ECHA
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GUIDANCE

Guidance on information requirements and Chemical Safety Assessment

Chapter R.7a: Endpoint specific guidance

Draft Version x.0

March 2015

LEGAL NOTICE

Guidance on information requirements and chemical safety assessment
Chapter R.7a: Endpoint specific guidance

Reference: ECHA-14-G-03-EN
ISBN: 978-92-9244-749-6
Publication date: xxxxx 2015
Language: EN

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Preface

This document relates to the REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006[1].

This document describes the information requirements under REACH with regard to substance properties, exposure, uses and risk management measures, and the chemical safety assessment. It is part of a series of guidance documents that are aimed to help all stakeholders with their preparation for fulfilling their obligations under the REACH Regulation. These documents cover detailed guidance for a range of essential REACH processes as well as for some specific scientific and/or technical methods that industry or authorities need to make use of under REACH.

The initial guidance documents were drafted and discussed within the REACH Implementation Projects (RIPs) led by the European Commission services, involving stakeholders from Member States, industry and non-governmental organisations. After acceptance by the Member States Competent Authorities the guidance documents had been handed over to ECHA for publication and further maintenance. Any updates of the guidance are drafted by ECHA and are then subject to a consultation procedure, involving stakeholders from Member States, industry and non-governmental organisations. For details of the consultation procedure, please see:

http://echa.europa.eu/documents/10162/13608/mb_63_2013_revision_consultation_procedure_guidance_en.pdf

The guidance documents can be obtained via the website of the European Chemicals Agency

http://echa.europa.eu/web/guest/guidance-documents/guidance-on-reach

[1]. "Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396 of 30 December 2006, p. 1; corrected by OJ L 136, 29.5.2007, p. 3)"

Version	Changes	Date
Version 1.0	First edition	May 2008
Version 2.0	Full revision of the Introduction and Section R.7.1 "Physicochemical properties" within Chapter R.7a: "Endpoint specific guidance" addressing structure and content. The Introduction and Section R.7.1 have been revised by updating, correcting or deleting mistakes and inconsistencies related to actual interpretation and application of generic aspects of the REACH Regulation (EC No 1907/2006) and the overall process for determining physicochemical information requirements in order to fulfil the registration requirements for a substance under the REACH Regulation. The content has been reworked with the aim to help registrants to establish a link between the REACH Regulation and the CLP Regulation (EC No 1272/2008) and guide them on how to comply with both of these Regulations when preparing a chemical safety assessment. As some physicochemical properties – notably explosive, flammable and oxidising properties – are intimately linked to physical hazards and there is thus a link between the physical hazards classification and the respective information requirements on explosive, flammable and oxidising properties it was decided to incorporate the content of the former IR&CSA Guidance Chapter R.9: "Physico-chemical hazards" into relevant sub-sections of Section R.7.1 "Physicochemical properties" of the present document. The original Chapter R.9: "Physico-chemical hazards" of the IR&CSA Guidance will therefore be obsoleted when the present document is published. For the purposes of structuring the updated Guidance document according to CLP but nevertheless allowing the assignment to the respective information requirements of Annexes VII to XI to REACH, an updated and completely revised structure of Section R.7.1 has been implemented. Furthermore, to give the registrants further guidance when applying the general rules for adaptation of the standard testing regime set out in Annexes VII to X of the REACH Regulation a specific sub-section covering further guidance on this topic has been included in the revised text for every endpoint. Similarly an additional sub-section giving advice on how to provide Endpoint specific information in the registration dossier/IUCLID has been included in each relevant section. Information already covered by technical manuals, content falling under the scope of other guidance document or other internationally recognised recommendations has been removed and link to it has instead been provided. The update includes the following: • revision of section Introduction, by eliminating and amending out of date information. • revision of section R.7.1 Physicochemical properties, by reorganising the text in order to reflect the	November 2012

	Guidance structure update. The order of subsections has been modified and several sub-sections added if deemed necessary or deleted where information was identified as redundant. • Addition of a Table showing correlations between the Information requirements as specified in Annexes VII to IX to REACH and corresponding test methods according to the Test Method Regulation and CLP. • Complete revision of content and structure of sections R.7.1.2 – R.7.1.18. • Addition of new sections R.7.1.19 and R.7.1.20 in order that a link with new Appendices addressing recommendations for nanomaterials applicable to physicochemical properties could be established. • Addition of a new section R.7.1.21 in order to remind registrants which further information for classification and labelling in hazard classes of the substance in accordance with Article 10 (a) (iv) of REACH must be included in a REACH registration dossier. • Deletion of Appendices R.7.1-1 "Comments on thermodynamic consistency of physico-chemical properties", R.7.1-2 "pH correction of partition coefficients for ionisable substances" and R.7.1-3 "Temperature correction" and an update of Appendix R.7.1-1 [before R.7.1-4] "Henry's law and evaporation rate".	
Version 2.1	Corrigendum covering the following: • Addition of a new footnote 8 on page 26 with a reference to a comprehensive review paper with the title: "QSPR prediction of physico-chemical properties for REACH" in sub-chapter R.7.1.1.3 Evaluation of available information on physicochemical properties.	August 2013
Version 2.2	Corrigendum correcting the page numbers within the reference in footnote 8 on page 26.	August 2013
Version 2.3	Corrigendum covering the following: • new formatting for the entirety of the R.7a guidance; • new pathfinder figure on the p.6; • addition of a title for a table R.7.1-2: 'CLP Regulation hazard classes for which the REACH Regulation does not require the generation of information'; • a new footnote below tables R.7.1-1, R.7.1.-2, R.7.1.-7 and R.7.1.-15 reminding the reader about changes introduced by the 4th ATP No 487/2013; • a new footnote in chapters R.7.1.10.1 and R.7.1.21.2 reminding the reader about changes introduced by the 4th ATP No 487/2013; • updated *Guidance on the Application of the CLP Criteria* references to reflect the changes of the Version 4.0 published in November 2013.	December 2013

Version 2.4	Corrigendum correcting a value for water density in chapter R.7.1.4.2 and a reference to REACH Annex in chapter R.7.1.16.6 and R.1.18.6.	February 2014
Version 3.0	Full revision addressing the content of sub-sections R.7.7.1 to R.7.7.7 related to Mutagenicity. The update includes the following: • Update of the information on non-testing methods in sub-section R.7.7.3.1, in particular with regard to the prediction models for mutagenicity and the OECD QSAR toolbox; • Update of the information on new/revised OECD test guidelines for genotoxicity testing in sub-section R.7.7.3.1, in particular with regard to the Transgenic rodent (TGR) somatic and germ cell gene mutation assays and the in vivo comet assay; • Amendment of sub-section R.7.7.4 on Evaluation of available information on mutagenicity based on the updated information on non-testing and testing methods; • Amendment of sub-section R.7.7.6 on Integrated Testing Strategy (ITS) for mutagenicity to take into account the new/revised OECD test guidelines for genotoxicity testing, in particular with regard to the recommended follow-up in vivo genotoxicity tests; • Clarification of the similarities and differences between this Guidance and other authoritative Guidance documents with regard to the recommended testing strategy for genotoxicity testing; • Clarification of the Registrant's obligation to submit a testing proposal to ECHA for any test mentioned in REACH Annex IX or X independently from the registered tonnage; • Clarification of the use of genotoxicity test results for Classification and Labelling; • Update of Figure R.7.7-1 on the recommended mutagenicity testing strategy in line with the amended Guidance text; • Update of table R.7.7-5 with addition of a missing title, insertion of a new row presenting a new example case, amendment of outdated information in line with the amended Guidance text; • Update of hyperlinks to ECVAM and ECVAM DB-ALM webpages in different sections across Chapter R.7a.	August 2014
Version x.0	Update to R.7 Structure of Chapter R.7a to reflect revised structure of human health sections. Update to section R.7.6 Reproductive toxicity. The section has been fully revised as follows: • xx •	

Comment [SJ1]: ECHA will elaborate this text at the end of the update process.

Convention for citing the REACH and the CLP Regulations

Where the REACH and the CLP Regulations are cited literally, this is indicated by text in italics between quotes.

