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PART 4/16

COMMISSION STAFF WORKING DOCUMENT

IMPACT ASSESSMENT

Defining criteria for identifying endocrine disruptors in the context of the implementation of the plant protection product regulation and biocidal products regulation

Annex 3 out of 16

Accompanying the document

COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL

on endocrine disruptors and the draft Commission acts setting out scientific criteria for their determination in the context of the EU legislation on plant protection products and biocidal products

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ANNEX 3

SCREENING METHODOLOGY TO IDENTIFY ENDOCRINE DISRUPTORS ACCORDING TO DIFFERENT OPTIONS IN THE CONTEXT OF AN IMPACT ASSESSMENT

Contents

1. INTRODUCTION

As specified in the roadmap[1], and in Section 4 of the main impact assessment report, four different policy options are outlined for identifying endocrine disruptors (EDs). To determine which substances would be tentatively identified as ED under the different options, the methodology summarised below has been developed by the Joint Research Centre of the European Commission (JRC). The method is being applied by an external SANTE contractor to approximately 600 substances selected from the total lists of substances subject to the Regulations on Plant Protection Products (PPP Regulation), Biocidal Products (BP Regulation), Chemicals (REACH), Cosmetic Products and priority substances under the Water Framework Directive (WFD).

2. AIM AND SCOPE OF THE METHODOLOGY

The screening methodology was developed to assess in a limited amount of time the potential ED properties for approximately 600 substances previously selected (see Annex 4). Therefore, the methodology was applied to existing data only.

The development of this methodology comprised the following steps:

- Identification of data sources.
- Selection of relevant data types to be collected and relevant to inform on the potential ED properties of a substance.
- Definition of a data analysis procedure to categorise substances under the four policy options.

Each step comprises a well-defined set of activities, which are elaborated in the following sections; Figure 1 provides a schematic representation of the methodology.

The assessment focused 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. As the understanding regarding the disturbance of the endocrine system of many invertebrate species is limited, the effects on wildlife were limited to the effects observed in mammals, fish, amphibians, and to a very limited extent in birds.

The endocrine relevant effects were limited to effects on the estrogenic, androgenic, thyroid and steroidogenesis (EATS) pathways, as these are relatively well understood and consensus guidance on the interpretation of effects observed in OECD Test Guidelines is available from the OECD Guidance Document (GD) 150.[2] Perturbations of other non-EATS pathways –

[1] European Commission. 2014. Defining criteria for identifying Endocrine Disruptors in the context of the implementation of the PPP Regulation and BP Regulation. Retrieved from: http://ec.europa.eu/smart-regulation/impact/planned_ia/docs/2014_env_009_endocrine_disruptors_en.pdf

[2] OECD. 2012. Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Environmental Health and Safety Publications, Series on Testing and Assessment n°150, Organisation for Economic Cooperation and Development, Paris. Retrieved from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282012%2922&doclanguage=en

while potentially relevant for ED - were beyond the scope of this methodology. Human epidemiological and *in silico* data (such as (Q)SAR predictions) were also not considered.

Existing data on the EATS pathway may also 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 not 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.

As a result of the limitations in its scope, this screening methodology is neither equivalent to nor intended to replace an in-depth assessment process as usually carried out for regulatory purposes. 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.

