to these limitations, this screening methodology is neither equivalent to nor intended to replace an in-depth assessment process as usually carried out for regulatory purposes. Hence, the outcome of the screening does not pre-empt in any way the formal regulatory conclusions that may eventually be made under different pieces of EU legislation.

3 Information gathering: data sources, collection and organisation

In order to assess the evidence on the endocrine disrupting potential of a substance according to the four policy options in the Roadmap, different types of data were needed. This information was extracted from a variety of sources, including regulatory documents and scientific literature. After extraction, these data were codified in a structured way to support the data analysis in order to categorise each substance under the four policy options. Detailed information on the used data sources and data collection is provided in section 3.1 and section 3.2 respectively. A schematic representation of this process is given in Figure 2.

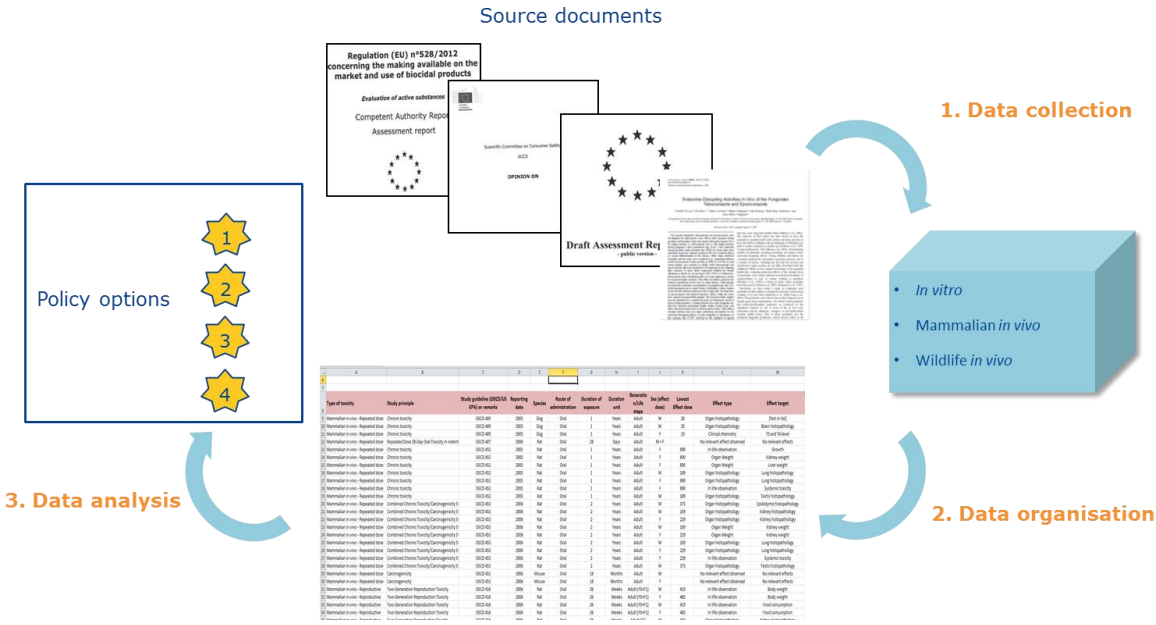


Figure 2. Schematic representation of the workflow from identification of data sources to data analysis.

Different types of information are required to categorise a substance into each of the four policy options (see Figure 3). For option 1, which is based on the interim criteria, information is needed regarding the hazard classification according to the Classification, Labelling and Packaging (CLP) Regulation and information on toxicity to endocrine organs. For options 2, 3, and 4, which are based on the IPCS/WHO definition (IPCS/WHO, 2002) of an endocrine disrupting substance, *in vivo* and/or *in vitro* mechanistic data (endocrine activity) along with data on adverse effects which may be endocrine-mediated (ED Adversity) are required. In addition, data on the presence or absence of general overt toxicity (non-ED Adversity) are needed, to be able to judge whether observed endocrine effects are possibly a non-specific secondary consequence of other toxic effects. A plausible link between the available mechanistic and adverse effect data was drawn on the basis of the consensus interpretation in OECD GD 150 (OECD, 2012b) regarding linkage of each adverse effect to one or more of the EATS pathways. In order to apply option 4, the doses/concentrations at which effects were first observed (LOAEL/LOEC) are required. In the following sections, a more detailed description of the data sources used is given.

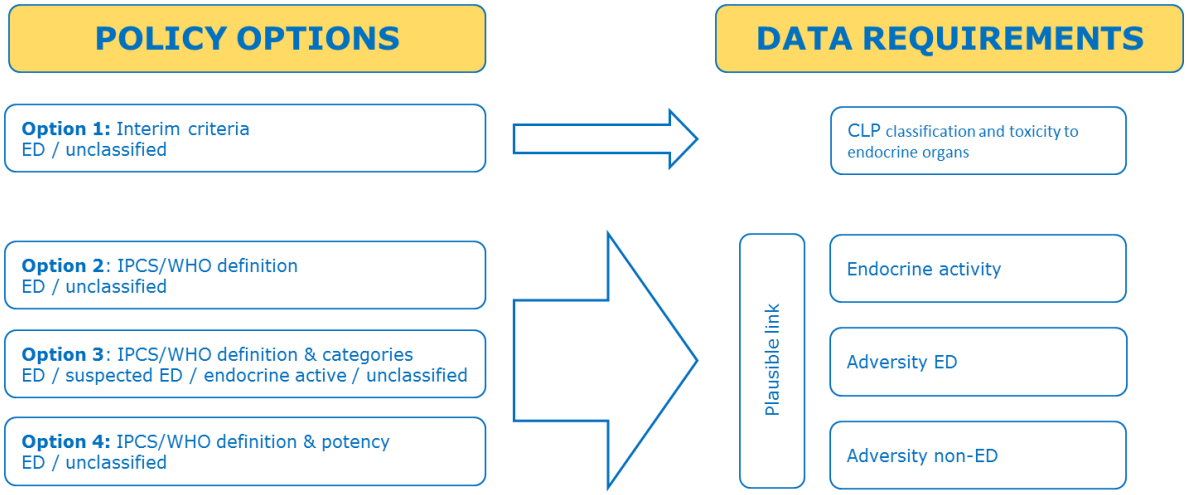


Figure 3. Types of data required to apply the four policy options in the Roadmap.

3.1 Data sources

For the categorisation of substances under the four policy options, the following data sources were used.

3.1.1 Source documents for policy options 1 to 4

3.1.1.1 EU Regulatory documents

All compounds subject to this IA fall under the scope of at least one specific EU Regulation/Directive (PPPR, BPR, REACH, CPR, WFD) and the relevant regulatory documents have been used as the primary source to extract the information relevant for this IA. As the data from these documents have been assessed independently (*e.g.* by the Member States Competent Authorities (MSCA), EU Regulatory Agencies, *etc.*), they were assumed to be of high quality and relevant by default and no additional quality checks were performed.

Depending under which EU Regulation a screened substance falls, the (eco)toxicological data, mostly obtained from laboratory animals (*in vivo*), were collected from the following regulatory assessment reports:

- Pesticides (PPPR)[12]
    - Member State Draft Assessment Report (DAR) or Renewal Assessment Report (RAR) (Evaluation by the Rapporteur Member State) and peer review (by all Member States) of information supplied by the applicant (including scientific literature when available).
    - EFSA conclusion (Peer review of the DAR/RAR by EFSA and all Member States).
    - For PPP substances for which the risk assessment had been performed by the Commission, the DAR is not publically available in the EFSA website and thus was retrieved from the confidential area of the European Commission's CIRCABC for PPPs. The Review Report, containing the final List of EndPoints, was downloaded from the EU Pesticide Database[13].

[12] http://dar.efsa.europa.eu/dar-web/provision
[13] http://ec.europa.eu/sanco_pesticides

The screening methodology described in this report was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The results obtained by applying the screening methodology, published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these two Regulations.

- Biocides (BPR)[14]
    - Member State Competent Authority Report (CAR) (Evaluation by the Rapporteur Member State and peer review (by all Member States) of information supplied by the applicant plus any other information considered relevant for the assessment)
    - ECHA Assessment Report (peer review of the CAR by ECHA and all Member States)
    - For BP substances for which the CAR was not available in the ECHA website this was retrieved from the confidential area of the European Commission's CIRCABC for BPs.

For substances that are both pesticides and biocides, all the above EU regulatory documents were used for data collection. For pesticides or biocides that are also REACH registered, the REACH registrant's submissions were also consulted. For substances that have been selected under the REACH criteria, the regulatory documents were used as specified below.

- REACH

Apart from the REACH substances already selected owing to their use as pesticides or biocides, the selection of further REACH substances was done according to a stepwise rationale based on 5 criteria (see Appendix A for more details). Therefore, depending under which criterion a REACH substance was selected, the following EU regulatory documents were used:

- *Criterion 1:*
    - Member State Committee opinions and support documents (available via the link to the candidate list)[15].
- *Criterion 2:*
    - Member State Committee opinions and support documents[16].
- *Criterion 3:*
    1. From the candidate list, the support document for identification of the substance as a substance of very high concern is used[17]. If no information was available, documents from the next step were used.
    2. Background Harmonised Classification and Labelling (CLH) dossier and/or the Committee for Risk Assessment (RAC) opinion on the CLH dossier[18]. If no information was available, documents from the next step were used.
    3. Risk Assessment Reports carried out and finalised under the Existing Substances Regulation (EEC 793/93)[19]. If no information was available, documents from the next step were used.
    4. Annex XV transitional reports for those substances where the work was started under the Regulation 793/93, but not finalised before REACH came into force[20].
    5. If no information was available from the above sources, the registrants' submissions were used.