Table of Terms and Abbreviations

See Chapter R.20

Pathfinder

The figure below indicates the location of part R.7(a) within the Guidance Document

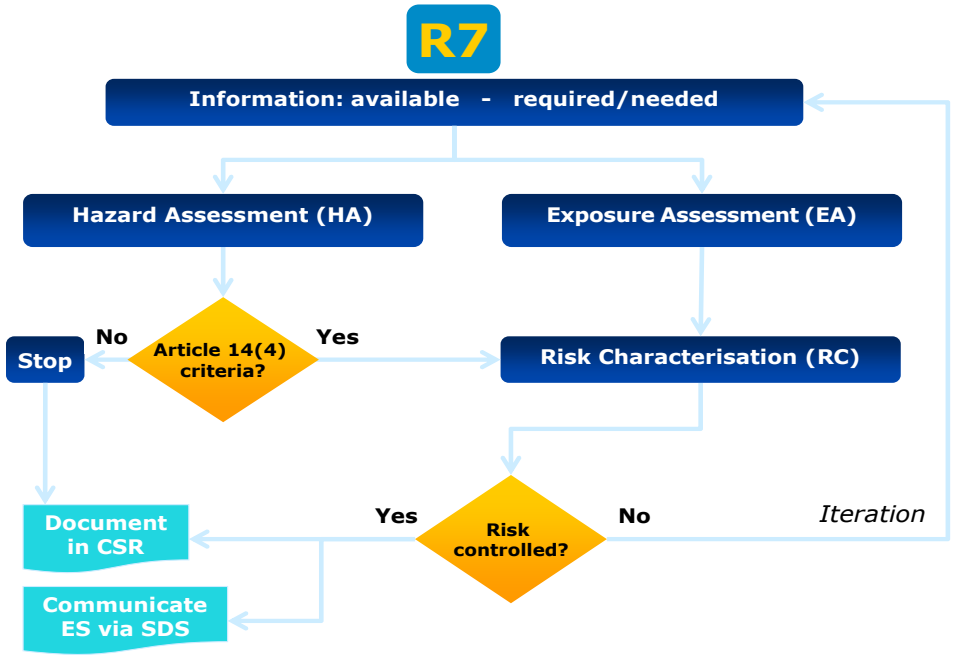

Table of Contents

Comment [SJ2]:
NOTE 1: PLEASE DO **NOT** UPDATE the lists of **Table of Contents, Tables, Figures or Appendices** because all the section numbering will be lost/changed. This will be done at the end of the update. Thank you
NOTE 2: R.7.6: the list of sections is the current published structure – this will be revised during the update.

Tables

Figures

Comment [SJ3]: ECHA will address formatting at the end of the update process

Appendices

Comment [SJ4]: ECHA will address formatting at the end of the update process

R.7 Endpoint specific guidance

Introduction

The previous sections of the Guidance on information requirements and chemical safety assessment (IR/CSA) provide advice on the interpretation and application of generic aspects of the Regulation describing the overall process that should be followed in finding, assembling and evaluating all the relevant information that is required for the registration of a chemical under Regulation (EC) No 1907/2006 (the REACH Regulation). The chapters also describe factors that may have an influence on the information requirements and give advice on how the information collected from different sources could be integrated and used in an ~~weighed evidence based~~ approach to allow a conclusion on whether or not the available information is sufficient for regulatory purposes, i.e. hazard assessment and risk assessment. Under Regulation (EC) No 1272/2008 (CLP Regulation or CLP), this approach is called a weight of evidence determination (WoE). According to CLP, an evaluation by applying WoE determination (i.e. all available information relevant for the evaluation of the specific hazard is considered together) using expert judgment, must always be carried out where the criteria cannot be applied directly (Article 9(3), CLP). This weight of evidence (WoE) determination should not be confused with the use of Weight of Evidence according to Annex XI, 1.2 of REACH, an adaptation rule for standard information requirements where sufficient weight of evidence may allow the conclusion/ assumption that a substance has or has not a particular dangerous property.

The guidance given thus far is applicable across the field and comprises the general rules that should be followed.

Structure of Chapter R.7a

In this chapter, specific guidance on meeting the information requirements set out in Annexes VI to XI to the REACH Regulation is provided. The information requirements relate both to those physicochemical properties that are relevant for exposure and fate considerations as well as to physical hazards, human health hazards and environmental hazards. The guidance for each specified property or hazard has been developed as a specific "sub-chapter" (referred to as a Section) in this guidance, addressing the aspects of collection, generation and evaluation of information to help registrants provide adequate and relevant information for registration under REACH.

All data sources, including non-testing data, have to be taken into account when doing the chemical safety assessment. Most of the reports follow a logical common format that complements the generic guidance and the general decision making frameworks detailed in first paragraph above.

R.7.1 Physicochemical properties

This first "sub-chapter", underwent a guidance revision process between 2011 and 2012 and therefore follows a revised structure. The Section R.7.1 covers both classification and non-classification related properties, where the sections covering the physicochemical properties each have six or seven "sub-sections", depending on the need for information on references and the sections covering the physical hazards have seven "sub-sections" (also referred to as sections).

In the physicochemical properties sections

- the first section details the type of property;
- the second section provides the definition of the property;
- the third lists the preferred test method(s);

Comment [SJ5]: This general introduction is **not in the scope of the guidance update** except to check and amend any editorial errors and incorrect facts.

This section has ONLY been edited to provide a more user friendly format for easier reading and added some text for clarification.

NOTE for the Consultation Procedure: Commenting on this section is not required.

- the fourth section deals with adaptation of the standard testing regime, namely adaptation options that can be explored under each specific physicochemical property;
- the fifth section deals with impurities and uncertainties and the last section outlines what kind of property-specific information should be given in the registration dossier (note that sometimes an additional section is added where relevant references are provided);

By contrast the physical hazard sections

- start with the definition section;
- followed by a second section on classification criteria and relevant information;
- the third section explores various adaptations options, namely how the standard testing regime can be adapted;
- the fourth section outlines the impurities and uncertainties;
- the fifth section aims to help in concluding on the Directive 67/548/EEC (Dangerous Substances Directive - DSD) classification, repealed by Regulation (EC) No 1272/2008 (CLP Regulation or CLP);
- the sixth section outlines the physical hazards-specific information to be included in the registration dossier and in IUCLID;
- the seventh section gives relevant further information and used references.

R.7.2 Human health properties or hazards

Chapters tackling human health properties or hazards in R.7a remain generally unchanged using a similar structure. However as each section is updated the information may be re-organised to be presented in a clearer and more constructive order. In these chapters there are seven main sections to the guidance on each property or hazard;

- the introduction section (R.7.X.1 Introduction) provides an introduction in which the property or hazard is described, further defined and an explanation given as to its importance in the context of human health, or environmental fate and effect of a given substance;
- the second section (R.7.X.2 Information requirements and testing approaches for …..) details the specific information requirements for the endpoint of interest; these will depend on the tonnage band of the substance, its usage pattern and other considerations including data on other endpoints and on related substances. Endpoint[2] specific guidance can be thought of as logical steps that should be taken to assemble the information that is detailed under the second section; thus,
- the third section (R.7.X.3 Information sources on) provides an inventory of all the types of data that could potentially provide useful information on the endpoint of interest and, most importantly the sources of that information;
- in the fourth section (R.7.X.4 Evaluation of available information for ….) on how to evaluate the information that might be available for a given substance; this advice focuses on providing the criteria to aid in the judgement and ranking of the

[2] REACH uses the term "endpoint" both to denote a physicochemical property (example: Annex VII to REACH, Column 1 standard information required: 7.3 Boiling point, and 7.4 Relative density) and to denote hazardous properties (example: Annex VII to REACH, Column 1 standard information required: 7.11 Explosive properties and 7.13 Oxidising properties) which are subject to classification according to the applicable EU legislation. In the following, the wording of Part 7(a) of this guidance document will differentiate between these different types of properties where this appears appropriate, in order to facilitate the identification of properties which serve the regulatory purpose of classification.

available data for their adequacy and completeness. This section may also provide an indication of the remaining uncertainty inherent in the different types of data for the given endpoint;

- The fifth section (R.7.X.5 Conclusions on …..) describes how conclusions may be drawn for a given substance on the suitability of the available information for regulatory purposes. Chemical safety assessment within REACH is fundamentally dependent on an adequate conclusion on classification and PBT/vPvB assessment since exposure assessment and risk characterisation are triggered by classification and fulfilment of PBT/vPvB criteria. Therefore data need to be adequate for both classification & labelling and for chemical safety assessment if the latter is required;
- The sixth section (R.7.X.6 Integrated Testing Strategy (ITS) for …) comprises an Integrated Testing Strategy (ITS) for the given endpoint(s), providing guidance on how to define and generate relevant information on substances in order to meet the requirements of REACH. It is noteworthy that all experiments using vertebrate animals shall be designed to avoid distress and unnecessary pain and suffering to experimental animals, in accordance with Article 7(4) of Directive 86/609/EEC.

  The proposed testing strategies are guidance for data generation in a stepwise approach. The strategies build on the concept that if the available information is not sufficient to meet the regulatory needs, further gathering of information at a succeeding step in the testing strategies is needed. On the other hand, if the available information is adequate and the standard information requirements are met, no further gathering of information is necessary. Standard information requirements will not need to be fulfilled by standard tests, where the available information is judged to be sufficient to adapt the standard information requirement in accordance with Annex XI of REACH or an applicable Column 2 provision of Annexes VII to X of REACH.
- The seventh and final section (R.7.X.7 References) lists all used references on the given endpoints.

