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

Endocrine disrupting chemicals in the EU legal frameworks

Human health perspective

RIVM Letter report 2016-0137
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Colophon

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Synopsis

Endocrine disrupting chemicals in the EU legal frameworks
Human health perspective

The EU Commission published the draft criteria for the identification of endocrine disrupting chemicals (EDC). Currently the data requirements of the relevant legal frameworks are mostly insufficient to comply with these criteria.

An EDC is defined as a substance which causes an adverse effect, like decreased fertility or effects on the developing nervous system. Additionally it must be shown that the adverse effect is biologically related to a disturbance of the function of the hormonal system. This is, in most cases, difficult.

Legislation requires information for a safe use of a substance, among which animal studies are also required. Animal studies are, however, not specifically developed to show adverse effects caused by endocrine disruption (ED). These studies are also not developed to elucidate endocrine mechanisms of action (MOA). Currently the best study to investigate effects caused by ED is the extended one generation study (EOGRTS). Neither the EOGRTS nor studies specifically developed to investigate MOA mediated effects are currently required in most of legal frameworks.

RIVM recommends to update several animal studies that can detect MOA mediated adverse effects and to oblige the EOGRTS in the data requirements of the relevant legal frameworks. These adoptions are relatively easy to fulfil and are important for the detection of EDC. Additionally it is recommended to develop test guidelines that can detect different endocrine MOAs and the related effects. Finally, it is imperative to develop a balanced guidance to evaluate which information fulfils the proposed criteria.

Keywords: endocrine disrupting chemicals, criteria, legislation, regulatory framework, extended one generation reproductive toxicity study

Publiekssamenvatting

Hormoonverstorende stoffen in Europese regelgeving
Bezien vanuit de gezondheid van de mens

De Europese Commissie heeft concept criteria opgesteld waarmee kan worden bepaald of stoffen hormoonverstorend zijn. Het blijkt dat binnen de huidige wet- en regelgeving veelal de gegevens ontbreken om stoffen aan deze criteria te toetsen.

Om aan te tonen of een stof hormoonverstorend is, moet hij een nadelig (of schadelijk) effect veroorzaken, zoals een verminderde vruchtbaarheid of mogelijke verstoring van het zich ontwikkelende zenuwstelsel. Vervolgens moet worden aangetoond dat het schadelijke effect een biologisch verklaarbaar gevolg is van een verstoring van het hormoonsysteem. Dit is doorgaans lastig aan te tonen.

De regelgeving vereist informatie die aangeeft dat een stof veilig kan worden gebruikt. Deze informatie kan onder andere in de vorm van gestandaardiseerde dierproeven worden verkregen. De vereiste proefdierstudies zijn echter niet ontwikkeld om alle nadelige effecten als gevolg van hormoonverstoring aan te tonen. Deze studies zijn evenmin ontwikkeld om het achterliggende biologische mechanisme te achterhalen. De studie die op dit moment het beste in staat is om nadelige effecten als gevolg van een mogelijk hormonaal werkingsmechanisme op te pikken is de zogenoemde *extended one generation*-studie. Deze studie is echter nog lang niet in alle wettelijke kaders verplicht. Hetzelfde geldt voor gestandaardiseerde studies om het biologische werkingsmechanisme te testen.

Om beter te kunnen beoordelen of een stof hormoonverstorend is op basis van de voorgestelde criteria pleit het RIVM ervoor om enkele dierproefstudies aan te passen en de *extended one generation*-studie te verplichten. Daarnaast wordt voorgesteld om de gestandaardiseerde studies naar werkingsmechanismen verplicht te stellen in de huidige regelgeving. Deze aanpassingen in de regelgeving zijn relatief eenvoudig uit te voeren en zijn belangrijk om stoffen met een hormoonverstorende werking op te sporen. Ook zijn nieuwe methoden voor studies nodig om de nog ontbrekende effecten en mechanismen relevant voor hormoonverstoring aan te tonen. Ten slotte is het belangrijk dat er een evenwichtige beoordelingsstrategie wordt ontwikkeld om te evalueren of informatie voldoet aan de voorgestelde criteria.

Kernwoorden: hormoonverstoorders, criteria, wet en regelgeving, extened one generation reproductive toxicity study

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Summary

Endocrine Disrupting Chemicals (EDC, also known as Endocrine disrupting substances, EDS) are suspected of having severe health and environmental impacts. Therefore, EDC have been included in several pieces of European Union (EU) legislation. The EU Commission has adopted the WHO definition on EDC and published the draft proposal for scientific criteria for the definition of EDC on 15 June 2016. This identification of EDC is based on information on adversity, endocrine modes of action (MOAs) and the biological plausibility of the causal link between the two. The regulation of EDC differs in wording and in regulatory consequences among EU legal frameworks. EDC are specifically indicated in REACH, PPPR (Plant Protection Products Regulation), BPR (Biocidal Products Regulation) and Cosmetic Regulation but are not specifically indicated in FCM (Food Contact Materials) regulation. Identification of EDC is needed for REACH, PPPR and BPR. Due to a ban of animal testing, if a substance is only used as a cosmetic product, it is not possible to identify it as an EDC because *in vivo* animal testing is needed on the basis of the WHO definition and the draft criteria. A hazard and a risk approach for regulating EDC are applicable depending on the legal frameworks. For PPPR, a hazard approach is applicable (EU, 2014). However, this approach may be shifted to a risk approach based on the draft legal text and guidance (EU, 2016). Depending on the uses of biocides, a hazard- and a risk-approach is applicable for the BPR. Regulatory decisions will be taken based on the results of the risk assessment or socio economic analysis for REACH, depending on whether EDC are considered to act via threshold or non-threshold mechanism. If a substance is only used in cosmetic products, due to a ban of animal testing, a potential EDC will be treated similarly to EDC in the other legal frameworks. Although they are not specified in the FCM regulation, EDC are treated similarly to other chemicals of concern on the basis of risk assessment.

From the regulatory perspective, it is of primary importance to clearly define what types of information is needed and how much information is needed in order to conclude that a substance fulfills the WHO/draft COM criteria for EDC. Data requirements between the different frameworks differ largely. No ED relevant data are required for the cosmetic regulation, the FCM regulation at a migration level of <0.05 mg/kg and REACH requirements for 1-10 tpa. A limited set of studies are required for the FCM regulation at a migration level of 0.05- 5 mg/kg, and REACH requirements for 10-100 tpa require. More ED relevant tests are required for PPPs, biocides, FCMs >5 mg/kg migration and REACH chemicals at >100 tpa.
On the basis of the current information requirements in all above mentioned legal frameworks, it is very difficult to fully establish a biologically plausible causal relationship between adverse effects and the underlying MOAs because these information requirements focus mainly on adversity. Clearly defining the amount of evidence needed for identification and risk assessment of EDC would be critical to regulating EDC in time. Furthermore, there is a need for an intelligent and integrated testing strategy for the identification of EDC based on weight

of evidence. Inclusion of both in vitro and in vivo screening studies that detect relevant key events for ED effects, inclusion of the extended one generation study and update of repeated dose studies would facilitate identification and risk assessment of EDC. However, consensus is needed on which study results would trigger further testing. 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 to facilitate establishing cause and effect is of importance for identification and risk assessment of EDC. Finally guidance is needed to address uncertainties in the data interpretation of information requirements and ED relevant pathways.

