



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Endocrine disrupting chemicals within EU legal frameworks: environmental perspective

RIVM Letter report 2016-0145
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Colophon

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Z. Dang (author), RIVM
E. Smit (author), RIVM
P. van Vlaardingen (author), RIVM
C. Moermond (author), RIVM
C. Bodar (author), RIVM

Contact:
Zhi-Chao Dang
zhichao.dang@rivm.nl

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This is a publication of:
**National Institute for Public Health
and the Environment**
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

Endocrine Disrupting Chemicals in the EU Legal Frameworks: environmental perspective

The European Commission recently proposed the criteria for identifying endocrine disrupting chemicals for both human health and the environment. This is an important step forward, but RIVM points out that the environmental data requirements in the current legislation will not supply enough information for this identification. There is a need for an intelligent testing strategy that allows to focus quickly on the chemicals of concern. Moreover, more harmonization between the various legal frameworks is necessary.

Endocrine disrupting chemicals (EDCs) pose a threat to humans and the environment. Current data requirement in different legal frameworks focus on the adverse effects of chemicals. However, they supply little information on the mechanisms of action of chemicals and on how the observed effects are mediated. These two elements are essential to identify a chemical as an EDC in accordance with the Commission's proposal. RIVM considers that these data gaps will hamper authorities in tackling EDCs. The European Commission also proposes that all available scientific data should be considered in a systematic manner when evaluating a chemical. According to RIVM, however, the current regulatory process of substance evaluation is not suited to do so.

Endocrine disruption has now been recognized as a concern in several European legal frameworks that aim to reduce the risks of chemicals: REACH, biocides, plant protection products, and (veterinary) medicines. RIVM notes that there are differences among these frameworks. Risk management measures for EDCs are more stringent in some legal frameworks compared to others. RIVM therefore seriously calls for a better harmonization of legal frameworks at the European level.

The current environmental risk assessment and hazard classification of chemicals is mainly based on population relevant effects on organisms in ecosystems, such as mortality, growth and reproduction. Studies on endocrine disruption also determine other effects, such as changes in the protein vitellogenin that is essential for the development of yolk sac in fish. Whether such effects threaten the entire population is often not fully clear but RIVM strongly suggests that they should also be considered when evaluating chemicals.

Keywords: chemicals, endocrine disruption, risk assessment, legal frameworks

Publiekssamenvatting

Hormoonverstorende stoffen in Europese wet- en regelgeving: milieu-aspecten

De Europese Commissie stelde onlangs criteria voor op basis waarvan hormoonverstorende stoffen als zodanig kunnen worden geïdentificeerd. De criteria betreffen niet alleen hormoonverstoring bij de mens, maar ook in het milieu. Dit is een stap vooruit, maar het RIVM constateert dat de datavereisten in de huidige wet- en regelgeving hier niet goed op aansluiten. Het pleit voor een slimme teststrategie zodat snel kan worden ingezoomd op de stoffen die het eerst moeten worden aangepakt. Bovendien is een betere afstemming tussen de diverse wettelijke kaders noodzakelijk om het gewenste doel te bereiken.

Hormoonverstorende stoffen vormen een bedreiging voor mens en milieu. De huidige, verplichte dossiervereisten zijn erop gericht schadelijke effecten van stoffen te bepalen. Ze zeggen echter meestal weinig over het precieze werkingsmechanisme van de stof en de manier waarop de waargenomen effecten tot stand komen. Deze twee elementen zijn juist nodig om een stof volgens het commissievoorstel als hormoonverstorend te identificeren. Het RIVM verwacht dat dit kennishiaat overheden zal belemmeren om deze stoffen snel te kunnen aanpakken. De Europese commissie stelt ook dat alle beschikbare wetenschappelijke gegevens op een systematische manier moeten worden meegewogen bij het beoordelen van een stof. Volgens het RIVM is het huidige proces van stofbeoordeling daar echter niet op ingericht.

Hormoonverstoring is inmiddels als aandachtspunt opgenomen in diverse Europese wettelijke kaders die erop zijn gericht de risico's van chemische stoffen te beperken: REACH, biociden, gewasbeschermingsmiddelen en (dier)geneesmiddelen. Het RIVM constateert dat er verschillen zijn tussen deze kaders. De beperkingen voor het gebruik van een hormoonverstorende stof zijn in het ene beleidskader soms aanmerkelijk strenger dan voor dezelfde stof in een ander kader. Het RIVM pleit nadrukkelijk voor een betere harmonisatie op Europees niveau.

De huidige milieurisicobeoordeling en gevaarsindeling van stoffen is vooral gebaseerd op effecten die direct doorwerken op de populaties van organismen in ecosystemen, zoals sterfte, groei en voortplanting van organismen. Studies naar hormoonverstoring meten ook andere effecten, zoals veranderingen in de eiwitten die nodig zijn voor de ontwikkeling van de eidooier in vissen. Bij dit soort effecten is het niet eenvoudig te bepalen of ze de hele populatie bedreigen. Het RIVM vindt echter dat deze bredere effecten ook moeten worden meegewogen bij de beoordeling van stoffen.

Kernwoorden: chemische stoffen, hormoonverstoring, risicobeoordeling, wettelijke kaders

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Summary

Endocrine disrupting chemicals (EDCs) pose a threat to humans and the environment. The European Commission recently proposed the criteria for identifying endocrine disrupting chemicals. From an environmental viewpoint, this study focuses on the implications of this proposal and on other EDC-related aspects within the various EU legal frameworks on chemical risk management.

Endocrine disruption has now been recognized as a concern in several European legal frameworks that aim to reduce the risks of chemicals: REACH, biocides, plant protection products, and (veterinary) medicines. RIVM notes that there are differences among these frameworks. Risk management measures for EDCs are more stringent in some legal frameworks compared to others. RIVM therefore seriously calls for a better harmonization of legal frameworks at the European level.

Current environmental data requirements in the above-mentioned frameworks target on the adverse effects of chemicals. However, they supply little information on the mechanisms of action of chemicals and on how the observed effects are mediated. These two elements are essential to identify a chemical as an EDC in accordance with the Commission's proposal. RIVM considers that these data gaps will hamper authorities in tackling EDCs.

The European Commission also proposes that all available scientific data should be considered in a systematic manner when evaluating a chemical on its potential EDC properties. However, the current regulatory process of substance evaluation in these legal frameworks is not suited to do so. RIVM concludes that there is a need for a smart testing strategy that allows to focus quickly on the chemicals of concern

The current environmental risk assessment and hazard classification of chemicals is mainly based on population relevant effects on organisms in ecosystems, such as mortality, growth and reproduction. Studies on endocrine disruption also determine other effects, such as changes in the protein vitellogenin that is essential for the development of yolk sac in fish. Whether such effects threaten the entire population is often not fully clear, but RIVM strongly suggests that they should also be considered when evaluating chemicals.

In case there is a broad consensus that additional information for a chemical with endocrine disrupting properties is needed, it is a serious challenge now to actually make the necessary steps in further testing. Procedural limitations in the current legal frameworks interfere with smoothly obtaining further, conclusive information. In REACH, for example, it often takes around one year to include a chemical of interest in the substance evaluation process. Then about one year is needed for agreement on a testing proposal, followed by at least two years for executing, reporting and evaluating tests. The above-mentioned smart testing strategy may hopefully contribute to a more fluent process on the regulation of EDCs

1 Introduction

1.1 Endocrine disrupting chemicals in a legal context

An Endocrine Disrupting Chemical (EDC) is referred to as “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (WHO, 2002). EDCs are suspected of having severe health and environmental impacts. Therefore, EDCs have been included in several pieces of European Union (EU) legislation. Examples of the legal frameworks are the regulation on industrial chemicals (Registration, Evaluation, Authorization and restriction of Chemicals, EC 1907/2006, REACH), the Plant Protection Products Regulation (EC 1107/2009, PPPR), and the Biocidal Products Regulation (EU 528/2012, BPR). As a general rule for BPR and PPPR, a chemical identified as an EDC is banned on the basis of hazard, although in some cases derogations, considering risks or socio-economic issues, may apply (EC, 2016a). A chemical identified as an EDC under REACH could be subject to authorisation, where a risk assessment or socio-economic analysis is needed depending on whether a threshold (safe level) or non-threshold approach is to be applied. Apparently, identification and risk assessment form a basis for regulating EDCs, but the question is if the focus, approaches and consequences are consistent within the different EU legal frameworks.