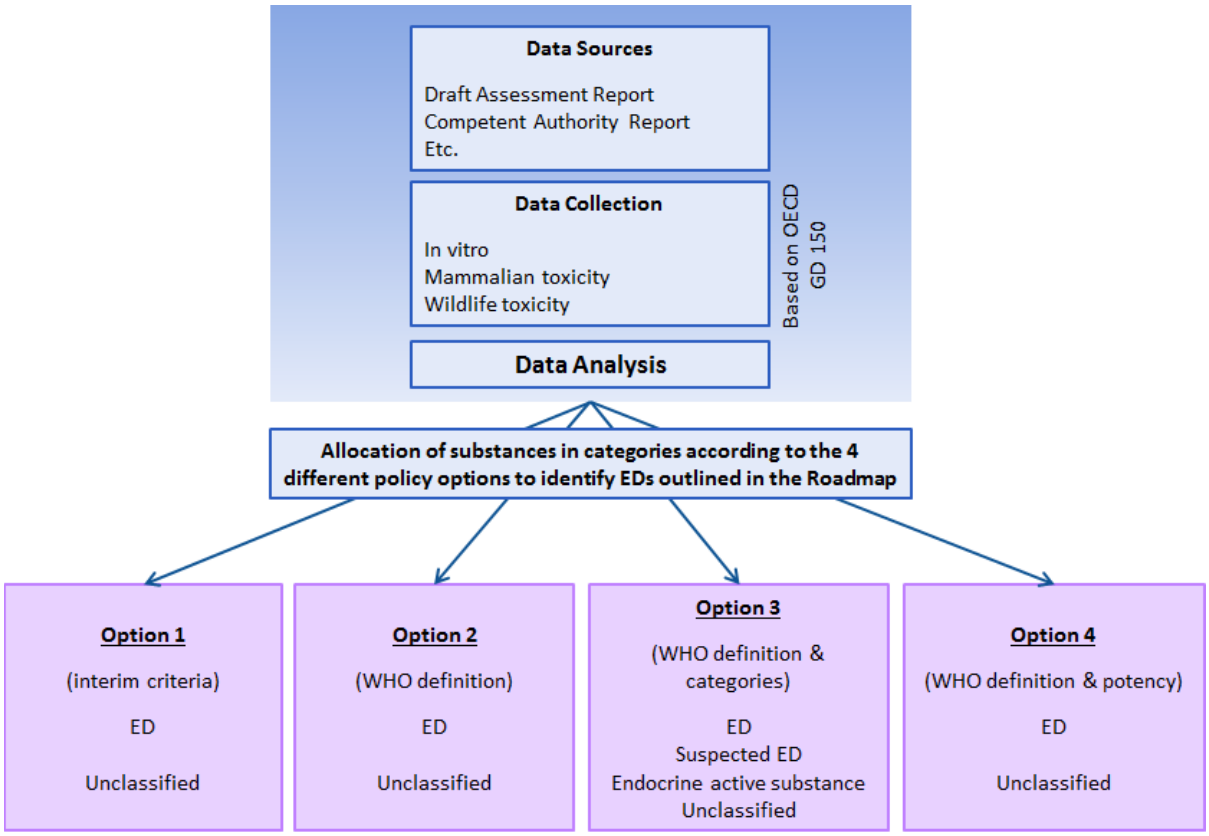

Figure 1. Schematic representation of the screening methodology to tentatively identify which substances would be identified as EDs under four policy options

3. SUBSTANCE SELECTION

Substances were selected as described in Annex 4. This information was also published on the DG SANTE website[3] in December 2015.

[3] European Commission. 2015. Selection of substances to be screened in the context of the impact assessment on criteria to identify endocrine disruptors. Retrieved from: http://ec.europa.eu/health/endocrine_disruptors/docs/impactassessment_chemicalsubstancesselection_en.pdf

4. DATA COLLECTION

Figure 2 provides a schematic representation of which data sources were used to collect relevant data which were then organised in a template to support the data analysis in order to categorise each substance under the four policy options.