1 Introduction

1.1 Endocrine disrupting chemicals in a legal context

An Endocrine Disrupting Chemical (EDC) is referred to as "an exogenous chemical 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). Wording of EDC differs between organisations and legislation. In this report in general "chemical" is used, however in some cases also "substance" (for example substance of concern, active substance, etc.) is used. Both are replaceable.
EDC are suspected of having severe health and environmental impacts. In addition, the issue of EDC and their potential risks have received attention in relation to consumer products and food contact materials (FCMs) that are relevant to the daily life of people.
Therefore, EDC 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 (EC 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 EDC, but the question is if the focus, approaches and consequences are consistent within the different EU legal frameworks.

1.2 Identification of EDC

The European Commission has developed draft scientific criteria to identify EDC in PPPR and BPR. The criteria conform to the WHO definition and may be applicable to REACH or other legal frameworks (EU, 2016). The WHO definition and the EU criteria include three key elements: adverse health effects (adversity) in an intact organism, endocrine mode or mechanism of action (MOA) and the underlying causal relationship between these two. All these key elements need to be supported by experimental data in intact animals, some of which are requested by the minimal information requirements of EU legal frameworks. Depending on the legal framework, additional testing is possible when there is an indication of concerns. This report focusses on the question whether standard information requirements of legal frameworks are sufficient for the identification of EDC and if needed, whether these minimal information requirements will provide sufficient indication of concern for triggering further testing.

1.3 Risk assessment of EDC

Current scientific knowledge concerning EDC has recently been evaluated by many EU and international organisations including EU

Commission, JRC, EFSA, EEA, OECD, and WHO/UNEP[1]. The WHO report shows that EDC are found widespread in the environment, food, humans and animals. The presence of EDC could be associated with many human disorders *e.g.* the reproductive disorders, neurotoxicity and metabolic disorders (WHO, 2013). Although endocrine disruption is one of the most intensively studied toxicological fields, its possible impact on public health remains controversial. Many questions have been raised in terms of risk assessment of EDC. These questions include low dose effects, non-monotonic dose response relationships (NMDR), developmental causes of adult disease, and mixture effects, which challenge the current hazard/risk assessment paradigm (EFSA, 2013, JRC, 2013).

1.4 Aim and scope of this report

With the focus on the human health, this report intends to summarise the EDC-related aspects of EU legal frameworks for industrial chemicals, pesticides, biocides, cosmetics and FCMs (a comparable report is available for environmental perspectives, see Dang *et al*, 2016). The FCM framework is included because some chemicals e.g. phthalates, bisphenol A are present in FCMs and have gained particular attention.. We analyse the challenges of regulating EDC, and present recommendations for policy and future research. Chapter 2 describes the OECD mammalian screening and testing methods, which form a basis for detecting chemicals with endocrine disrupting properties. Chapter 3 gives an overview of the status of EDC in several EU legal frameworks, including whether or not minimal information requirements in the these frameworks are adequate to identify EDC. Chapter 4 discusses challenges of regulating EDC, with the focus on key issues related to identification and risk assessment in different legal frameworks. Recommendations are made in Chapter 5 for how to face challenges for identification and regulating EDC.

[1] EU commission: European Commission, JRC: Joint Research Centre, EFSA: European Food Safety Authority, OECD: Organisation for Economic Co-operation and Development, WHO/UNEP: World Health Organization/United Nations Environment Programme.

2 Mammalian screening and testing methods for detecting EDC

2.1 OECD Conceptual Framework

The OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters was initially proposed in 2002 and then revised in 2012. The differences between these two versions are three-fold: 1) no exposure information is included in the version of 2012; 2) update and adopted test guidelines (TGs) are added to the 2012 version; 3) Mammalian and non-Mammalian toxicology have been specified. It is important to indicate that both mammalian and non-mammalian information can be used for the identification of EDC. This report focuses only on mammalian tests. The non-mammalian toxicity tests can be found in another report (Dang et al., 2016).
The CF lists the OECD TGs and standardized test methods available, under development, or proposed, which can be used to evaluate chemicals for endocrine disruption. These test methods are organized into five levels (Table 1).

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 includes the physico-chemical properties of a chemical of interest; *in silico* predictions like QSARs, read across; and ADME predictions. Data from literature studies on toxicological tests that are not included at levels 2-5 can be included at Level 1. Information at level 1 can be used for a weight of evidence analysis.

Level 2 contains *in vitro* assays providing data about selected endocrine mechanisms or pathways. As shown in Table 1, TGs are available for oestrogen receptor (ER) binding and transactivation assays as well as for the steroidogenesis (S) *in vitro* assay. TGs for androgen receptor (AR) binding and transactivation assays are under development and approved in vitro TGs for the thyroid pathways are not available at this stage. Another *in vitro* test, the MCF-7 cell proliferation assay, has been listed at level 2 of the CF. The development of the test method, however, has been stopped from the OECD program because of the lead country has withdrew from this project. Overall, the current *in vitro* tests available are only for elucidating EAS pathways. Other pathways may be considered once the test methods are adopted by the OECD.

Level 3 includes *in vivo* assays that provide data about selected endocrine mechanisms or pathways. Two assays, the Uterotrophic assay and the Hershberger assay, are available for mammalians; the former detects the ER-mediated pathways and the latter detects AR-mediated pathways. Both assays are considered to elucidate MOAs but are not used for risk assessment because the experimental animals need to be operated in these assays. In summary, two mammalian tests are available for detecting E and A pathways, respectively.