1.2 Identification of EDCs

The European Commission recently proposed to endorse the WHO definition and published criteria to identify EDCs in the field of plant protection products (PPP) and biocides (EC, 2016b, 2016c). In line with the WHO definition, the identification criteria embody three key elements: adverse effects (adversity), endocrine mode or mechanism of action (MOA) and the underlying biological plausible relationship between these two. These key elements need to be supported by experimental data in intact animals, some of which are requested by EU legal frameworks. In some cases, additional testing is possible when there is an indication of concerns. So far, it is unknown whether standard information requirements of legal frameworks are enough for the identification of EDCs and if needed, whether these data will give enough indication of concerns for additional testing.

1.3 Risk assessment of EDCs

Risk assessment of chemicals typically falls into two areas: a risk assessment for human health and an environmental risk assessment (ERA). Both assessments are almost exclusively based on adverse apical¹ responses in test organisms. The human health risk assessment

¹ Apical endpoint:

- Traditional, directly measured whole-organism outcomes of exposure in in vivo tests, generally death, reproductive failure, or developmental dysfunction.
- Observable effects of exposure to a toxic chemical in a test animal. The effects reflect relatively gross changes in animals after substantial durations of exposure.
- An observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant.

relies on data from mammalian studies (rats, mice, rabbits, monkeys, dogs, etc) to draw inference about the potential hazard to humans. For ERA, data from laboratory toxicity tests with fish, daphnids and algae are usually used. Whereas the human health risk assessment is aimed at the protection of individuals, ERA aims at protecting ecosystems through the protection of populations and communities. Only population relevant endpoints, like survival, growth, development, and reproduction endpoints in tested organisms are used when deriving predicted no effect concentrations (PNEC)² from single species laboratory tests. Testing for endocrine MOAs often reveals sensitive endpoints for which the relationship with population level effects is unclear. However, considering that interference with the endocrine system is a major concern, the question is raised whether such endpoints should be used for risk assessment. In the past decades, several other issues have been identified in relation to EDCs that challenge the current procedures for toxicity testing and risk assessment. Among these are mixture toxicity, low dose effects, non-monotonic dose response relationships, and delayed and transgenerational effects (Munn and Goumenou, 2013; EFSA, 2013; Matthiessen et al., 2016).

1.4 Aim and scope of this report

With the focus on the environment, this report intends to summarise the EDC-related aspects of EU legal frameworks and accompanying guidelines, to analyse the challenges of regulating EDCs, and to make recommendations for policy and future research. -In parallel to this environmental study a comparable RIVM report is published on human health perspectives of EDC (Graven *et al*, 2016)-. Chapter 2 gives an overview of the status of EDCs in several EU legal frameworks. Chapter 3 discusses challenges of regulating EDCs, with the focus on key issues related to identification, classification, PBT (persistence, bioaccumulation, and toxicity) assessment, risk assessment and testing. Finally, recommendations are made for how to face challenges for regulating EDCs (Chapter 4).

² The term varies in different legal frameworks. For the sake of consistency, it is used throughout the report.

2 EU legal frameworks for regulating EDCs

2.1 Regulation of EDCs in EU frameworks

Several pieces of EU chemicals legislation and accompanying guidelines address EDCs from the environmental perspective. There are, however, differences in wording and in regulatory consequences in relation to EDCs among these legal frameworks. The recent publication of draft legal texts setting out criteria for the determination of ED properties for implementation in the BPR and PPPR framework brings new elements and consequently brings new regulatory consequences. These are summarised in Table 1 and described in the following sections.

2.1.1 REACH

Chemicals with endocrine disrupting properties are targeted with REACH. If a chemical is an endocrine disruptor it may be considered to give rise to an equivalent level of concern (ELoC) for both human health and the environment as the other criteria for substances of very high concern (SVHC) listed in Article 57f. This implies that identification of EDC and ELoC consideration are needed for inclusion of EDCs in the SVHC list. An EDC of ELoC that is once placed on the SVHC list is then subject to the Authorisation. Currently, there are still uncertainties on whether a safe level or threshold can be determined for EDCs. Without safe levels an authorisation of EDCs may only be granted if, the exposure is as low as possible and if it is shown that socio economic benefits outweigh the impact to human health or the environment arising from the use of the chemical. Consideration of suitable alternative chemicals or technologies is part of the socio economic analysis. According to the recently published Communication, the Commission will finalise and present the review whether or not a threshold is applied to EDCs by the end of 2016 (EC, 2016a). In summary, both identification and risk assessment of EDCs are essential for regulatory actions under REACH. If EDCs are considered as non-threshold chemicals, a socio economic analysis would be needed.

2.1.2 Plant Protection Products Regulation(PPPR)

The PPPR explicitly addresses chemicals with endocrine disrupting properties from the environmental perspective (Table 1). In the draft amendment to the Regulation published this year (EC, 2016b), point 3.8.2 is replaced by the identification criteria in non-target organisms and key points for the interpretation of these criteria (Table 1). One important point in the draft legal text is that for non-target organisms the definition of adversity is focused at the population level. There is an unless-clause on negligible exposure of non-target organisms under the proposed use conditions. This negligible exposure is replaced by negligible risk in the draft Regulation. However, there is no guidance that further explains what is meant by negligible risk. This derogation option is in line with the BPR, where negligible risk is also included. According to the Communication published by the Commission in accompany with the draft Regulation, a chemical identified as an EDC is banned on the basis of hazard, although in some cases derogations, considering risks or socio-economic issues, may apply (EC, 2016a).

2.1.3 *Biocidal Products Regulation (BPR)*

It is noted that the current BPR does not specifically refer to EDCs from the environmental perspective. However, the BPR refers to REACH in the exclusion criterion dealing with EDCs, and the latter includes environmental EDCs. As industrial chemicals are not designed to target specific biological MOAs, the reference on EDCs in the BPR to REACH appears vague in view of biocides that in most cases have specific and intended MOAs to serve their purpose, and possibly also specific endocrine MOAs e.g. targeting ecdysteroid and juvenile hormones. In the draft amendment for the Regulation, endocrine disrupting properties with respect to non-target organisms is defined by including identification criteria and key points for interpretation of these criteria (Table 1). This specification, similar to that of PPPR, highlights the importance of EDCs from the environmental perspective. Similar to REACH and PPPR, identification of EDCs is critical to the implementation of BPR, as a positive identification is an exclusion criterion for approval of active chemicals in biocides. The unless clauses in the draft amendment to the Regulation are the same as before. Similar to PPPR, a chemical identified as an EDC is banned on the basis of hazard, although in some cases derogations, considering risks or considering socio-economic issues, may apply (EC, 2016a).

Table 1. Overview of legal frameworks addressing EDCs from an environmental perspective.