[14] http://echa.europa.eu/information-on-chemicals/biocidal-active-substances
[15] http://echa.europa.eu/candidate-list-table
[16] http://echa.europa.eu/role-of-the-member-state-committee-in-the-authorisation-process/svhc-opinions-of-the-member-state-committee
[17] http://echa.europa.eu/candidate-list-table
[18] http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling
[19] http://echa.europa.eu/en/information-on-chemicals/information-from-existing-substances-regulation
[20] http://echa.europa.eu/information-on-chemicals/transitional-measures/annex-xv-transitional-reports

- *Criterion 4:*
  1. Final background document on a substance adopted by RAC as well as the opinion adopted by RAC (both documents are listing study overviews[21]. If the restrictions for substances in question were old ones and therefore no information was available, documents from the next step were used.
  2. Risk Assessment Reports carried out and finalised under the Existing Substance Regulation (EEC 793/93)[22]. If no information was available, documents from the next step were used.
  3. Annex XV transitional reports (for those substances where the work was started under the Regulation 793/93, but not finalised before REACH came into force)[23].
- *Criterion 5:*
  1. From the Community Rolling Action Plan (CoRAP) list[24], the following documents were used:
  2. In case the process to evaluate substance under CoRAP had been concluded, then the available dossier was used.
  3. In case the process had not been concluded (most cases), the documents/presentations/factsheets prepared by the MSCA and presented to ECHA Endocrine Disruptor Expert Group (ED EG) were used if available and not containing potentially confidential information.
  4. In case no MSCA document was available the registrants' submissions were used.
- Cosmetics (CPR)
  - Opinions of Scientific Committee on Consumer Safety, SCCS[25] were used.
- Water Framework Directive (WFD)
  - No additional sources were used for the data collection of substances regulated under the WFD since these substances were already subject to one of the other legislative frameworks (PPPR, BPR, REACH or CPR) and the regulatory documents available from these other regulatory frameworks listed above were used.

In specific cases documents (peer reviewed Opinions) identified in the EFSA website have been used (*e.g.* for substances which are also considered food contaminants).

3.1.1.2 EU Additional sources

In addition, data were collected from the following sources, focusing on endocrine effects (particularly as a source of mechanistic data) including non-regulatory studies:

- ToxCast: US Environmental Protection Agency (EPA) database: Selection of ED relevant *in vitro* assay data related to estrogen, androgen- and thyroid-receptor and steroidogenesis (see Appendix C for more details).
- ToxCast ER (Estrogen Receptor) prediction model. Computational model that integrates results from 18 estrogen receptor ToxCast high-throughput screening assays, in order to discriminate bioactivity from assay-specific interference and cytotoxicity. The model predicted results of EDSP Tier 1 guideline and other uterotrophic studies with 84% to 100% accuracy (Browne et al., 2015). Prediction values of individual substances, where available, were

[21] http://echa.europa.eu/previous-consultations-on-restriction-proposals
[22] http://echa.europa.eu/en/information-on-chemicals/information-from-existing-substances-regulation
[23] http://echa.europa.eu/information-on-chemicals/transitional-measures/annex-xv-transitional-reports
[24] http://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table
[25] http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm)

kindly provided by the US EPA. In case the result of the ToxCast ER prediction model was not available for a certain substance, then the individual ToxCast *in vitro* assays related to the estrogen receptor were used as indicated in the previous bullet point (see Appendix C for more details).

- Endocrine Disruptor Screening Program (EDSP) (US EPA) WoE analyses (summarised data) of ED relevant *in vitro* and *in vivo* assays, focusing on EATS pathways. The analysis is based on data from the current registration documents, scientific literature and additional ED relevant tests that were performed specifically for the EDSP.

When substances were present in any of the following sources, the mentioned references were used for data collection:

- JRC's Endocrine Active Substances Information System (EASIS)
- The Endocrine Disruption Exchange (TEDX)
- Substitute It Now (SIN): List of substances identified by the non-governmental organisation ChemSec as Substances of Very High Concern, specifically substances listed because of ED concerns, including their evaluation by the Danish Centre on Endocrine Disruptors (Danish Centre on Endocrine Disruptors, 2012).
- Public consultation (JRC, 2015): References supplied by the public consultation that were considered.

An inventory of screened substances was compiled to indicate where substances are listed (potentially in more than one source from those listed above) in the EU Regulatory documents and additional sources. All relevant sources were used for data collection (as shown in figure 4).

Chemical Name	CAS	Pesticides	Biocides	REACH	Cosmetics	WFD	EASIS	SIN	Tedx	ToxCast	EDSP
Chemical 1	1111111	1	1				1		1	1	1
Chemical 2	2222222	1	1				1		1	1	1
Chemical 3	3333333	1	1				1		1		
Chemical 4	4444444	1	1				1		1	1	1
Chemical 5	5555555	1	1				1		1	1	
Chemical 6	6666666	1	1				1		1	1	
Chemical 7	7777777	1	1				1		1	1	
Chemical 8	8888888	1	1				1		1	1	
Chemical 9	9999999	1		1			1		1	1	

Figure 4. Example of inventory of substances screened with indication of the available data-sources for each substance.

3.1.1.3 Scientific literature

In the case of substances not appearing in any of the above-mentioned 'additional sources' a search of the open literature (limited to publications in English) was performed by using both the following search-engine tools:

- SCOPUS: querying compound name & endocrine
- SciFinder: querying "endocrine disruption" & substance identifier based on CAS RN

All references were further screened for relevance, *e.g.* removing invertebrate studies which are not within the scope of this screening methodology. Only studies that have been performed using single substances (or the active ingredient in the case of PPPs and BPs) were considered: formulations were beyond the scope of this screening exercise. In the case of more than 50 references per substance a pragmatic approach was applied to

identify the most pertinent focussing on the most recent (e.g. published after most recent EU regulatory assessment). Considering time constraints, literature searching was only performed on pesticides and biocides.

All data obtained from the additional sources and literature searching were considered to be reliable by default, unless there were clear indications to the contrary. Thus, no additional systematic quality check was performed on these data. Data from these databases and the published scientific literature gathered in the targeted searches are considered valuable because they are specifically designed to investigate whether a substance has activity towards the endocrine system (EATS pathways).

3.1.2 Additional source documents for policy option 1

The categorisation of a substance as ED under Option 1 is based on the interim criteria set out in the PPPR (article 3.6.5) and BPR (article 5[3]):

- substances that are or have to be classified as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.
- substances such as those that are or have to be classified as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.

Therefore, the additional sources used to categorise substances under policy option 1 are EU regulatory documents where information on hazard classification is available. Particularly, both harmonised classification (when available) and the proposed classification (when relevant) were considered and the sources used were:

- For the harmonised classification
    - classification as included in Annex VI of Regulation (EC) 1272/2008 (CLP Regulation) [26], which is available in the C&L inventory of the ECHA website[27];
- For the proposed classification (when the proposal was more recent than the decision for the harmonised C&L or no harmonised classification was available)
    - classification proposal concluded during the peer review process under PPPR (EFSA Conclusion or DAR/RAR) and/or under BPR (ECHA Assessment Report/CAR).
    - Background Harmonised Classification and Labelling (CLH) dossier and/or the Committee for Risk Assessment (RAC) opinion on the CLH dossier[28] when the proposal has not been yet adopted.
    - Classification proposed in the regulatory documents (when available) for miscellaneous chemicals (*e.g.* Member State Committee Opinions).
    - REACH Registrant's proposal where relevant.

For option 1, together with CLP classification, also information on the toxicity to endocrine organs is required which was obtained from the data sources described in Section 3.1.1.

[26] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures

[27] http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

[28] (http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling

3.2 Data collection

In order to categorise all substances under the four policy options, different types of data were collected to then capture all relevant information needed to support the identification of EDs. Four main categories of data were collected as follows:

1. **General substance information**: chemical name; CAS Registry Number; current CLP classification; specific remarks in the regulatory source documents relevant to ED assessment.
2. **Study information**: type of toxicity test (*in vitro, in vivo*, mammalian, fish, amphibians, and to a limited extent birds and reptiles); the study principle, including the protocol used (*e.g.* OECD or US EPA TGs and deviations from these guidelines; non-GLP study); the source of the data (*e.g.* EU regulatory document, scientific literature, ToxCast, EASIS, SIN, *etc.*), including the primary reference given within this source and the reporting date.
3. **Study details**: test species and strain (for *in vitro* assays, the test system used as cell line, receptor, *etc*.); number of animals per group: doses administered; route and method of administration; duration of exposure; the purity of the substance.
4. **Effect details**: sex, generation and/or life stage for which the effect was observed; the lowest dose/concentration at which the specific effect was observed (LOAEL/LOEC), including the direction of the effect (increase, decrease) and optional additional details to further specify the observation. In the case of *in vitro* studies, the lowest effect concentration is generally not reported, so EC50/AC50/IC50 values derived from the concentration-response relationships were captured instead.

From the data sources listed in section 3.1.1, the relevant effects were collected from non-acute toxicity *in vivo* studies, especially from studies on developmental toxicity, reproductive toxicity, carcinogenicity and (sub)acute and (sub)chronic repeated dose toxicity. The selection of the effects was primarily based on the OECD GD 150 (OECD, 2012b), as this document represents a consensus interpretation of the evaluation of effects that indicate endocrine pathway interference. OECD GD 150 provides a list of effects (*in vitro* and *in vivo*) which are related specifically to the EATS pathways. Therefore, for this screening methodology, all potentially ED-mediated effects from the studies listed in OECD GD 150 were captured from the toxicological study reports. Similarly, effects from mechanistic *in vivo* and *in vitro* tests, either from the regulatory documents or from additional sources (*e.g.* ToxCast, EASIS, SIN, TEDX, scientific literature), were captured as well (more details regarding the selection of tests can be found in Appendix C).

In addition to the EATS-specific effects, some other effects were captured that are not directly linked to endocrine disruption, *e.g.* effects considered to be secondary to general toxicity (such as decreased body weight and food consumption, *etc.*). These endpoints are important for the interpretation of the specificity of the potentially endocrine-mediated effects, especially if they are occurring at the same dose as (or lower than) the EATS-specific effects. A complete list of endpoints is included in Appendix D.

3.3 Data organisation

All collected data were captured in a template (Excel file), which was specifically developed by DG JRC for this screening methodology. Table 1 provides a detailed description of the columns of the template.

Table 1. Description of the columns of the template.

Column name	Type of column	Column description
Type of toxicity	pick list	Type of toxicity study (*in vitro*, mammalian *in vivo* or wildlife *in vivo*)
Study principle	pick list	The type of protocol used for the toxicity study (*e.g.* two-generation study, 90-days repeated dose, *etc*.)
Study ID Matrix	free text	Number to identify study for further data-analysis within this methodology
Study reference ID	free text	Identification code related to the source used to gather the data filled in the template
Study guideline (OECD/US EPA) or remarks	free text	The guideline used for the study design, if given (*e.g*. OECD 416). Also other remarks regarding the guideline can be given here
Source	free text	Source used for the toxicity data (*e.g*. EFSA DAR, ECHA database, EASIS, ToxCast, *etc*.)
Reference (citation)	free text	Reference given within the source (*e.g*. the study unique identifier, or scientific paper)
Reporting date	free text	Reporting date, if available, of when the study was performed. If the reference is a scientific paper, this can be the date of publishing
Species	pick list	Species used for the toxicity study. For *in vitro* test systems, this field can be used to specify the cell system model
Strain or *in vitro* model	free text	The specific strain used for the toxicity study, when applicable.
Animals/sex/group	free text	Number of animals used for each dose group
Sex (administration)	pick list	Sex of the animals administered in a particular study
Purity (%)	free text	Purity of the substance (% of active ingredient) administered
Route of administration	pick list	The route of exposure that is used for exposing the animals (oral, inhalation, dermal, direct or other). For *in vitro* test systems, the exposure is normally from the cell medium
Method of administration	pick list	The method that is used to expose the animal to the test compound (*e.g*. feed, gavage, whole-body, capsule, water, topical, subcutaneous, intravenous or other)
Doses tested	free text	The range of the doses applied within the test or a listing of all the individual doses (*e.g*. 1, 3, 5, 10), excluding the 0 or control concentration
Lowest dose tested	free text	The lowest dose used within the test (excluding the 0 exposure or control)

Table 1 (continued). Description of the columns of the template.