In vivo assays that provide data on adverse effects on endocrine relevant endpoints are listed at Level 4. There are 10 tests included for

mammalians. All assays at this level include apical endpoints and are initially designed for hazard and risk assessment. Due to the great impact of EDC, OECD has been developing tests or updating existing test methods for detecting endocrine activities. For mammalian tests, the 28-day Repeated Dose Toxicity Test (TG 407) and the Reproduction/Developmental Toxicity Screening Test (TG 421) have been updated with ED parameters in 2008 and 2015, respectively. The updates include parameters suitable to detect EATS mediated activity (triggers). However, the sensitivity of the updated assays is not be sufficient to identify all EATS-mediated EDC (OECD 2012). The update of TG 414 with endocrine sensitive parameters is currently under development. Three assays do not have OECD test guidelines but have been included in the US endocrine disruptor screening program (EDCP). These three assays are male pubertal assay, female pubertal assay and intact adult male endocrine screening assay, which can detect the potential of the chemical to interact with EATS pathways (OECD 2012). Other tests at level 4 have limited capacity to indicate EATS mediated activity (triggers); these include TG 409, TG 451-3, and TG 426. Taken together, although these tests in principle were not developed to detect EDC, they can be responsive to one or more of the EATS pathways, and may be sensitive to some additional endocrine pathways (*e.g.* those involving the corticosteroid axis, somatotropic axis, vitamin D signalling, retinoid signalling, pancreatic system, or PPAR pathway – as described in OECD GD 150, or other endocrine glands/structures). For example, in the repeated dose toxicity studies (OECD TG 407, 408, 451-3), histopathological investigations of the adrenal gland could provide data on endocrine relevant endpoints involving the corticosteroid axis; or in the reproductive toxicity studies (OECD TG 414, 415, 421, 422, 426), growth evaluation could provide information on apical endpoints involving the somatotropic axis and the retinoid signalling pathway. However, it is to be stressed that these TGs provide insufficient information to conclude on the EDC status of a chemical.

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. There are two tests for mammalians, the Extended One-Generation Reproductive Toxicity Study (EOGRTS) and the two-generation toxicity test (Table 1). The two-generation toxicity test (TG 416) was initially designed to detect adverse effects of a chemical and was not considered as a sensitive test method to detect endocrine activity. The most recent version of this test was adopted in 2001. In this version, some endocrine endpoints like estrous cyclicity and primordial follicle counts were included. However, it does not include some other endocrine sensitive endpoints like nipple retention. No further update is planned in the OECD. Instead, OECD has developed an EOGTRS (TG 443), in which endocrine sensitive endpoints in the juvenile and adult F1 have been included. The EOGRTS is preferable for detecting endocrine disruption because more parameters are included. The basic study design of the EOGRTS focuses on the evaluation of the fertility of parental animals and of defined parameters on postnatal development of F1 animals until adulthood. It does not include mating of F1 animals (producing F2 generation) or cohorts for DNT (developmental neurotoxicity) or DIT (developmental

immunotoxicity). Conditions for triggering an extension of F2, DNT and DIT varies with the different legal frameworks. Although both TG 416 and TG 443 are considered to detect the interference of some of the EAST pathways. the EOGRTS has several advantages compared to TG 416.

- Compared to OECD TG 416 a significant number of animals can be saved.
- Many more parameters addressing fertility and reproductive toxicity are included (*e.g.* clinical-chemical parameters as normally addressed in repeat-dose studies; developmental immunotoxicity and developmental neurotoxicity in case the cohorts (*i.e.* F2, DNT and DIT) are included).
- It includes several parameters sensitive to endocrine disruption that are not included in the updated version of TG416, such as nipple retention, ano-genital distance at birth, vaginal patency and balano-preputial separation.
- It has increased statistical power with respect to parameters for reproductive toxicity.
- There is the possibility for modification *e.g.* to include new endpoints for the assessment of endocrine active chemicals disrupting the hypothalamus-pituitary-gonad (HPG) axis, the somatotropic axis, the retinoid signalling pathway, the hypothalamus:pituitary:thyroid (HPT) axis, the vitamin D signalling pathway and the peroxisome proliferator-activated receptor (PPAR) signalling pathway.

The mammalian level 5 tests provide more comprehensive data on endocrine-relevant apical endpoints (predominantly mediated by the EATS modalities but also provide some information by other endocrine modalities such as those involving the corticosteroid axis, somatotropic axis, vitamin D signalling, retinoid signalling, PPAR pathway or other endocrine glands/structures) over more extensive parts of the life cycle of the organism, although old age or senescence is generally not covered.

Taken together, tests listed at levels 2 to 5 of the OECD CF are considered as the most important test methods for detecting endocrine disruption (OECD 2012). Some of these tests can be used for detecting adverse effects, endocrine MOAs and the causal relationship between adversity and MOAs. But they have some limitations (see section 2.2). At this stage these tests cover only EATS pathways. Test methods for other pathways that are relevant to EDC-induced diseases, e.g. obesity and diabetes, are not included in the CF because such a type of test methods has not yet been validated. The CF is a toolbox but not a testing strategy. This means that any test at any level can be conducted and it is not necessary to follow the CF in a linear manner.

Table 1. OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors

Levels	Tests	TG	MOAs
Level 1 Existing data and non-test information	• 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 in silico predictions, and ADME model predictions.		
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s)/pathways(s)	• Estrogen receptor binding affinity • Estrogen receptor transactivation • Androgen receptor binding affinity • Androgen transactivation • thyroid transactivation • Steroidogenesis in vitro • MCF-7 cell proliferation assays • Other assays as appropriate	493 455 dev. dev. n.a. 456 stop	E E A A T S E
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s)/pathway(s)	• Uterotrophic assay • Hershberger assay	440 441	E A
Level 4 *In vivo* assays providing data on adverse effects on endocrine relevant endpoints	• 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	407 408 415 EPA EPA EPA 414 451-3 421-2 426	
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	• Extended one-generation reproductive toxicity study • 2-Generation reproduction toxicity study	443 416	

2.2 Limitations of the tests included in the OECD CF

Current endocrine disrupting testing focuses on the potential of chemicals to interact with the estrogen, androgen, thyroid, or steroidogenesis (EATS) signalling pathways that are related to two axes, the hypothalamus-pituitary-gonad (HPG) axis, and the hypothalamus-pituitary-thyroid (HPT) axis. These axes affect reproductive development and function. Even for the available tests there are limitations as summarized below:

The *in vitro* methods at level 2 have the following limitations:

1) Only *in vitro* test methods for detecting EAS pathways are available. No standard *in vitro* test methods are available for the T pathway.
2) None of the listed *in vitro* tests include metabolic systems. Lack of metabolic systems in *in vitro* assays may lead to false negatives for chemicals that are biotransformed to endocrine active metabolites but may potentially also lead to false positives for endocrine active chemicals that are very quickly transformed to endocrine inactive metabolites.

Limitations of *in vivo* tests at Level 3 are:

1) The available methods are used for detecting EA pathways. No *in vivo* human health methods for testing S and T pathways.
2) Current *in vivo* tests for human health cover partly the HPG axis. Some essential targets like inhibins and activins that influence the HPG axis are not included in the current test methods.

