A. Introduction

A.1. Background

1. The OECD initiated a high-priority activity in 1998 to revise existing, and develop new, test guidelines (TGs) for the screening and testing of endocrine disrupting chemicals. Since then a number of potential assays have been developed into test guidelines and others are in development. The screens and tests are contained within the OECD Conceptual Framework for the Screening and Testing of Endocrine Disrupting Chemicals (CF) which was developed in 2002 by the Endocrine Disrupters Testing and Assessment Advisory Group (EDTA AG), modified and updated in 2012 and again in 2017. The 2017 revised version of the CF is shown in Section A.2. A workshop on "OECD Countries' Activities Regarding Testing, Assessment and Management of Endocrine Disrupters" was held in Copenhagen on 22-24 September 2009 (OECD, 2010b). One output from this workshop was a recommendation that a guidance document (GD) on the assessment of chemicals for endocrine disruption should be developed by the EDTA AG. This was supported by the EDTA AG at its meeting on 17-18 May 2010. The objectives and scope of the GD were defined such that the document would be a tool to support regulatory authorities by helping to interpret assay results and suggesting possible additional studies for reducing uncertainty. The guidance should not prejudge or constrain what regulatory actions may be taken by a member country and should not suggest a testing strategy. The guidance should also support, but not duplicate, other GDs (e.g. guidance on hazard assessment). It should be noted that the use of many of these tests for determination of toxicity due to endocrine disruption (hazard identification/characterisation) for mammals and non-mammals was still rather new, and therefore the guidance given was considered to be subject to changes based on new evidence. The guidance was intended to be a "living" document to be updated as the science in this area evolves, and the present publication represents the first such update. In particular, this update takes into account the many new validated assay/test methods developed since the 2012 version of the GD (see Table A.1 and discussion in Section B). This document also provides additional guidance on evaluation of each validated assay/test method (Section C).

A.2. The OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals

2. The OECD Conceptual Framework lists the OECD TGs and standardised test methods available, under development or proposed, that can be used to evaluate chemicals for endocrine disruption. It is not an exhaustive list and will be updated as new assays are developed. Assays other than those described in the list may also be valuable for assessing chemicals for endocrine disruption and could be assigned to a level based on the level descriptors. The CF is intended to provide a guide to the tests available which can provide information on assessment of endocrine disruption, but is not intended to be a testing strategy. Furthermore, the CF, as revised in 2017, does not include evaluation of exposure as it is intended for hazard identification/characterisation (see definitions in Section A.3).

The OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals, revised 2017

Mammalian and non-mammalian toxicology		
Level 1 Existing data and existing or new non-test information	– Physical and chemical properties, e.g. molecular weight reactivity, volatility, biodegradability – All available (eco)toxicological data from standardised or non-standardised tests – Read-across, chemical categories, quantitative structure activity relationships and other *in silico* predictions, and absorption, distribution, metabolism and excretion model predictions	
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s)/ pathway(s) (mammalian and non-mammalian methods)	– Estrogen (OECD TG 493) or androgen receptor binding affinity (US EPA TG OPPTS 890.1150) – Estrogen receptor transactivation (OECD TG 455, ISO 19040-3), yeast estrogen screen (ISO 19040-1 & 2) – Androgen receptor transactivation (OECD TG 458) – Steroidogenesis *in vitro* (OECD TG 456) – Aromatase assay (US EPA TG OPPTS 890.1200) – Thyroid disruption assays (e.g. thyroperoxidase inhibition, transthyretin binding) – Retinoid receptor transactivation assays – Other hormone receptors assays as appropriate – High-throughput screens	
	Mammalian toxicology[3]	**Non-mammalian toxicology[3]**
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s)/ pathway(s)[1]	– Uterotrophic Assay (OECD TG 440) – Hershberger assay (OECD TG 441)	– Amphibian metamorphosis assay (AMA) (OECD TG 231) – Fish short-term reproduction assay (FSTRA) (OECD TG 229)[2] – 21-day fish assay (OECD TG 230) – Androgenised female stickleback screen (AFSS) (OECD GD 148) – EASZY Assay. Detection of Substances Acting through Estrogen Receptors using Transgenic cyp19a1b GFP Zebrafish Embryos (draft OECD TG) – *Xenopus* embryonic thyroid signalling assay (XETA) (draft OECD TG) – Juvenile medaka anti-androgen screening assay (JMASA) (draft OECD GD) – Short-term juvenile hormone activity screening assay using *Daphnia magna* (draft OECD TG) – Rapid androgen disruption adverse outcome reporter (RADAR) assay (draft OECD TG)
Level 4 *In vivo* assays providing data on adverse effects on endocrine-relevant endpoints[2]	– Repeated dose 28-day study (OECD TG 407) – Repeated dose 90-day study (OECD TG 408) – Pubertal development and thyroid function assay in peripubertal male rats (PP male assay) (US EPA TG OPPTS 890.1500) – Pubertal development and thyroid function assay in peripubertal female rats (PP female assay) (US EPA TG OPPTS 890.1450) – Prenatal developmental toxicity study (OECD TG 414) – Combined chronic toxicity and carcinogenicity studies (OECD TG 451-453)	– Fish sexual development test (FSDT) (OECD TG 234) – Larval amphibian growth and development assay (LAGDA) (OECD TG 241) – Avian reproduction assay (OECD TG 206) – Fish early life stage (FELS) toxicity test (OECD TG 210) – New guidance document on harpacticoid copepod development and reproduction test with *Amphiascus* (OECD GD 201)[2] – *Potamopyrgus antipodarum* reproduction test (OECD TG 242)[4] – *Lymnaea stagnalis* reproduction test (OECD TG 243)[4]

The OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals, revised 2017 (*continued*)