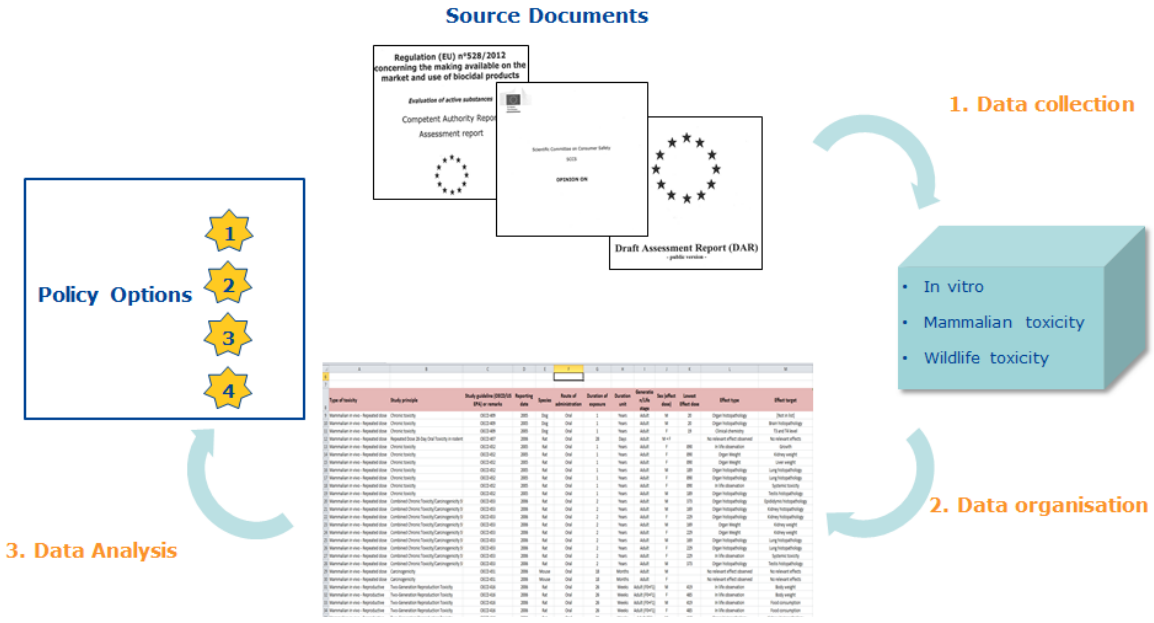

Figure 2. Schematic representation of the workflow from identification of data sources to data analysis

1.1. Information on adverse effects

To determine whether a substance would classify as an ED under each of the four different policy options, different types of information were needed (See Figure 3):

- **Option 1 (interim criteria):** assessment based on the CLP classification (as carcinogen category 2 or toxic for reproduction category 2, harmonised and proposed) and toxicity to endocrine organs. As "endocrine organ" is not defined in the interim criteria, for the purpose of this impact assessment it constitutes 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.
- **Option 2, 3 and 4 (all based on the WHO definition):** all relevant effects are captured that provide information on potential interference with the endocrine system, according to the interpretation given in OECD GD 150. Results are obtained from existing studies on developmental toxicity, reproductive toxicity, carcinogenicity and (sub)acute and (sub)chronic (repeated dose) toxicity.