Framework	Specified in	Quotations from the legal text or guideline document
REACH	EC 1907/2006, Article 57, Substances to be included in Annex XIV, paragraph 57(f)	The following substances may be included in Annex XIV (...): Substances - such as those having endocrine disrupting properties (...) for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern (...).
	Article 138, paragraph 7, Review	The Commission shall carry out a review to assess whether or not, taking into account latest developments in scientific knowledge, to extend the scope of Article 60 (3) (socio-economic route) to substances identified under Article 57 (f) as having endocrine disrupting properties. On the basis of that review the Commission may, if appropriate, present legislative proposals.
Plant Protection Products Regulation	EC 1107/2009, Article 23, Approval criteria for basic substances, paragraph 1b, Annex II, Procedure and criteria for the approval of active substances, safeners and synergists, point 3.8.2	<p>(...) a basic substance is an active substance which (...) does not have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects.</p> <p>An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible.</p>
	Draft amendment point 3.8.2 of Annex II (EC, 2016 b)	<p>Point 3.8.2. is replaced by the following:</p> <p>1. As of [Date of EIF], an active substance, safener or synergist shall be identified as having endocrine disrupting properties with respect to non-target organisms if it is a substance that meets all of the following criteria:</p> <p>(1) it is known to cause an adverse effect for non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences, considered relevant at the population level;</p> <p>(2) it has an endocrine mode of action;</p> <p>(3) the adverse effect relevant for the non-target organism at the population level is a consequence of the</p>

Framework	Specified in	Quotations from the legal text or guideline document
		<p>endocrine mode of action.</p> <p>2. The identification of an active substance, safener or synergist as having endocrine disrupting properties in accordance with point 1 shall be based on all of the following:</p> <p>(1) all available relevant scientific evidence:</p> <p>(a) primarily performed according to internationally agreed study protocols (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo, in vitro and mechanistic studies informing about endocrine modes of action), in particular, on those internationally agreed study protocols listed in the Commission Communications in the framework of setting out the data requirements for active substances and plant protection products, in accordance with Regulation (EC) No 1107/2009,</p> <p>(b) applying a systematic review methodology, in particular following guidance listed in the Commission Communications in the framework of setting out the data requirements for active substances and plant protection products, in accordance with Regulation (EC) No 1107/2009, to analyse other relevant scientific information.</p> <p>(2) a comparison of the weight of the scientific evidence on endocrine mediated adverse effects with the criteria set out in point 1, considering whether or not the effects are adverse, the mode of action, together with the biological plausibility of the causal link between the adverse effect and the endocrine mode of action.</p> <p>(3) in applying the weight of evidence determination referred in point 2, using expert judgement and internationally agreed guidelines, all of the following elements shall be considered:</p> <p>(a) The assessment of quality, reliability, reproducibility and consistency of the scientific evidence shall consider all of the following factors:</p> <p>i. Both positive and negative results shall be considered together in a single weight of evidence determination, discriminating between taxonomic groups (e.g. mammals, birds, fish) where relevant.</p> <p>ii. The weight of evidence should consider the relevance of the study designs, for relevance of the adverse effects at the population level, and for the evaluation of mechanistic information. Generally, evidence from field studies shall have precedence over other data. Nevertheless positive results from well-conducted laboratory studies shall be considered even in the case of lack of positive results in field studies.</p> <p>iii. The adverse consequences on reproduction and growth/development, as these are the effects most likely to impact on populations. Adequate, reliable and representative higher tier experimental studies and/or results from reliable population models shall be considered where available for assessing the relevance of the adverse effect at the population level.</p> <p>iv. The biological plausibility of the link between the adverse effects and the endocrine mode of action, and its relevance for populations of non-target organisms.</p> <p>v. The quality and consistency of the data shall be given appropriate weight, considering the pattern and coherence of the results within and between studies of a similar design and across different taxonomic groups.</p>

Framework	Specified in	Quotations from the legal text or guideline document
		<p>vi. The concept of the limit dose and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity.</p> <p>(b) Adverse effects or endocrine modes of action that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms.</p> <p>(c) Where there is information demonstrating that the adverse effects are clearly not relevant at the population level for non-target organisms, the substance should not be considered a endocrine disruptor with respect to non-target organisms. 3. An active substance, safener or synergist shall only be approved if it is not identified as having endocrine disrupting properties according to the criteria specified above, unless the risk from exposure of the non-target organisms to that active substance, safener or synergist in a plant protection product, under realistic worst case proposed conditions of use, is negligible."</p>
Biocidal Products Regulation	EU 528/2012, Article 5, Exclusion criteria, paragraph 1d, 2a-c	<p>(..) the following active substances shall not be approved: active substances which are considered as having endocrine-disrupting properties that may cause adverse effects in humans or which are identified in accordance with Articles 57(f) and 59(1) of Regulation (EC) No 1907/2006 as having endocrine disrupting properties; active substances (...) may be approved if it is shown that at least one of the following conditions is met: (a) the risk to humans, animals or the environment from exposure to the active substance in a biocidal product, under realistic worst case conditions of use, is negligible (...) (b) it is shown by evidence that the active substance is essential to prevent or control a serious danger to human health, animal health or the environment; or (c) not approving the active substance would have a disproportionate negative impact on society when compared with the risk to human health, animal health or the environment arising from the use of the substance.</p>
	Section B - Endocrine disrupting properties with respect to non-target organisms (EC, 2016c)	<p>1. An active substance shall be identified as having endocrine disrupting properties with respect to non-target organisms if it is a substance that meets all of following criteria: (1) it is known to cause an adverse effect for non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences, considered relevant at the population level; (2) it has an endocrine mode of action; (3) the adverse effect relevant for the non-target organism at the population level is a consequence of the endocrine mode of action.</p> <p>2. The identification of an active substance as having endocrine disrupting properties in accordance with point 1 shall be based on all of the following:</p>

Framework	Specified in	Quotations from the legal text or guideline document
		<p>(1) all available relevant scientific evidence:</p> <p>(a) primarily performed according to internationally agreed study protocols (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo, in vitro and mechanistic studies informing about endocrine modes of action) and on Guidance on the implementation of Regulation (EU) No 528/2012, issued by the European Chemicals Agency;</p> <p>(b) applying a systematic review methodology to analyse other relevant scientific information.</p> <p>(2) a comparison of the weight of the scientific evidence on endocrine mediated adverse effects with the criteria set out in point 1, considering whether or not the effects are adverse, the mode of action, together with the biological plausibility of the causal link between the adverse effect and the endocrine mode of action.</p> <p>(3) in applying the weight of evidence determination referred in point 2(2), using expert judgement and internationally agreed guidelines, all of the following elements shall be considered:</p> <p>(a) the assessment of quality, reliability, reproducibility and consistency of the scientific evidence shall consider all of the following factors:</p> <p>(i) both positive and negative results shall be considered together in a single weight of evidence determination, discriminating between taxonomic groups (e.g. mammals, birds, fish) where relevant.</p> <p>(ii) the weight of evidence should consider the relevance of the study designs for the relevance of the adverse effects at the population level and for the evaluation of mechanistic information. Generally, evidence from field studies shall have precedence over other data. Nevertheless positive results from well-conducted laboratory studies shall be considered even in the case lack of positive results in field studies.</p> <p>(iii) the adverse consequences on reproduction and growth/development, as these are the effects most likely to impact on populations. Adequate, reliable and representative higher tier experimental studies and/or results from reliable population models shall be considered where available for assessing the relevance of the adverse effect at the population level.</p> <p>(iv) the biological plausibility of the link between the adverse effects and the endocrine mode of action, and its relevance for populations of non-target organisms.</p> <p>(v) the quality and consistency of the data shall be given appropriate weight, considering the pattern and coherence of the results at different doses or exposure levels within and between studies of a similar design and across different taxonomic groups.</p> <p>(vi) the concept of the limit dose and international guidelines on maximum recommended doses and for assessing confounding effects of excessive toxicity.</p> <p>(b) adverse effects or endocrine modes of action that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms.</p> <p>(c) where there is information demonstrating that the adverse effects are clearly not relevant at the population level for non-target organisms, the substance should not be considered a endocrine disruptor with respect to non-target organisms.</p>

Framework	Specified in	Quotations from the legal text or guideline document
Water Framework Directive	2000/60/EC, ANNEX VIII, Indicative list of the main pollutants, point 4 Technical guidance for deriving environmental quality standards, section 2.9.1, Mode of action section 3.3.3.1, derivation of EQS	<p>Substances and preparations, or the breakdown products of such, which may affect steroidogenic, thyroid, reproduction or other endocrine-related functions in or via the aquatic environment should be in the indicative list of the main pollutants.</p> <p>If there are indications of adverse effects via endocrine activity (e.g. bioassays) or other specific effects that have not been adequately reflected in bird or mammals studies (...), an additional assessment factor may be considered to cover the anticipated effects</p> <p>When there are indications that a substance may cause adverse effects via disruption of the endocrine system of mammals, birds, aquatic or other wildlife species, the assessor should consider whether the assessment factor would be sufficient to protect against effects caused by such a mode of action, or whether a larger AF is needed</p>
Community code* relating to medicinal products for human use	Guideline on the ERA of Medicinal products for human use, Chapter 3 Chapter 4	<p>Certain substances, such as highly lipophilic compounds and potential endocrine disruptors, may need to be addressed irrespective of the quantity released into the environment.</p> <p>In some cases, the action limit may not be applicable. Some drug substances may affect the reproduction of vertebrate or lower animals at concentrations lower than 0.01 µg/L. These substances should enter Phase II and a tailored risk assessment strategy should be followed that addresses its specific mechanism of action. In these cases, the Applicant should justify all actions taken.</p>
Community code* relating to veterinary medicinal products	Guideline for veterinary medicinal products, Introduction	Some VMPs that might otherwise stop in Phase I may require additional environmental information to address particular concerns associated with their activity and use. These situations are expected to be the exception rather than the rule and some evidence in support of the concern should be available.
Classification, Labelling and Packaging Regulation	EU 1272/2008	no reference to EDCs

* the code brings together all the existing provisions in force on the sale, production, labelling, classification, distribution and advertising of medicinal products in the EU.