Column name	Type of column	Column description
Highest dose tested	free text	The highest dose used within the test
Dose unit	pick list	The unit for the dose applied in the test
Duration of exposure	free text	Duration of exposure
Duration unit	pick list	The unit for the duration of exposure
Generation/Life stage	pick list	The generation or life stage for which the reported effect is given (*e.g.* Adult (P1), Foetus, Offspring F1, Offspring F2, Embryo, Egg, Larval, *etc.*)
Sex (effect dose)	pick list	The sex for which the observed effect is reported
Lowest Effect dose	free text	The actual dose at which the effect is observed. In case for each of the doses tested in the study there is a description of effect on a particular endpoint, capture only the effect observed at the lowest dose since it is assumed that at higher doses the effect is still present and possibly more severe. For *in vitro* assays (*e.g.* ToxCast) this refers to EC50
Effect type	pick list	Broad categories of effects defined by the JRC to better organise the different effects to be captured in this template. These broader categories are: In life observations; organ weight, organ histopathology, clinical chemistry, related to reproduction or development or abnormalities observed.
Effect target	pick list	The specific effect observed (specifying what exactly is targeted from the broader categories given above: *e.g.* which organ, which hormone *etc.*)
Effect classification	pick list	Each effect target is grouped in 5 groups: A) *in vitro* mechanistic, B) *in vivo* mechanistic, C) EATS specific adversity, D) Non-specific adversity (may or may not be indicative of EATS) and E) Adversity - General
Effect description	free text	A more detailed description of what is actually observed for a certain effect
Effect determination	free text	Field to state whether the effect determination (*e.g.* weight gain) is relative or absolute
Effect direction	pick list	Indicating whether the observed effect is increased, decreased, or not changed
NOAEL/NOEL/NOEC	free text	NOAEL/NOEL/NOEC values
LOAEL/LOEL/LOEC	free text	LOAEL/LOEL/LOEC values
Unit	pick list	The unit of NOAEL/NOEL/NOEC/LOAEL/LOEL/LOEC values
Additional remarks	free text	Any relevant remark to assist with data interpretation

4 Data analysis

The data collected and organised in the template were then analysed in order to categorise all substances screened under the four policy options.

Since it was anticipated that the data set might be too limited to allow a conclusion to be drawn with respect to ED Cat I, II or III for many substances, an additional category, not defined in the Commission Roadmap, of "unclassified" was introduced to categorise those substances that either cannot be assessed because there is no data to inform on ED properties or where the available data indicate a lack of activity towards the endocrine system. The term "unclassified" is used rather than "not ED" considering the incomplete coverage of available assays and the possibility that new data generated in the future may indicate ED activity (*e.g.* from other non-EATS endocrine MoAs). The additional category "unclassified" has been introduced for all options.

The workflow to perform the data analysis was different for each option, since each requires different types of data to support the categorisation. Therefore, in the following sections, the data analysis is described separately depending on whether it was used to assess policy option 1 or options 2/3 or option 4.

4.1 Categorisation under option 1 (ínterim criteria)

As described in previous section 3.1.2, the categorisation of a substance under option 1 is first triggered by its classification according to the CLP Regulation, harmonised (when available) or proposed (when more recent), as toxic for reproduction Category 2. Then, if accompanied also with classification as carcinogen Category 2 no further information is required to categorise the substance. If the substance is only classified as toxic for reproduction Category 2 then there is the need to evaluate if the substance also causes toxicity to endocrine organs.

Figure 5 provides a schematic representation of the data-analysis process under option 1.

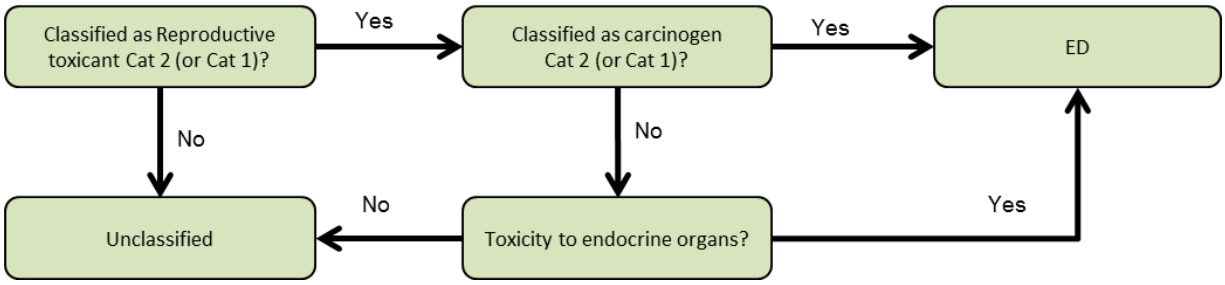


Figure 5. Decision tree leading to categorisation as ED according to the interim criteria as stated in the PPPR and the BPR.

As no definition is given in the interim criteria for which organs should be regarded as endocrine organs, for the purpose of this IA, the endocrine organs were considered to be the organs that secrete hormones as well as the target organs that express the receptors for the sex hormones and thyroid hormones and are included in the OECD GD 150 (mammary gland; accessory sex glands *e.g.* Cowper's gland, seminal vesicles, prostate gland, bulbourethral glands, glans penis; testis; epididymis; penis; cervix; uterus, endometrium; vagina; hypothalamus; pituitary; thyroid; adrenals; ovaries; placenta; levator ani/bulbocavernosus muscles (LABC)). This information is obtained from the toxicity data sources that are described in section 3.1.1.

According to the criteria set out in the CLP Regulation the classification of a substance as carcinogenic or toxic for reproduction is only relevant to humans. Therefore, option 1 is not applicable to vertebrate wildlife.

4.2 Categorisation under options 2 and 3

In this methodology, about 180 endpoints were selected for data collection covering all those listed in the OECD GD 150 and supplemented with some others, which are frequently reported in toxicological studies related to systemic toxicity (including body weight and food intake), that are informative only in the context of whether potentially ED-mediated effects are secondary to other non-ED modes of action (for more details refer to section 3.2 and Appendix D).

As also explained in the OECD GD 150, not all the effects provide the same type of information towards supporting if a substance causes an adverse effect through an EATS pathway. In fact, some effects are more specific towards EATS pathways while some others might provide less specific information which can be relevant only in combination with mechanistic data.

Additionally, among the about 180 endpoints considered in this methodology, some are related to *in vitro* studies and others to *in vivo* studies. In general, *in vitro* effects provide information on the mechanism through which a substance could potentially cause adversity (*e.g.* binding to a receptor). *In vivo* effects, instead, provide information which is related to the manifestation of adversity (*e.g.* damage to an organ). In some specific toxicological studies, *in vivo* endpoints can also provide mechanistic information (*e.g.* changes in plasma vitellogenin in fish).

Therefore the confidence in concluding that a substance is a potential ED increases with multiple types of information provided by mechanistic data, apical adverse effects data and ultimately by deriving a biologically plausible link between the two (as required to fulfil the IPCS/WHO definition).

In conclusion, the effects collected for a certain substance were evaluated in terms of the types of information they provide to support an assessment of ED properties. Figure 6 shows a schematic representation of the step-wise approach used for the data analysis. In the following sections, a more detailed description is provided regarding data processing and the application of the decision tree.

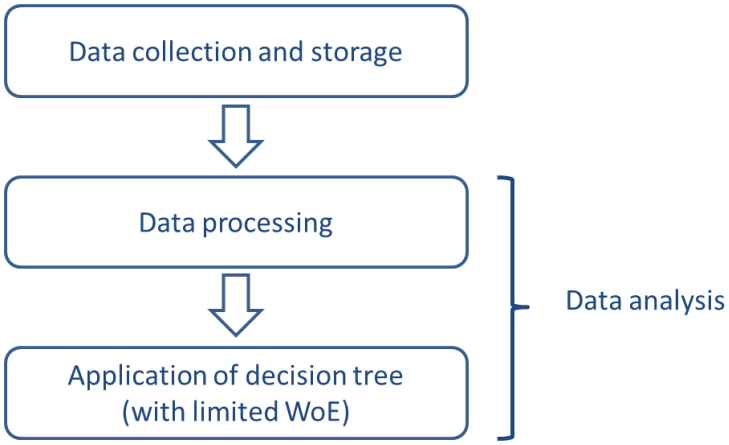


Figure 6. Stepwise approach used in the data analysis.

4.3 Data processing

The data processing is based on the grouping of effects (among the 180 endpoints selected for the data collection) according to the type of information they provide to support whether a substance causes adverse effects *via* an endocrine MoA. Five groups were defined in relation to two components: 1) level of specificity of adverse effects

towards EATS pathways based on the interpretation provided in OECD GD 150 and 2) mechanistic/mode of action information, which may be generated *in vitro* or *in vivo*. Figure 7 illustrates these 5 groups (A-E) with a brief description of each.

Group		Description of type of information
A	*In vitro* mechanistic	*In vitro* information on mechanism of action.
B	*In vivo* mechanistic	*In vivo* mechanistic assays designed to be informative on a specific mode of action (towards EATS) as well as hormone levels from *in vivo* assays. *In vivo* evidence is regarded as stronger evidence of an ED mode of action, compared to *in vitro* assays.
C	EATS specific adversity	*In vivo* endpoints considered to be specific of adversity caused through EATS pathways
D	Non-specific adversity (may or may not be indicative of EATS)	*In vivo* endpoints considered potentially sensitive to, but not specific of, adversity caused through EATS pathways
E	General adversity	*In vivo* endpoints related to systemic toxicity and not related to EATS pathways (*e.g.* mortality, body weight change, *etc.*)

Figure 7. Grouping of types of information for data processing.