Additional considerations

The following additional considerations apply generally to the endpoint specific guidance given in this chapter:

Information requirements in the light of the applicable classification regime

The main regulatory purpose of the information requirements set out in Annexes VI to X to the REACH Regulation is to assess hazards and risks related to substances and to develop and recommend appropriate risk management measures, as highlighted in Recital 19 of the REACH Regulation. According to Recital 26: '*in order to undertake chemical safety assessments of substances effectively, manufacturers and importers of substances should obtain information on these substances, if necessary by performing new tests'*. The chemical safety assessment (CSA) should be performed in accordance with the provisions set out in Annex I of the REACH Regulation. According to Section 0.6 of Annex I, the first three steps of the CSA require the carrying out of a human health hazard assessment, a human health hazard assessment of physicochemical properties and an environmental hazard assessment, including determining the classification of substances. When the REACH Regulation was adopted, the DSD was the applicable classification regime (see, more in particular, the transitional provisions set out in Article 61 of Regulation (EC) No 1272/2008). Accordingly, many REACH information requirements are inspired by the categories of danger under DSD such as points 7.10., 7.11. and 7.13. in Column 1 of Annex VII of REACH (*i.e.* flammability, explosive properties and oxidising properties, respectively).

On 20 January 2009 Regulation (EC) No 1272/2008 (CLP Regulation or CLP) entered into force. The CLP Regulation has amended certain parts of the REACH Regulation (see Article 58 of CLP for amendments applicable from 1 December 2010 and Article 59 of CLP for amendments applicable from 1 June 2015). Nevertheless, the terminology used in REACH currently still comprises terms which were used under the DSD (for substances) and still apply (for mixtures until 1 June 2015) under Directive 1999/45/EC (Dangerous Preparations Directive – DPD). With respect to the updated physicochemical part of this guidance and the section dealing with the exploration of adaptation possibilities of the standard testing regime, the term 'dangerous' can be interpreted in a broader context (particularly, in certain contexts within this document, to include 'hazardous' as defined under CLP) as it does not refer strictly to the DSD.

According to the requirements of Article 10(a)(iv) of the REACH Regulation, the technical dossier required for registration purposes includes the classification and labelling of the substance as specified in Section 4 of Annex VI to REACH, resulting from the application of Titles I and II of CLP Regulation. From 1 December 2010 until 1 June 2015 substances must be classified in accordance with both DSD and CLP and they must be labelled and packaged in accordance with CLP (Article 61(3) of CLP). Similarly, until 1 June 2015 Safety Data Sheets (SDS) must include information on classifications according to both CLP and DSD for substances and component substances in mixtures until 1 June 2015 (see updates to REACH via Commission Regulation (EU) No 453/2010 and the ECHA guidance on the compilation of Safety Data Sheets: http://echa.europa.eu/documents/10162/13643/sds_en.pdf).

Use of data derived from EU or other international standardised test methods

For the purposes of determining whether any of the physical hazards referred to in Part 2 of Annex I of CLP apply to a substance (or a mixture), the manufacturer, importer or downstream user must perform the tests required by the above mentioned Part 2, unless there is adequate and reliable information available (see Article 8(3) of CLP). Further in this guidance for each relevant physical hazard a reference to the corresponding test according to UN Recommendations on the Transport and Dangerous Goods, Manual of Test and Criteria (UN-MTC), starting with a UN test method name will be provided. According to Article 8(5) of CLP, where new tests for **physical hazards** are carried out for classification and labelling purposes, they must be performed in compliance with a relevant recognised quality system (e.g. GLP) or by laboratories complying with a relevant recognised standard (e.g. with EN ISO/IEC 17025), at the latest from January 2014.

For the purpose of determining whether a substance or mixture fulfils the criteria for classification in any of the **human health and/or environmental hazard classes** (and differentiations within a hazard class, if applicable), there is no similar testing requirement. If there is already adequate and reliable information available (see Article 8(2) of CLP), this must be used. Provided that the manufacturer, importer or downstream user has exhausted all other means of generating information, new tests may however be performed (Article 8(1), CLP).

Where new tests for **human health or environmental hazards** are carried out for classification purposes, they must be performed in compliance with a relevant recognised quality system (e.g. GLP) or by laboratories complying with a relevant recognised standard (e.g. with EN ISO/IEC 17025), at the latest from January 2014. (Article 8(5), CLP). Further requirements for tests performed for the purpose of CLP are given in Article 8, CLP.

Further, according to Article 13(3) of REACH, tests for generating information on intrinsic properties of substances must be conducted in accordance with the test methods laid

down in Commission Regulation (EC) 440/2008 (Test Method Regulation)[3] or in accordance with other international test methods recognised by the Commission or the Agency as being appropriate, such as European Standards (EN) (www.cen.eu) or the OECD guidelines (www.oecd.org). Regulation (EC) 440/2008 lays down the test methods to be applied for the purposes of REACH. Thus, in the following sections on specific endpoints, references given for each test method will include the OECD Test Guideline (TG) number and, where available, the test method number, as defined in the Test Method Regulation.

According to Recital 37 of the REACH Regulation, if tests are performed, they should comply with the relevant requirements for protection of laboratory animals, as set out in Council Directive 86/609/EEC[4]. Article 13(4) of REACH states that ecotoxicological and toxicological tests and analyses must be carried out in compliance with the principles of good laboratory practice (GLP) provided for in Directive 2004/10/EC[5] or other international standards recognised as being equivalent by the Commission or the Agency and with the provisions of Council Directive 86/609/EEC, if applicable.

Interdependence of endpoints in hazard assessment

Although guidance is provided for each specific endpoint separately, it should be remembered that different endpoints are related to each other. Information collected within one endpoint may influence hazard/risk assessment of other endpoints, e.g. information on rapid primary degradation of a parent compound may result in including the degradation products in the overall assessment of the toxicity of a substance. Regarding the physicochemical properties of a substance, for example boiling point and flash point are properties used for the classification of flammable liquids, and therefore these properties are important for physical hazard assessment. Similarly, information on toxicity/specific mode of action in one endpoint may indicate possible adverse effects for organisms considered for assessment of other endpoints, e.g. endocrine disrupting mode of action in mammals may indicate the same mode of action in fish. Another example may be when data on toxic effects measured in one group of organisms may be directly used in more than one endpoint, e.g. data from a repeated dose toxicity study may also be used in assessment of risk for secondary poisoning of mammals exposed via the food chains.

Adequacy of methods for generating additional information

Before (proposing) additional animal testing, use of all other options should be considered. It is important to emphasise that testing on vertebrate animals must only be conducted or proposed as a last resort, when all other data sources have been exhausted (see Recital 47 of the REACH Regulation, Article 25 of REACH and Step 4 of Annex VI to REACH). Therefore, it is important to first consider all issues that may impact upon this decision whether and how to perform the testing, such as:

- applicable information requirements pursuant to REACH;
- adaptation possibilities of Annex XI and Column 2 of Annexes VII to X, e.g.:

[3] Council Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [OJ L 142, 31.5.2008, p. 1].

[4] Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC).

[5] Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.

- classifications that may allow for adaptations;
- available data on a category, a group or on individual substances for which the physicochemical and toxicological properties are likely to be similar;
- assumption/conclusion on presence or absence of a particular dangerous property of a substance in a weight of evidence approach based on several independent sources;
- Absence or no significant exposure based on exposure scenarios.

- substance properties;
- available *in vitro* and *in vivo* data;
- available toxicokinetic and toxicodynamic information;
- any trigger/alert that may require testing going beyond the applicable minimum information requirements;

All these issues should be considered, not only to design fit for purpose *in vivo* tests, but also for justifying why an *in vivo* study is not needed under certain circumstances. Animal tests must comply with the provisions laid down in Council Directive 86/609/EEC[6].

Degradation products and metabolites

In the context of evaluating substances for their effects, it is important to note that, once released into the environment or taken up by animals, a substance may be transformed through degradation or metabolism. These processes and their outcome may need to be taken into account in the overall assessment.

Degradation products may be formed as a result of transformation processes in the environment, either biotic or abiotic. For distinguishing the substance undergoing degradation from the degradation products, the former is often referred to as the parent compound.