2.1.4 *Water Framework Directive (WFD)*

The Water Framework Directive (Directive 2000/60/EC) focuses on chemicals with significant risk to the aquatic environment. EDCs belong to this targeted category and may be listed as priority chemicals. It is not the focus of the WFD to identify EDCs and to perform a risk assessment. Instead, EDCs identified in REACH or other legal frameworks may be considered as priority hazardous chemicals in the WFD. Elimination of the emissions of such chemicals is the ultimate aim. In case of an EDC, it should be considered whether the default assessment factor (AF) would be sufficient to protect against effects caused by such an endocrine MOA, or a larger AF is needed when deriving environmental quality standards (EQS). The current WFD technical guidelines literally state that indications of adverse effects via endocrine activity (e.g. bioassays) should also be taken into account when deciding on the assessment factors that are used to derive such standards (EC, 2011).

2.1.5 *Pharmaceuticals*

The Directive 2001/83/EC and Directive 2001/82/EC specify the request of ERAs for human and veterinary pharmaceuticals, respectively. These directives, however, do not specify details in terms of standard data requirements and risk assessment where it concerns the environment. Instead, the corresponding guidelines (EMA, 2000; 2005, 2006, 2008, EMA 2011) give detailed instructions. In general, an ERA shall be performed to assess the potential harmful effects to the environment that may be caused by the use of the human or veterinary medicinal product. For both human and veterinary pharmaceuticals, an ERA is usually performed in a stepwise approach, which starts with an initial screening phase (Phase I) where the environmental exposure level is estimated with a simple model. If the predicted exposure level is above the action limit or if specific concerns are identified due to chemical specific characteristics, a number of studies should be submitted to enable an environmental risk assessment (Phase II) and the modelled exposure concentrations may be refined. Different from the aforementioned legal frameworks, there are no specific provisions related to EDCs for both human and veterinary pharmaceuticals. However, potential EDCs have been explicitly indicated in the guideline for human pharmaceuticals; a risk assessment has to be performed for potential EDCs regardless of their modelled exposure concentration. In relation to this aspect, the Question and Answers document for the EMEA guideline (EMA, 2011) specifies some tests that focus on the adverse effects on reproduction, and are needed for potential 'sexual endocrine disrupting' chemicals. It is noted that this explicit reference to sexual endocrine disruption is not made in the guideline itself (see Table 1). This specific phrasing to sexual endocrine disruptors is not used in other legal frameworks. In the guideline for veterinary pharmaceuticals, potential EDCs are not explicitly indicated but may be included in the clause on particular concerns (Table 1). For both human and veterinary pharmaceuticals, ED properties are one of the particular concerns that can be addressed on a case by case basis, irrespective of the emissions to the environment. Product authorisation of veterinary pharmaceuticals may require specific risk reduction measures or may even be refused on grounds of predicted environmental risks. In contrast, a potential environmental risk is not part of the benefit/risk

assessment with the authorisation of human pharmaceuticals, although the legal text states that 'specific arrangements to limit it shall be envisaged'. In summary, ED properties can be considered as part of risk assessment for both human and veterinary pharmaceutical frameworks. Formal identification of EDCs on the basis of studies in the dossier is not necessary, but the risk assessment can address the concern.

2.1.6 *Classification, Labelling and Packaging Regulation*

EDCs are not explicitly indicated under the Classification, Labelling and Packaging (CLP) Regulation (EC) 1272/2008. Similar to other chemicals, they are classified and categorised according to population relevant adverse effects on aquatic organisms.

2.2 **Summary**

The regulation of EDCs differs among various EU legal frameworks that deal with the environmental impact of chemicals (Table 2). Formal identification of EDCs is necessary to take action under REACH, PPPR and BPR. Recently, draft identification criteria were presented by the Commission to be used under the PPPR and BPR. As a general rule for BPR and PPPR, a chemical identified as an EDC is banned on the basis of its hazard, although in some cases derogations may apply (EC, 2016a). In principle, it is possible that for the same chemical the conclusion regarding derogations will differ under the PPPR and BPR, because of the differences in exposure-related risks.

The proposed criteria are being set to fulfil legal obligations under the PPPR and BPR. Whether the same criteria will be used for REACH remains unknown. According to the Commission the fact that identification of EDCs under REACH has already been carried out according to the WHO definition, implicitly effectuates consistency between frameworks. Under REACH, if a chemical is identified as an EDC of EoC and as such placed on Annex XIV, it could be authorized on the basis of either risk assessment or socio-economic analysis approach depending on whether threshold or non-threshold approach is to be applied to EDCs. According to the Commission's Communication (EC, 2016a), whether or not such threshold approach is applicable to EDCs will be reviewed and concluded at the end of this year. In addition, chemicals identified as EDCs may be subject to restriction.

For human and veterinary pharmaceuticals, a concern for ED properties may be a trigger for further environmental risk assessment, but not for the identification of EDCs. The outcome of the risk assessment is not taken into account in the risk-benefit analysis for human pharmaceuticals, but is considered for veterinary pharmaceuticals. The Question and Answers document to the EMEA guideline for human pharmaceuticals potentially narrows EDC down to sexual disrupting chemicals. The WFD has no provisions for the identification of EDCs. However, identification as an EDC in other frameworks may have consequences under the WFD in terms of the identification of priority hazardous substances and setting environmental quality standards. EDCs are not specially addressed in the CLP regulation, although toxicity induced by EDCs is considered.

Table 2 Differences in regulating EDCs among EU legal frameworks

Legal framework	Identification	Risk assessment	Note
REACH	yes	yes**	SEA may be applicable if EDCs are considered as non-threshold.
PPPR	yes	yes *	Once identified, EDCs are not approved.
BPR	yes	yes *	General public uses are not approved and risk assessment is not performed. Other uses will undergo risk assessment.
WFD	no	no	Identification [#] of priority hazardous chemicals followed by derivation of environmental quality standards.
Hum. Pharm.	no	yes	No consequences of environmental risk for EDCs.
Vet. Pharm.	no	yes	
CLP	no	no	

* Only for active substances for which the 'unless clause' applies and in some cases, risk or socio-economic issues may apply.

** A risk based approach is only applicable if a threshold can be demonstrated.

Identification is not part of the assessment under the WFD, but information from other frameworks is taken into account.

3 Challenges for regulating EDCs

3.1 Challenges for the identification of EDCs

In Chapter 2 it was shown that EDCs are covered in several pieces of EU legislation. Although the observed differences in 'EDC status' between regulations and guidelines may lead to inconsistencies, there does seem to be a theoretical or formal platform for regulating EDCs. In this chapter the focus is on actual challenges when bringing the implementation of these regulatory frameworks into practice. The recently released identification criteria include three key elements: adversity of effects, endocrine MOAs and the biologically plausible link between those two in intact organisms (EC 2016). The following sections discuss if and how the different elements are addressed in the respective frameworks.