A detailed description of each group is as follows:

A. *In vitro* mechanistic

This group captures all *in vitro* information relevant to EATS pathways collected from the selected sources. Examples of *in vitro* mechanistic information include measurements of receptor binding, inhibition of enzyme activity, cellular proliferation, or changes in steroid hormones levels in a cell system.

B. *In vivo* mechanistic (including *in vivo* hormone levels)

This group mainly refers to *in vivo* assays which are designed to provide mechanistic information (towards EATS pathways). For this methodology, as a default assumption, the evidence derived from these effects was regarded as stronger evidence of an ED MoA, compared to *in vitro* effects. The reasoning is that *in vivo* mechanistic effects, unlike *in vitro* methods, incorporates absorption, distribution, metabolism and excretion. In addition, effects observed *in vivo* are generally more "downstream" events along the pathway leading to ED adversity than *in vitro* endpoints. Therefore, they are more closely linked to the manifestation of the adversity. The following tests according to OECD GD 150 provide *in vivo* mechanistic information: Hershberger, uterotrophic, male and female pubertal assays, adult male assay, amphibian metamorphosis assay, and fish short-term reproduction assay. In addition, some of the other OECD TGs in levels 4 & 5 of the OECD Conceptual Framework (OECD, 2012a) provide information on both adverse effects and mechanistic information. For example, changes in vitellogenin levels in fish assays, as well as changes in *in vivo* hormone levels in mammalian assays can also be regarded as informative on the MoA, as they indicate perturbations of specific endocrine pathways. Fluctuations in hormone levels can be observed within certain limits without adverse consequences, so the changes cannot be considered adverse on their own. The point at which these fluctuations become significant cannot be generally defined

and would in an actual assessment always require a case-by-case decision which goes beyond the scope of this methodology. Therefore, for the purpose of this screening methodology, all significant changes in hormone levels were regarded as biomarkers informative of a specific MoA. Details on which hormones are considered are provided in Appendix D.

C. EATS specific adversity

The question of whether an observed effect can be considered adverse requires an assessment of data that goes beyond the screening methodology applied here. From a practical point of view, it is much easier and straightforward to conclude a change in *e.g.* morphology as directly adverse, without the requirement to assess in addition whether the change would actually lead to an impairment of function. Consequently, for the purpose of this screening, this group includes all those effects which are considered in OECD GD 150 indicative of E, A, T or S pathway. All these effects are then considered to be adverse effects. For more details refer to Appendix D.

D. Non-specific adversity (may or may not be indicative of EATS)

This group refers to the effects that are considered in the OECD GD 150 potentially indicative of, but not specific to, EATS pathways. Therefore they are considered informative only in combination with mechanistic-effects and/or EATS-specific effects (groups A to C) requiring a case-by-case decision to conclude if they can inform on ED properties.

E. General adversity

This group refers to *in vivo* effects which are only related to general systemic toxicity (*e.g.* changes in body weight, food consumption, signs of animal stress, mortality, *etc.*). Therefore these effects do not provide information on endocrine disrupting MoA. However, for the purpose of this screening methodology, they were collected and analysed to help with the interpretation of how specific the adverse effects in groups "C" and "D" are to inform on the likelihood of being endocrine-mediated.

In fact, effects (those grouped above under C and D) that are observed in presence of general adversity are considered to arise as a consequence of general toxicity (when the whole biological system is perturbed) and therefore, by default for this screening, were not considered specific or informative on endocrine disrupting MoA.

4.3.1 Application of a decision tree with limited WoE analysis

4.3.1.1 Decision tree

The next step of the data analysis was to evaluate the collected information organised in the five groups (A to E) in order to decide if a substance is a potential ED.

For groups A to D, it can be asked whether a substance causes any of the effects falling in that specific group. Group E (General adversity) is not associated with a question, since this group only serves to put the other effects into context (as explained in section 4.2.1).

Ultimately, a question was posed regarding the likelihood of a biologically plausible link between the adverse effects (groups C and D) and the mechanistic information (groups A and B). If so, both adverse effect(s) on the one hand and the mechanistic evidence on the other hand should correspond to a disruption of the same pathway, *i.e*. E, A, T or S.

Therefore a total of five questions were posed with each of these having two possible answers "yes" or "no" (Table 2).

Table 2. Questions of the decision tree developed for the data processing for options 2 and 3. The actual decision tree is given in figure 8.

Question	Answer
Is there evidence of adverse effect(s) that may or may not be EATS-specific in an intact organism, or its progeny, or in a (sub) population?	Yes / No
Is there evidence of adverse effect(s) – EATS specific in an intact organism, or its progeny, or in a (sub) population?	Yes / No
Is there evidence of *in vivo* mechanistic information?	Yes / No
Is there evidence of *in vitro* mechanistic information?	Yes / No
Is there evidence of a plausible link between *in vitro/in vivo* mechanistic information and the observed EATS-specific or non-specific adverse effect(s)?	Yes / No

Considering the different combinations of answers to the five questions, all the possibilities are captured in a decision tree (Figure 8) which allows for a potential categorisation, as Cat I, II or III. Substances for which not enough data are available to place the substance in any of the aforementioned categories are regarded as being unclassified.

In this decision tree, "EATS specific adversity" is considered as a strong indication of ED related adversity, leading to the "higher" potential categorisation of the substances as "ED (Cat I) or (Cat II)". In contrast, "Non-specific adversity (may or may not be indicative of EATS)" is considered as indication of ED related adversity, which may lead to "lower" potential categories, mostly Cat II, III and "Unclassified" in the absence of "EATS specific adversity".

In vivo mechanistic data (which may or may not be supported by *in vitro* mechanistic data) are considered as strong indication of endocrine MoA. In case *in vivo* mechanistic data are available and a plausible link is determined with either "EATS specific adversity" or "Non-specific adversity (may or may not be indicative of EATS)" the substance is classified as Cat I.

In vitro mechanistic data, in the absence of *in vivo* mechanistic data, are generally considered as weak indications of endocrine MoA. In this case, a substance could be categorised as Cat I, Cat II or Cat III depending on the type of adversity observed (EATS specific adversity or non-specific adversity) and on whether or not a plausible link to the observed adversity is established. The possibility that a substance is categorised as Cat I on the basis of *in vitro* mechanistic data is limited to the cases where there is clear and strong evidence of EATS specific adversity and a plausible link with equally clear and strong *in vitro* mechanistic data is established. However, in applying the decision tree, presented in figure 8, it was often difficult to establish a direct plausible link to adverse effects and thus a Cat II in case of presence of EATS specific effects or Cat III in presence of non-specific adverse effects was more likely.

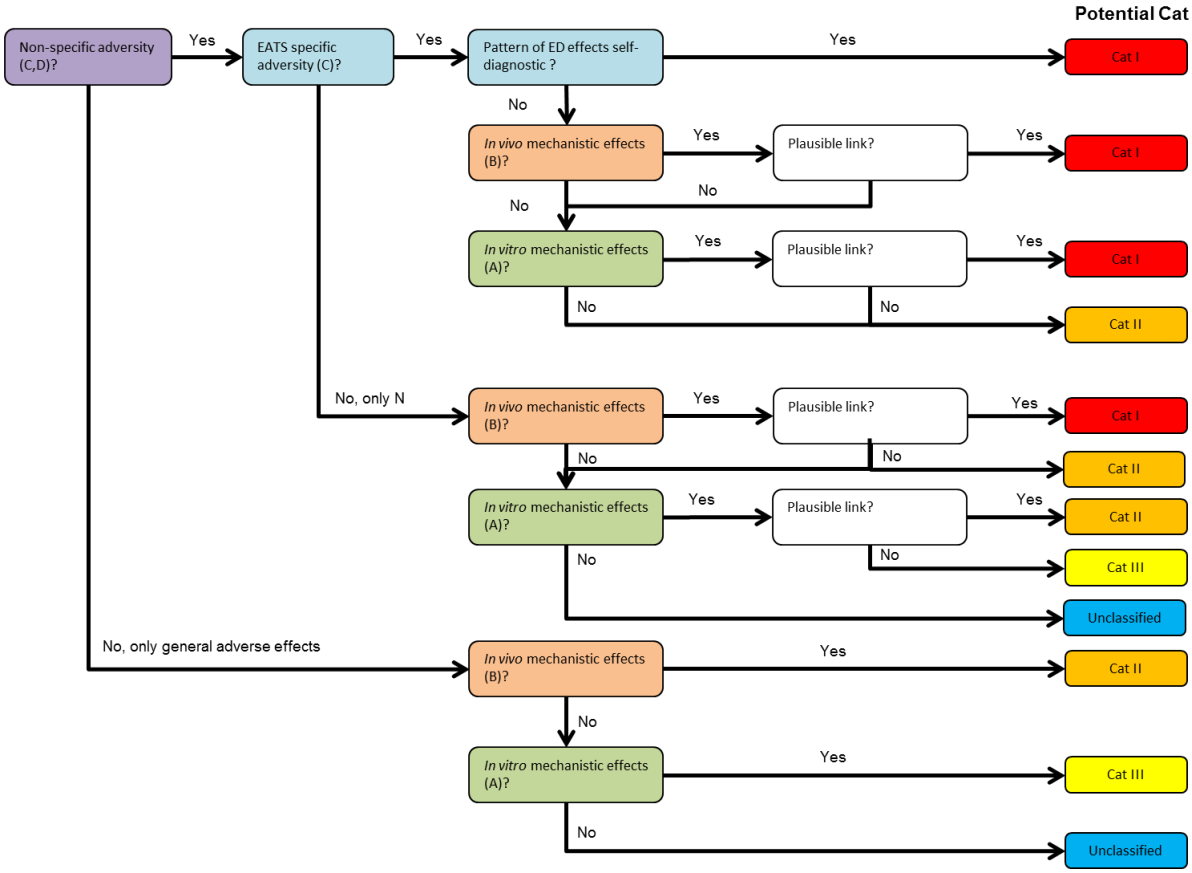


Figure 8. Decision tree leading to the different potential ED classifications according to options 2 and 3. Substances that are classified as ED Cat I are considered to be EDs under option 2.