	Mammalian toxicology[3]	**Non-mammalian toxicology**[3]
Level 4 (*continued*)	– Reproduction/developmental toxicity screening test (OECD TG 421) – Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) – Developmental neurotoxicity study (OECD TG 426) – Repeated dose dermal toxicity: 21/28-day study (OECD TG 410) – Subchronic dermal toxicity: 90-day study (OECD TG 411) – 28-day (subacute) inhalation toxicity study (OECD TG 412) – Subchronic inhalation toxicity: 90-day study (OECD TG 413) – Repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409)	– Chironomid toxicity test (OECD TG 218-219) [4] – *Daphnia magna* reproduction test (with male induction) (OECD TG 211)[4] – Earthworm reproduction test (OECD TG 222)[4] – Enchytraeid reproduction test (OECD TG 220)[4] – Sediment water *Lumbriculus* toxicity test using spiked sediment (OECD TG 225)[4] – Predatory mite reproduction test in soil (OECD TG 226)[4] – Collembolan reproduction test in soil (TG OECD 232)[4]
Level 5 *In vivo* assays providing more comprehensive data on adverse effects on endocrine-relevant endpoints over more extensive parts of the life cycle of the organism[2]	– Extended one-generation reproductive toxicity study (EOGRTS) (OECD TG 443)[5] – Two-generation reproduction toxicity study (OECD TG 416, most recent update)	– Fish life cycle toxicity test (FLCTT) (US EPA TG OPPTS 850.1500) – Medaka extended one-generation reproduction test (MEOGRT) (OECD TG 240) – Avian two-generation toxicity test in the Japanese quail (ATGT) (US EPA TG OCSPP 890.2100/740-C-15-003) – Sediment water chironomid life cycle toxicity test (OECD TG 233)[4] – *Daphnia* multigeneration test for assessment of EDCs (draft OECD TG)[4] – Zebrafish extended one-generation reproduction test (ZEOGRT) (draft OECD TG)

Notes:

1. Some assays may also provide some evidence of adverse effects. 2. Some endpoints can be sensitive to more than one mechanism and may be due to non-endocrine mechanisms. 3. Depending on the guideline/protocol used, the fact that a substance may interact with a hormone system in these assays does not necessarily mean that when the substance is used it will cause adverse effects in humans or ecological systems. 4. At present, these invertebrate assays solely involve apical endpoints which are able to respond to some endocrine active substances and some non-endocrine active substances. Those in Level 4 are generally partial life cycle tests, while those in Level 5 are full or multiple life cycle tests. 5. The EOGRTS (OECD TG 443) is preferable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints in the juvenile and adult F1, which are not included in the two-generation study (OECD TG 416) adopted in 2001.

Notes to the OECD Revised Conceptual Framework:

Entering at all levels and exiting at all levels is possible and depends on the nature of existing information and needs for testing and assessment. The assessment of each chemical should be made on a case-by-case basis, taking into account all available information. The framework should not be considered as all inclusive at the present time. It includes assays that are either available, or for which validation is under way. With respect to the latter, these are provisionally included, and a few assays (e.g. the avian two-generation test) have only been validated at national level. At Level 2 some assays are not (yet) proposed for validation but are included because they may provide information on important molecular interactions.

A.3. Definitions and terms used

3. In the context of this document, the following terms have been defined according to published and generally well-accepted definitions. The definitions from the Berlin Worshop Consensus Statement (Solecki et al., 2017), where several scientists in the endocrine disruption field agreed statements, are also shown and accepted in this document.

Term	Definition	Comments	Reference
Endocrine disrupter (ED)	An ED is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or (sub)populations.	It is acknowledged that many other definitions exist (e.g.Weybridge Conference, 1996), but the WHO/IPCS (2002) definitions have been used as working definitions for this document because they cover both human health and non-mammalian populations. Accepted by Solecki et al. (2017) and within the European Union (EC, 2016).	WHO/IPCS (2002)
Potential endocrine disrupter	A potential ED is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, its progeny or (sub)populations.		WHO/IPCS (2002)
Endocrine active substance (EAS)	A substance having the inherent ability to interact or interfere with one or more components of the endocrine system resulting in a biological effect, but need not necessarily cause adverse effects.		EFSA (2013)
Adverse effect	A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.	Widely accepted as the definition of "adverse effect" to accompany the WHO/IPCS (2002) definition of an ED, and also by Solecki et al. (2017).	WHO/IPCS (2009)
Adverse outcome pathway (AOP)	An AOP is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organisation relevant to risk assessment.	AOPs can be very helpful in establishing the links between an endocrine mechanism and its potential apical effects.	Ankley et al. (2010)
Intact organism	The term "intact organism" is understood to mean that the effect would occur *in vivo*, either observable in a test animal system, epidemiologically or clinically. However, it does not necessarily mean that the adverse effect has to be demonstrated in an intact test animal, but may be shown in adequately validated alternative test systems predictive of adverse effects in humans and/or wildlife. The importance of mechanistic data derived from experimental systems (*in vitro* or *in vivo* in which the animals have been surgically or genetically altered as part of a focused experiment) was also recognised.	"Intact organism" to accompany the WHO/IPCS (2002) definition of an ED.	Solecki et al. (2017)
Hazard identification	The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment.	GD 150 only covers assessment of hazard, not risk. Exposure is not considered. The term hazard identification/characterisation is used in relevant places and may encompass elements of both of these definitions.	IPCS/WHO (2004)

Term	Definition	Comments	Reference
Hazard characterisation	The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties. Hazard characterisation is the second stage in the process of hazard assessment and the second of four steps in risk assessment.		IPCS/WHO (2004)
Mode of action (MOA)	A set of key events and processes starting with the interaction of an agent with a cell, through physiological and tissue or organ changes, potentially resulting in an adverse outcome.	"Mode" of action is contrasted with "mechanism", which implies a more detailed biochemical and molecular description of causality. These definitions are implicit in the IPCS Human Relevance Framework and Adverse Outcome Pathways (AOP)	Dellarco and Fenner-Crisp (2012) Boobis et al. (2006; 2008) Ankley et al. (2010) OECD (2016)
Unknown or variable composition substances, complex reaction products or biological materials (UVCBs)	These are substances where the number of constituents is relatively large, the composition is largely unknown, or the variability of composition is high or unpredictable.		Substance identity – UVCB substances ECHA workshop 2 Feb. 2012
Weight of evidence (WOE)	A process in which all of the evidence considered relevant for a hazard identification/characterisation is evaluated and weighted.	This concept is central to the evaluation of endocrine active substances and endocrine disrupters.	WHO/IPCS (2009)

4. The above definitions implicitly refer not only to the chemical in question, but also to its endocrine-active impurities. For multi-constituent substances, UVCBs and mixtures, the definitions refer to relevant constituents. Furthermore, when reference is made to a chemical in this context, it implicitly also covers its relevant environmental transformation products and its metabolites that are formed in exposed organisms.