Limitations of *in vivo* tests at level 4 and 5 are:

1) For human health assays, a validation has only been done in TG407 with the available endpoints for detecting endocrine disruption. It is to be stressed that this is a screening study using only 5 animals per dose level, in view of its limited power, window of exposure and the parameters tested, only positive results can be interpreted as being conclusive, whereas negatives are not indicative for no effect. Updates of this study with endocrine screening parameters have also been made in other test guidelines *e.g.* TG421/422, TG416 and TG443.
2) As the same endpoint may be modulated via different MOAs, indication of endocrine MOAs has some uncertainties. Sensitivity of some assays is neither sufficient to identify all EATS-mediated EDC nor to exclude such a mode of action.
3) Current mammalian tests do not cover certain endpoints that might be induced by exposure during foetal or pubertal development but do not emerge until later in life, *e.g.* certain cancers and effects on reproductive senescence.
4) Most of these tests can only detect adverse effects but do not provide an indication to MOAs.

In summary, the currently available standardised guideline tests have been updated with parameters for EATS pathways. The existing assays, however, are not adequate to exclude effects on various endocrine-sensitive endpoints and there are limitation in the test guidelines on *e.g.* sensitivity, sensitive window, certain effects and effects manifested in later life stages caused by early exposure. Standard test methods for

other pathways like peroxisome proliferator-activated receptor (PPAR) signalling pathway essential for regulating obesity have not yet been developed.

3 EU legal frameworks for regulating EDC

3.1 REACH

Under REACH endocrine disruption is mentioned in Art 57 (f) in relation to substances of very high concern (SVHC). EDC that exert serious effects to human health or the environment that give rise to an equivalent level of concern (EloC) to those effects listed under Article 57 (a)-(e) can be identified on a case-by-case basis as Article 57(f) chemical and as such can be placed on the list of SVHC substances (the Candidate list of REACH). This means that identification as EDC and assessment of EloC is needed for inclusion of EDC in the SVHC list. An EDC of EloC in the Candidate list is then subject to the Authorisation. Currently, there are still uncertainties on whether a safe level can be determined for EDC. According to the recently published Communication, the Commission will finalize and present the review whether or not threshold is applied to EDC by the end of 2016. Without safe levels, an authorisation of EDC may only be granted if it is shown that socio economic benefits outweigh the risks 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. In summary, both identification and risk assessment of EDC are essential for regulatory actions under REACH.

3.2 Plant protection products (PPP)

The PPPR explicitly addresses chemicals with endocrine disrupting properties. Annex II of Regulation (EC) No 1107/2009 states in point 3.6.5 that "An active substance, safener or synergist shall only be approved if, …, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible".

Recently the Commission set out scientific criteria for the determination of endocrine disrupting properties amending Annex II to Regulation (EC) 1107/2009. In this amendment it is stated that an active substance, safener of synergist shall be considered as having endocrine disrupting properties with respect to humans if it is a chemical that meets the following criteria: 1) it is known to cause an adverse effect (relevant for human health), 2) it has an endocrine mode of action and 3) the adverse effect is a consequence of the endocrine mode of action.

It is further stated that an active substance, safener or synergist shall not be approved if it meets the criteria unless the risk from exposure is negligible. Without guidance, negligible exposure has been considered difficult. Currently, the Commission is drafting a technical guidance on negligible exposure which is divided into dietary and non-dietary exposure. Dietary exposure is negligible *"where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value of* 0.01 mg/kg. Non-dietary negligible exposure can be calculated by applying an additional safety factor to the chronic toxicological reference values, establishing an exposure level which is far below the level which is of no risk even for the most vulnerable groups.

In addition, non-dietary negligible exposure can be assumed where levels to which humans are exposed are equal to or lower than natural background levels (EU, 2016). Apparently, this may lead to a consideration of not only a hazard but also a risk of EDC. It should be noted though that this guidance is provisional and not yet adopted. Priority of this guidance is low, and currently the Commission did not announce when or whether this guidance will be further discussed and adopted.

3.3 Biocidal Product Regulation (BPR)

In the BPR a chemical that is considered to have 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 should be excluded unless the risk is negligible, the active substance is essential to prevent/control health, or exclusion would have a disproportionate impact on society when compared to the risk.
Recently the Commission set out scientific criteria for the determination of endocrine disrupting pursuant to Regulation (EC) 528/2012. In this annex to the Regulation it is stated that an active substance shall be considered as having endocrine disrupting properties with respect to humans if it is a chemical that meets the following criteria: 1) it is known to cause an adverse effect (relevant for human health), 2) it has an endocrine mode of action and 3) the adverse effect is a consequence of the endocrine mode of action.
Depending on the uses of biocides, a hazard- and a risk-approach is applicable for the BPR. Overall, both identification and risk assessment of EDC play critical roles in implementation of BPR.

3.4 Cosmetics Products Regulation

According to the regulation, the Commission shall review this Regulation with regard to chemicals with endocrine-disrupting properties when the identification criteria are available. Due to the suspended obligatory deadline for defining identification criteria of EDC, the review has not yet been carried out. Instead, the Scientific Committee on Consumer Safety (SCCS) has issued a memorandum (SCCS/1544/14) to clarify its position on chemicals with endocrine disrupting properties when used as cosmetic ingredients: they should be treated like most other chemicals of concern for human health and be subject to risk assessment and not only hazard assessment. In addition, the SCCS published Notes of guidance, which addresses cosmetic ingredients suspected to have endocrine disrupting properties (Reference). Due to the ban on animal testing for cosmetic ingredients effective since 2013, it is not possible to identify a chemical as an EDC based on the draft EU criteria. If a chemical is only used in cosmetic products, it will be extremely difficult to differentiate between a potential EDC and EDC.

3.5 Food contact materials Regulation

EDC are not explicitly indicated under the FCM Regulation. In principle, EDC should be treated like most other chemicals of concern for human health and be subject to risk assessment.

3.6 Summary

This report summarizes five pieces of EU chemicals legislation that address EDC from the human health perspective. The regulation of EDC differs in wording and in regulatory consequences among EU legal frameworks. Except the FCM Regulation, EDC is specifically indicated in REACH, PPPR, BPR and Cosmetic Regulation. Identification of EDC is needed for REACH, PPPR and BPR. However, the available draft criteria are as yet only applicable to PPPR and BPR. Whether the same criteria should be used for REACH remains to be decided. In view of the fact that the already identified EDC has been carried out according to the WHO definition, it suggests the same criteria could be used under REACH. Due to a ban of animal testing, if a chemical is only used as a cosmetic product, it is not possible to identify it as an EDC because in vivo animal testing is needed for identification of EDC. Both a hazard and a risk approach have been implemented in various types of legislation for regulating EDC. For PPPR, a hazard approach was chosen (EU, 2014) . However, based on the draft guidance and the Communication (EU, 2016a, and b) this approach may be changed on a case by case basis to a risk or social economic approach. Depending on the uses of biocides, a hazard- and a risk-approach are applicable for the BPR. For REACH, regulatory decisions will be taken based on the results of socio economic analysis, or in case a threshold can be proven, risk assessment. If a chemical is only used in cosmetic product, in principle a risk approach is followed. For the FCM regulation, EDC are not specifically indicated and will be treated similarly to other chemicals of concern on the basis of risk assessment.