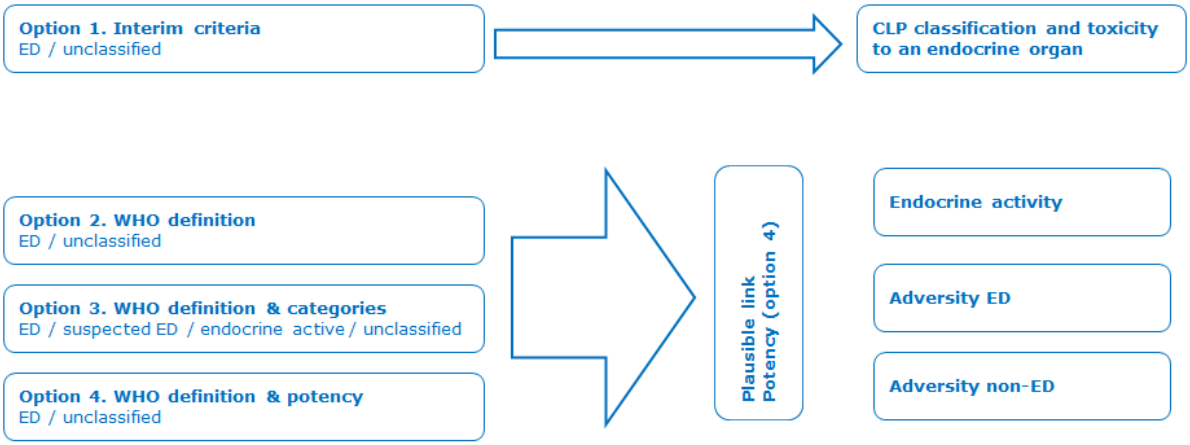

Figure 3. Data requirements for the four different policy options. For option 1, data is required on the CLP classification and the toxicity to an endocrine organ. For option 2, 3, and 4, in vivo and in vitro data are required that show a likelihood of endocrine mediated effects (in the absence of general overt toxicity).

1.2. Information sources

For option 1 (interim criteria), the hazard classification of a substance according to Regulation (EC) No 1272/2008[4] (CLP Regulation) was obtained from the ECHA Classification & Labelling Inventory. If no harmonised classification was available, but a classification was proposed in the regulatory documents (e.g. EFSA Conclusions), then the proposed classification was used. If the proposed classification was more recent than the harmonised classification, both were recorded.

The (eco)toxicological data, mostly obtained from laboratory animals (*in vivo*), was initially collected from evaluated data from the existing regulatory assessment reports, including: EFSA conclusions, MS Draft Assessment Reports, MS Competent Authority Reports, REACH restriction dossiers, Support documents for identification of SVHC and opinions of the SCCS. As the data in these documents have been assessed independently by the MS Competent Authorities, they are assumed to be of high quality and relevant by default.

This information was then supplemented by additional information, gathered from databases focusing on endocrine effects including non-regulatory studies, including:

1. Endocrine Active Substances Information System (EASIS): JRC Database of study reports on substances related to endocrine activity;
2. Substitute It Now (SIN) list: substances that have been identified by the NGO ChemSec as being substances of concern. Endocrine disrupting activity is included as a category for reason of concern;

[4] Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation), OJ L 353 31.12.2008, p. 1. Retrieved from: http://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A02008R1272-20150601

3. The Endocrine Disruption Exchange (TEDX) list: potential Endocrine Disruptors; developed by the US Organisation TEDX;
4. ToxCast Database (including ToxCast ER prediction model): data for substances tested in one of the 26 *in vitro* assays that are considered to be relevant for the EATS pathways, developed by US EPA.

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

Data that inform on how a substance exerts its toxic effects are described as *mechanistic* or *mode of action* data. Such data may be derived from *in vivo* or *in vitro* studies. In the case of endocrine disruption, these data are needed as evidence that a substance alters the endocrine system in accordance with the WHO definition.

1.3. Data extraction and organisation

All effect data from *in vitro* and *in vivo* studies that are potentially informative on ED action were captured. The list of relevant effects was based on a list provided in the OECD GD 150, supplemented with effects from similar *in vivo* and *in vitro* tests, also focusing on the EATS pathways. Some additional effects were captured that are not directly linked to endocrine disruption, e.g. effects occurring at the same dose as (or lower than) the endocrine effects, which help with the interpretation of the specificity of the endocrine related effects.

The data captured included the following information:

- general substance information, including chemical name, CAS Registry Number, current CLP classification (harmonised and proposed), and specific remarks in the regulatory source documents relevant to ED assessment;
- study information, including the type of toxicity test (*in vitro*, *in vivo*, mammalian, fish, birds, amphibians), the study principle including the protocol used (e.g. OECD or US EPA test guidelines and deviations from these guidelines), and the source of the data (e.g. the specific database from which the regulatory document was retrieved), including the primary reference given within this source and the reporting date;
- study details, including the test species and strain (for *in vitro* assays, the test system used), number of animals per group, the doses administered, the route and method of administration, duration of exposure and the purity of the substance;
- effect details, including the sex, generation and/or life stage for which the effect was observed. The lowest dose at which the specific effect was observed, including the direction of the effect and classification of the effect (optional additional details to further specify the observation). In the case of *in vitro* studies, generally the lowest effect dose is generally not reported, so median values (EC50/AC50/IC50) derived from the concentration-response relationships were captured instead.