5. The following "concepts" related to endocrine disruption were also agreed at the Berlin Workshop (Solecki et al., 2017) and are reproduced below as they are central to an understanding of endocrine disruption:

- Alterations of the function of the endocrine system may arise from interaction with hormone receptors; changes in circulating levels of the hormone; and from the impact of chemical(s) on hormone synthesis, transport, metabolism and other factors.
- Certain hormones interact with their receptors according to an equilibrium reaction. Accordingly, the concentrations of both free hormone and free receptor are important variables controlling hormone action, explaining why different cells and tissues at different times during development are differentially sensitive to the hormone. These factors also vary between species.
- Experimental work has led to a better understanding of the role of hormones in development and during the maintenance of physiological functions. Disruption of the programming role of hormones during prenatal and postnatal development can cause adverse effects that do not become evident until later in life.
- Interference with the role of many hormones during the maintenance of physiological functions in adult life can also lead to adverse effects.

A.4. Objectives

6. The objectives of this guidance document are to:

- Provide guidance on assays that might indicate the potential for endocrine disruption, endpoints within these assays and interpretation of their results.
- Support regulatory authorities' decisions on the hazard of specific chemicals and toxicologically relevant metabolites when they receive test results from a TG, draft TG or other standardised assay for the screening/testing of chemicals for endocrine disrupting properties. The context for these decisions will vary, depending on local legislation and practice, so the advice is worded in such a way as to permit flexible interpretation.
- Provide guidance on how to interpret the outcome of individual tests and how to strengthen the weight of the evidence on whether or not a substance may be an endocrine disrupter (ED). Testing strategies or guidance on interpretation from a suite of tests are not given.

7. Hazard assessment methods in this document are arranged in a two-step process, with the intention of minimising animal testing globally through application of the 3Rs (replace, reduce and refine the use of laboratory animals in testing):

- Use of a harmonised framework for assessing test results together with existing information on likely or known hazards should avoid unnecessary animal testing.
- Recommendation of a test method that may be performed if regulatory authorities need more evidence. The test method is defined precisely to facilitate the mutual acceptance of data and to avoid unnecessary duplication of testing. The recommended test method will utilise non-animal tests where possible, although a few alternative scenarios are considered depending on existing information.
- Because hormone receptors and pathways are highly conserved across the vertebrates, cross-species extrapolations should be considered as a way to reduce vertebrate testing.

A.5. General approach

8. The general approach taken by this GD is primarily to consider the possible results that might be obtained from each endocrine disruption-responsive assay,[1] and to provide guidance about how these results might be interpreted in the light of data that may or may not already be available from other *in vitro* or *in vivo* assays. This should include all available data such as publications in the peer-reviewed literature as well as TGs. In order to inform this interpretation, background data on the assays addressed, non-testing approaches and other considerations relevant to the assays are discussed. These include cross-species extrapolations, read-across and multiple modes of action (MOA). The nature, quantity and quality of the existing and new data in each of the scenarios for the endocrine disruption-responsive assays should be evaluated systematically in a weight of evidence (WOE) approach (WOE and examples are also discussed), and there is generally no single "right" answer. Use of other technologies (for example gene expression analysis or "omics" data) may help in understanding the link between endocrine-related mechanisms and apical effects in a WOE approach. This GD should therefore be used flexibly in the light of local regulatory needs. The key questions addressed concern likely mechanisms of endocrine

action and any resulting apical effects that can be attributed to such action. Given the widely agreed definition of endocrine-disrupting chemicals (WHO/IPCS, 2002), the advice suggests that a chemical is an ED if an adverse *in vivo* effect can be plausibly linked to an endocrine MOA.

9. This document provides advice on the next step in testing (if any) which might be appropriate for a regulatory authority to take, given the various data scenarios. It should be noted that it has only been possible to cover the most likely scenarios. Advice on further testing which may be needed to assist in deciding if a chemical is an ED is generally limited to a single next step, and this GD therefore does not present an entire hazard testing strategy for endocrine disruption.

10. The key advice for each assay is given in tabular format listing a series of scenarios (see Section C). These scenarios describe combinations of different assay results, provide advice on interpreting assay results and on further testing. However, each table should be read in conjunction with the preceding text that explains issues related to the assay and for which there is insufficient space in the tabular format. Once again, it is important to note that these tables (so-called "building blocks") are purely advisory, so individual regulatory authorities are not in any way bound to follow the advice. This is all the more important given that the guidelines for testing for endocrine disruption are still relatively new and the field will probably develop further.

A.6. Scope and limitations

A.6.1. Assays and endocrine modalities covered

11. The scope of the main section of the GD is limited to providing guidance on how to interpret results from assays included in the OECD Conceptual Framework (CF) for testing and assessment of EDs (see Section A.2). As the field of endocrine disruption is still developing, the CF will be subject to periodic revisions. In fact, during the updating of the GD, the CF was revised for the second time. The assays discussed are most of those included in the original CF plus some additional assays added in 2017 that were considered relevant to assessment of endocrine disruption. Some other assays have been added to the CF that are not included in this GD but may be useful when new assays for EDs are considered and validated in the future. Guidance is provided on the endpoints for the assays discussed, with respect to the endocrine modalities listed below. This is followed by guidance on how to strengthen the WOE that a chemical is/is not an ED based on the result from the assay under consideration and other existing relevant information. Various scenarios are considered and the guidance suggests different considerations and the next test that may be performed in a single step.

12. Detailed guidance is given for the most relevant and fully validated assays in the CF from the perspective of ED identification, while more limited guidance is provided for newer assays which are still in the process of being validated. The GD is limited to endocrine mechanisms and hazard assessment. Information on chemical exposure (e.g. on use, volume, fate, levels, duration and route) is not considered.