In some cases, a substance can be classified as ED Cat I even if there is only information on adversity without mechanistic data available. In these cases, the adverse effects observed are considered also indicative of an endocrine disrupting MoA, through EATS pathways. Therefore, they are considered to be diagnostic for endocrine disruption. Two examples of such effects have been provided by the JRC Report of the Endocrine Disrupters Expert Advisory Group (ED EAG) (JRC, 2013):

- Example 1: "*In ecotoxicological assessment a change in sex ratio of fish was seen as both adverse and, according to the majority of the experts, highly likely to be a marker of endocrine disruption. An example was given of the OECD fish sexual development TG (OECD TG 234) in which consistent co-observation of a change in sex ratio accompanied by a change in vitellogenin level (biomarker of endocrine activity) has been observed in certain fish species (OECD, 2012b). For other fish species than those recommended in the TG the basis for using sex ratio as diagnostic of endocrine disruption was unclear. The degree of change in sex ratio would also be a factor in weighing the strength of evidence as a complete feminisation/masculinisation could be considered as diagnostic while only a small change or a delay in sexual differentiation might not.*"
- *Example 2: "In humans a pattern of effects known as testicular dysgenesis syndrome including hypospadias, cryptorchidism and decreased sperm quality which can also be replicated in laboratory mammals by certain chemicals (including hypo- and a-spermatogenesis, atrophy of the seminal vesicles and prostate, nipple retention, hypospadias, penis malformations, vaginal pouches, ectopic testes and decreased anogenital distance), was seen as highly likely to be mediated by an anti-androgenic mode of action.*"

4.3.1.2 Limited WoE analysis

Although the decision tree (figure 8) leads to only a "yes/no" qualitative answer to the 5 questions, the strength and WoE available was also considered.

In general, weight of evidence (WoE) analysis is an approach widely used to support decision-making in different scenarios in the field of chemical risk assessment. Several guidance documents exist under different regulatory programmes to describe the principles of WoE analysis and assist experts in its practical use to support specific purposes. However, while the WoE analysis can be a structured and systematic process that can rely on a set of clearly defined and prescriptive criteria, the final conclusion can still be considered subjective, as it is rather based on expert judgement and expert knowledge. Therefore, its practical implementation requires an *ad-hoc* definition of the criteria to be used when applying it within a specific context. Furthermore WoE analysis involves an extensive evaluation of all available evidence which is necessarily an extensive time demanding process.

As summarised in the 2013 JRC ED EAG Report (JRC, 2013), WoE analysis is described as weighing all available evidence, both positive and negative in order to reach a conclusion. Factors that are identified as important include quality, reliability and relevance of the individual studies as well as the consistency and reproducibility of reported effects, the pattern of effects across and within studies, number of species showing the same or similar effects, time of onset of effects and life stage affected, dose-concentration dependence and the biological plausibility of a causal relationship between the induced endocrine activity and the adverse effect(s). Concerning specifically study reliability, the report states that quality criteria are necessary to accept the validity of reported findings, referring to the approach described by Klimisch *et al.* (1997). The report goes on to propose that whilst recognising the value of studies conducted according to OECD TGs (or equivalent) non-guideline data (*e.g.* from academic laboratories) following good scientific principles in design, conduct and reporting and employing appropriate statistics, should be judged on their scientific merit and not automatically considered of lower quality to a TG conducted by a GLP accredited facility.

In the frame of this screening methodology a limited WoE analysis was carried out, while applying the decision-tree (figure 8), in order to find the right balance between a fast screening of substances (due to time constraints) and the need to evaluate all available information.

Studies considered reliable and scientifically sound by EU regulatory bodies within the consulted regulatory assessment reports were also considered reliable within the context of this screening methodology. Hence, the critical effect(s), target organ(s) and tissues(s) identified, the dose-response relationships and NOAEL(s)/NOEC(s) and LOAEL(s)/LOEC(s) for the critical effects were adopted for this screening. Considering time-constraints, the study quality of the scientific data available in the open literature, which had not been independently evaluated by a regulatory body, could only be assessed to a limited extent. Thus, all peer reviewed studies used for data collection were considered reliable by default. However, the results of poorly presented papers were discounted or given a lower weight of evidence.

In cases where, for the same effect, contrasting evidence was available from different studies on the same chemical (*e.g.* one study observing decreased organ weight and another study observing an increase), expert judgment was used to reach an overall conclusion.

Lastly, in case of conflicting evaluations of substance categorisation (*e.g.* different views between experts), a conservative worst-case approach was followed to decide on the categorisation of a substance. For this screening methodology, the worst-case approach was defined as placing the substance in the higher category (*e.g.* Cat I instead of Cat II)

A more detailed description of the criteria used for the limited WoE analysis applied in the context of this screening methodology, as developed with the contractor during the screening, is given below:

1. Effects that are considered to be secondary to general systemic toxicity rather than specific to EATS pathways, were not considered relevant to inform on ED and therefore were not used for substance categorisation.
2. Reproducibility of the effects was evaluated by observing if relevant effects (those in groups A to D from previous section 4.2.1) are observed across different studies, performed under similar experimental conditions.
3. When the same adverse effect (measured in both sub-chronic and chronic studies) was observed only in sub-chronic studies (*e.g.* 13-week) and not in any of the available chronic studies (*e.g.* 52-week or 104-week) conducted in the same species, using the same route of administration and relevant doses, this effect was disregarded or at least considered as weak evidence. In other words, these effects were not considered reproducible.
4. Consistency of the effects was assessed by evaluating if changes in the same effect were reported by different studies, if performed under similar experimental conditions, and followed the same direction (*e.g.* all studies observing increase of an organ weight).
5. *In vivo* mechanistic data were considered to provide stronger evidence than *in vitro* studies, in the identification of an endocrine-related MoA (see also section 4.2).
6. Plausible link was assessed by evaluating if information from mechanistic effects (group A and B) is biologically and mechanistically linked to the relevant adverse effect(s) (group C and D).
7. Where relevant, proposed Adverse Outcome Pathways (AOPs) in the OECD AOP Knowledge Base[29] were used to support a plausible link between the available mechanistic data and the adverse effects observed *e.g.*:
    - Androgen receptor agonism leading to reproductive dysfunction
    - Aromatase inhibition leading to reproductive dysfunction (in fish)
    - Estrogen receptor antagonism leading to reproductive dysfunction
    - PPARα activation in utero leading to impaired fertility in males
    - PPARγ activation leading to impaired fertility in adult females
    - Xenobiotic induced inhibition of thyroperoxidase and subsequent adverse neurodevelopmental outcomes in mammals
8. Histopathological findings in rat thyroid and increased thyroid weight in presence of liver histopathology (including liver enzyme induction) were attributed to a liver-mediated mechanism not considered to be ED-mediated. Since in the frame of this screening methodology enhancement of the metabolism and excretion of thyroid hormones by the liver was not considered as an endocrine MoA, such effects were not considered relevant to conclude on ED. The same reasoning was applied to effects on sex steroid hormones observed together with liver enzyme elevation.
9. The organ weight values were reported, if available, as both absolute and relative weights (organ-to-body-weight ratios). However, only absolute testis weight was used for the evaluation since testis weight, like brain weight, is normally conserved despite body weight loss (Holson *et al.*, 2011). Also, the evaluation was based on

[29] http://aopkb.org/

the principle that organ-to-body weight ratio is predictive for evaluating liver and thyroid gland weights, and organ-to-brain weight ratio is predictive for evaluating ovary and adrenal gland weights (Bailey *et al.*, 2004).

10. A rather restrictive approach was followed for the evaluation of adrenal effects. An increase of adrenal gland weight and hypertrophy of the adrenal cortex are often altered in response to subacute and chronic stress. In toxicity studies where there is an increase in adrenal gland weights, it is important to differentiate adrenal gland hypertrophy due to stress from degenerative changes of the adrenal cortex (cellular hypertrophy and vacuolation) due to disruption of steroidogenesis (Everds *et al.*, 2013). Therefore, particular emphasis was given to degenerative effects on adrenal gland, whilst changes in adrenal weights were disregarded or at least considered of low weight of evidence. Moreover, those effects observed in the absence of others from different endocrine organs or at high dose levels accompanied by generalised toxicity were in most cases disregarded in the evaluation.
11. For vertebrate wildlife evaluation, only the adverse effects that are considered to be population relevant were taken into account for the categorisation. Considering studies in mammals, these effects include (but are not limited to) the following: effects on reproductive organs (ovaries, testis, etc.), developmental effects (litter size, litter weight, sex ratio, teratological effects, etc.), reproductive effects (abortions, pre- and post-implantation losses, gestation length, embryo/fetal viability *etc.*), effects on survival, sexual maturity, *etc.*
12. Since it is scientifically accepted that the thyroid dysfunction can adversely affect reproduction and development, for the purpose of this screening methodology, thyroid effects in mammalian studies were considered to be population relevant only when they were accompanied by reproductive/developmental effects in the same species.
13. In case of substances showing reproductive and/or developmental adverse effects but not classified as "Repr. Cat. 2 or 1B or 1A" (see CLP Regulation), these effects were considered in most cases to be secondary to maternal toxicity and were therefore not used in the evaluation/categorisation procedure. However, in some cases severe adverse effects on pup/foetus (*e.g.* resorptions, malformations such as hydrocephaly, reduced pup/foetal viability or total litter loss) although observed at maternally toxic doses, might not be exclusively attributable to maternal toxicity and therefore would not necessarily be disregarded.
14. In case of substances showing reproductive and/or developmental adverse effects and classified as "Repr. Cat. 2 or 1B or 1A", effects were used even if observed in the presence of maternal toxicity since according to the criteria in the CLP Regulation, a substance is classified as toxic for reproduction, only when the reproductive/developmental adverse effects are considered not to be a secondary non-specific consequence of maternal/parental toxicity.
15. During the evaluation procedure, tumours in endocrine organs were considered as "EATS specific adversity" to be consistent with the consideration of histopathological findings in the same organs. This approach overrides the general approach of classifying tumours as "Non-specific adversity (may or may not be indicative of EATS)", by considering them as EATS specific when occurring in an endocrine organ.
16. In cases where the only relevant adverse and/or mechanistic effect/s was observed in a unique study in one species and there was no study of longer duration with the same species available (*e.g.* 2-year rat or 52-week dog study) or no other study of the same type of investigation (*e.g.* a unique multigenerational reproductive study

in rat), these effects were not disregarded, since in case of limited evidence a worst case approach was generally followed.

17. The weight of evidence approach applied for the observed adversity was also applied for the available *in vitro*/*in vivo* mechanistic data. If an effect was observed only in one mechanistic study, but it was the only mechanistic study available, then it was not disregarded in the framework of this screening methodology, where in case of limited evidence a worst case approach was generally followed. Where there were more than one *in vivo* or *in vitro* mechanistic studies reported but with different effect direction (*e.g.* increase/decrease), then the evidence was considered equivocal and not used in the evaluation process.
18. The potency of the available *in vitro* mechanistic data was taken into consideration for the evaluation/categorisation procedure and for the possibility to establish a plausible link between them and the adverse effects observed (*e.g.* in case the only in vitro mechanistic data available was a signal of low potency in an agonist assay, then this information was disregarded or at least considered as weak evidence for a plausible link).