5. DATA ANALYSIS AND EVALUATION

All effects captured were codified as providing one of the following types of evidence: *in vitro* mechanistic [A], *in vivo* mechanistic (including hormone levels)[B], EATS specific adverse effects [C], non-specific adverse effects (may or may not be related to EATS) [D] and general adverse effects (not ED-related).

In addition, the consensus interpretation regarding linkage of each effect to one or more of the EATS pathways is indicated. Because of the limited scope of the screening and absence of relevant data for many substances, it is not possible to conclude that a substance is not an ED, hence all substances that cannot be categorised on the available information are considered to be *Unclassified*.

For Option 1 (interim criteria), the identification as ED is based on the interim criteria and depends on the answers to the questions shown in Figure 4 below.

Both the harmonised classification (when available) and the proposed classification (when relevant) have been considered for the substance categorisation under Option 1.

The final categorisation considering the available harmonised and/or proposed classification for each substance as ED or not (unclassified) was based on the scheme shown in Figure 4 below:

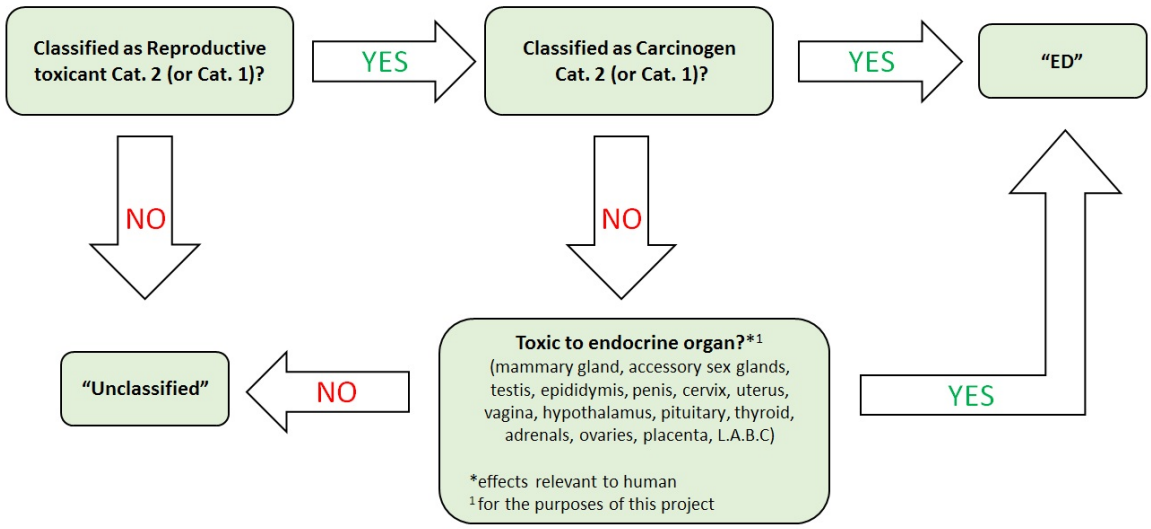

Figure 4. Decision tree, leading to the different ED categorisations according to the interim criteria as stated in the PPP Regulation and the BP Regulation.

Regarding the interpretation of "toxic to endocrine organs", endocrine organs were considered to be those 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. This includes: 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).