3.1.1 *Scope of the endocrine mode of action*

In the current regulatory context, the 'endocrine or hormonal system' referred to in the WHO-definition is mainly discussed in relation to the potential of chemicals to interact with the estrogen, androgen, thyroid and steroidogenesis (EATS) signaling pathways. This focus on EATS pathways is due to the availability of OECD test guidelines (EC, 2016). The EATS signalling pathways are related to two axes, the hypothalamus-pituitary-gonad (HPG) axis, and the hypothalamus-pituitary-thyroid (HPT) axis. These axes affect growth, development and reproduction. However, there are more endocrine pathways and axes that are relevant from the environmental perspective, and our concept of 'endocrine' is being broadened by the discovery of chemical regulators secreted from many other organs, such as heart, body fat, muscle, liver, intestines, and kidneys (WHO, 2002). The OECD has developed a detailed review paper on other pathways, such as the hypothalamus-pituitary-adrenocortical (HPA) axis, the somatotrophic axis, the retinoid signaling pathway, the vitamin D signaling pathway, and peroxisome proliferator-activated receptors (PPARs) signaling pathway (OECD, 2012b). Test guidelines addressing some of these pathways are under development. There are even more endocrine pathways than those mentioned in the OECD detailed review paper (Kortenkamp et al., 2011). When discussing the scope of the endocrine MOA, it is noted that, in the Q&A document, the human pharmaceuticals framework explicitly refers to 'sexual endocrine disruptors' that focus on reproduction effects. Care should be taken that this specification should not be interpreted as a reason to narrow the scope of endocrine MOAs because reproduction is regulated by EATS pathways as well as other pathways, e.g PPARs (Bogacka et al., 2015). Moreover, it is noted that the WHO definition also includes EDCs that affect other endpoints than reproduction. From an environmental perspective, some invertebrate-specific endocrine systems, e.g. ecdysteroids and moulting hormones, are of particularly interest because they are not present in vertebrates and are a target of specially designed insecticides. As indicated in the Commission's Communication, the scope of endocrine MOAs centers on the hormonal systems of EATS, but the draft measures are not limited to

these hormonal systems (EU, 2016a). Other pathways should be taken into account when identifying EDCs.

3.1.2 *Limitations of current standard information requirements*

Even with the current focus on EATS-related endocrine systems, the identification of EDCs is hampered by a lack of adequate information. Under current EU legal frameworks, testing for chemicals with endocrine disrupting concern should be target-driven and be triggered by relevant concerns or indications thereof. Without such indications, additional testing would not be possible for these legal frameworks. The question is whether the current standard information requirements³ give enough information to either identify EDCs or to trigger additional testing to do so. The next sections discuss for each framework whether or not the standard information requirements are sufficient to address EDCs and to make the regulatory decisions as outlined in Table 1. For this, the standard information requirements are compared with tests included in the OECD conceptual framework (CF) for testing and assessment of endocrine disrupters (OECD, 2012a). This OECD CF is a toolbox including assays for both endocrine MOAs and/or for adverse effects in long-term/chronic toxicity tests divided into five levels (see Annex 1). The following description focuses only on chronic toxicity tests because only these chronic tests are relevant for *in vivo* testing of chemicals with endocrine disrupting properties (OECD 2012a). Discussion topics include whether or not results of these tests address the key elements of adversity, endocrine MOA and the underlying biological plausibility for identification of EDCs. The CLP regulation and the WFD do not have their own standard information requirements, but depend on data generated in other EU-frameworks and open literature. Both CLP and WFD will therefore not be further discussed in the following sections on standard information requirements and testing. For both human and veterinary pharmaceuticals, instead of testing according to the standard information requirements, targeted testing on the basis of initial endocrine disrupting concerns would be possible (EMA, 2011). However, risk assessment rather than identification of EDCs is the purpose of additional testing for pharmaceuticals. Therefore, both human and veterinary pharmaceuticals will only be further discussed in section 3.2 on risk assessment.

3.1.2.1 *Industrial chemicals (REACH)*

In REACH, the standard information requirements are dependent on the tonnage per year (tpa) of the chemical under registration, specified in Annex VII (≥ 1 tpa), Annex VIII (≥ 10 tpa), Annex IX (≥ 100 tpa), and Annex X (≥ 1000 tpa), respectively. Chronic aquatic toxicity tests are only required for tonnages of ≥ 100 tpa: one *Daphnia* reproduction test and one fish chronic toxicity test should be provided. The suggested chronic fish toxicity tests are: fish early life stage test (FELS; TG210), fish short term toxicity embryo and sac fry (TG212), and fish juvenile growth test (TG212). However, none of these fish tests is included in the OECD CF. Long term toxicity to sediment organisms and long term or

³ In the EU legal frameworks, different terms like 'standard' or 'core data' or 'information requirements' have been used when referring to the data that should always be included in a dossier. For the sake of consistency, this report uses the term 'standard information requirements' for those data that should be provided for all chemicals within a specific framework.

reproductive toxicity to birds are required at the tonnage level of ≥ 1000 tpa. All aforementioned toxicity tests only supply information on adverse effects, but not on endocrine MOAs and the underlying biological plausibility.

3.1.2.2 *Biocides (BPR)*

The BPR sets out rules on information requirements, which are specified for active substances in Annex II, and for the respective biocidal products in Annex III. Standard information requirements only include acute toxicity tests. Chronic toxicity tests are considered as additional, but are always needed for certain types of biocides. Therefore, some of additional ecotoxicity tests are in fact 'standard information requirements'. Similar to REACH, the BPR recommends TG210, TG212, and TG215 as chronic fish toxicity tests when a chemical has the potential to bioaccumulate (TG210), or has a high affinity to fat expressed in a log octanol-water partition coefficient (K_{ow}) < 4 (TG212) or a log $K_{ow} < 5$ (TG215). Both TG212 and TG215 are considered as alternatives to the FELS test (TG210). In addition, the *Daphnia* reproduction test (TG211) is also performed for these biocides. Except for the *Daphnia* test, none of the tests is listed in the OECD CF. Both fish and *Daphnia* tests can only supply information on adverse effects, but not on endocrine MOAs and the biological plausibility of the link between these two.

3.1.2.3 *Plant protection products (PPPR)*

Commission Regulation (EU) 283/2013 lays down the data requirements for the dossier to be submitted for approval of active substances contained in PPPs. Commission Regulation (EU) 284/2013 lists the data requirements for the authorisation of PPPs. Standard information requirements include only acute toxicity tests on fish, *Daphnia* and algae. A second invertebrate is needed for insecticides or chemicals with insecticidal activity, and macrophyte testing is needed for herbicides and fungicides with herbicidal action. Chronic ecotoxicity tests are considered additional, but are always needed for certain types of pesticides or certain conditions. Therefore, some of the additional ecotoxicity tests are in fact "standard information requirements". Two tests, the fish early life stage toxicity test (TG210) and the *Daphnia* reproduction toxicity test (TG211) are considered as 'standard information requirements' where exposure of surface water is possible and the substance does not hydrolyse instantly ($\text{DegT}_{90} > 1$ d). A fish full life cycle toxicity (FFLC) test is required where the bioconcentration factor (BCF) is > 1000 L/kg, the elimination during the 14-d depuration phase in the bioconcentration study is < 95 % or the substance is stable in water or sediment ($\text{DegT}_{90} > 100$ d). If long-term exposure is expected based on the predicted field exposure profile, an FFLC study might be required as well. If the test chemical is designed to or suspected of interfering with moulting hormones, or has other effects on insect growth and development, an additional study on chronic toxicity shall be carried out using relevant non-crustacean species such as *Chironomus* spp (TG 218-219). In summary, four chronic ecotoxicity tests may be available for PPPs. These tests are the FELS test (TG210), the *Daphnia* reproduction toxicity test (TG211), the FFLC and *Chironomus* toxicity tests (TG218-219). Except TG210, these tests are included in the OECD CF. All four tests can only give information on

adversity, but not on endocrine MOAs and the underlying biologically plausible link between these two.

3.1.3 *Options for additional testing*

As shown in the previous sections, standard information requirements currently set out in the EU legal frameworks only give information on adversity. Due to these limitations on flagging endocrine disrupting concern from standard information requirements, additional testing, with the purpose of identifying EDCs, may not be triggered from these tests. This is especially true for newly developed chemicals and for data-poor chemicals. For PPPs and biocides, information on the MOAs in target organisms may be available, but does not necessarily cover endocrine MOAs. If such an indication is not available, additional testing of a potential EDC may not be triggered by the standard information requirements and should therefore be based on other relevant *in silico*, *in vitro* and *in vivo* information. However, procedural and scientific limitations hamper the use of this information.