Degradation products may be formed as a result of abiotic environmental processes such as hydrolysis, direct or indirect photolysis or oxidation. They may also be formed as a result of aerobic or anaerobic biodegradation, i.e. due to microbial activity. Degradation products require further investigation if the Chemical Safety Assessment indicates the need, i.e. if stable degradation products are formed in the environment within a relevant time frame, as deduced from the test system, or if they fulfil the PBT/ vPvB criteria. Likewise it may be considered to assess whether degradation products fulfil the environmental hazard classification criteria (see Section R.7.9 in Chapter R.7(b): Endpoint specific guidance).

Metabolites refer to transformation products, which are formed due to biodegradation (and then the term metabolite is synonymous with the term biodegradation product) or formed as a result of biotransformation (metabolism) within exposed organisms after uptake of the parent compound. Metabolic pathways and hence the identity of metabolites may or may not be fully known. The latter is frequently the case. Moreover for the same substances metabolic pathways may or may not differ between various organisms belonging to different phyla and/or trophic levels. However, the toxicity of metabolites formed within the duration of laboratory tests will be reflected by their parent compound, with the exception of delayed effects which are only evident after the observation time of the tests. Knowledge of metabolic pathways and metabolites may increase planning and focussing of toxicity testing and understanding of toxicological

[6] Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes [OJ L 358, 18.12.1986, p. 1].

findings (see Section R.7.12 in Chapter R.7(c): Endpoint specific guidance). Therefore, in some cases it may be possible to use grouping approaches for structurally closely-related substances, which undergo similar metabolic transformation (see Section R.6.2, Chapter R.6: Guidance on QSARs and grouping of substances).

When biotransformation processes include oxidation, metabolites are often less hydrophobic than the parent compound. This is a very general rule of thumb and may not always apply; however, when it does, often this has implications for the hazard profile of the metabolites. For example more polar metabolites created after oxidation processes have normally a lower adsorption potential, and thus the relevance of the metabolites for the soil and sediment compartments is normally lower than that of the parent compound. Such less hydrophobic metabolites also tend to be excreted more rapidly from organisms than the parent compound. Hence both their bioaccumulative potential and narcotic toxicity tend to be lower.

Similarities in metabolic pathways of structurally-related substances may serve as an indication for waiving for further investigation, depending on the case and nature of the metabolites.

It should be noted that metals, and in particular metal compounds, do not degrade in the environment in the same way as organic substances. They transform usually through dissolution to the dissolved form.

Selection of the appropriate route of administration for toxicity testing

Having established the need for additional toxicity testing to meet the requirements of REACH for a given substance, for certain endpoints, notably acute or repeated dose toxicity but also reproductive toxicity, chronic toxicity and carcinogenicity, a decision must be made on which route(s) of exposure is(/are) most appropriate. The overall objective of such testing is to determine the potential hazard of the test substance to human beings. Humans may normally be exposed to substances by one or more of three routes: inhalation, dermal and oral. In general, the final decision on which route of exposure is to be considered in a particular test should be taken in the light of requirements for the particular endpoint concerned, the recommendation given in the respective test methods, all available information including physicochemical properties of the substance, human exposure, structure-activity relationships (SAR) or the data from available toxicity tests on the substance itself.

If no adequate experimental effect data using the relevant route of administration is available, route-to-route extrapolation might be an alternative method for evaluating the hazard. However this approach should only be used for systemic effects, and not for local effects such as irritation of the lungs following inhalation of a substance. Route-to-route extrapolation is recommended only under conditions where route specific effects are not expected. Therefore, route-to-route extrapolation should be considered on a case-by-case basis taking into account the additional uncertainties. It is to be noted that route-to-route extrapolation is associated with a high degree of uncertainty and should be conducted with caution relying on expert judgment. In a subsequent risk assessment the uncertainties introduced through route-to-route extrapolation should be taken into account, for example by adjusting the assessment factor in the determination of the DNEL (see Section R.8.4.3, Chapter R.8: Characterisation of dose [concentration]-response for human health). Further guidance on this strategic approach to toxicity testing is given in Chapter R.8 Characterisation of dose [concentration]-response for human health.

Assessment of the environmental impact of a substance

With regard to the evaluation of the environmental impact of a substance, the interaction of that substance with the environment is an important consideration. The fate and behaviour of a substance are largely governed by its inherent physicochemical properties. The knowledge of the physicochemical properties of the substance, together

with results from multimedia fate and transport models (e.g. Mackay level 3 models), enables the identification of the environmental compartment(s) of primary concern. Such information will also determine the prioritisation of higher tiered tests. More extensive guidance and considerations on this aspect are given in Chapter R.16: Environmental Exposure Estimation.

R.7.6 Reproductive toxicity

NOTE for the Consultation Procedure

The draft new structure of R.7.6 is proposed as follows: please note that only the first "two levels" of sub-sections are listed here for reference.

Also to note is that this text is only temporary for the consultation procedure and will be removed at the end of the consutlaitons when the Table of Contents for the R7a Guidance document will be updated accordingly.

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R.7.6.1 Introduction

For the general population, reproductive hazards of chemicals are of obvious concern. Similarly, to the individual, an impairment of the ability to reproduce and the occurrence of developmental disorders are self-evidently serious health constraints. Therefore it is important that the potential hazardous properties and risks with respect to reproduction are established for substances. The REACH information requirements have two core objectives:

- to have adequate information in order to decide whether classification and labelling, including categorisation, as a reproductive toxicant is warranted;
- to have sufficient information for the purpose of risk assessment.

The terminology used in various legislation and in context related to reproductive toxicity differs. In this guidance document "reproductive toxicity" is used to cover both the effects on fertility and development and the terms used are "fertility" and "developmental toxicity". Fertility is seen as a broad concept covering all the effects on the reproductive cycle except for developmental toxicity as defined in the text below.

In REACH, the Chemical Safety Report (CSR) format includes the terms "effects on fertility" and "developmental toxicity" under the main heading of "toxicity to reproduction". Also in other texts in REACH, such as in the REACH Annexes, reproductive toxicity is divided into fertility and developmental toxicity[7]. It is worth noting that in IUCLID the main heading for reproductive toxicity (7.8) is "Toxicity to reproduction", the subheading for fertility (7.8.1) is "Toxicity to reproduction" and the subheading for developmental toxicity (7.8.2) is "Developmental toxicity / teratogenicity".

In Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation) as defined in Annex I, the term "*reproductive toxicity*" is used to describe the adverse effects induced (by a substance) on sexual function and fertility in adult males and females, the development of the offspring and adverse effects on or mediated via lactation. Thus, in the CLP Regulation, the differentiation within reproductive toxicity differs from the one stipulated in REACH, namely that lactation effects are considered separately. Hence, for the purpose of classification, reproductive toxicity is divided into three main differentiations, which relate to (i) impairment of male and female reproductive functions or capacity (*fertility*), (ii) the induction of non-heritable harmful effects on the progeny (*developmental toxicity*), and (iii) effects on or via lactation, respectively.

It is necessary to distinguish as far as possible effects on fertility and developmental toxicity for a substance and information on both types of effects is required by REACH above certain tonnage levels. The term "*fertility*" is used in the present guidance document instead of "*sexual function and fertility*" as explained above in order to follow the terminology used in REACH. The term "*sexual function and fertility*" is not used in REACH, however, in specific places, where classification and labelling is discussed, "*sexual function and fertility*" is used as a hazard class in the same context as "*fertility*" used alone. It is to be noted that fertility (as a REACH endpoint) covers functional fertility, morphological and histological changes related to reproductive organs in males and females as well as the ability to produce offspring and to nurse them.

In the following text, endpoints for fertility and developmental toxicity are explained based on the description provided in the CLP Regulation. In practical terms, reproductive toxicity is characterised by multiple diverse endpoints, which relate to impairment of male and female reproductive functions or capacity (fertility), the induction of non-

[7] in Column 2 (see REACH Annexes VIII, IX and X, 8.7.1, Column 2).

heritable harmful effects on the progeny (developmental toxicity), and effects on or via lactation.

Adverse effects on sexual function and fertility include any effect of a substance that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive (oestrus) cycle normality, sexual behaviour, fertility, gestation length, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system.

Developmental toxicity includes, in its widest sense, any effect interfering with normal development of the organism, before or after birth and resulting from exposure of either parent prior to conception, or exposure of the developing organism during prenatal development, or postnatal development, to the time of sexual maturation – thus generally speaking, these effects can be manifested at any point in the life span of the organism. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.[8]

This guidance provides advice on how the registrant can address the reproductive toxicity of the substance and how the information requirements of REACH can be met, thereby providing data on the hazardous properties that can be used for classification purposes and in the risk assessment.

R.7.6.2 Information requirements and testing approaches for reproductive toxicity

Article 10 of REACH specifies the information that is to be submitted for general registration purposes. This information includes minimum information requirements on physicochemical, toxicological and ecotoxicological properties, which are dependent on the tonnage of the registration (Article 10(a)(vi) and (vii) read with Article 12(1) of REACH).