13. The GD mainly covers the same endocrine modalities as the original CF, i.e.:

- estrogen mediated (E)
- androgen mediated (A)
- thyroid hormone mediated (T)

- steroidogenesis interference (S).

However, some assays covering apical responses to the juvenile hormone and ecdysteroid (Ec) modalities in arthropods are now included, although none have specific mechanistic endpoints for these modalities. Possible effects on the retinoic acid pathway are also included, following the publication of the *Detailed Review Paper on the State of the Science on Novel* In Vitro *and* In Vivo *Screening and Testing Methods and Endpoints for Evaluating Endocrine Disrupters* (OECD, 2012) and the draft "Detailed review paper on the retinoid system" (OECD, 2017).

14. Although the assays in this guidance are applicable to most types of EDs and endocrine active substances (EASs) which are currently known (i.e. those operating via estrogen/androgen/thyroid/steroidogenesis [E,A,T,S] modalities), it should be recognised that the assays may not be responsive to certain poorly understood chemical types or MOA. For example, it is unlikely that EDs that damage the corticosteroid system of non-mammalian species will be covered (Trenzado, Carrick and Pottinger, 2003) although the adrenals are examined in many mammalian assays, therefore providing an alert. Several modalities in vertebrates are also not covered by available assays. Equally, there are no validated assays available for assessing mechanisms of endocrine activity in invertebrates, so it is not at present possible to conclude that a chemical is an ED in invertebrates.

Epigenetic effects

15. There is a growing body of evidence that some EDs may operate through epigenetic mechanisms (although such effects are not confined to EDs). Such potential effects have been reviewed and discussed *inter alia* by Crews et al. (2014) and Nilsson and Skinner (2015), and a more extensive analysis of human data, with experimental systems is provided in recent reviews by Marczyo, Jacobs and Gant (2016) and Jacobs et al. (2017). In essence, an epigenetic effect is a change in phenotype or gene expression, inherited over rounds of cell division and sometimes transgenerationally, caused by mechanisms other than alterations in gene sequence (e.g. histone modifications, DNA methylation, RNAi mediated gene silencing). It has been suggested that epigenetic changes may result in transgenerational phenotypic effects that even occur in the absence of continued environmental exposures. It is currently unclear whether the long-term assays available for testing for endocrine disruption (e.g. insect, fish, avian and rodent life cycle tests) would reveal the full range of potential epigenetic responses. For vertebrate wildlife, the information is more scarce; for example, Brown, Schultz and Nagler (2009) failed to observe heritable reproductive defects in the offspring of male rainbow trout exposed to a strong estrogen. In contrast, two recent studies do correlate ED effects. A first report shows increased intragenic DNA methylation of the follicle stimulating hormone receptor (Fshr) gene within the gonad tissue of juvenile female European eels (*Anguilla anguilla*) from highly polluted compared with lightly polluted French waters: correlated with increased levels of gonadal persistent organic pollutants (POPs) and metals, decreased Fshr mRNA, and reduced gonad development in the highly polluted eels (Pierron et al., 2014). The second measured reproductive impairments in 75-85 differentially methylated DNA regions in the red blood cells sampled from adult male American alligators (*Alligator mississippiensis*) living in POP and metal contaminated lakes (Guillette et al., 2016). Genes associated with the differentially methylated DNA regions were within pathways of endocrine relevance. *In vitro* and *in vivo* testing methods of epigenomic endpoints for evaluating endocrine disruption were reviewed as part of the OECD *Detailed Review Paper on the State of the Science on Novel* In Vitro *and* In Vivo *Screening and Testing Methods and Endpoints for Evaluating Endocrine Disrupters* (OECD, 2012; Greally and Jacobs,

2013) and in the last five years more relevant *in vitro* test systems, and human cancer data are now available (Jacobs et al., 2017; Parfett and Desaulniers, 2017; Alavian-Ghanini and Rüegg, 2017) and are being integrated into OECD work on non-genotoxic carcinogenesis, including endocrine modes of carcinogenesis. In summary, there is scope for epigenetic factors to have a role in some types of endocrine disruption. Assays for these effects are currently being discussed but have not yet been standardised by the OECD.

A.6.2. Scope of assessment and restriction to single assays

16. This GD does not present a testing strategy as it is restricted to a single step when further testing is recommended or proposed for consideration. It only recommends the most appropriate assay that could be performed if authorities need more evidence to support a regulatory decision. The proposed guidance is not meant to encourage animal testing. It encourages the maximal use of all existing information consistent with the OECD's integrated approaches to testing and assessment (OECD, 2008).

17. The level of confidence about whether or not a compound impacts endocrine function will increase with combined lines of pertinent evidence from multiple studies and endpoints across taxa, and which encompass different life stage effects and a range of doses. The amount of evidence needed to decide whether a substance is an ED in a regulatory context will depend on different authorities' policies/frameworks and the regulatory decision context. For example, results from a particular test or building block may suffice when taking a decision for priority setting but may not be adequate for more predictive hazard identification/characterisation.

18. Detailed guidance is not given on the conduct of WOE evaluations, or the relevance for human health of results from the assays considered. Section B.5 provides a summary of current WOE guidance that may be helpful for endocrine assessment. It is acknowledged that some mechanisms of action in rodents may not be relevant for humans, but the human relevance of specific mechanisms is not discussed.

19. Furthermore, the guidance does not consider exposure; however, this should be included when deciding whether further testing is needed in order to avoid unnecessary animal tests. This may be particularly relevant to non-mammalian wildlife where environmental hazard assessment aims at deriving a safe exposure level in the form of a predicted no-effect concentration (PNEC) or an environmental quality criterion/ environmental quality standard for the chemical. Traditionally the approach aims at the protection of all species in the relevant environmental compartment. For this purpose it is relevant to compare the sensitivity of several species in the compartment in question. If data are available on potential ED effects in more than one species/taxon, further testing may first be performed with the most sensitive species/taxon provided that it is possible to identify this taxon based on available information. Lastly, as in any evaluation, it is essential that the degree of confidence and uncertainty be communicated in the characterisation of the conclusions.