First of all, most frameworks have a clear routing for a dossier with strict deadlines for submitting information. For example, the approval process for biocides and PPPs starts with a completeness check by the evaluating competent authority (eCA). If at that stage a dossier is considered complete, it is hard to request additional information during the course of the assessment, which has also to do with the strict legal timelines that are set for the process. Consultations between the eCA and the applicant on the dossier should take place in a very early stage and the eCA has to have a strong case for requesting additional data. The options for the eCA to include own information from outside the dossier are limited since under the PPPR and BPR, the principal role of the eCA is to comment on the risk assessment of the applicant. Furthermore, most frameworks have a tradition of using only original study reports from accredited laboratories and the use of information from the scientific literature has only recently started to become more usual. For industrial chemicals, additional testing for endocrine disrupting properties is often requested in the substance evaluation process. However, without an indication of concerns from the standard information requirements, open literature and *in silico*, requesting additional testing would not be possible for the substance evaluation under REACH. The situation may be different for pharmaceuticals, where a 'particular concern' can be a reason to request further information (see Table 1). There may be indication of concerns because information is available on MOAs and on target organisms, e.g. mammals, before the ERA is performed. However, there is no guidance to define which concerns trigger additional ED testing.

Apart from these procedural issues, there are also scientific limitations to the use of relevant *in silico*, *in vitro* and *in vivo* information. *In silico* data include information derived from Structure Activity Relationships (SARs), Quantitative Structure Activity Relationships (QSARs), read-across and chemical category approaches. However, current SARs and QSARs models are only available for estrogenic receptor (ER) and androgenic receptor (AR) pathways, but not for other pathways. *In vitro* OECD screening assays are only available for estrogen, androgen, and steroidogenesis (EAS) pathways. However, even these *in vitro* tests are

not included in the standard information requirements of any EU legal framework. The lack of these requirements hampers the triggering of additional testing. There are also gaps in the available OECD test methods in detecting effects on the thyroid pathway and components of the HPG and HPT axes. In the literature, there may be an extensive amount of data available for EAS and other endocrine pathways like progesterone receptors, PPARs, retinoid X receptor (RXR), and thyroid hormone receptor (TR). However, such literature information is only available for the most thoroughly studied chemicals, but not for newly developed chemicals as well as for data-poor chemicals. When information from the literature is available, it is often not included in the registration dossier. The evaluating CA has to put a lot of time, efforts and resources to find and underpin potential triggers for EDs. This is, in practice, often not realistic and manageable. It is further noted that the majority of *in vitro* information is derived from mammalian cell lines and receptors. The usefulness of read across from the results of mammals to fish, amphibians and other vertebrates and *vice versa* remains largely unknown for most pathways. An even more difficult question is the relevance of such information for invertebrate species, because knowledge on the endocrine system of these animals is scarce. This lack of knowledge hinders the understanding of chemical induced endocrine effects on invertebrates and the development of relevant test methods.

Another scientific limitation is that the effects on certain pathways and adverse effects on apical endpoints in general are the result of a complex multifactorial sequence of events which can be influenced by chemical, non-chemical, and biological factors alone and their interactions (Dang, 2016). Both chemical specific and non-specific effects can be observed in the same *in vivo* experiment. Defining chemical specific effects on both mechanistic and apical endpoints is critical for the identification of EDCs. For this, testing at different biological levels is needed including both *in vitro* and *in vivo* evidence. At this stage, however, it has not yet been specified which biological key events are needed or can be used for testing in order to identify EDCs in the EU chemical legal frameworks for industrial chemicals, plant protection products, and biocides.

3.1.4 *Identification: summary and conclusions*

The current identification of EDCs is mainly based on information on adversity, endocrine MOAs and the underlying biological plausibility. The current standard information requirements seem to be only sufficient to address the aspect of adversity. Regarding endocrine MOAs, the current scope is focused on EATS pathways, while even for these pathways only a limited number of test guidelines are available. Neither EATS nor the other endocrine MOAs can be deduced from the current standard information requirements. Supplemental non-standard data on endocrine MOAs may be available, but the use of this information is hampered by procedural and scientific limitations. This results in dilemmas for testing beyond the standard requirements in all aforementioned legal frameworks, because of an absence of flagging endocrine disrupting concerns. Of the three key-elements for identification of EDCs, biological plausibility is considered as the most challenging. The biological plausibility is difficult to be directly demonstrated on the basis of the test guidelines, and it is questionable if

full proof is possible at all. It may be feasible to address the biological plausibility by demonstrating in a weight of evidence approach. However, guidance is needed for the interpretation of biological plausibility and the weight of evidence analysis. The application of the concepts of the adverse outcome pathways (AOP) and Toxicity Testing in the 21st Century (Cote et al., 2016) may be useful in this respect.

3.2 Challenges for risk assessment of EDCs and associated issues

3.2.1 Challenges for the use of testing data

Risk assessment, classification, PBT assessment and establishing environmental quality standards for chemicals are based on data available from regulatory dossiers and open literature. As indicated in section 1.3 and the proposed criteria, the focus is primarily on changes in adverse apical endpoints like survival, development, growth, and reproduction that are considered to have impact at population level (EC, 2016b,c). However, EDCs may induce changes in many non-apical endpoints, from subcellular to organism level, and these non-apical endpoints may even show effects at lower concentrations than apical endpoints. Among these are changes in enzyme activity, histology changes in organs like liver and kidney, biomarkers like vitellogenin (VTG) and secondary sex characteristics (SSC), and behavioural changes. Some of these potentially sensitive endpoints, e.g. VTG, have been included in the OECD test guidelines.

Until now, non-apical endpoints are generally not used in environmental risk assessment and hazard assessment in the context of classification, the PBT assessment, and the derivation of environmental quality standards. The rationale for this is that the link between these endpoints and adverse population level effects is often unknown (see also 3.2.3). The question arises if the identification as EDC should lead to a different approach in risk assessment. More specifically: should the endpoints that led to the identification as EDC be used in the derivation of PNECs, risk limits or hazard assessments? And, if the answer is yes, how can this be done? The underlying question is: is the absence of an established link between effects on non-apical endpoints and population level effects a reason not to include them in risk assessment or quality standard derivation? This question is not only relevant for specific EDC-related endpoints and biomarkers, but also for all non-apical endpoints such as effects on behaviour, feeding and movement, whether or not mediated through underlying endocrine mechanisms.

3.2.2 Options for risk assessment of EDCs

One approach would be to add an additional safety factor for a chemical of interest to cover all remaining uncertainty that is associated with potential endocrine disrupting properties. However, if the PNEC is lowered because of ED properties and a potential risk is identified, options for refinement would be demanded. From a scientific point of view, the main drawback of a fixed assessment factor for EDCs is that it ignores the experimental data and the knowledge that is obtained from the available tests. As indicated in Table 1, under the WFD an additional assessment factor may be considered if anticipated effects are not covered by the default factor. The main question should therefore be if remaining uncertainties from EDC is sufficiently covered. There are situations in which endocrine disrupting properties of a chemical can be

established on the basis of information from a particular taxon, while there still remains large uncertainties regarding the effect on other endpoints and taxa.

Considering the use of experimental data, the most fundamental approach would be to use the lowest available endpoint, no matter whether it is expressed at the population level or not. However, this may lead to an over-regulation of chemicals that do not impair organisms at a population level. In the context of PPP authorisation, the overall aim is to protect aquatic plants and animals at the population level, but the protection goal for aquatic vertebrates aims at the individual level to avoid mortality and suffering due to acute toxicity (EFSA, 2013). It should be noted that a protection goal at the individual level may probably be considered acceptable for endangered vertebrates.