The standard information requirements for the lowest tonnage level are given in Annex VII of REACH. Whenever a higher tonnage level is reached, the minimum requirements of the corresponding Annex (i.e. the Annex for the higher tonnage level) have to be fulfilled in addition to those in all preceding Annexes (see Annex VI of REACH).

For reproductive toxicity, as for any endpoint, all available information must be collected, including data from literature searches. This should then be evaluated with regard to its reliability and relevance, and whether it fulfils the information requirements and their adaptations (triggers and waivers), as well as its use for the purpose of classification, risk assessment and risk management measures.

R.7.6.2.1 REACH information requirements

To examine effects on reproduction, REACH requires information on fertility and developmental toxicity via the "standard information requirements" which are specified in Column 1 of the respective REACH Annexes.

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[8] As written in 3.7.1.3 and 3.7.1.4 in Annex I, CLP (the definition for developmental toxicity is shortened here).

These standard information requirements are minimum information requirements. If there are concerns ("triggers" or "conditions") further testing might be needed to assure availability of appropriate information for chemical safety assessment (including risk characterisation, classification and labelling and other risk management measures).

The term "triggers" is used here as a general term instead of various other possible terms (such as an alert, condition, indication, indication of concern, serious concern, a particular concern) which are used in the REACH Regulation and this Guidance document. A discussion on the evaluation of triggers is given in Appendix 5. For clarification purposes when reading this Guidance document, the terms are used as follows:

- triggers: general terms covering all other terms describing findings/conditions which raise concerns
- alerts: previous term used in this guidance, means the same as triggers but may also include aspects regarding waiving
- conditions: a specific term used e.g. in Annex IX/X for triggering the extension of Cohort 1B, includes aspects which are not findings.

Certain specific adaptation rules described in Column 2 for reproductive toxicity specify when further testing is needed or may be needed at that tonnage level.

REACH information requirements can also be fulfilled by adaptations that reduce the requirement for testing. Adaptation possibilities are either specified in Column 2 of the information requirement or in Annex XI.

An approach on how to fulfil the information requirements is presented in Section R.7.6.2.3 "*Testing approaches and adaptations*".

The information requirements specified in Column 1 (standard information requirements) are generally cumulative with increasing tonnage levels. Column 2 adaptations are linked with the corresponding Column 1 requirement in the respective Annex and should be considered together with the Column 1 requirement. For reproductive toxicity the standard information requirements (Column 1) combined with specific Column 2 adaptations that require different or further testing are as follows:

Annex VIII (applicable for any registration of 10 tonnes or more per year)

- *Screening for reproductive/developmental toxicity[9], one species (OECD TGs 421 or 422[10]) if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from in vitro methods that the substance may be a developmental toxicant;*

In cases where there are serious concerns about the potential for adverse effects on fertility or development, the registrant may propose:

an extended one-generation reproductive toxicity study (B.56 of the Commission Regulation on test methods as specified in Article 13(3) or OECD TG 443) in cases where there are serious concerns about the potential for adverse effects on fertility or peri-postnatal development;

or

a prenatal developmental toxicity study (B.31 of the Commission Regulation on test methods as specified in Article 13(3) or OECD TG 414) in cases where there

[9] Later referred also as a screening study

[10] To date there are no corresponding EU test methods available.

are serious concerns about the potential for adverse effects on prenatal development[11];

Annex IX (applicable for any registration of 100 tonnes or more per year)

- *Prenatal developmental toxicity study, one species, most appropriate route of administration, having regard to the likely route of human exposure[12] (B.31 of the Commission Regulation on test methods as specified in Article 13(3) or OECD TG 414);*

and in cases where Column 2 of REACH Annex IX, Section 8.7.2 applies:

Prenatal developmental toxicity study, second species (B.31 of the Commission Regulation on test methods as specified in Article 13(3) or OECD TG 414);

- *Extended one-generation reproductive toxicity study (B.56 of the Commission Regulation on test methods as specified in Article 13(3) or OECD 443), basic test design (cohorts 1A and 1B without extension to include a F2 generation), one species, most appropriate route of administration, having regard to the likely route of human exposure[12], if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity.*

see Annex IX, Section 8.7.3., Column 2 for the triggers (conditions) when to extend the Cohort 1B to mate the F1 animals and produce the F2 generation, and the triggers (conditions) when to include the Cohorts 2A/2B and/or Cohort 3. For further information on study design see Appendix 2

and in cases where Column 2 of REACH Annex IX, Section 8.7.3. apply for a second species/strain:

Extended one-generation reproductive toxicity study on a second strain or a second species (exceptional cases only).

It should be noted that regarding to the requirement of a second species, the EU B.45, OECD TG 433 prefers the rat and notes that if another species is to be used, justification should be given and appropriate modifications to the protocol will be necessary. There is currently (at the time of publication July 2015) still very limited experience of the protocol and only in rats. This will of course change in the future and Registrants should check for new protocols and updates. It is stated in the OECD TG 443 paragraph 9 that "*When a sufficient number of studies is available to ascertain the impact of this new study design, the Test Guideline will be reviewed and if necessary revised in light of experience gained*."

Annex X (applicable for any registration of 1000 tonnes or more per year)

- *Developmental toxicity study, one* [additional] *species, most appropriate route of administration, having regard to the likely route of human exposure[12] (OECD TG 414);*
- *Extended one-generation reproductive toxicity study (B.56 of the Commission Regulation on test methods as specified in Article 13(3) or OECD 443), basic test design (cohorts 1A and 1B without extension to include a F2 generation), one species, most appropriate route of administration, having regard to the likely*

[11] It is strongly recommended that the registrant considers conducting a screening study in addition to the prenatal developmental toxicity study to cover the fertility and early peri/post natal development

route of human exposure[12], unless already provided as part of Annex IX requirements.

see Annex X, Section 8.7.3., Column 2 for the triggers (conditions) when to extend the Cohort 1B to mate the F1 animals and produce the F2 generation, and the conditions when to include the Cohorts 2A and 2B and/or Cohort 3. For further information on study design see Appendix 2.

and in cases where Column 2 of REACH Annex IX, Section 8.7.3. apply for second species/strain:

Extended one-generation reproductive toxicity study on a second strain or a second species, in exceptional cases if not already provided as part of Annex IX requirements. (for further explanation see Annex IX above)

A simplified summary of the information requirements for reproductive toxicity is presented in the following Table R.7.6-1.. The standard information requirements of REACH Annexes VIII to X, Section 8.7. Column 1 are indicated combined with specific Column 2 adaptations that require different or further testing.

Table R.7.6-1. Summary of information requirements for reproductive toxicity in REACH (Annexes VII to X).

Comment [SJ6]: ECHA will check numbering/ formatting at the end of the update process

Study	Annex VII (<10 t/yr)	Annex VIII (≥10 t/yr)	Annex IX (≥100 t/yr)	Annex X (≥1000 t/yr)
Screening test for reproductive /developmental toxicity (OECD TG 421 or 422)		Required	Strongly recommended if no higher tier study (such as OECD TG 443) is/will be available to address fertility and peri/post natal development	(a higher tier study is required)
Prenatal developmental toxicity study (EU B.31, OECD TG 414)		May be proposed in case of serious concern[1] for prenatal developmental toxicity instead of the screening study. However, it is strongly recommended to consider conducting a screening study in addition to the prenatal developmental toxicity[2] study	Required in one species; second species may be triggered[3]	Required in two species
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)[4]		May be proposed in case of serious concern for fertility instead of the screening study[1]	Required in one species if triggered[5]; second species/strain may be triggered in exceptional cases	Required in one species unless already conducted at previous Annex level; second species/strain may be triggered in exceptional cases

NOTES for Table R.7.6-1

[1] Column 1 and Column 2 provisions at Annex VIII, 8.7.1, need to be considered together. Serious concern reflects a high likelihood for adverse effects on reproductive health.

[2] See discussion at Stage 4.3 (i) Reproduction/developmental toxicity screening test under Section R.7.6.2.3.2

[3] For discussion on triggers see Stage 4.4 (ii), Prenatal developmental toxicity study under Section R.7.6.2.3.2.

[4] Basic study design addressing fertility and developmental toxicity effects manifested after birth with Cohort 1A and Cohort 1B without extension of Cohort 1B, see Stage 4.4 (iii) and Stage 4.5 (ii) Extended one-generation reproductive toxicity study under Section R.7.6.2.3.2 for overview and Appendix 2 and 3 for details

[5] For description of triggers see Stage 4.4 (iii), extended one-generation reproductive toxicity study under Chapter R.7.6.2.3.2.