For Option 2 (WHO definition) **and Option 3** (WHO definition + categories), all effects were collated to determine whether there was sufficient evidence that the substance "alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations".[5]

Depending on the evidence, substances were categorised as Cat I, II III, or Unclassified according to the decision tree in Figure 5. Higher weight was given to EATS specific adverse effects compared to non-specific adverse effects and, in relation to mechanistic data, higher weight was given to *in vivo* mechanistic data than to *in vitro* mechanistic data. Although not covering every situation, generally the type of evidence leading to categorisation into one of the four categories was as follows:

- Cat I: confirmed ED. Adverse effects with plausible link (i.e. same pathway) to mechanistic (endocrine mode of action) information or, in some specific cases, the pattern of adverse effects may be diagnostic of an ED mode of action
- Cat II: suspected ED. Specific adverse effects indicating endocrine disruption but without supporting mechanistic evidence, or *in vivo* mechanistic evidence without evidence for adverse effects
- Cat III: endocrine active. No *in vivo* evidence indicating endocrine adverse effects but mechanistic information *in vitro*
- Unclassified: No (existing) *in vivo* or *in vitro* data that indicate endocrine adverse effects.

[5] WHO/IPCS. 2002. Global Assessment of the State-of-the-science of Endocrine Disruptors. World Health Organization/International Programme on Chemical Safety. WHO/PCS/EDC/02.2, 180 pp. Retrieved from: http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/