Table 2. Overview of the minimal data requirements for the regulatory frameworks (for more details see appendices).

Test guidelines relevant for ED (OECD CF)	Biocides	PPP	Food contact materials 5-60 mg/kg/food	Food contact materials 0.05-5 mg/kg/food	Food contact materials <0.05 mg/kg/food	REACH Annex VII (1-10 tpa)	REACH Annex VIII (10-100 tpa)	REACH Annex IX 100-1000 tpa)	REACH Annex X (> 1000-tpa)	Cosmetics
Level 1										
Existing Data and non-test information		X				X	X	X	X	X
Level 2										
OECD 455	No studies required in any legislation									
Androgen or thyroid transactivation										
OECD 456										
MCF-7										
Level 3										
OECD 440	No studies required in any legislation									
OECD 441										
Level 4										
OECD 407	X	X					X	X	X	
OECD 408	X	X	X	X				X	X	
OECD 415										
Male pubertal										

Test guidelines relevant for ED (OECD CF)	Biocides	PPP	Food contact materials 5-60 mg/kg/food	Food contact materials 0.05-5 mg/kg/food	Food contact materials <0.05 mg/kg/food	REACH Annex VII (1-10 tpa)	REACH Annex VIII (10-100 tpa)	REACH Annex IX 100-1000 tpa)	REACH Annex X (> 1000-tpa)	Cosmetics
assay										
Female pubertal										
intact adult male endocrine screening										
OECD 414	X	X	X					X	X	
OECD 451 - 3	X	X	X							
OECD 421							X[3]	X[3]	X[3]	
OECD 422							X[3]	X[3]	X[3]	
OECD 426										
Level 5										
OECD 443	X[1]	X[1]						X[4]	X	
OECD 416	X[1]	X[1]	X							

[1] OECD 443 or OECD 416.

[2] OECD 443 is only required

[3] either a 421 or 422 could be considered

[4] only in case of indications of relevant effects in-vivo studies

4 Challenges for regulating EDC

In Chapter 3 it was shown that EDC are addressed in several EU legislations. This chapter focuses on challenges faced upon actually regulating EDC under these regulatory frameworks. Identification of EDC is based on information on adversity, endocrine MOAs and the causal relationship in intact organisms. As causality is hard to prove, COM proposes that evidence of biological plausibility would be suitable for identification of EDC. (EU, 2016). The current EU legal frameworks specify that testing for chemicals with a concern for endocrine disrupting properties should be target-driven and be triggered by relevant concerns or indications thereof. Without such indications for a possible effect on the endocrine system, additional testing is currently not required.

This chapter addresses the question whether the current standard information requirements[2] of the different EU regulatory frameworks give enough information to either identify EDC or to provide triggers to request additional testing (to clarify concern for ED properties). The standard information requirements of the EU regulatory frameworks are compared to the tests included in the OECD Conceptual Framework for testing and assessment of endocrine disrupters (OECD, 2012a). Based on this comparison, section 4.2 discusses to what extent the standard information requirements and the results of the tests that may be used to comply to these requirements address the key elements for the identification of EDC of adversity, endocrine MOA and the underlying biological plausibility.

4.1 Standard information requirements (Table 2)

4.1.1 Industrial chemicals (REACH)

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) of REACH, respectively. The information required under each Annex is additional to all the information required in the previous Annex(es). Information on repeated dose toxicity is only required for tonnages of ≥10 tpa of Annex VIII. At the tonnage level of 10-100 tpa, information on 28-day repeated dose toxicity (TG 407), and reproductive toxicity screening (screening, one species, TG 421/422) is required. At the tonnage level of 100-1000 tpa, information on 90-day toxicity (TG 409), prenatal developmental toxicity (PDNTS) in one species (OECD 414), and, if triggered, an EOGRTS (TG 443) in one species is required. At the tonnage level of >1000 tpa a PDNTS in a second species (OECD 414) could be requested. An EOGRTs (TG 443), if not already requested at Annex IX, is required at Annex X level. Under REACH, TG 443 is implemented without a standard request for the cohorts for F2, and developmental neuro and immunotoxicity (DNT/DIT). These cohorts are

[2] 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.

triggered only in case of specific and well substantiated concern. Carcinogenicity information is only required in very rare cases on basis of specific triggers (more details in Appendix III).

4.1.2 Plant protection products (PPPs)

Regulations (EU) 283/2013 (EU, 2013a) and 284/2013 (EU, 2013b) set out the standard information requirements for active substances and plant protection products, respectively. Short-term and long-term repeated dose toxicity in mammals (TG 407, 408, and one of the TG 451, 452 or 453) as well as developmental and reproductive toxicity studies (TG 414, TG 416) are included in the standard information requirements of PPPR (more details in Appendix I). The extended one-generation reproductive toxicity study (EOGRTS; TG 443) is considered as an alternative to the two-generation reproductive toxicity study (TG 416). Additional data (OECD CF level 2/3), can be requested if considered necessary by the competent authority based on information/results from the minimal required data, as specifically mentioned in Regulation (EU) 283/2013.

4.1.3 Biocides

Similar to PPP, the short- and long-term repeated dose toxicity in mammals (TG 407, 408, and one of the TG 451, 452 or 453) as well as the developmental and reproduction toxicity studies (TG 414, TG 416 or TG 443) should be provided (more details in Appendix II). Similar to PPP, additional data can be requested if considered necessary by the competent authority based on information/results from the minimal required data.

4.1.4 Food contact materials

For food contact materials, data requirements are divided into three classes based on migration levels higher than 0.05 mg/kg food (see appendix IV). In case of high migration (5-60 mg/kg food), an extensive data set is required. The required studies relevant for the detection of EDC contain repeated dose toxicity tests (TG408), developmental (TG414), reproduction (TG416) and carcinogenicity studies (TG 451/452/453). It should be noted that only few chemicals migrate into food in this highest class of migration. For chemicals that migrate in levels between 0.05 – 5 mg/kg food, only a 90 day toxicity study (TG408) in two species is mandatory. For food contact materials that migrate in levels of <0.05 mg/kg food no studies relevant for the detection of endocrine mediated effects are mandatory (more details in Appendix IV).

4.1.5 Cosmetics

Since 2013, it is prohibited to test cosmetic products and cosmetic ingredients on animals; *in vivo* tests thus cannot be performed under the cosmetics framework. None of the *in vitro* tests of the Conceptual Framework are included in the standard information requirements. In the SCCS Notes of Guidance, OECD 455 is referred to as an additional *in vitro* test that may be used to detect estrogen receptor antagonists and/or agonists, but it is not obligatory.