Another option could be to further invest in research to unravel the relationship between non-apical endpoints and effects at the population or ecosystem level. In a recent literature review, Postma and Keijzers (2014) found many studies demonstrating a correlation between behavioral effects and individual fitness (growth, reproduction, survival), but studies focusing on the relationship with population sustainability or a generalisation of these individual studies are more scarce. Based on the available literature they conclude that for the parameters 'movement' and 'feeding' a link with population-level effects is at least plausible and effects on these parameters should not be ignored in risk assessment or environmental quality standard setting. The question remains if the effect levels should be treated in the same way as for the 'traditional' apical endpoints, i.e. taking the lowest value with an assessment factor, and if so, whether the same assessment factors should be used. Postma and Keijzers (2014) make a plea for a 'trial and error' approach. Comparing the outcome of risk assessments with and without the behavioral parameters will provide insight in the magnitude of such an effect as well as on the size of the AF which might be applied.

In addition, the AOP concept and the related AOP networks are important to understand the relationship between non-apical and apical endpoints (Knapen et al., 2015). Initial studies have showed that using ED-related biomarker responses as the basis for PNEC-derivation is a promising approach. For example, Ankley et al. (2008) showed that changes in plasma concentrations of 17 β -estradiol or testosterone in the females of fathead minnow positively correlated with the effects on fecundity. Dang et al. (2011) showed that the sensitivity of VTG changes, fecundity and gonad histology is comparable to the reproduction toxicity tests of zebrafish, medaka and fathead minnow and suggested that changes in VTG should be used, similar to fecundity, for risk assessment. However, it is noted that the interpretation of non-apical endpoints like fish biomarkers is not straightforward and rather complex (Dang, 2016). It is often not known how much change of non-apical endpoints would be indicative of adverse effects. During a recent SETAC Pellston® workshop, it was therefore suggested that non-apical endpoints should not be used for risk assessment until the linkage

between these non-apical endpoints and population relevant endpoints is established (Matthiessen et al., in review).

In line with the recommendations of Postma and Keijzers (2014), it is suggested, as a first step for evaluating EDCs, that non-apical endpoints should not be excluded beforehand from the dataset. Listing all available endpoints, including the non-apical ones is important to get a complete overview of all effect concentrations at different levels of biological organisation. A next step could then be to check whether effects on non-apical endpoints are covered when a PNEC is derived according to common practice, i.e. by using the lowest known population relevant endpoint with the appropriate assessment factor. If non-apical endpoint effects are seen at levels below the PNEC, or in species for which no 'traditional' endpoints are available, this may be a reason for a higher assessment factor. The transparent listing of all effects may also offer the possibility to draw links between effects at different levels of organisation, and to substantiate the request for additional studies. However, it is acknowledged that this approach can only have an added value when the procedural and scientific limitations outlined in section 3.1.3 are overcome. This will mean that risk assessors should have the opportunity to look beyond the standard information requirements.

3.2.3 *Challenges for classification and labelling*

Environmental hazard classification of a chemical into aquatic chronic categories is determined by data generated from acute and chronic aquatic toxicity tests. Similar to risk assessment, the environmental classification is generally based on apical endpoints observed in chronic ecotoxicity tests. In ECHA/RAC discussions on classification and labelling, questions have been raised for several substances whether non-apical endpoints like VTG should be used for classification (see section 3.2.1). In the case of classification of triadimenol with a NOEC of 0.17 mg/L on growth and a NOEC of 0.03 mg/L on VTG, the classification of "Aquatic chronic 2" would be changed into "Aquatic chronic 1" if the basis is the effect on VTG (ECHA, 2015). Similar to what is outlined above for risk assessment, it remains an open question whether EDCs should be classified on the basis of the most sensitive endpoint, e.g. biomarker or non-apical endpoints.

Currently, classification is mainly based on the test results obtained from fish, crusteans and algae. These organisms may not be sensitive to some EDCs. Other species, e.g. molluscs or amphibians, are not routinely included in the classification exercise and may be more sensitive to these EDCs. Data from these sensitive species have been considered on a case by case basis, but should be more regularly used for classification purposes.

3.2.4 *PBT assessment*

The assessment of whether a chemical fulfills the T criterion with respect to aquatic organisms (long-term NOEC < 0.01 mg/L) is usually based on results from standard long-term toxicity testing. Similar to the discussion in section 3.2.3. on classification, a chemical of interest would be labelled fulfilling 'T' if the NOEC < 0.01 mg/L for non-population relevant endpoints is used. At this stage, it remains a question whether or not such non-apical endpoints can be used for the T criterion. Another

point is that chemicals classified as CMR or chemicals having other evidence of chronic toxicity, e.g. STOT category 1 and 2, are considered to meet the T criterion. Following a similar line of reasoning, it may be discussed if identification as an EDC should also be reason to consider a chemical as being T. As chemicals with endocrine disrupting properties may cause severe effects similar to CMRs, it may be discussed if EDCs, once identified, should be considered to fulfil the T criterion.

3.2.5

Risk assessment and associated issues: summary and conclusions

OECD test guidelines have been shifted from detecting apical endpoint responses to capturing responses of both apical endpoints and non-apical mechanism related endpoints. The question has been raised whether these non-apical endpoints should be used in risk assessment, classification, PBT assessment, and EQS derivation. In view of the fact that these non-apical endpoints can be concentration-related and chemical specific, it is important to investigate the relationship between non-apical and apical endpoints. Addition of other taxonomic groups to the standard dataset would be important for the removal of other uncertainties.

4 Conclusions and recommendations

Chemicals with endocrine disrupting properties are broadly pointed out as chemicals of concern. Risk management tools for EDCs have already been conceptually implemented in a number of EU legal frameworks. This study, focusing on EDC issues from an environmental perspective, shows that there are differences in the regulatory consequences among these legal frameworks. This may lead to situations that, for example, an EDC is directly phased out in one legal framework, whereas it may still be used in others. In the view of RIVM these inconsistencies in regulating EDCs among legal frameworks need serious consideration. Current EU activities that are focusing on further harmonizing legal frameworks should also address the disparities on EDCs revealed in this study (EU, 2016b).

The availability of the draft EDC identification criteria will undoubtedly remove some uncertainties. However, a number of important challenges for regulating EDCs will remain, irrespective of the above-mentioned inconsistencies among the various frameworks at a conceptual level. A major challenge is how to obtain sufficient information from the available test data to 'flag' a chemical with endocrine disrupting properties. This study shows that standard environmental testing requirements in most legal frameworks will not be adequate for this. The reason is that current EU regulations almost exclusively rely on evaluation of chemical-induced adverse apical responses that are population relevant, but not on demonstrating endocrine modes of actions (MOAs). Supportive information can be available from other sources, but the use of such information is hampered by procedural (see below) and scientific limitations (see 3.1.3).

Thus, although the draft identification criteria and conceptual frameworks for regulating EDCs are present, 'the supply chain' of data for actually identifying, assessing and regulating EDCs will still remain a bottle neck for many chemicals.

Tests for identifying particular endocrine MOAs have for almost all of these MOAs not yet been included in standard information requirements. According to its Communication (EC, 2016a), the Commission intends to take all necessary steps to ensure that data requirements are enshrined in the relevant legal frameworks. In view of the long process of development of OECD test guidelines, the number of endocrine MOAs and the complexity of biological responses in intact organisms, there is a need for developing an integrated testing strategy for the identification of EDCs, which is able to filter out candidates for further testing. Legal frameworks should include information requirements that allow for the assessment of MOAs, which could then trigger the need for further studies at a higher level of biological organisation. It is important to note that the current OECD conceptual framework is not a testing strategy and the tests listed are limited to detecting EATS pathways.

From the regulatory perspective, it is also of primary importance that it is clearly defined what types of tests are needed and how much testing is sufficient for EDCs. On the basis of the current test guidelines, it is

almost impossible to fully establish biological plausibility between adverse effects and the underlying MOAs. RIVM emphasizes that clearly defining the kind of evidence needed for identification and risk assessment of EDCs would be critical for regulating EDCs in time. Furthermore, the current OECD test guidelines only cover a limited number of endocrine pathways and mostly only include certain parts of these pathways. Developing new OECD test guidelines and updating existing test guidelines that cover more endocrine components and pathways is of importance for identification and risk assessment of EDCs.

In case there is a broad consensus that additional information for a chemical with endocrine disrupting properties is needed, another challenge will be to actually make the necessary steps in further testing. Procedural limitations in the current legal frameworks interfere with smoothly obtaining further, conclusive information. In REACH, for example, it often takes around one year to include a chemical of interest in the substance evaluation process. Then about one year is needed for agreement on a testing proposal, followed by at least two years for executing, reporting and evaluating tests.