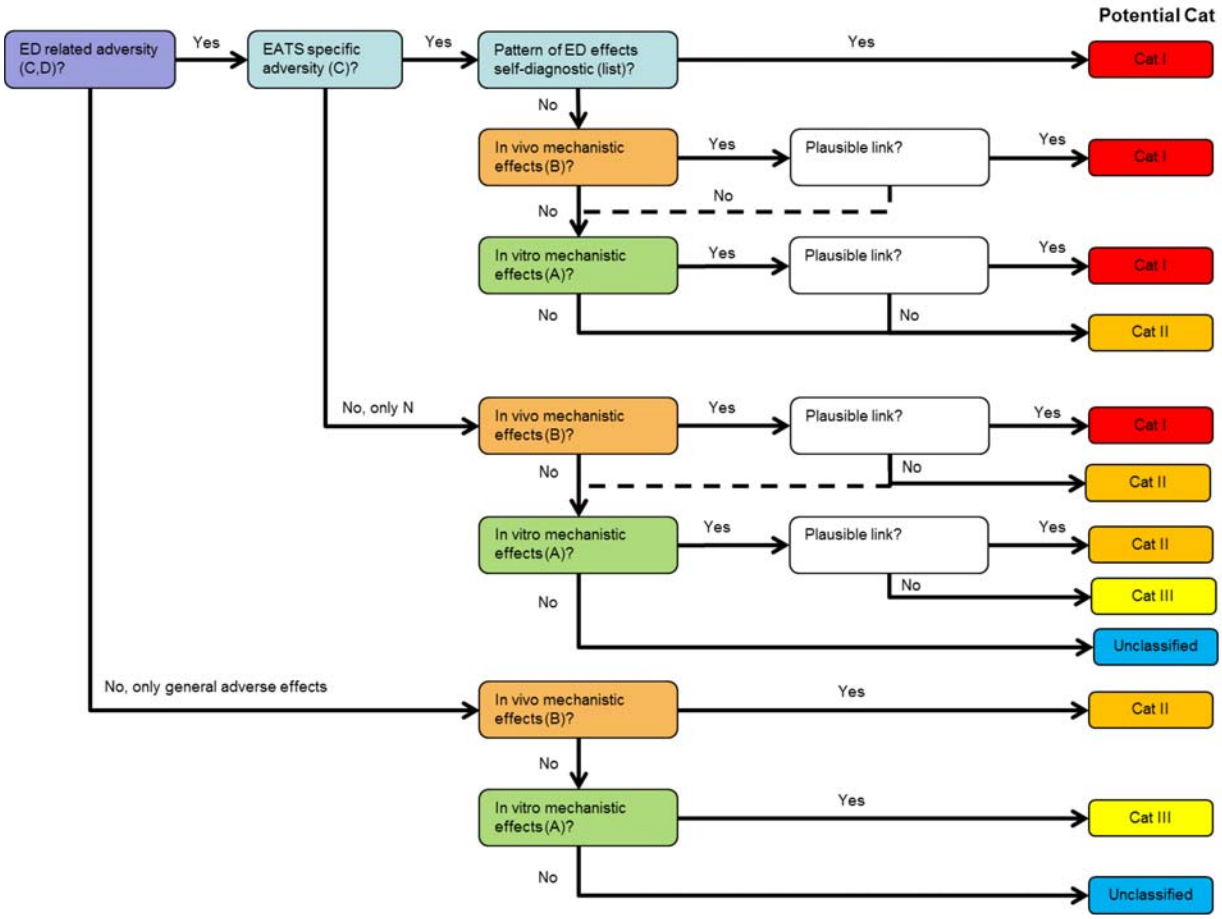

Figure 5. Decision tree for policy options 2 and 3: endocrine disruption according to the WHO definition. A limited weight of evidence based on expert judgement was applied at the Yes/No decision points.

If the decision tree is applied independently of the weight of evidence supporting each of the elements in the decision tree, it may lead to an overestimation of the number of substances identified as EDs. Therefore, a limited weight of evidence approach was applied at the Yes/No decision points in the decision tree.

This limited weight of evidence approach was based, among others, on the following considerations:

a) the magnitude and nature of the adverse effects;
b) the pattern and coherence of adverse effects observed at different doses within and between studies of a similar design and across different species;
c) the weight of certain studies with respect to others: e.g. long term/chronic/repeated-dose studies versus short term/acute studies; *in vivo* tests versus *in vitro* tests; studies with clear study-design versus poorly detailed studies;
d) the biological plausibility of a causal relationship between the induced endocrine activity and the adverse effect(s);
e) the presence of overt toxicity together with the potential ED-related effects;
f) the data available on the human relevance of the effects and mode of action observed.

Thus an isolated effect of low magnitude in one species not observed in other studies of similar design with the same species (provided the effect had been measured) would have lower weight than a case where a clear pattern of effects was seen across a number of studies and in more than one species. As this largely depends on expert judgement, this part could not be codified into the decision tree.

When potential ED-related effects were observed in the presence of overt toxicity, these effects were not considered to be informative of an endocrine mode of action.

Identification as ED under Option 4 (WHO definition + potency) takes into account the potency aspect. Potency depends on the endpoint, but also on the dose, on the duration and timing of exposure.[6]

Option 4 applies only to those substances that are identified under Option 2 or 3 Category I. To categorise a substance under Option 4 for the purpose of this impact assessment, it was agreed to use a trigger value as cut-off value.

The potency of a substance was assessed in this methodology by evaluating if the dose at which an endocrine-related-effect was observed (effect used to categorise that substance in Option 2 or 3 Category I) was above or below a relevant cut-off value. If the ED-related endpoint was below this cut-off value, the substance was considered to satisfy the potency criteria under option 4 and it was thus considered an ED. If it was above the potency cut-off, it was considered as unclassified.

In this methodology, potency-based STOT-RE Cat 1 trigger values from the Regulation (EC) No 1272/2008[7] are proposed as cut-off criteria to evaluate potency. The most sensitive endocrine specific endpoint was compared to the potency cut-off values taken from the STOT-RE, according to the route of exposure (oral, dermal, inhalation). As the duration of *in vivo* assays is variable, the doses were time-adjusted to a 90-day study. However, the same value was used for all species and no further adjustment was applied to take into account the different sizes (body weights) or life spans of different species.

The following decision tree was used to categorise substances under Option 4 by using the defined cut-off value (Figure 6).

[6] EFSA. 2013. 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. [84 pp.] doi: 10.2903/j.efsa.2013.3132.