It should be mentioned that when applying the decision tree for each substance (figure 9) under options 2 and 3, the WoE of the observed types of adversity and endocrine MoA was taken into account for each step followed. When the WoE of the observed effects was considered inadequate the path followed was similar to cases where no effects were observed.

4.4 Categorisation under option 4

Option 4 of the Roadmap applies the IPCS/WHO definition with inclusion of potency as an element of hazard characterisation. Potency depends not only on the endpoint but also, on the dose, on the duration and timing of exposure (EFSA, 2013).

Option 4 applies only to those substances that are categorised as ED under option 2 or ED Cat I under option 3. For categorising a substance under option 4, a trigger cut-off value was used. Although the application of a cut-off based on potency for endocrine disrupting substances is widely debated (Kortenkamp *et al.*, 2011; JRC, 2013), potency-based cut-off values were taken from the DE-UK joint position paper which proposed to use the Specific Target Organ Toxicity - Repeated Exposure (STOT-RE) Cat 1 trigger values (from CLP Regulation; ECHA, 2015) (see Table 3). The following decision tree was used to categorise substances under option 4 by using these defined cut-off values (Figure 9).

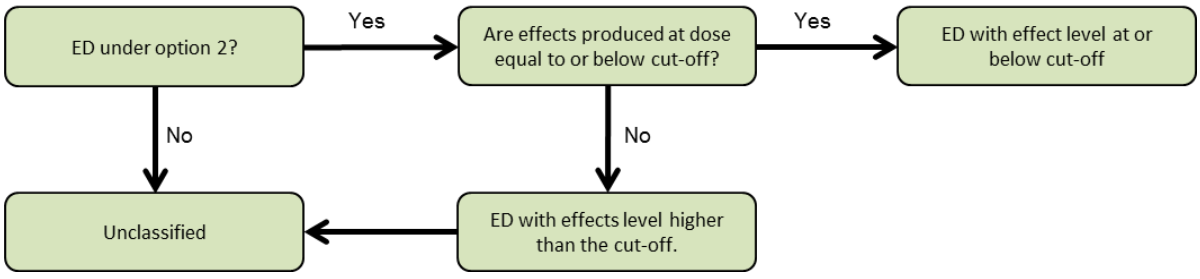


Figure 9. Decision tree leading to the different ED classifications according to option 4.

Table 3 shows the potency-based STOT-RE Cat 1 trigger values for different routes of exposure that were used as cut-off values in this screening methodology.

Table 3. Guidance values for STOT-RE Cat I, based on a 90-day rat toxicity study.

Route of exposure	STOT-RE Cat 1
Oral (rat)	10 mg/kg bw/day
Dermal (rat or rabbit)	20 mg/kg bw/day
Inhalation (rat) gas	50 ppmV/6h/day
Inhalation (rat) vapour	0.2 mg/l/6h/day
Inhalation (rat) (dust/mist/fume)	0.02 mg/l/6h/day

The STOT-RE Cat 1 trigger values presented in Table 3 refer to effects seen in a standard 90-day rat toxicity study. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of longer or shorter duration. In particular, dose/exposure time extrapolation can be conducted by using an approach similar to Haber's rule for inhalation. This rule states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure. This leads to: *e.g.* an increase by a factor of 3 of the guidance values reported in Table 3 for a 28-day study; or a decrease by a factor of 8 of the guidance values for a 2-year study. Based on the approach followed by the RAC, similar extrapolation factors for rat, mouse and dog studies were used[30].

Having used such extrapolations, substances categorised as potential ED under "Option 2" or Cat I under "Option 3" on the basis of mammalian data remain categorised as potential EDs for humans under "Option 4" if the effect used for the plausible link was observed at dose levels equal to or below the adjusted potency cut-off value. When the effect used for the plausible link was observed at dose levels above the adjusted potency cut-off value the substance was categorised as "unclassified".

For evaluation of vertebrate wildlife (ecotoxicological assessment), substances categorised as potential ED under "Option 2" or Cat I under "Option 3" primarily on the basis of non-mammalian data (avian, fish, amphibian), were also categorised as potential ED under "Option 4" by applying a virtual very high potency cut-off value to the non-mammalian data. If the categorisation under "Option 2" and "Option 3" were established on the basis of mammalian data only, then the same cut-off values as used in the human health evaluation were used under "Option 4" for vertebrate wildlife.

[30] RAC Opinion ECHA/RAC/CLH-O-0000002970-73-01/F, September 2012

The screening methodology described in this report was developed in the context of an impact assessment to evaluate the impacts associated with options for criteria to identify endocrine disruptors under the regulations on plant protection products and biocidal products. The results obtained by applying the screening methodology, published separately under a contract for DG SANTE (SANTE/2015/E3/SI2.706218), do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these two Regulations.

5 Summary and conclusions

This screening methodology was developed to assess, in a limited amount of time, the potential endocrine disrupting properties of approximately 600 substances. The substances were selected from the total lists of substances subject to different pieces of EU legislation related to management of risks to human health and environment, including the PPPR, BPR, REACH, CPR and substances subject to the WFD. In developing this screening methodology, it was foreseen that the results for pesticide and biocidal active substances would serve as an input to the assessment and comparison of the impacts of the different policy options on substances falling under the PPPR and the BPR.

Bearing in mind the time constraints on the study, the methodology was designed to be feasible, scientifically robust and transparent, and to allow traceability of data and conclusions. It was necessary to limit the screening, as described above, to the MoA and adverse effects that are better understood, for which there exist relevant TGs, and for which guidance is available on the interpretation of relevance of observed effects to an endocrine disrupting MoA. In practice, this meant that the focus was on the Estrogenic, Androgenic, Steroidogenesis and Thyroid (EATS) pathways of the endocrine system. The OECD GD 150 was used as basis for selection of endpoints, for interpretation of test/assay results and for supporting the establishment of a possible link between the mode(s) of action and the adverse effect(s).

Every effort was made to codify the data collection and evaluation process, and document all assumptions made, while recognising that any chemical assessment inevitably involves a degree of expert judgement that cannot be codified. As a consequence of the constraints of this study, which was designed to support an IA carried out in a limited amount of time, the screening methodology is neither equivalent to nor intended to replace an in-depth assessment process, and the results obtained are not intended to pre-empt in any way the formal regulatory conclusions that may eventually be made under different pieces of EU legislation.

References

Bailey, SA, Zidell, RH, Perry, RW. "Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?". *Toxicologic Pathology* 32 (2004); 448–466.

Browne, P, Judson, RS, Casey, WM, Kleinstreuer, NC, Thomas, RS. "Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model". *Environmental Science & Technology* , vol. 49 (2015); 8804−8814.

Danish Centre on Endocrine Disruptors. *Evaluation of 22 SIN List 2.0 substances according to the Danish proposal on criteria for endocrine disruptors.* 2012.

ECHA, "Guidance on the Application of the CLP Criteria", version 4.1, June 2015.

EFSA Scientific Committee. *Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment*. EFSA Journal 2013;11(3):3132; 2013. doi: 10.2903/j.efsa.2013.3132.

Everds, NE, Snyder, PW, Bailey, KL, Bolon, B, Creasy, DM, Foley, GL, Rosol, TJ, Sellers, T. "Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment"-. *Toxicologic Pathology* 41(4) (2013);560-614.

Holson, JF, Nemec, MD, Stump, DG, Kaufman, LE, Lindström, P, Varsho, BJ. *Significance, Reliability, and Interpretation of Developmental and Reproductive Toxicity Study Findings.* Chapter 9 In: *Developmental and Reproductive Toxicology*. Ed. Hood R.D., Taylor & Francis (2nd Edition), 2011.

IPCS/WHO, *Global assessment of the state-of-the-science of endocrine disruptors*. Geneva: World Health Organisation; 2002.

JRC. *Public consultation on defining criteria for identifying endocrine disruptors in the context of the implementation of the Plant Protection Products regulation and the Biocidal Products regulation. Analysis of responses relevant for a toxicological impact assessment.* Technical report; 2015.

JRC. *Key scientific issues relevant to the identification and Characterisation of endocrine disrupting substances-Report of the Endocrine Disrupters Expert Advisory Group.* Scientific and Policy Reports; 2013.

Klimisch, HJ, Andreae, M, Tillmann, U. "A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data", *Regulatory Toxicology and Pharmacology* 25(1) (1997);1–5.

Kortenkamp, A, Martin, O, Faust, M, Evans, R, McKinlay, R, Orton, F, Rosivatz, E. State of the Art Assessment of Endocrine Disrupters - Final Report; 2011.

OECD.(2012a) Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012); 2012. http://www.oecd.org/env/ehs/testing/OECD%20Conceptual%20Framework%20for%20Testing%20and%20Assessment%20of%20Endocrine%20Disrupters%20for%20the%20public%20website.pdf

OECD (2012b), *Guidance Document on standardised test guidelines for evaluating chemicals for endocrine disruption, Series on Testing and Assessment No. 150,* 2012.

Rotroff, DM, Dix, DJ, Houck, KA, Knudsen, TB, Martin, MT, McLaurin, KW, Reif, DM, Crofton, KM, Singh, AV, Xia, M, Huang, R, Judson, RS. "Using in Vitro High Throughput Screening Assays to Identify Potential Endocrine-Disrupting Chemicals", *Environmental Health Perspectives* 121 (2013);7-14.