4.1.6 Conclusion

The possibility to detect EDC properties within a framework depends on the number and type of studies and or information that is minimally required. The cosmetic regulation, food contact material regulation (at a migration level of <0.05 mg/kg) and REACH requirements at Annex VII (1-10 tpa) require no ED relevant data. Although in principle, EDC properties according to level 1 of the OECD CF could be detected based on read across, (public) literature available and physico-chemical properties, but this is highly dependent on high quality public literature. The food contact material regulation at a migration level of 0.05- 5 mg/kg and REACH at Annex VIII (10-100 tpa) requires general toxicity studies and reproduction screening studies. The ability to detect endocrine properties is limited as a few set of ED relevant parameters are measured. Moreover, some ED properties can only be detected when test animals are exposed during their critical window in development, which may not be present in 28-day and/or 90-day repeated dose toxicity studies.

The legislations for PPPs, biocides, food contact materials (>5 mg/kg migration) and REACH chemicals at Annex IX and X (>100 tpa) require a more extensive dataset. Studies where test animal is exposed during the critical window of development are available (2-gen study or the EOGRTS). However, information on the EOGRTS may not be available in PPP and Biocide dossiers because this test is a more expensive alternative to TG416.

This report shows that currently minimal required data are insufficient for identification of EDC according to the criteria proposed by the EU Commission.

Table 3 Potential responsiveness of the various studies

Endocrine modalities/axes/pathways	Assays														
	Uterotrophic assay (OECD 440)(Level3)	Hershberger assay (OECD 441)(Level 3)	Enhanced 28-day study (OECD 407)(Level 4)	90-day study (OECD 408) (Level4)	1-generation study (OECD 415) (Level 4)	Male pubertal assay (US EPA OPPTS890,1500) (Level4)	Female pubertal assay (US EPA OPPTS 890,1450) Level 4)	Intact adult male assay (no TG available) (Level 4)	Intact adult male assay (No TG available) (Level 4)	Prenatal dev tox study (OECD 414) (Level 4)	Chronic tox and carcinogenicity studies (OECD 451-3)(Level4)	Enhanced reproductive screening assay (OECD 421) (Level4)	Developmental neurotoxicity study (OECD 426)(Level 4)	Extended 1-generation study (OECD 443)(Level 5)	2-generation study (OECD 416)(Level 5)
Oestrogen	M		A	A	A		A		A	A	A	A	A	A	A
Anti-Oestrogens	M		A	A	A		A		A	A	A	A	A	A	A
Androgen	M	M	A	A	A	A		A	A	A	A	A	A	A	A
Anti-androgen		M	A	A	A	A		A	A	A	A	A	A	A	A
Thyroid		M	(M)[1] /A	A	A	A	A	A	A	A	A	M/A	A	M/A	M/A
Anti-thyroid			(M)[1] /A	A	A	A	A	A	A	A	A	A	A	M/A	M/A
Steroidogenesis			A	A	A	A	A	A	A	A	A	A	A	A	A
HPA/Corticosteroid axis			P	P	P				P	P	P	P	P	P	P
Vitamin D			P	P	P				P	P	P	P	P	P	P

signaling															
Retioid signaling			P	P	P				P	P	P	P	P	P	P
PPAR pathway			P	P	P				P	P	P	P	P	P	P
Other potential endocrine modalities			P	P	P				P	P	P	P	P	P	P

In vivo mammalian toxicity screens and tests listed in the OECD CF, showing their known or potential responsiveness to various (selected and not exhaustive) endocrine modalities/axes/pathways. For each test, its level of the CF is shown: those at Level 3 are suitable for identification of endocrine activity, while those at Levels 4 and 5 are more suitable for hazard identification and characterisation. **M**: screen providing some mechanistic information (not adequate to substantiate no effect); **A**: screen or test providing some apical information; **P**: apical endpoints potentially responsive, but not yet fully evaluated (EFSA, 2013 with some amendments).

[1] Not obligatory.

4.2 General challenges for regulating EDC

From the regulatory perspective, it is of primary importance to clearly define what types of information is needed and how much information is sufficient for establishing whether or not a chemical is an EDC and how to deal with uncertainty if information on various endpoints and mechanisms is lacking.

As shown in the previous sections, the majority of standard information requirements currently set out in the EU legal frameworks rarely supply information on both adversity and MOAs, leading to limitations for flagging an ED concern. An important reason 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. As for any toxicological endpoint, both chemical specific and non-specific effects can be observed in the same *in vivo* experiment. This complicates flagging a certain chemical as EDC. Defining chemical specific effects on both mechanistic and apical endpoints is critical for the identification of EDC. However, currently none of the OECD CF level 2 and 3 tests are implemented in any of the legislations. Tests at levels 2 and 3 of the OECD CF can elucidate MOAs in more detail however the causality between effect and MOA should be considered with care. And even if endocrine parameters show changes in the presence of observed adverse health effects in an intact organism, the former may be the consequence of the latter, complicating the interpretation of biological plausibility of the causality. Additionally, i*n vitro* OECD screening assays are only available for EAS pathways, but not for other pathways for which more and more scientific information of their importance becomes available. For example there is a lack of OECD test methods in detecting the thyroid pathway and components of the HPG and HPT axes (Table 3). In the scientific literature, an extensive amount of data is available for EAS and other endocrine pathways like progesterone receptors, PPARs, retinoid X receptor (RXR), and thyroid hormone receptor (TR), however for these tests no OECD protocol is (yet) available.

In the last few years there has been a lot of debates regarding the questions whether the EDC criteria should include potency. In terms of identification of EDC, it has been recently concluded that potency should not be considered in the identification of a chemical as an EDC (BfR report, 2016, EU, 2016). Besides, uncertainties and challenges for risk assessment of EDC have been addressed (Kortenkamp et al., 2011; JRC 2013 a and b, EFSA, 2013; BfR 2016). These challenges include how to handle low dose effects, non-monotonic dose response, critical windows of exposure, early exposure and late life effects, lack of robust test methods for all EDC associated endpoints, transgenerational effects, epigenetic effects and mixture toxicity. In view of the trend towards a risk based assessment of EDC it is important that methods should be developed to address these uncertainties inherent to data gaps.

Besides scientific limitations also procedural limitations hamper the use of additional information (additional information besides the standard required information). Most frameworks have a clear routing for a

dossier with strict deadlines for submitting information. For example, the authorization process of biocides and PPPs starts with a completeness check by the competent authority (CA). 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 timelines that are set for the process. Consultations between the CA and the applicant on the dossier should take place in a very early stage and the CA has to have a strong case for requesting additional data. Under the PPPR and BPR the options for the CA to include information from outside the dossier are limited since, the principal role of the CA 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 become more common. On the other hand, the quality of the open scientific literature and its variety in study designs require special attention. For industrial chemicals, that have like other regulatory frameworks a lack of basic information requirements on a endocrine MoA, additional testing may be requested in the chemical evaluation process of REACH. However, without an indication of concerns from the standard information requirements, open literature and *in silico* data, additional testing cannot be requested for the chemical evaluation under REACH, because this process asks for a clear substantiation of the concern.