A.6.3. Rationale for assay inclusion

20. Detailed guidance is provided on the validated[2] and/or mainly widely accepted assays in the 2017 revised CF; these are listed in Parts A and B of Table A.1. Those assays listed under (A) are established methods, either with endocrine active substance (EAS)-specific endpoints or with non-specific sensitivity to EASs, which have been validated and published as OECD test guidelines. Assays listed under (B) have not received full validation by the OECD, or are in the process of OECD validation, or are guidelines which

have been validated and published by other organisations. The terms "validation" and "validated assays" are used as defined in the OECD GD on the "Validation and international acceptance of new or updated test methods for hazard assessment" (OECD, 2005) (see also Glossary). Validation may have been conducted by the OECD or other organisations (e.g. the Interagency Coordinating Committee on the Validation of Alternative Methods [ICCVAM]). Note that the word "assay" is used here to be consistent with the terminology used in the CF and describes a "test method" as defined in OECD (2005): "a test method is an experimental system that can be used to obtain a range of information from chemical properties through the adverse effects of a substance. The term 'test method' may be used interchangeably with 'assay' for ecotoxicity as well as for human health studies". The word "screen" is used in this document to describe *in vitro* or *in vivo* assays which primarily provide information on an endocrine disruption mechanism, and also occasionally information on adverse effects for use in hazard identification/characterisation. However, some regulatory authorities may wish to use positive screening tests for preliminary hazard identification/characterisation. Screens are generally rapid, and often simple, test methods and may have a truncated response range. On the other hand, the word "test" covers *in vivo* assays which can provide evidence to support a conclusion that a chemical is an endocrine disrupter that may cause adverse effects in an intact organism. An example of a screen would be the estrogen binding assay which only measures receptor binding activity *in vitro*, whereas an example of a test would be the Medaka Extended One-Generation Reproduction Test (MEOGRT), which measures reproductive success in intact fish. "Screen" and "test" are also broadly defined in OECD (2005), but here the word "test" is used more precisely, see the Glossary for all terms.

21. All of the assays in Parts A and B of Table A.1 are now included in the 2017 revised CF. Assays with non-specific sensitivity to EAS (e.g. OECD TG 408 repeated dose 90-day oral toxicity study in rodents and OECD TG 451-3 combined chronic toxicity/carcinogenicity studies) contain relevant endocrine endpoints (e.g. weights and histopathology of sex organs), and are used as such for REACH (OECD TG 408) and pesticide dossier evaluation in the EU, for example. OECD TG 453 (combined chronic toxicity/carcinogenicity studies) provides information on carcinogenicity in endocrine tissues and is therefore very important for endocrine assessment of chemicals. OECD TG 421 (reproduction/developmental toxicity screening test) and OECD TG 422 (combined repeated dose toxicity study with the reproduction/developmental toxicity screening test) provide information on reproduction in addition to effects on endocrine organs and are also used for REACH.

22. The TGs in the CF are also included in regional testing frameworks, such as the United States Environmental Protetion Agency's (US EPA) Endocrine Disruptor Screening Program (EDSP), although it should be noted that the two-tier structure of the EDSP differs from the CF paradigm. All of the US EPA TGs within this tiered testing strategy utilise the CF assays. Further information on the EDSP can be found in Section B.6.

23. Assays in Parts A and B of Table A.1 and the modalities they can detect (of E, A, T, S) are shown in Table A.2. This table is intended to be a guide only and does not reflect which assays are most relevant or have the most endpoints for detecting these modalities. A more detailed listing of endpoints and their responses can be found in Table B.1.

A.6.4. Rationale for assay exclusion

24. Assays mentioned in the 2017 revised CF but not covered in this document are listed in Part C of Table A.1. Guidance for these assays has generally been omitted either

because they have not yet started validation (e.g *in vitro* assays for determining disruption of thyroid function), there is insufficient experience in their use (e.g. invertebrate life cycle assays), they are thought not to offer significant advantages over existing tests (e.g. fish hepatocyte vitellogenin assay) or because they failed validation (e.g. MCF-7 cell proliferation assay). The list has been expanded to include other non-TG *in vitro* assays in common use, regardless of their validation status.

25. Progress has been made in the development of *in vitro* screening assays for disruption of thyroid function (OECD, 2006). The thyroid scoping document (OECD, 2014) reviews several key biological mechanisms of thyroid system disruption for their "state of readiness" as candidates for validation: short term (A), intermediate (B) or long term (C). *In vitro* assays identified as A or B are listed in Part C of Table A.1. *In vitro* assays with long-term validation plans will not be further discussed here. The thyroperoxidase (TPO) assay is now included in the high throughput screens (HTS) conducted by the US EPA (https://actor.epa.gov/dashboard; https://pubchem.ncbi.nlm.nih.gov), and a QSAR model predicting TPO inhibition based on a comprehensive training set of this HTS has recently been published (Paul et al., 2014; Rosenberg et al., 2016). The US EPA has now developed several high throughput thyroid screening assays and the EU Joint Research Centre is currently initiating a validation study involving EURL ECVAM's European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) to assess the performance of a number of assays for disturbance of thyroid hormone function.

26. *In vitro* and *in vivo* assays for disruption of the function of the thyroid hormone system were discussed in a recent workshop on thyroid disruption. The output of the workshop is available and has helped to inform further assay development (EU, 2017).

27. The yeast estrogen (YES) and yeast androgen screens have also not been included in Parts A or B of this OECD guidance, although they are commonly used as *in vitro* screens in ecotoxicology (Routledge and Sumpter, 1996; Sohoni and Sumpter, 1998). They may suffer from limitations such as problems with materials that have fungicidal activity or inhibit cell proliferation, solubility, permeability or transport issues across the cell wall (ICCVAM, 2003). It has also been reported that the YES assay is not sensitive for anti-estrogenic chemicals (Fang et al., 2000) The detailed review paper on "Environmental endocrine disrupter screening: The use of estrogen and androgen receptor binding and transactivation assays in fish" (OECD, 2010a) also describes these assays.