Key objectives and information produced by the test methods referred to in the REACH Regulation for reproductive toxicity are explained in short below in the text and in Table R.7.6-2. More information on how these studies are to be used in a REACH context and important aspects to consider during planning and evaluation are described in Section R.7.6.4.2.

Annex IX and X level studies and other studies considered not to be screening level studies, require a testing proposal.

R.7.6.2.2 Key objectives and information produced by the test methods referred to in REACH

R.7.6.2.2.1 Reproduction/Developmental Toxicity Screening Test

The purpose of the reproduction/developmental toxicity screening tests (OECD TGs 421 and 422) is to provide initial information of the effects on male and female reproductive performance such as gonadal function, mating behaviour, conception and parturition and histopathological information on reproductive organs. Initial information on the offspring is limited to mortality, abnormal behaviour and body weight of pups after birth and a macroscopic examination. These screening tests are not meant to provide complete information on all aspects of reproduction and development.

R.7.6.2.2.2 Prenatal developmental toxicity study

The prenatal developmental toxicity study (EU B.31, OECD TG 414) provides a focused evaluation of potential effects following prenatal exposure, although only effects that are manifested before birth can be detected. More specifically, this study is designed to provide information on substance-induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses.

R.7.6.2.2.3 Extended one-generation reproductive toxicity study (EOGRTS)

The extended one-generation reproductive toxicity study (EOGRTS, EU B.56, OECD 443) allows evaluation of effects of the test substance on the integrity and performance of the adult male and female reproductive system, prenatal effects manifested postnatally and postnatal effects of chemicals on development as well as a thorough evaluation of systemic toxicity in pregnant and lactating females and young and adult offspring. EOGRTS is a modular study design with various investigational options. A check list for information that should be presented in the dossier in order to establish the existence or the nonexistence of the conditions and triggers specifying the study design for EOGRTS regarding the extension of Cohort 1B, inclusion of Cohort 2 and/or Cohort 3 is provided in Appendix 1. More detailed information and examples of triggers and conditions for extension of Cohort 1B and the need to include Cohort 2 and/or Cohort 3, are presented in Appendix 2.

The focus of the study in the REACH Annexes is on fertility[12], which should be considered in the study design of the EOGRTS Thus, as a starting point, a 10-week premating exposure duration and a highest dose level with the aim to induce some toxicity for all variant study designs of EOGRTS should be proposed, however based on substance specific justifications the premating exposure duration may be shorter than 10 weeks but should not be shorter than two weeks. Discussion on the premating exposure duration and considerations for a shorter than 10 weeks period are provided in Appendix 3. Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked.

The basic study design, which is the standard information requirement at Annexes IX and X[13], focuses on evaluation of the fertility of parental animals (F0 animals) and of defined parameters on postnatal development of F1 animals until adulthood (see the test method, EU B.56, OECD TG 443) . The basic study design does not include mating of F1 animals (extension of Cohort 1B) or cohorts for developmental neurotoxicity (Cohorts 2A and 2B) or developmental immunotoxicity (Cohort 3). The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) provides information on the fertility of the offspring, (i.e. the F1 generation), which has been exposed already during primordial germ cell and germ line formation, pre-implantation, *in utero* and postnatal periods. The fertility of Cohort 1B animals, if mated, is evaluated after exposure of full spermatogenesis.

Cohorts 2A and 2B provide information on developmental neurotoxicity and Cohort 3 on developmental immunotoxicity; this information is not covered by any other study within REACH requirements.

Conditions for triggering extension of Cohort 1B and Cohorts 2 and 3 are adaptations to the standard information requirement described in Column 2 and must be proposed by the registrant if the triggers (conditions) described in Column 2 are met (see Appendix 1 and 2).

Table R.7.6-2 Overview of *in vivo* EU test methods and OECD test guidelines for reproductive toxicity referred to in REACH

Test	Design	Focus of examination
Reproduction/ developmental toxicity screening test (OECD TG 421 and	Exposure from 2 weeks prior to mating (P) until a specified post-natal day (F1)	*Parental (P) generation:* Growth, survival, fertility (limited) Pregnancy length and litter size

[12] Recital (7) of Commission Regulation (EU) 2015/282 of 20 February 2015. amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended one-generation reproductive toxicity study: *"It should be ensured that the reproductive toxicity study carried-out under point 8.7.3 of Annexes IX and X to Regulation (EC) No 1907/2006 will allow adequate assessment of possible effects on fertility. The premating exposure duration and dose selection should be appropriate to meet risk assessment and classification and labelling purposes as required by Regulation (EC) No 1907/2006 and Regulation (EC) No 1272/2008 of the European Parliament and of the Council."*

[13] Recital (6) of Commission Regulation (EU) 2015/282 of 20 February 2015. amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended one-generation reproductive toxicity study: *"The standard information requirement in Annexes IX and X to Regulation (EC) No 1907/2006 should be limited to the basic configuration of EOGRTS. Nevertheless, in certain specific cases, where justified, the registrant should be able to propose and the European Chemicals Agency (ECHA) should be able to request the performance of the F2 generation, as well as the DNT and DIT cohorts."*

Test	Design	Focus of examination
422)	3 dose levels plus control Preferred species rat Preferred route oral[1] N = 10 mating pairs per dose group	Histopathology and weight of reproductive organs Histopathology and weight of major non-reproductive organs (OECD TG 422 only) *Offspring (F1):* Growth and survival until a specified post-natal day
Prenatal developmental toxicity study (EU B.31, OECD TG 414)	Maternal exposure at least from implantation to one or two days before expected delivery 3 dose levels plus control Preferred species rat and rabbit Preferred route oral[1] N = 20 pregnant females per dose group	*Maternal animals:* Growth, survival, (effects on implantation only if dosing is started before implantation), maintenance of pregnancy *Offspring:* Resorptions, foetal deaths foetal growth Morphological variations and malformations (external, skeletal and visceral)
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) REACH requires a "basic study design" with a focus on fertility and defines specific conditions for the extension of Cohort 1B and/or inclusion of Cohorts 2A and 2B and/or Cohort 3 (see section R.7.6.4.2.3 and Appendix 2 of this Guidance)	Exposure of 10 weeks prior to mating[2] (P) until post-natal day 90-120 (Cohorts 1A and 1B). If the extension of Cohort 1B is triggered, then until post-natal day 4 or 21 (F2)[3]. 3 dose levels plus control; highest dose level must be chosen with the aim to induce some toxicity. Preferred species rat Preferred route oral[1] N = sufficient mating pairs to produce 20 pregnant animals per dose group (P generation) N = 20 mating pairs (extension of Cohort 1B, if triggered) N = 10 males and 10 females per dose group (Cohorts 2A, 2B and 3, if triggered)	*Parental (P) generation:* Growth, survival, fertility Oestrus cyclicity and sperm quality Pregnancy length and litter size Histopathology and weight of reproductive and non-reproductive organs Haematology and clinical chemistry *Offspring (F1):* Growth, survival and sexual maturation Histopathology and weight of reproductive and non-reproductive organs (Cohort 1A) Weight of reproductive organs and optional histopathology (Cohort 1B) Haematology and clinical chemistry Fertility of F1 animals to produce F2 generation (extension of Cohort 1B) under certain conditions Developmental neurotoxicity (Cohorts 2A and 2B or a separate study) in case of a particular concern Developmental immunotoxicity (Cohort 3 or a separate study) in case of a particular concern

NOTES for Table R.7.6-2

[1] See Stage 4.1 (iv) for discussion on route of administration (Section R.7.6.2.3.2).

[2].Unless data to support a shorter pre-mating period (see discussion in Appendix 3)

[3] According to the test method EU B.56 (OECD TG 443) the F2 generation may be terminated on postnatal day 4 or 21. For further details see R.7.6.4.2.3.6, Extended one-generation reproductive toxicity study, Further aspects to consider related to extension of the Cohort 1B.

R.7.6.2.3 Testing approaches and adaptations

R.7.6.2.3.1. Overview

This section describes how to use testing approaches and adaptations to achieve the core objectives of REACH (to fulfil information requirements for adequate risk assessment and classification and labelling purposes) with effective use of the gathered information and for designing potential actions needed to fulfil information requirements and to ensure the safe use of substances.

While Column 1 describes the standard information requirements, Column 2 sets certain rules if further or different information is triggered or if information may be omitted – thus, Column 2 specific adaptation rules should be considered together with Column 1 standard information requirements. Adaptation may mean further or less information needs than specified in Column 1 standard information requirements. In case where the specific adaptation rules in Column 2 or general adaptation rules in Annex XI are not met, the standard information requirements must be fulfilled.

The Registrant is guided in a step-by-step tiered manner on how to meet the information requirements within the production tonnage and influenced by triggers (or conditions) which may increase the need for information or conditions which may allow adaptation of standard information requirements by means of replacing, omitting or adapting in another way. Adaptations of information requirements always need to be clearly stated and supported by adequate justification demonstrating the fulfilment of applicable conditions established by REACH.