5 Conclusions and recommendations

This report, focusing on EDC issues for human health, points out that the identification of EDC and regulatory consequences work out differently among various EU legal frameworks (See chapter 3). June 15 2016, the EU Commission has proposed draft criteria for identification of EDC for PPP and biocides.
This report shows that currently, minimal required data are insufficient for the identification of EDC based on the criteria proposed by the EU Commission (for details see chapter 4).

Current EU regulations extensively rely on evaluation of chemical-induced adverse apical responses but not on endocrine MOAs. Some *in vivo* tests are not sensitive enough/ not developed to identify EDC. Supportive information can be available from other sources, but the use of such information is hampered by procedural limitations. Information on MOAs has to be obtained from the required repeated dose and reproduction studies on animals, as none of the legal frameworks requires specific studies to collect information on mode of action (level 2/3 CF) as a basic requirement although it is possible to request additional information based on information from the minimal required data in most frameworks if there are specific triggers). None of the repeated dose and reproduction studies are specifically designed to elucidate mode of action. Obtain sufficient information from the available test data to 'flag' a chemical with ED concern would be a challenge. This applies for all evaluated legal frameworks PPP, biocides, REACH, food contact materials. In terms of the cosmetics regulation, no testing is allowed for intact animals. In this case, the information needed to establish a chemical as an EDC will never be generated. To increase the ability of a framework to obtain information on mode of action, additional studies or additional measurements in study protocols should be required. This would imply inclusion of the mechanistic studies (level 2/3 CF), and more comprehensive/obligatory testing of ED related parameters in repeated studies. A recommendation would be to replace TG 416 by TG 443, as the TG 443 includes more ED relevant endpoints. Even if studies currently mentioned in the OECD CF are included as standard requirements in frameworks it should be realised that there is still a lack of OECD methods detecting all ED relevant pathways/modalities (for example *in vitro* OECD screening assays are only available for estrogen, androgen, and steroidogenesis (EAS) pathways, and no mechanistic study in in the OECD CF on other non EATS pathways like PPAR, retionoid X, etc.). Knowledge gaps exist both in mechanistic aspects as well as in their relation to toxicity pathways and adverse health effects in *in vivo* studies. In addition, to fully understand EDC action more information is needed on how chemicals interact within the body and what are their potential effects in humans and/or animals.
In view of the number of endocrine pathways and the long process of developing test guidelines, extending the standard data requirements may not be the best way forward, as it would severely increase the burden for test animals, industry and regulators. Searching other ways is needed in the field of regulating EDC. Therefore it is stressed out that

there is a need for an intelligent and integrated testing strategy for the identification of EDC, which would be able to filter out candidates for further testing. To minimize the need for animal data and at the same time minimize human health risk , such a concept would ask for the development and the mandatory inclusion of (in vitro) screening studies that detect relevant key events for ED effects. The application of the concepts of the adverse outcome pathways (AOP) may play a relevant role here. As noted these concepts only work in the various legal frameworks when information on relevant key events and/or MoA becomes obligatory.

Designation of a chemical as an EDC under WHO definition and EU criteria is a hazard assessment. The subsequent risk assessment of EDC is considered challenging as there are a lot of uncertainties which currently have no scientific consensus. These include low dose effects, non-monotonic dose response, critical windows of exposure, early exposure and late life effects, transgenerational effects, epigenetic effects and mixture toxicity. There should be a clear guidance if and how risk assessment should account for these uncertainties and uncertainties as a result of lack of data.

In summary, the major challenges for regulating EDC begin with the need for consensus methods for establishing biologically plausibility of the cause and effect relationships between endocrine modulation and adverse health effects. Such methods are scarce, and those that are available have only been included in the various legal frameworks to a certain extent. Furthermore up to now these methods have inherent difficulties of proving cause and effect. Therefore, a scientifically based strategy should be developed on how existing endocrine screening tests can be employed for flagging endocrine properties of chemicals. Novel methods should be developed and included in the various legal frameworks where MOA and adverse effect gaps exist. In addition, the strategy should include aspects to be considered in the interpretation of such flags in terms of the biological plausibility of the causal relationship between the endocrine mode of action and the adverse effect. This is a considerable challenge, as under-triggering of endocrine hazard identification should be avoided, but also over-triggering is undesirable. Subsequent risk assessment of EDC should include consideration of aspects such as exposure scenarios and information on dose response. Further guidance on how to deal with potential information gaps and uncertainties in risk assessment needs further attention.

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8 Appendix

The minimum data requirements (studies) in the different legal frameworks is outlined in Table 2 below, more details about the data requirements can be found in the appendixes. Minimal required data means that these studies are required as a minimum for regulatory purposes. Some studies are required only if there is reason for further investigation of a certain effect. For example, neurotoxicity studies are not part of the minimal required data and are mentioned in some frameworks as required data if there is certain indication. These studies are therefore not considered as minimal required data.
Additionally, only studies which are relevant with respect to endocrine disruption (for example genotoxicity studies are not considered relevant for EDC detection) are outlined, and are identical to the studies outlined in the OECD CF.
Some legal frameworks have subdivision in their requirements. For example REACH requirements are based on produced tonnage per year (tpa, tonnage per annum), Food contact materials have requirements based migration levels. For Veterinary medicines are different for products used on food producing animals and non-food producing animals.

8.1 Appendix I (Plant Protection Products)

Specific data requirements are available in Commision Regulation (EC) 283/2013 and commission communication 2013/C 95/01.

Mammalian toxicology

Annex point	**Study type**	**CDS / ADS**	**Protocol**	**Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.**
Regulation (EC) 283/2013 and communication 2013/C 95/01 5.3.1	28 day toxicity study	CDS	OECD 407	
Commission communication 2013/C 95/01 5.3.3	28 day toxicity study	ADS	OECD 410 OECD 412	Additional non-oral studies shall be considered on a case by case basis.
Commission communication 2013/C 95/01 5.3.2	90 day toxicity study	CDS	OECD 408 OECD 409 OECD 411 OECD 413	
Commission communication 2013/C 95/01	90 day toxicity study	CDS	OECD 411 OECD 413	Additional non-oral studies shall be considered on a case by case basis.