28. However, in spite of these limitations, they are widely used as they are easy to handle and require no sophisticated lab equipment. Furthermore, in the absence of complex gene-regulating networks in the yeast cells, no cross talk is possible between other hormonal pathways and only the respective hormonal signalling (i.e. receptor binding) is captured. Variants of the yeast-based assays (*Saccharomyces cerevisiae* and *Arxula adeninivorans*) carrying the human ERα-receptor have recently been standardised within the International Organization for Standardization (ISO): ISO 19040 series: Determination of the estrogenic potential of water and waste water,[3] together with human cell line based transactivation assays.

- ISO 19040-1. Water quality – Determination of the estrogenic potential of water and waste water – Part 1: Yeast estrogen screen (YES, *Saccharomyces cerevisiae*)
- ISO 19040-2. Water quality – Determination of the estrogenic potential of water and waste water – Part 2: Yeast estrogen screen (A-YES, *Arxula adeninivorans*)
- ISO 19040-3. Water quality – Determination of the estrogenic potential of water and waste water – Part 3: *In vitro* human cell-based reporter gene assay.

29. The three ISO standards have a similar core. This core covers issues such as scope, sample handling, glassware, etc. ISO 19040-1 and ISO 19040-2 use two different yeast species. Various properties differ between these species, e.g. ISO 19040-2 is more salt tolerant (used for seawater). They also differ in media and processing requirements, etc. ISO 19040-3 is a generic standard that covers human cell lines (not yeast). Any human cell line that meets the criteria of the standard (e.g. the EC50 of the reference), would be a valid cell line for this standard.

30. The YES assays will be finalised as ISO standards in 2018. The YES is therefore included in the CF. ISO may take on the validation of the androgen receptor transactivation assays (including the yeast androgen screen [YAS] assays) in the future. If this happens, this process will likely take several years to complete.The YES and YAS assays could be considered to be the functionally similar to the ER and the AR stably transfected transactivation assay (STTA) assays and many of the possible next steps to be taken would be the same. These "building blocks" could therefore be used cautiously to provide guidance for the YES and YAS assays, but noting the limitations described above. The guidance for the ER STTA (OECD TG 455) would cover the YES assay and is given in Section C.1.2. The guidance for the AR STTA would cover the YAS assay and is given in Section C.1.3.

31. Other nuclear hormone receptor-based transactivation assays have also become more commonly used in research, including the aryl hydrocarbon receptor, the peroxisome proliferator-activated receptor (alpha and gamma), the liver X receptor, the vitamin D receptor, retinoid receptors (retinoid X receptor, retinoic acid receptor), the constitutive androstane receptor, the pregnane X receptor and the growth hormone receptor. Although none of these assays have been formally validated, many of them are included in high throughput screens conducted by the US EPA and have now become publically available.[4]

32. Guidance about tests that are based on the induction of proliferation (e.g. the E-screen where proliferation in estrogen-responding cells, particularly in the MCF-7 human breast cancer cell line), is used to detect estrogenic activity (Soto and Sonnenschein, 2001) is also not included. Proliferation assays are not recommended by the ICCVAM (2003) because cell proliferation can be mediated through pathways other than those involving transcriptional activation of estrogen responsive genes. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods in the United States co-ordinated an international interlaboratory validation study of a MCF-7 cell proliferation test method. The validation study was completed in 2011. Although accuracy of the ER agonist protocol was high at the lead laboratory and sufficient in the partner labs, interlaboratory reproducibility was insufficient for the method to proceed further. The study indicated that the test method protocols, especially the antagonist protocol, required additional development before this method could be considered validated (https://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/endocrine-disruptors/index.html).

Table A.1. **Screens and tests for which guidance is provided in this document**

The definitions of "screen" and "test" are given in Section A.6.3 and the Glossary.

Those assays listed under (A) are established methods, either with endocrine active substance-specific endpoints or with non-specific sensitivity to endocrine active substances, which have been validated and published as OECD test guidelines. Assays listed under (B) have not received full validation by the OECD, or are in the process of OECD validation, or are guidelines which have been validated and published by other organisations. Guidance for both assay types can be found in Section C of this guidance document, but it is important to note that this guidance is not yet definitive for those assays still undergoing validation. Assays listed in this table under (C) are largely those appearing in the Conceptual Framework (as revised in 2017) but for which no guidance is provided because they are not yet the subject of a guideline, or assays where there is insufficient experience in their use with endocrine active substances. Guidance for assays under (C) will be written when the validation of the assays is more advanced. All assays have been sorted according to which level they should occupy in the CF, although some do not yet appear in it.

It is important to bear in mind that the CF (see Section A.2) is not a testing strategy to be followed linearly from Level 1 through to Level 5, although in cases where little or no information is available (i.e. for new chemicals), it could provide guidance about where to start testing. Level 1 gathers together existing information, Level 2 covers *in vitro* mechanistic assays, Level 3 covers *in vivo* assays providing some data about selected endocrine mechanism(s)/pathway(s) (in some cases they also provide information about generally recognised hazard endpoints which, however, in some cases with other data may be robust enough for regulatory decision making), Level 4 covers *in vivo* assays providing some data on adverse effects of endocrine-relevant endpoints, and Level 5 covers *in vivo* assays which provide more comprehensive data on adverse effects over more extensive parts of an organism's life cycle.

It should be noted that the invertebrate assays generally report on apical effects of various types, and do not allow conclusions about mechanism (with the possible exception of the short-term juvenile hormone activity screening assay using *Daphnia magna*). At present, it is therefore not possible in most cases to reach conclusions about whether chemicals are endocrine disruptors in invertebrates.