List of abbreviations and definitions

A	Androgenic pathway
AC50	Half maximal active concentration
AOP	Adverse Outcome Pathway
BPR	Biocidal Products Regulation
CAS RN	Chemical Abstract Service Registry Number
CAR	Competent Authority Report
CIRCABC	Communication and Information Resource Centre for Administrations, Businesses and Citizens
CLH	Harmonised Classification and Labelling
CLP	Classification, Labelling and Packaging
CPR	Cosmetic Products Regulation
CoRAP	Community Rolling Action Plan
DAR	Draft Assessment Report
DG	Directorate General
E	Estrogenic pathway
EASIS	Endocrine Active Substances Information System
EATS	Estrogen, Androgen, Thyroid and Steroidogenesis
ECHA	European Chemicals Agency
ECHA ED EG	European Chemicals Agency European Chemicals Agency Endocrine Disruptor Expert Group
EC50	Half maximal effective concentration
ED	Endocrine disruptor
EDSP	Endocrine Disruptor Screening Program
EFSA	European Food Safety Authority
EU	European Union
GD	Guidance Document
IA	Impact assessment
IC50	Half maximal inhibitory concentration
IPCS	International Programme on Chemical Safety
JRC	Joint Research Centre
LABC	Levator ani/bulbocavernosus muscles
LOEC	Lowest Observed Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
MoA	Mode of action
MS	Member State
MSCA	Member State Competent Authority

NGO	Non-governmental organisation
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PPPR	Plant Protection Products Regulation
QSAR	Quantitative Structure-Activity Relationship
RAC	ECHA Risk Assessment Committee
RAR	Renewal Assessment Report
REACH	Registration, Evaluation, Authorisation and Restriction of CHemicals
S	Steroidogenesis pathway
SCCS	Scientific Committee on Consumer Safety
SIN	Substitute It Now
STOT-RE	Specific Target Organ Toxicity - Repeated Exposure
SVHC	Substance of Very High Concern
T	Thyroid pathway
TEDX	The Endocrine Disruptor eXchange
TG	Test Guideline
ToxCast	Toxicity Forecaster (Database of *in vitro* assay data from US EPA)
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organisation
WoE	Weight of Evidence

List of figures

List of tables

Appendix A. Criteria used for substance selection

Substances to be screened were selected according to the criteria described in the document "Selection of chemical substances to be screened in the context of the IA on criteria to identify ED" published on the DG SANTE website (http://ec.europa.eu/health/endocrine_disruptors/docs/impactassessment_chemicalsubstancesselection_en.pdf). The following rationale was used:

- **CHEMICAL SUBSTANCES REGULATED UNDER THE PLANT PROTECTION PRODUCTS REGULATION (PPPR) AND THE BIOCIDAL PRODUCTS REGULATION (BPR)**
  All relevant chemicals approved by 11 May 2015 at European level to be used in plant protection products and biocidal products were considered as a starting point. The screening was then focused by excluding those substances that are considered to be out of scope. The stepwise rationale followed for excluding active substances from the screening is:
  1) Microorganisms (living organisms, no chemical substances).
  2) Basic substances, defined in Article 23 of Regulation (EC) No 1107/2009 as being substances of no concern and no inherent capacity to cause endocrine disrupting effects, and where the approval procedures follow particular rules.
  3) Low risk substances, defined in Annex II to Regulation (EC) 1107/2009 as, among others properties, not deemed to be an endocrine disruptor.
  4) Natural extracts, mixtures, or repellents
  5) Attractants (pheromones) or plant hormones
  6) Others (*e.g.* inert substances, salts, acids)

Following this rationale, 324 substances falling under the PPPR and 95 substances falling under the BPR were selected. Among the 95 BPs there are also some chemicals not yet approved but where the corresponding opinions were already adopted by the Biocidal Products Committee of the European Chemical Agency (ECHA). 23 PPPs and 3 BPs were not selected following this rationale but appear on the list because they were substances screened during the earlier phase of the project.

- **CHEMICAL SUBSTANCES REGULATED UNDER THE REACH REGULATION**
  Substances were selected for the screening exercise according to the following stepwise rationale:
  1) All substances on the Candidate List already identified as Substances of Very High Concern (SVHC) because of ED concerns under Art. 57(f)
  2) All substances for which an SVHC opinion on the identification of the substance as SVHC due to its endocrine disrupting properties was provided by the ECHA Member State Committee ;
  3) All substances on the Candidate list identified as SVHC because of reproductive toxicity 1A/1B;
  4) All substances listed in Annex XVII for restrictions due to an ED concern or because of having a harmonised classification as toxic for reproduction 1A/1B;
  5) All substances placed on the community rolling action plan (CoRAP) due to ED concern;

  Following this procedure, 149 REACH chemical substances were selected. Furthermore, 52 substances registered under REACH also appear on the list of screened chemicals but were selected following the rationales applied for other legislative frameworks (i.e. they are either PPPs/BPs or substances used in cosmetic products) or because they were substances screened during the earlier phase of the project.

- **CHEMICAL SUBSTANCES REGULATED UNDER THE COSMETIC PRODUCTS REGULATION (CPR)** Substances used in cosmetic products were selected based on the following criteria:
    1) Substances for which an opinion of the Scientific Committee on Consumer Safety (SCCS) was provided, which contained a discussion but not necessarily a conclusion on their endocrine disrupting potential;
    2) Substances for which an SCCS opinion was provided due to the potential or de facto classification as carcinogenic, mutagenic, or toxic for reproduction (CMR)1A/1B or CMR2 under the Classification, Labelling and Packaging (CLP) Regulation;
    3) Substances not classified as CMR but for which SCCS expressed some concern on toxicity endpoints;
    4) Substances for which concern was raised by stakeholders / Member States on potential endocrine disrupting properties;

  Following this procedure, 45 chemical substances falling under the CPR were selected. A further 6 substances falling under the CPR also appear on the list of screened chemicals because they were selected following the rationales applied for other legislative frameworks (i.e. they are either PPPs /BPs or REACH substances.)

- **CHEMICAL SUBSTANCES REGULATED UNDER THE WATER FRAMEWORK DIRECTIVE (WFD)**

For the WFD, no specific selection criteria were applied to identify substances for the screening. However, some of the substances on the screening list, selected following the rationales applied for other legislative frameworks (i.e. PPPs/BPs, Cosmetics or REACH), are listed individually or fall under a group (*e.g.* lead and its compounds) in the list of priority substances under the WFD.

Appendix B. Policy options defined in the Commission Roadmap

The following text is copied from the Roadmap (http://ec.europa.eu/smart-regulation/impact/planned_ia/docs/2014_env_009_endocrine_disruptors_en.pdf).

Option 1: No policy change (baseline). No criteria are specified. The interim criteria set in the BPR and the PPPR could continue to apply.

Option 2: IPCS/WHO definition to identify endocrine disruptors (hazard identification).

Endocrine disruptors are identified as:

a) Substances which are:
    i) known or presumed to have caused endocrine-mediated adverse effects in humans or population-relevant endocrine-mediated adverse effects in animal species living in the environment or
    ii) where there is evidence from experimental studies (*in vivo*), possibly supported with other information (*e.g.* (Q)SAR, analogue and category approaches) to provide a strong presumption that the substance has the capacity to cause endocrine-mediated adverse effects in humans or population-relevant endocrine-mediated adverse effects on animal species living in the environment;
b) The experimental studies used to determine if a substance is an endocrine disruptor shall provide clear evidence of endocrine-mediated adverse effects in the absence of other toxic effects, or if occurring together with other toxic effects, the endocrine-mediated adverse effects should not be a non-specific secondary consequence of other toxic effects;
c) An adverse effects 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, as stated in (IPCS/WHO; 2002);
d) Where there is (*e.g.* mechanistic) information demonstrating that the effects are clearly not relevant for humans and not relevant at population level to animal species living in the environment, then the substance should not be considered an endocrine disruptor;
e) The identification shall follow a step by step procedure as follows:
    i) gather all available data;
    ii) assess the data quality, reliability, reproducibility and consistency;
    iii) consider adversity and MoA together in a weight of evidence approach based on expert judgement
    iv) evaluate whether endocrine disruption is due to a specific endocrine-mediated MoA and not to a non-specific secondary consequences of other toxic effects;
    v) evaluate human and wildlife relevance;
    vi) Final (eco)toxicological evaluation indicating, where possible, whether the adverse effect is in relation to human health or environment (vertebrates and/or invertebrate populations), and where possible which are the axes or mechanisms concerned (*e.g.* estrogenic, androgenic, thyroid and/or steroidogenic axes).

Option 3: IPCS/WHO definition to identify endocrine disruptors and introduction of additional categories based on the different strength of evidence for fulfilling the IPCS/WHO definition.

Category I: endocrine disruptors (as defined in 2a-2d)

Category II: suspected endocrine disruptors

a) Substances where there is some evidence for endocrine-mediated adverse effects from humans, animal species living in the environment or from experimental studies, but where the evidence is not sufficiently strong to place the substance in Category I. If, for example, limitations in the study (or studies) make the quality of evidence less convincing, Category II could be more appropriate.
b) Endocrine-mediated adverse effects should be observed in the absence of other toxic effects, or if occurring together with other toxic effects, the endocrine-mediated adverse effects should not be a non-specific secondary consequence of other toxic effects;
c) the points c) and d) for Category I remaining valid as well.

Category III: endocrine active substances

a) Substances for which there is some *in vitro* or *in vivo* evidence indicating a potential for endocrine disruption mediated adverse effects in intact organisms and where the evidence is not sufficiently convincing to place the substance in Category I or II.

The allocation to categories shall follow a step by step procedure as follows:

i) gather all available data;
ii) assess the data quality, reliability, reproducibility and consistency;
iii) consider adversity and MoA together in a weight of evidence approach based on expert judgement
iv) evaluate whether endocrine disruption is due to a specific endocrine-mediated MoA and not to a non-specific secondary consequences of other toxic effects;
v) evaluate human and wildlife relevance;
vi) final (eco)toxicological evaluation and decision on categorisation indicating, where possible, for Categories I and II whether the adverse effect is in relation to human health or environment (vertebrates and/or invertebrate populations), and where possible which are the axes or mechanisms concerned (*e.g.* oestrogenic, androgenic, thyroid and/or steroidogenic axes).

Option 4: IPCS/WHO definition to identify endocrine disruptors and inclusion of potency as element of hazard characterisation (hazard identification and characterisation).

Appendix C. Selection of assays for the screening methodology

The selection of tests and assays considered for this screening methodology. The selection was based on the OECD GD 150, with additional *in vitro* assays selected from the US EPA ToxCast program. Rotroff *et al*. (2013) was used as further guidance to assist with the selection of relevant assays. As explained in section 3.1.1.2 the ToxCast ER prediction model (Browne *et al.,* 2015) was used, when available, instead of all ToxCast individual assays related to the estrogen receptor.

Table C1. *In vitro* assays considered for the screening methodology, selected from the OECD GD 150.

		US EPA		OECD	
Assay	**Pathway**	**Guideline (OPPTS)**	**Tier**	**Guideline**	**CF level**
ER Binding Assay	Estrogen	890.1250	1	-	2
Estrogen receptor transactivation assay	Estrogen			TG 455 TG 457	2
AR Binding Assay	Androgen	890.1150	1	-	2
H295R Steroidogenesis Assay	Steroidogenesis	890.1550	1	TG 456	2
Aromatase Assay	Steroidogenesis	890.1200	1	-	2
MCF-7 proliferation assays	Estrogen				

Non-standard or guideline *in vitro* methods with comparable endpoints published in scientific literature were also included, *e.g.* specific reporter gene assays, proliferation assays and binding assays.