Annex point	**Study type**	**CDS / ADS**	**Protocol**	**Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.**
5.3.3				
Commission communication 2013/C 95/01 5.5	Long-term repeated dose toxicity	CDS	OECD 451 OECD 452 OECD 453	
Commission communication 2013/C 95/01 5.6.1	Two-generation reproduction toxicity study or extended one generation toxicity study	CSD	OECD 416 or 443	
Commission communication 2013/C 95/01 5.6.2	Developmental toxicity study	CDS	OECD 414	In two species.
Commission communication 2013/C 95/01 5.6.2	Developmental neurotoxicity study	ADS	OECD 426	
Commission communication 2013/C 95/01 5.8.3	Endocrine disruption	ADS	OECD 456 OECD 441 OECD 455 OECD 440 OCSPP 890.1500 OCSPP 890.1450 US EPA 15-day intact adult male rat assay.	*Endocrine disrupting properties* If there is evidence that the active substance may have endocrine disrupting properties, additional information or specific studies shall be required: — to elucidate the mode/mechanism of action, — to provide sufficient evidence for relevant adverse effects. Studies required shall be designed on an individual basis and taking into account Union or internationally agreed guidelines, in the light of the particular parameters to be investigated and the objectives to be achieved.

8.2 Appendix II (Biocides)

Specific datarequirements referring to the data set for active substances are available in Regulation (EC) 528/2012.

Annex point	**Study type**	**CDS / ADS**	**Protocol**	**Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.**
Regulation (EC) 528/2012 active substance data requirement 8.9.1	28 day toxicity study	CDS	OECD 407 OECD 410 OECD 412	Does not need to be conducted if: Reliable sub-chronic (90 day) study is available, exposure indicates that longer term studies are appropriate, other available data indicate that the substance may have a dangerous property that cannot be detected in a short-term toxicity study, or appropriately designed toxicokinetic studies reveal accumulation of the substance or its metabolites in certain tissues or organs which would possibly remain undetected in a short term toxicity study but which are liable to result in adverse effects after prolonged exposure
Regulation (EC) 528/2012 active substance data requirement 8.9.2	90 day toxicity study	CDS	OECD 408 OECD 409 OECD 411 OECD 413	Does not need to be conducted if: a reliable short-term toxicity study (28 days) is available showing severe toxicity effects according to the criteria for classifying the substance as H372 and H373 (Regulation (EC) No 1272/2008), for which the observed NOAEL-28 days, with the application of an appropriate uncertainty factor allows the extrapolation towards the NOAEL-90 days for the same route of exposure, and a reliable chronic toxicity study is available, provided that an appropriate species and route of administration were used, or the substance is unreactive, insoluble, not bioaccumulative and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-day 'limit test', particularly if such a pattern is coupled with limited human exposure.
Regulation (EC) 528/2012	Long-term repeated dose toxicity	CDS	OECD 452 OECD	Does not need to be conducted if: Long-term exposure can be excluded and no effects have been seen at the

Annex point	Study type	CDS / ADS	Protocol	Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.
active substance data requirement 8.9.3/ 8.11.1			453	limit dose in the 90-day study or a combined long-term repeated dose/ carcinogenicity study (8.11.1) is undertaken. Both rat and mouse (8.11.2).
Regulation (EC) 528/2012 active substance data requirement 8.10.1	Developmental toxicity study	CDS	OECD 414	The study shall be initially performed on one species
Regulation (EC) 528/2012 active substance data requirement 8.10.2	Two-generation reproduction toxicity study or an extended one generation toxicity study	CSD	OECD 416 or 443	If OECD 415 is used justification shall be provided.
Regulation (EC) 528/2012 active substance data requirement 8.13.2	Developmental neurotoxicity study	ADS	OECD 426	
Regulation (EC) 528/2012 active substance data requirement 8.13.3	Endocrine disruption	ADS	OECD GD 178 and 150	If there is any evidence from in vitro, repeat dose or reproduction toxicity studies, that the active substance may have endocrine disrupting properties then additional information or specific studies shall be required to: — elucidate the mode/mechanism of action — provide sufficient evidence for relevant adverse effects For evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route (see Guidance below).

8.3 Appendix III (REACH)

The information requirements under REACH are set out in the Annexes VII to X, and depend on the tonnage level of the substance; the higher the tonnage, the more data is required. In addition, Annex XI is relevant for example for conditions for waiving or the use of alternatives to animal testing.

tpa	**Study type**	**Protocol**	**Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.**
>10 tpa	28 day toxicity study	OECD 407	The short-term toxicity study (28 days) does not need to be conducted if: — a reliable sub-chronic (90 days) or chronic toxicity study is available, , or — relevant human exposure can be excluded in accordance with Annex XI Section 3
>100 tpa	90 day toxicity study	OECD 408	The sub-chronic toxicity study (90 days) does not need to be conducted if: — a reliable short-term toxicity study (28 days) is available showing severe toxicity effects according to the criteria for classifying the substance as R48) for further explanation se column 2 Annex IX,.
-	Long-term repeated dose toxicity	OECD 451 OECD 452 OECD 453	Only if triggered
>10 tpa	Screening reproductive/developmental toxicity	OECD 421 OECD 422	This study does not need to be conducted if: — the substance is known to be a genotoxic carcinogen, or — the substance is known to be a germ cell mutagen and appropriate risk management measures are implemented, or — relevant human exposure can be excluded Annex XI section 3, or — a pre-natal developmental toxicity study (Annex IX, 8.7.2) or a two-generation reproductive toxicity study (Annex IX, Section 8.7.3) is available.
> 100 tpa	extended one generation toxicity study	OECD 443	At Annex IX this study is only requested in case of triggers from in vivo studies. For Annex IX and X the study does not need to be conducted if: — the substance is known to be a genotoxic, or — the substance is known to be a germ cell mutagen and appropriate risk management measures are implemented, or — or of it can be proven the substance is of low concern or no significant human exposure. See column 1 and 2 Annex IX and X
> 100 tpa	Developmental toxicity study	OECD 414	If applicable second species at Annex X

8.4 Appendix IV (Food contact materials)

All data requirements are mentioned in the FCM note for guidance: http://www.efsa.europa.eu/en/efsajournal/doc/21r.pdf

As a general principle, the greater the exposure through migration, the more toxicological information will be required.
(a) In case of high migration (5 - 60 mg/kg food), an extensive data set is needed to establish the safety.
(b) In case of migration between 0.05 – 5 mg/kg food, a reduced data set may suffice.
(c) In case of low migration (<0.05 mg/kg food), only a limited data set is needed (genotoxicity testing only).

Migration	**Study type**	**CDS / ADS**	**Protocol**	**Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.**
a, b	90 day toxicity study	CDS	OECD 408 OECD 409	
a	Two-generation reproduction toxicity study	CSD	OECD 416	One species
a	Developmental toxicity study	CDS	OECD 414	Normally in two species
a	Carcinogenicity study / combined chronic toxicity/carcinogenicity study	CDS	OECD 451 OECD 452 OECD 453	Normally in two species

If the above-mentioned studies or prior knowledge or structural considerations indicate that other biological effects such as peroxisomal proliferation, neurotoxicity, immunotoxicity or **endocrinological** events may occur, additional studies may be required.

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