Conceptual Framework level	*In vitro* screens	Mammalian *in vivo* screens and tests	Non-mammalian *in vivo* screens and tests
A. OECD test guidelines with endocrine active substance-specific endpoints or with non-specific sensitivity to endocrine active substances			
2	– OECD TG 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) *In Vitro* Assays to Detect Chemicals with ER Binding Affinity – OECD TG 455: Performance-Based Test Guideline for Stably Transfected Transactivation *In Vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists – OECD TG 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals – OECD TG 456: H295R Steroidogenesis Assay	Not applicable	Not applicable
3	Not applicable	– OECD TG 440: Uterotrophic Bioassay in Rodents (UT Assay) (including OECD GD 71 on the use of the assay to screen for anti-estrogenicity) (screen) – OECD TG 441: Hershberger Bioassay in Rats (H Assay) (including OECD GD 115 on the Weanling Hershberger Bioassay) (screen)	– OECD TG 229: Fish Short-Term Reproduction Assay (FSTRA) (screen/test) – OECD TG 230: 21-Day Fish Assay (screen) – OECD TG 231: Amphibian Metamorphosis Assay (AMA) (screen)
4	Not applicable	– OECD TG 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents (test) – OECD TG 408: Repeated Dose 90-Day Oral Toxicity Study (test) – OECD TG 451-3: Combined Chronic Toxicity/Carcinogenicity Studies (test) – OECD TG 421: Reproduction/Developmental Toxicity Screening Test – OECD TG 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (test) – OECD TG 414: Prenatal Developmental Toxicity Study (test) – OECD TG 426: Developmental Neurotoxicity Study (test) – OECD TG 410: Repeated Dose Dermal Toxicity: 21/28-Day Study (test) – OECD TG 411: Subchronic Dermal Toxicity: 90-Day Study (test) – OECD TG 412 28-Day (Subacute) Inhalation Toxicity Study (test) – OECD TG 413: Subchronic Inhalation Toxicity: 90-Day Study (test) – TG 409: Repeated dose 90-Day Oral Toxicity Study in Non-Rodents (test)	– OECD TG 242: *Potamopyrgus antipodarum* Reproduction Test (test) – OECD TG 243: *Lymnaea stagnalis* Reproduction Test (test) – OECD TG 218-219: Chironomid Toxicity Test (test) – OECD TG 211: *Daphnia* Reproduction Test (with Male Induction) (test) – OECD TG 210: Fish Early Life Stage Toxicity Test (test) – OECD TG 234: Fish Sexual Development Test (FSDT) (test) – OECD TG 241: Larval Amphibian Growth and Development Assay (LAGDA) (test) – OECD TG 206: Avian Reproduction Test (test)

Conceptual Framework level	*In vitro* screens	Mammalian *in vivo* screens and tests	Non-mammalian *in vivo* screens and tests
5	Not applicable	– OECD TG 443: Extended One-Generation Reproductive Toxicity Study (EOGRTS) (test) – OECD TG 416: Two-Generation Reproduction Toxicity Study (test)	– OECD TG 233: Sediment Water Chironomid Life Cycle Toxicity Test (test) – OECD TG 240: Medaka Extended One-Generation Reproduction Test (MEOGRT) (test)
B. Guidelines that have not received full validation by the OECD, or are in the process of OECD validation or which have been validated and published by other organisations			
2	– AR Binding Assay (US EPA OPPTS 890.1150) – Aromatase Assay (US EPA OPPTS 890.1200)	Not applicable	Not applicable
3	Not applicable	No assays in this category	– Short-Term Juvenile Hormone Activity Screening Assay Using *Daphnia magna* (draft OECD TG) (screen) – OECD GD 148: Androgenised Female Stickleback Screen (AFSS) (screen) – EASZY Assay. Detection of Substances Acting Through Estrogen Receptors Using Transgenic cyp19a1b GFP Zebrafish Embryos (draft OECD TG) (screen) – Juvenile Medaka Anti-Androgen Screening Assay (JMASA) (draft OECD GD) (screen) – *Xenopus* Embryonic Thyroid Signalling Assay (XETA) (draft OECD TG) (screen) – Rapid Androgen Disruption Adverse Outcome Reporter (RADAR) Assay (draft OECD TG) (screen)
4	Not applicable	– Pubertal Development and Thyroid Function Assay in Peripubertal Male Rats (Male PP Assay) (US EPA OPPTS 890.1500) (screen) – Pubertal Development and Thyroid Function Assay in Peripubertal female Rats (Female PP Assay) (US EPA OPPTS 890.1450) (screen)	– New Guidance Document on Harpacticoid Copepod Development and Reproduction Test with *Amphiascus* (OECD GD 201)[2] (test)
5	Not applicable	No assays in this category	– *Daphnia* Multigeneration Test for Assessment of EDCs (draft OECD TG) (test) – Fish Life cycle Toxicity Test (FLCTT) (US EPA OPPTS 850.1500) (test) – Zebrafish Extended One-Generation Reproduction Test (ZEOGRT) (draft OECD TG) (test) – Avian Two-Generation Toxicity Test in the Japanese Quail (ATGT) (US EPA OCSPP 890.2100/ 740-C-15-003) (test)

Conceptual Framework level	*In vitro* screens	Mammalian *in vivo* screens and tests	Non-mammalian *in vivo* screens and tests
C. Assays corresponding to those in the Conceptual Framework (original or revised 2017) for which no guidance has been written at present			
2	See Section A.6.4 for more details. – Thyroperoxidase inhibition – Transthyretin binding – Thyroid binding globulin binding – Sodium-iodine symporter modulation – Thyroid hormone receptor and thyroid stimulating hormone receptor modulation – T3 deiodinase inhibition – Thyroid hormone receptor transactivation – Thyroid gland explant – Stably Transfected Human Thyroid Receptor Transactivation Assay (TR STTA) – Retinoid receptor transactivation assays (retinoic acid receptor, retinoid X receptor) – Liver receptor ransactivation assays (aryl hydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, peroxisome proliferator-activated receptor, liver X receptor, vitamin D receptor) – Other hormone receptors (e.g. glucocorticoid receptor, growth hormone receptor) – Fish hepatocyte vitollegenin assay – Yeast transactivation assays (yeast estrogen screen and yeast androgen screen) – Proliferation-based screens e.g. MCF-7 cell proliferation assay (estrogen receptor ant/agonist) – High-throughput screens (see OECD Guidance Document No. 211 for Describing Non-Guideline *In Vitro* Test Methods)	Not applicable	Not applicable
3	Not applicable	No assays in this category	No assays in this category
4	Not applicable	No assays in this category	– Fish Reproduction Partial Life Cycle Test (when/if TG is available) (test) – Earthworm Reproduction Test (OECD TG 222) (test) – Enchytraeid Reproduction Test (OECD TG 220) (test) – Sediment Water *Lumbriculus* Toxicity Test Using Spiked Sediment (OECD TG 225) (test) – Predatory mite reproduction test in soil (OECD TG 226) (test) – Collembolan Reproduction Test in Soil (OECD TG 232) (test)
5	Not applicable	No assays in this category	No assays in this category