A final issue is how to fit non-apical endpoints, e.g. VTG level, from current or newly developed tests that are used to identify an EDC into the regulation processes of risk assessment, PBT assessment, classification and labelling, and EQS derivation. In general, RIVM advocates that non-apical endpoints are not excluded beforehand from the ecotoxicological dataset. Transparent listing of all available endpoints will increase transparency on effect concentrations at different levels of biological organisation, and will give the option to check whether effects on non-apical endpoints are covered when deriving PNECs according to current practice. If not, a higher assessment factor may be considered to cover residual uncertainty related to non-standard effects.

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Annex 1- OECD conceptual framework

Endocrine disrupting properties of a chemical are based on the relevant information available on its hazardous properties and mode of action properties. This information can include experimental data generated in toxicological tests and ecotoxicological tests, information generated in *in vitro* tests, (Quantitative) Structure Activity Relationships ((Q)SAR), "read across", or category approaches. Such information is organized into five levels and listed as an OECD conceptual framework (CF) for testing and assessment of Endocrine Disrupters.

Level 1 includes existing data and non-test information. All existing information that is not included at levels 2 to 5 should be collated at this level. Such information can be used for a weight of evidence analysis.

Level 2 contains *in vitro* assays providing data about selected endocrine mechanisms or pathways. At this stage, test guidelines are available for estrogen receptor (ER) binding and transactivation assays as well as for the steroidogenesis *in vitro* assay. Test guidelines for androgen receptor (AR) binding and transactivation assays are under development and the test guideline for thyroid receptor transactivation is not available at this stage. The current assays available at level 2 can detect EAS pathways. There are, however, three limitations: 1) all test methods are only available for human health; 2) Lack of metabolic systems in *in vitro* assays may lead to false negatives for chemicals which are biotransformed to endocrine active metabolites but may potentially also lead to false positives for endocrine active chemicals which are very quickly transformed to endocrine inactive metabolites; 3) all assays use mammalian cells and receptors. The relevance to wildlife remains a discussion point.

Level 3 includes *in vivo* assays providing data about selected endocrine mechanisms or pathways. Five tests are available for non-mammalians, including 2 amphibian tests and 3 fish tests (Table 2). Two amphibian tests are used for detecting chemicals interfering with the Thyroid hormone pathway (T). The test, xenopus embryo thyroid signaling assay, is currently in Phase II validation. Three fish assays have been adopted by the OECD, with two assays of TG229 and TG230 for indicating EAS pathways; one assay of GD148 for the anti-androgenic pathway. The latter fish assay was developed due to the fact that the first two fish assays cannot consistently detect fish biomarker changes induced by anti-androgenic chemicals. The current test design of the OECD protocols at level 3 is purely for the screening purpose. However, the adopted non-mammalian test methods are performed in intact amphibians and fish, with some apical endpoints like fecundity that are population-relevant. With the modification of test design, e.g. an increase in the number of animals and test concentrations, the results of e.g. TG229 can be used for risk assessment and classification.

In vivo assays providing data on adverse effects on endocrine relevant endpoints are listed at Level 4. There are 12 tests for non-mammalians. Fish and amphibian tests (TG234 and TG241) are initially designed to

detect both adverse effects and MOAs. Therefore, both apical and biomarker endpoints have been validated during the test method development. TG234 can detect chemicals interfering with EAS pathways; TG241 can detect the EATS pathway interference. Different from fish and amphibians, TG206 is only used for detecting adverse effects. There are two mollusc assays which are expected to be adopted in April 2016. These two assays, however, include only apical endpoints due to lack of knowledge on invertebrate endocrinology. Similarly, the other assays on invertebrates, except the daphnia test, included at level 4 can only detect adverse effects. The Daphnia test may detect chemicals interfering with the Juvenile hormone pathway (Dang et al., 2012). However, this test for detecting this pathway has not yet been validated. Taken together, some tests at Level 4 can detect both adverse effects and the interference of EATS pathways. Other tests, however, can only detect adverse effects of a chemical of interest.

Level 5 consists of *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organisms. Medaka multi-generation test has been changed into medaka extended one generation test (MEORGTS), which was adopted in 2015. In addition to adverse effects, MEORGTS can detect changes in EAS pathways by including fish biomarkers. Mysid and Copepod assays have been removed from the OECD development program because the leading country stopped the project. TG233 for chironomid life cycle test is included at level 5. However, this assay is considered only for detecting adversity but not for MOAs. Again, in addition to adverse effects, tests included at level 5 can detect chemical-induced changes in EAS pathways.

Taken together, assays at levels 2 to 5 listed in the OECD CF are considered as the most important test methods for detecting endocrine disruption (OECD 2012). The OECD CF includes standard test methods that can be used for detecting both adverse effects, endocrine MOAs and the causal relationship between adversity and MOAs. At this stage, however, these tests cover only EATS pathways. This CF is a toolbox but not a testing strategy, which means that any test at any level can be conducted and it is not necessary to follow the CF in a linear manner.

Table 2 OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors

Levels	Tests	TG	MOAs
Level 1 Existing data and non-test information	<ul style="list-style-type: none"> Physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability. All available toxicological data from standardized or non-standardized tests. Read across, chemical categories, QSARs and other <i>in silico</i> predictions, and ADME model predictions. 		
Level 2 <i>In vitro</i> assays providing data about selected endocrine mechanism(s)/path	<ul style="list-style-type: none"> Oestrogen receptor binding affinity Oestrogen receptor transactivation Androgen receptor binding affinity Androgen transactivation thyroid transactivation Steroidogenesis <i>in vitro</i> 	493 455 dev. dev. n.a. 456	E E A A T S

Levels	Tests	TG	MOAs
ways(s)	<ul style="list-style-type: none"> MCF-7 cell proliferation assays Other assays as appropriate 	stop	E
Level 3 <i>In vivo</i> assays providing data about selected endocrine mechanism(s)/path way(s)	Mammalian Toxicology <ul style="list-style-type: none"> Uterotrophic assay Hershberger assay Non-Mammalian Toxicology <ul style="list-style-type: none"> Xenopus embryo thyroid signalling assay Amphibian metamorphosis assay Fish Reproductive Screening Assay Fish Screening Assay Androgenized female stickleback screen 	440 441 Dev 231 229 230 GD148	E A T T EAS EAS A
Level 4 <i>In vivo</i> assays providing data on adverse effects on endocrine relevant endpoints	Mammalian Toxicology <ul style="list-style-type: none"> Repeated dose 28-day study Repeated dose 90-day study 1-generation reproduction toxicity study Male pubertal assay Female pubertal assay Intact adult male endocrine screening assay Prenatal developmental toxicity study Chronic toxicity and carcinogenicity studies Combined 28-day/reproductive screening assay Developmental neurotoxicity Non-Mammalian Toxicology <ul style="list-style-type: none"> Fish sexual development test Fish Reproduction Partial Lifecycle Test Larval Amphibian Growth & Development Assay Avian Reproduction Assay Mollusc Partial Lifecycle Assays Chironomid Toxicity Test <i>Daphnia</i> Reproduction Test (with male induction) Earthworm Reproduction Test Enchytraeid Reproduction Test Sediment Water <i>Lumbriculus</i> Toxicity Test Using Spiked Sediment Predatory mite reproduction test in soil Collembolan Reproduction Test in Soil 	407 408 415 EPA EPA EPA 414 451-3 421-2 426 234 n.a. 241 206 Dev 218-9 211 222 220 225 226 232	
Level 5 <i>In vivo</i> assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism	Mammalian Toxicology <ul style="list-style-type: none"> Extended one-generation reproductive toxicity study 2-Generation reproduction toxicity study Non-Mammalian Toxicology <ul style="list-style-type: none"> Fish Life Cycle Toxicity Test Medaka Extended one-generation Test Avian 2 generation reproductive toxicity assay Mysid Life Cycle Toxicity Test Copepod Reproduction and Development Test Sediment Water Chironomid Life Cycle Toxicity Test 	443 416 n.a. 240 n.a. stop stop 233	