As an initial step, all available information relevant to reproductive toxicity must be collected for substances manufactured or imported at tonnage levels ≥1 t/y (REACH Annexes VII-X)(see Annex VI, Step 1). Information from literature may assist identifying the presence or absence of hazardous properties of the substance. In addition, information on exposure, uses and risk management measures should be collected. This information needs to be evaluated with regard to relevance and reliability and to decide if it is adequate for the purposes of risk assessment and classification for reproductive toxicity, including a comparison with the criteria for classification (Annex I, CLP); (see also Guidance on the *Application of the CLP criteria* and Guidance on *Information requirements and chemical safety assessment* Chapter R.3 on *Information gathering* and Chapter R.4 on *Evaluation of available information*). Considering all the information together, the registrant will be able to determine the need to generate further information in order to fulfil the information requirements.

Consistent with the information requirements defined within REACH Annexes VII to X, testing for reproductive toxicity is not required as a standard approach for registrations of chemicals for the manufacture or import at tonnage levels below 10 tonnes per year (Annex VII). At higher production volumes (i.e. ≥10 t/y, ≥100 t/y or ≥1000 t/y), standard information requirements are staggered according to tonnage levels of the registrations. Flexibility to adopt the most appropriate testing regime for any single substance is maintained by using adaptation rules provided by Column 2 and Annex XI. The adaptation rules are the key components of the testing approaches.

However, regardless of tonnage level, before any testing is carried out, careful consideration by the registrants of the following is required: all the available toxicological data, the classification for reproductive toxicity, carcinogenicity and germ cell mutagenicity (EU harmonised or self-classification), human exposure characteristics and current risk management procedures; these are necessary to ascertain whether the information requirements can already be met (see *Guidance on information requirements*

and chemical safety assessment Chapter R.5 on *Adaptation of information requirements*). If it is concluded that testing is required in order to fulfil the information requirements, for reasons such as due to triggers, data gaps which cannot be adapted (for the purpose of classification and/or risk assessment), increases in production volumes resulting in an Annex upgrade, then a series of decision points are defined and described below to help shape the scope of an appropriate testing programme. The REACH approach provides a four-stage process for clear decision-making, relevant for all tonnage levels.

Stage 1: Consider hazardous CMR properties meeting the classification criteria to Category 1A or 1B to decide on the need for further reproductive toxicity testing. Based on Column 2 adaptation of Section 8.7 in REACH Annexes further information on reproductive toxicity may be omitted in certain conditions described in Column 2. Therefore, dependent on the outcome of this analysis, it is possible that some chemicals may not progress beyond Stage 1.

Stage 2: Clarify the standard information requirements relevant for manufactured/imported tonnage level of a single registrant or a SIEF[14].

Stage 3: Evaluate the available toxicology database and consider reproductive toxicity findings and conditions that may serve as triggers or allow omitting further studies. This evaluation should also consider information from substances with a similar structure or causing toxicity via similar mechanisms/modes of action. The aim of this stage is to ensure that the applicable REACH information requirements are identified and to determine the scope of the reproductive toxicity testing necessary to adequately clarify the reproductive toxicity properties. Following this review in conjunction with the analysis in Stage 1 or if sufficient data for risk assessment/risk management and classification purposes are available allowing adaption based on Column 2 or Annex XI adaptation rules, it is possible that no further testing may be necessary.

If the specific adaptation rules in Column 2 or general adaptation rules in Annex XI are not met, the standard information requirements must be fulfilled. Thus, any scientific or other substance-specific justifications for adaptation, must follow Column 2 or Annex XI adaptation rules.

Stage 4: Plan and conduct a screening study or plan and propose a prenatal developmental toxicity study or an extended one-generation reproductive toxicity study or specific other studies in exceptional cases. In accordance with Article 12.1d-e/Article 22.1h of REACH, a testing proposal must be submitted to ECHA.

R.7.6.2.3.2 Procedure for testing approaches and adaptations

Collection of data

At all Annex levels, the available information from human, animal and non-animal studies and testing approaches need to be collected, including data from literature searches which needs to be evaluated and documented (see Annex I, Step 1 of REACH).

Stage 1: Genotoxic carcinogenicity, germ cell mutagenicity and reproductive toxicity (CMR- properties) to be considered before deciding whether any testing for reproductive toxicity potential is required (relevant for <u>all</u> tonnage levels)

If the answer at the Stage 1.1 and/or Stage 1.2 is yes, i.e. the substance has been already classified to Category 1 for any of the CMR property (as described below), no further testing for reproductive toxicity may be needed if the conditions are fulfilled and appropriate risk management measures are in place.

[14] SIEF is a substance information exchange forum

Stage 1.1: Has the substance already been classified[15] for effects on sexual function and fertility *and* developmental toxicity (Reproductive toxicity Category 1A or 1B (H360FD))?

If the answer is no, proceed to Stage 1.2. If the answer is yes and the available data are adequate to support a robust risk assessment, then no further testing may be necessary. However, if the substance is classified for fertility only, further testing for developmental toxicity must be considered and if the substance is classified for developmental toxicity only, further testing for fertility must be considered; then proceed to Stage 2 via Stage 1.2. If the available data are not adequate to support a robust risk assessment then proceed to Stage 2.

Stage 1.2: Is the substance known to be[16] a genotoxic carcinogen (Carcinogenicity Category 1A and at least Germ cell mutagenicity Category 2; or Carcinogenicity Category 1B and at least Germ cell mutagenicity Category 2) or as a germ cell mutagen (Germ cell mutagenicity Category 1A or 1B) and appropriate risk management measures are implemented?

If the answer is no, proceed to Stage 2. If the answer is yes, it is important to establish that appropriate risk management measures addressing potential carcinogenicity, genotoxicity and reproductive toxicity have been implemented and therefore further specific testing for reproductive and/or developmental toxicity will not be necessary.

Stage 2: Clarify the standard information requirements

At this stage it is relevant to understand what the standard information requirements are at the tonnage level relevant to the registrant. The registrant must fulfil the standard information requirements unless the Column 2 or Annex XI adaptions rules are met to omit the study. In addition to standard information requirements presented in Column 1, Column 2 adaptation rules may indicate triggers (or conditions) for further studies or if certain study design must be proposed.

Stage 3: Conduct a detailed review of the available relevant toxicological data to identify conditions to adapt standard information requirements for reproductive toxicity

At Stage 3, the available relevant data is examined to verify if any of the adaptations rules beyond "CMR classification adaptations" explained at Stage 1 are met. Adaptation rules may allow omitting the study or indicate when further information may be needed or must be proposed.

Before any testing is conducted, a thorough data review should be conducted.

Following the adaptation based on CMR classification considered in Stage 1, further general adaptation possibilities of Annex XI and specific adaptation possibilities for omitting the testing provided in column 2 of the REACH Annexes should be explored. These adaptation rules are described in Stage 3.1 in Appendix 4. These adaptation rules apply to substances for which standard information requirements apply because they passed the Stage 1.

It is important to consider both Column 2 and Annex XI adaptation possibilities because new tests on vertebrates must only be conducted or proposed as a last resort when all other data sources have been exhausted (REACH Annex VI, Step 4).

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[15] Harmonised classification or self-classification meeting the classification criteria

[16] Harmonised classification or self-classification meeting the classification criteria

If sufficient data are available to permit an adaptation according to Column 2 and/or Annex XI rules, then no further testing is required. If the rules for adaptation according to Column 2 or Annex XI are not met and there is a data gap, then the testing strategy for reproductive and/or developmental toxicity in Stage 4 should be followed.

Standard information requirements are described in Column 1 at each Annex. At Annex IX, if there are triggers for reproductive toxicity (fertility and postnatal development) an extended one-generation reproductive toxicity study must be proposed. For definition of triggers and how to evaluate them, see Appendix 5. The examples for triggers for extended one-generation reproductive toxicity study at Annex IX are described in this Section, under Stage 4.4 (iv), extended one-generation reproductive toxicity study.

If the data are insufficient, which study (or studies) is (are) most appropriate? This decision must take account of both the tonnage-related standard information requirements, the nature of the trigger(s) and total assessment of data.

REACH standard information requirements are minimum information requirements and triggers for reproductive toxicity may indicate a need for further information. Where there is an information gap that needs to be filled, new data must be generated (REACH Annexes VII and VIII) or a testing approach must be proposed (REACH Annexes IX and X). Note that other data sources need to be explored and new tests on vertebrates must only be conducted or proposed as a last resort when all other data sources have been exhausted (Annex VI, Step 4). Whether the registrant must or should or may propose/conduct further information beyond the standard information requirement depends on the Annex level and the provisions in Column 2 and any further concerns. These are further explained at Stage 3.2 and Appendix 5.