Table A.2. **List of assays for which guidance has been developed in GD 150, showing their responsiveness to various endocrine modalities**

Assay name	CF Level	Mode of action which may produce a response						
		E	A	S	T	JH	Ec	R
OECD TG 493: PBTG for Human Recombinant Estrogen Receptor (hrER) *In Vitro* Assays to Detect Chemicals with ER Binding Affinity	2	■						
OECD TG 455: PBTG for Stably Transfected Transactivation *In Vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists	2	■						
OECD TG 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals	2		■					
OECD TG 456: H295R Steroidogenesis Assay	2			■				
AR Binding Assay (US EPA OPPTS 890.1150)	2		■					
Aromatase Assay (US EPA OPPTS 890.1200)	2			■				
OECD TG 440: Uterotrophic Bioassay in Rodents	3	■						
OECD TG 441: Hershberger Bioassay in Rats	3		■		■			
OECD TG 229: Fish Short-Term Reproduction Assay	3	■	■	■				
OECD TG 230: 21-Day Fish Assay	3	■	■	■				
OECD TG 231: Amphibian Metamorphosis Assay	3				■			
OECD GD 148: Androgenised Female Stickleback Screen	3		■					
EASZY Assay: Detection of Substances Acting through Estrogen Receptors using Transgenic cyp19a1b GFP Zebrafish Embryos	3	■						
JMASA: Juvenile Medaka Anti-Androgen Screening Assay	3		■					
XETA: *Xenopus* Embryonic Thyroid Signalling Assay	3				■			
RADAR: Rapid Androgen Disruption Adverse Outcome Reporter Assay	3		■					
SJHASA: Short-Term Juvenile Hormone Activity Screening Assay Using *Daphnia magna*	3					▒		
OECD TG 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents	4	■	■	■	■			▒
OECD TG 408: Repeated Dose 90-Day Oral Toxicity Study	4	■	■	■	■			▒
OECD TG 451-3: Combined Chronic Toxicity/Carcinogenicity Studies	4	■	■	■	■			▒
OECD TG 421 and 422: Combined 28-Day Reproductive Screening Tests	4	■	■	■	■			▒
OECD TG 414: Prenatal Developmental Toxicity Study	4	■	■	■	■			▒
OECD TG 426: Developmental Neurotoxicity Study	4	■	■	■	■			▒
OECD TG 410: Repeated Dose Dermal Toxicity: 21/28-Day Study	4	■	■	■				▒
OECD TG 411: Subchronic Dermal Toxicity: 90-Day Study	4	■	■	■	■			▒
OECD TG 412: 28-Day (Subacute) Inhalation Toxicity Study	4	■	■	■	■			▒
OECD TG 413: Subchronic Inhalation Toxicity: 90-Day Study	4	■	■	■	■			▒
TG 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	4	■	■	■	■			▒
Pubertal Development and Thyroid Function Assay in Peripubertal Male Rats (US EPA OPPTS 890.1500)	4	■	■	■	■			▒
Pubertal Development and Thyroid Function Assay in Peripubertal female Rats (US EPA OPPTS 890.1450)	4	■	■	■	■			▒
OECD TG 210: Fish Early Life Stage Toxicity Test	4				▒			
OECD TG 234: Fish Sexual Development Test	4	■	■	■	▒			
OECD TG 241: Larval Amphibian Growth and Development Assay	4	■	■	▒	■			
OECD TG 206: Avian Reproduction Test	4	▒	▒	▒	▒			
OECD TG 242: *Potamopyrgus antipodarum* Reproduction Test	4							▒
OECD TG 243: *Lymnaea stagnalis* Reproduction Test	4							▒
OECD TG 218-219: Chironomid Toxicity Test	4					▒	▒	
OECD TG 211: *Daphnia* Reproduction Test (with male induction)	4					▒	▒	

Table A.2. **List of assays for which guidance has been developed in GD 150, showing their responsiveness to various endocrine modalities** (*continued*)

Assay name	CF Level	Mode of action which may produce a response						
		E	A	S	T	JH	Ec	R
OECD GD 201: New Guidance Document on Harpacticoid Copepod Development and Reproduction Test with *Amphiascus*	4							
OECD TG 416: Two-Generation Reproduction Toxicity Study	5							
OECD TG 443: Extended One-Generation Reproductive Toxicity Study	5							
OECD TG 240: Medaka Extended One-Generation Reproductive Toxicity Study	5							
FLCTT: Fish Life Cycle Toxicity Test (US EPA OPPTS 850.1500)	5							
ZEOGRT: Zebrafish Extended One-Generation Reproduction Test	5							
ATGT: Avian Two-Generation Toxicity Test in the Japanese Quail (US EPA OCSPP 890.2100/ 740-C-15-003)	5							
OECD TG 233: Sediment Water Chironomid Life Cycle Toxicity Test	5							
DMGT: *Daphnia* Multigeneration Test for Assessment of EDCs	5							

Notes: Dark blue = some endpoints responsive to and diagnostic of modality; light blue = endpoints responsive to but not diagnostic of modality. This table is intended to be a guide only and does not reflect which assays are most relevant or have the most endpoints responsive to these modalities. Modality abbreviations: E,A,T,S: estrogen/androgen/thyroid/steroidogenesis; JH: juvenile hormone, Ec: ecdysone, R: retinoid, -related modalities.

Notes

1. Endocrine disruption-responsive assays are those *in vitro* or *in vivo* assays whose endpoints are known to respond positively to endocrine disrupters and/or endocrine active substances.
2. These are assays which have been validated at the national or international level, especially as OECD TGs.
3. See, for example, https://www.iso.org/standard/64451.html.
4. See, for example, https://actor.epa.gov/dashboard and https://pubchem.ncbi.nlm.nih.gov.

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