[7] Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation), OJ L 353 31.12.2008, p. 1. Retrieved from: http://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A02008R1272-20150601

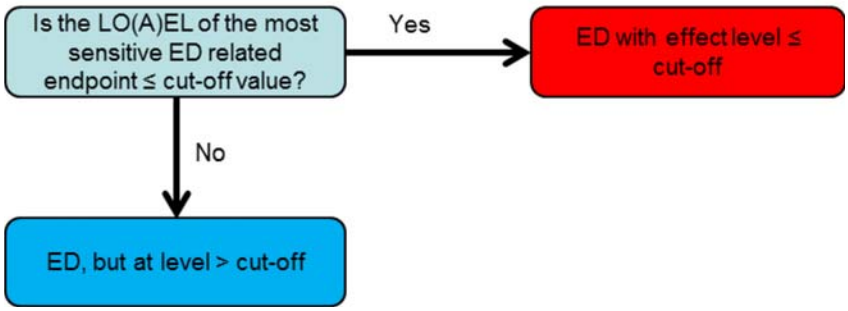

Figure 6. Decision tree, leading to ED categorisation according to option 4.

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

Table 1. Guidance values for STOT-RE Cat 1 for sub chronic and other medium-term studies.

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 assessment took into consideration the duration of exposure by applying commonly used extrapolation factors: e.g. for a 28-day study the guidance values reported in Table 1 were increased by a factor of three; for a 2-year study, the guidance values were decreased by a factor of eight. Based on the approach followed by the ECHA Risk Assessment Committee (RAC), the same guidance values for rat, mouse and dog studies were used.[8]

Having used such extrapolations, substances categorised as ED under Option 2 or under Option 3 Category I on the basis of evaluation of mammalian data remained classified as EDs for Human Health under Option 4 if the effect dose was lower than the adjusted potency cut-off value (Figure 6) or characterised as unclassified if the effect dose was higher than the adjusted potency cut-off value.

For the ecotoxicological evaluation under Option 4, substances categorised as ED under Option 2 or under Option 3 Category I were treated as follows.

If the plausible link was established on the basis of mammalian data only, then the same cut-off values as in human health assessment were used.

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

If vertebrate wildlife other than mammalian data (i.e. avian, fish, amphibian data) were used, these substances were categorised as ED under Option 4. In other words, the cut-off value was assumed to be very high.

Under Options 2, 3 and 4, the evidence was assessed for human health and for wildlife separately. For human health, all mammalian effects were assumed to be relevant. For wildlife, the data from fish, amphibians and birds were used in addition to the mammalian data. However, only the effects that are considered to have population relevance (i.e. developmental and reproductive effects) were used to categorise a substance.

6. SUMMARY AND CONCLUSIONS

A screening methodology was developed to assess, in a limited amount of time, the potential endocrine disrupting properties for 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 PPP Regulation, BP Regulation, Chemicals (REACH), Cosmetic Products and Water Framework Directive (WFD).

Bearing in mind the time and financial constraints on the study, the methodology was designed to be feasible, scientifically robust and transparent, allowing traceability of data and conclusions. It was necessary to limit the scope of the methodology, as described above, to the modes of action and adverse effects that are better understood and investigated in existing regulatory assessments. 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, this 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.

In developing this screening methodology, it was foreseen that the results for pesticide and biocidal active substances would serve as an input to a second study comparing the impacts of the different policy options on substances falling under the PPP Regulation and the BP Regulation.

GLOSSARY

A	Androgenic pathway
AC50	Half maximal active concentration
BP Regulation	Biocidal Products Regulation
CAR	Competent Authority Report
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, Mutagenic, Reprotoxic
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
EC50	Half maximal effective concentration
ED	Endocrine disruptor
EDSP	Endocrine Disruptor Screening Program
EFSA	European Food Safety Authority
EU	European Union
GD	Guidance Document
IC50	Half maximal inhibitory concentration
JRC	Joint Research Centre
MS	Member State
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PPP Regulation	Plant Protection Products Regulation
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
ToxCast	Database of *in vitro* assay data from US Environmental Protection Agency (EPA)
WFD	Water Framework Directive
WHO	World Health Organization
WoE	Weight of Evidence