Table C2. Additional *in vitro* assays selected from ToxCast[31].

Assay	Pathway
ACEA_T47D_80h	Estrogen receptor
ATG_ERa_TRANS	Estrogen receptor
ATG_ERE_CIS	Estrogen receptor
ATG_ERRa_TRANS	Estrogen receptor
ATG_ERRg_TRANS	Estrogen receptor
NVS_NR_bER	Estrogen receptor
NVS_NR_hER	Estrogen receptor
NVS_NR_mERa	Estrogen receptor
OT_ERa_ERb_1440_agonist	Estrogen receptor
OT_ERaERa_1440_agonist	Estrogen receptor
OT_ERbERb_1440_agonist	Estrogen receptor
OT_ER_ERaERa_0480	Estrogen receptor
OT_ER_ERaERb_0480	Estrogen receptor
OT_ER_ERbERb_0480	Estrogen receptor
OT_ERa_ERE_LUC_Agonist_1440	Estrogen receptor
OT_ERa_ERE_LUC_Antagonist_1440	Estrogen receptor
OT_ERa_GFPERaERE_0120	Estrogen receptor
OT_ERa_GFPERaERE_0480	Estrogen receptor
OT_ERb_ERE_LUC_Antagonist_1440	Estrogen receptor
ATG_AR_TRANS	Androgen receptor
NVS_NR_hAR	Androgen receptor
NVS_NR_rAR	Androgen receptor
OT_AR_ARE_LUC_Agonist_1440	Androgen receptor
OT_AR_ARSRC1_0480	Androgen receptor
OT_AR_ARSRC1_0960	Androgen receptor
NVS_NR_hTRa	Thyroid receptor
NVS_ADME_hCYP19A1	Steroidogenesis

[31] ToxCast data from 2014.

Table C3. Mammalian standard methods considered for the screening methodology.

		US EPA		OECD	
Assay	**Species**	**Guideline (OPPTS)**	**Tier**	**Guideline**	**CF level**
Uterotrophic Bioassay in Rodents (UT Assay)	Rodents		1	TG 440	3
Hershberger Bioassay in Rats (H Assay)	Rodents		1	TG 441	3
Male pubertal assay (PP Male Assay)	Rodents	890.1500	1		4
Female pubertal assay (PP Female Assay)	Rodents	890.1450	1		4
Adult Male Assay (=15 Day Adult Male assay)	Rat				4
Repeated Dose 28-Day Oral Toxicity Study in Rodents	Rodents	870.3050	-	TG 407	4
One-Generation Reproduction Toxicity Study	Rodents		-	TG 415	4
Extended One-Generation Reproductive Toxicity Study	Rodents		-	TG 443	5
Repeated Dose 90-Day Oral Toxicity Study	Rat	870.3100	-	TG 408	4
Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	Dog	870.3150	-	TG 409	
Reproduction/developmental toxicity screening test	Rat	870.3550		TG 421	4
Combined 28-Day Reproductive Screening Tests	Rat			TG 422	4
Two-Generation Reproduction Toxicity Study	Rodents	870.3800	2	TG 416	5
Combined Chronic Toxicity/Carcinogenicity Studies	Rat	870.4300	-	TG 451-3	4

Non-standard methods with comparable endpoints (e.g. *in vivo* studies specifically performed to detect endocrine effects) published in scientific literature were also included.

Table C4. Wildlife standard methods considered for the screening methodology.

		US EPA		OECD	
Assay	**Species**	**Guideline (OPPTS)***	**EDSP Tier**	**Guideline**	**CF level**
Amphibian Metamorphosis Assay (AMA)	*Xenopus laevis*		1	TG 231	Level 3
Fish Short Term Reproduction Assay (FSTRA)	Fathead minnow, Japanese Medaka, Zebrafish		1	TG 229	Level 3
Androgenised Female Stickleback Screen (AFSS)	Three-spined stickleback			GD 140	Level 3
Fish Sexual Development Test (FSDT)	Three-spined stickleback, Japanese Medaka, Zebrafish		-	TG 234	Level 4
21-Day Fish Assay	Fathead minnow, Japanese Medaka, Zebrafish		1	TG 230	Level 3
Avian Reproduction Test	Mallard duck Bobwhite quail Japanese quail			TG 206	Level 4
Fish Lifecycle Toxicity Test (FLCTT)	Fathead minnow or sheepshead minnow (marine)	850.1500	2		Level 5
Larval Amphibian Growth and Development Assay (LAGDA)	*Xenopus laevis*	Draft (December 2014)			Level 4
Medaka Extended One Generation Reproduction Test (MEOGRT)	Japanese Medaka	Draft (December 2014)			Level 5
Avian Two-Generation Test (ATGT)	Japanese Quail	890.2100			

* US EPA Office of Prevention, Pesticides and Toxic Substances

Non-standard methods with comparable endpoints (*e.g. in vivo* studies specifically performed to detect endocrine effects) published in scientific literature were also included.

Appendix D. List of endpoints considered for the screening methodology

List of endpoints and their classification from the tests listed in Appendix C.

Table D1. *In vitro* mechanistic endpoints

Estrogen receptor	Estradiol synthesis
Estrogen related receptor	Testosterone synthesis
Androgen receptor	Cellular proliferation
CYP19	Thyroid receptor
	Transthyretin (TTR)

Table D2. Mammalian *in vivo* effects – mechanistic

Cowper's glands weight (Hershberger)	Thyroid histopathology (Hershberger)
Glans penis weight (Hershberger)	Uterus histopathology (UT assay)
LABC weight (Hershberger)	Testosterone level
Prostate weight (Hershberger)	Estradiol level
Seminal vesicles weight (Hershberger)	Thyroid stimulating hormone (TSH) level
Uterus weight (UT assay)	T3 and T4 level
Keratinisation and cornification of vagina (UT assay)	Luteinizing Hormone (LH) level
Proliferation of endometrial epithelium (UT assay)	Follicle Stimulating Hormone (FSH) level

Table D3. Mammalian *in vivo* effects - EATS specific

Accessory sex glands weight	Keratinisation and cornification of vagina
Genital abnormalities	Male mammary gland histopathology
Coagulating gland weight	Mammary gland histopathology
Cervix weight	Ovary histopathology
Cowper's glands weight	Oviduct histopathology
Epididymis weight	Penis histopathology
Glans penis weight	Proliferation of endometrial epithelium
LABC weight	Prostate histopathology
Mammary gland weight	Seminal vesicles histopathology
Ovary weight	Testis histopathology
Prostate weight	Thyroid histopathology
Seminal vesicles weight	Uterus histopathology
Testis weight	Vagina histopathology
Thyroid weight	Vaginal smears
Uterus weight	Age at first estrus
Accessory sex organs histopathology	Age at preputial separation
Ano-Genital distance	Age at vaginal opening
Cervix histopathology	Estrus cyclicity
Coagulating gland histopathology	Sperm morphology

Colloid area	Sperm motility
Cowper's glands histopathology	Sperm numbers
Epididymis histopathology	Steroidogenesis (genes/enzyme changes)
Female mammary gland histopathology	Nipple Development
Follicular cell height	

Table D4. Mammalian *in vivo* effects - non-specific adversity (may or may not be indicative of EATS)

Pup mortality	Litter viability
Litter/pup weight	Number of implantations, *corpora lutea*
Adrenals weight	Number of live births
Pituitary weight	Number of ovarian follicles
Placental weight	Post implantation loss
Vagina weight	Pre implantation loss
Adrenals histopathology	Pup survival index
Pituitary histopathology	Reproduction
Placenta histopathology	Time to mating
Birth index	Resorptions
Dystocia	Live fetus
Fertility	Fetal development
Gestational interval	Fetal mortality
Gestation length	Fetal weight
Gestation Index	Maternal wastage
Intercurrent deaths	Aborted
Lactation index	Tumour types*
Litter size	Pup development

* If tumour of endocrine organ, considered to be EATS specific

Table D5. Mammalian *in vivo* effects - general adversity

Food consumption	Kidney weight
Body weight	Brain weight
Mortality	Lung weight
Maternal mortality	Spleen weight
Systemic toxicity	Liver histopathology
Growth	Kidney histopathology
Haematological parameters	Brain histopathology
Liver weight	Lung histopathology
	Spleen histopathology

Table D6. Fish *in vivo* effects – mechanistic.

Male 2nd sex characteristics in females	Levels of thyroid hormones
Male 2nd sex characteristics in males	Spiggin
Vitellogenin (VTG) in females	Testosterone level
Vitellogenin (VTG) in males	Estradiol level
Vitellogenin (VTG) in males and females	T3 and T4 level

Table D7. Fish *in vivo* effects – EATS specific.

Specific female gonad histopathology	Sex ratio (Female biased, no males)
Specific male gonad histopathology	Sex ratio (Male biased)
Sex ratio in fish	Sex ratio (Male biased, undifferentiated)
Sex ratio (Female biased)	Gonado-somatic index
Sex ratio (Female biased, intersex)	

Table D8. Fish *in vivo* effects – non-specific adversity (may or may not be indicative of EATS)

Behaviour	Fertility
Length	Time to maturity (time to first spawn)
Abnormal morphology and appearance	Reproduction (fecundity, fertility)
Gross morphology	Fecundity
Survival of embryos	Hatching success
Gonad	

Table D9. Fish *in vivo* effects – general adversity

Mortality	Hepatosomatic index
Growth	Liver effects
Body weight	Kidney effects
Survival	

Table D10. Avian *in vivo* effects – non-specific adversity (may or may not be indicative of EATS)

Gross pathology	Egg viability
Hatchability	Egg production
Eggshell thickness	Cracked eggs

Table D11. Avian *in vivo* effects – general adversity

Body weight	

Table D12. Amphibian *in vivo* effects – mechanistic.

Snout-vent length	Developmental stage
Hind limb length	Thyroid histopathology (amphibian)

Table D13. Amphibian *in vivo* effects – non-specific adversity (may or may not be indicative of EATS)

Mortality	Malformations

Table D14. Amphibian *in vivo* effects – general adversity

Behaviour	

LB-NA-27955-EN-N

JRC Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

EU Science Hub
ec.europa.eu/jrc

@EU_ScienceHub
EU Science Hub - Joint Research Centre
Joint Research Centre
EU Science Hub

Publications Office

doi:10.2788/73203

ISBN 978-92-79-58906-5