Stage 3.1 Substances for which the standard information requirement apply after Stage 1 – options for adaptation rules which may apply instead of conducting new studies

These are substances which are not classified for CMR properties as described in Stage 1 (are not genotoxic Category 1 carcinogens, germ cell Category 1 mutagens or Category 1 reproductive toxicants (fertility and development)).See Appendix 4 for details of adaptation possibilities for these substances. Stages 3.1.1-3.1.7 include Annex XI adaptations based on 1) existing information from non-GLP or test methods not referred in the test method regulation, 2) existing historical human data, 3) existing information in a weight of evidence approach, 4) non-animal approaches such as QSAR approaches and *in vitro* methods, 5) grouping and read across, 6) technical reasons, and substance-tailored exposure driven testing. Stage 3.1.8 describes adaptations based on Column 2 rules others than based on CMR classification described at Stage 1.

Stage 3.2 Substances for which there are triggers for further information needs beyond the standard information requirements (Column 1)

Whereas Column 1 describes the standard information requirements (and triggers for those), Column 2 includes triggers for further information needs (in addition to provision to omit studies which are described at Stage 3.1.8).

Column 2 triggers may have various levels of requirements/consequences:

1) the registrant must act
2) the registrant should act
3) the registrant may act

The consequence level, depends on the wording in Column 2. If there is further concern on reproductive toxicity beyond the information requirements (Column 1 and 2 provisions), it is the responsibility of the registrant to consider how to address the concern to ensure the safe use of that substance. The various triggers related to

reproductive toxicity and how to evaluate them are described in Appendix 5, Triggers for further information needs beyond the standard information requirements.

Stage 4. Reproductive toxicity tests triggered by tonnage level or by findings/conditions which raise concerns for further studies identified in Stages 1-3

Stage 4.1 Preliminary considerations

(i) Introduction

It has to be noted that if studies listed in REACH Annexes IX and X like the prenatal developmental toxicity study or the extended one-generation reproductive toxicity study are intended to be performed, a testing proposal has to be submitted to ECHA. Furthermore, before the result from a study for which a testing proposal is submitted to ECHA will be available, risk management measures have to be put in place, recorded in the chemical safety report and recommended to downstream users according to REACH Annex I, 0.5.

A brief description of the protocols for the studies listed in REACH Annexes are presented at Stages 4.2, 4.3 and 4.4 according to registration tonnage levels. When planning any reproductive toxicity studies, considerations such as the properties of the substance, dose levels, vehicle, adequate study design, route and animal species, are needed. Some of these considerations especially relevant for reproductive toxicity testing are presented below.

(ii) Range-finding studies

It is recommended that the dose range-finding studies are reported together with the main studies (in IUCLID) to provide sufficient information and justification for the doses selected for testing. The findings from a range-finding study may also support the interpretation of the results from the main study.

(iii) Selection of vehicle

Most of the test methods guide on selection of vehicle if that is needed. For use of all other vehicles except for water a justification is needed and has to be documented. The vehicle should not cause any adverse effects itself as that may interfere with the interpretation of the results and may invalidate the study. The vehicle must also not react with the substance or interfere with toxicokinetics of the substance or affect significantly the nutritional status of the animals. The Control group should receive the same vehicle and at the same dosing volume as the treated groups.

(iv) Route of administration for reproductive toxicity studies

REACH specifies that the reproductive toxicity studies should be conducted via the "*most appropriate route of administration, having regard to the likely route of human exposure"*. "*Likely routes of human exposure*" within REACH are oral, inhalation and dermal. The selection of the "most appropriate route of administration" focuses on identification of hazards (Section R.7.2-7) and depends on the most appropriate route for identification of the intrinsic properties of the substance for reproductive hazard.

According to the test methods for reproductive toxicity which focus on the detection of reproductive hazards, the oral (gavage, in diet, or in drinking water) route is the "default" route, except for gases. For the extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) dietary administration may be often an appropriate route to model human exposure. If another route of administration other than oral is used, the registrant should provide justification and reasoning for its selection. In practice, testing via the oral route is usually performed with liquids and dusts and testing via inhalation route is usually performed with gases and with liquids with very high vapour pressure. Testing via dermal route might be necessary under specific circumstances, for example for substances with high dermal penetration and indications for a specific toxicity

following dermal absorption. Dermal application or inhalation route using nose-only administration may need specific considerations to assure that the administration can be adequately conducted without causing confounding factors, e.g. cause additional stress to the pregnant animals. Case-specific deviations from the default approach must be justified, e.g. in case of available information on route-specific toxicity or toxicokinetics indicating that the use of oral administration of substance would not be relevant for assessing the human health hazards via inhalation, which would be the main route of exposure.

It is to be noted that corrosive or highly irritating substances should be tested preferentially via the oral route. The vehicle should be chosen to minimise gastrointestinal irritation. For some substances dietary administration may allow adequate dosing without irritation compared with oral gavage dosing. In certain cases, testing of neutral salts of alkaline or acidic substances may be appropriate and allows investigation of intrinsic properties at adequate dose levels. In case of immediate hydrolysis of a substance, it may be possible to provide information on all the cleavage products. For this read-across approach adequate justification and documentation is needed according to Annex XI, 1.5. For corrosive or irritating vapours or gases for which oral testing is not possible, the highest concentration for inhalation should be chosen carefully to induce some toxicity (or mild irritation).

(v) Selection of species

The most common species used for reproductive toxicity testing is the rat. There is good historical background information for various rat strains which may be used to support the interpretation of the results. The strain selected should have an adequate fecundity and not too high spontaneous malformation incidence or any other specific feature that may reduce the adequacy of the strain to study reproductive toxicity of a substance in question. In order to make integrated data interpretation including information from other studies, it is recommended to use the same strain both in reproductive toxicity testing as well as repeated dose toxicity studies.

For prenatal developmental toxicity studies, testing in two species is a standard information requirement for registrations at 1000 or more tonnes per year (and might be triggered at lower tonnage levels). According to the test methods (EU B.31, OECD TG 414), the rat is the preferred rodent species and the rabbit the preferred non-rodent species. The extended one-generation reproductive toxicity study may need to be conducted using a second strain or species in certain exceptional cases. (For details see Stage 4.5 (ii) under Section R.7.6.2.3.2.). The most sensitive species and/or strain should be used as a first species taking into account the human relevancy, if known.

However, in choosing the appropriate species or strain of animal, consideration must be given to the suitability of the species and strain for the test protocol, and the availability of background information on the species and strain for the test protocol. The species/strain selection should be justified if the default species referred to in a test method is not used.

(vi) Dose level selection

Like in repeated dose toxicity studies the highest dose level should be chosen with the aim to induce some toxicity unless limited by physical or chemical properties of the substance (e.g. flammability and explosivity limits). Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked. Generally at least three dose levels and a concurrent control must be used, except where a limit test (1000 mg/kg bw/day which is generally referred to as the oral limit dose level) is conducted. Expected human exposure may indicate the need for a higher dose level to be used than

a 1000 mg/kg bw/day[17]. The conditions for applicability of a limit test are provided in the individual test methods for reproductive toxicity. For inhalation exposure, OECD Guidance document 39 may be used.

Dose level selection is assisted by the information from existing studies as well as from specific dose range-finding studies that may need to be conducted. Toxicokinetic information may provide reasons to adjust e.g., the dosing route and regime. In addition, it should be considered that toxicity and toxicokinetics in pregnant animals may differ to that in non-pregnant animals. This may cause challenges in selecting the highest dose level for the study as at various phases of the study the sensitivity of the animals may differ.

For fertility as well as developmental toxicity it is important to investigate whether these reproductive toxicity effects are considered to be a secondary non-specific consequence of other toxic effects seen, such as, maternal toxicity, which may occur at the same dose level as the reproductive effects. However, in general, all findings on reproductive toxicity should be considered for classification purposes even if they are seen in the presence of parental toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must then be performed[18]. Thus, it is important to get information about the reproductive toxicity profile of a substance including the spectrum of reproductive toxicity effects related to different dose levels as well as information to allow evaluation of the potency for reproductive toxicity of a substance. Therefore, the highest dose level should be intended to produce some toxicity to provide adequate information on reproductive toxicity for the purpose of both classification (including categorisation within the Reproductive toxicity hazard class) and risk assessment. For further information and clarification see the CLP criteria for classification (Section 3.7, Annex I, CLP) and Section 3.7 in the Guidance on the Application of the CLP criteria.

In reproductive toxicity studies local irritating effects at the site of administration may not allow investigating the reproductive toxicity in relation to systemic toxicity. In addition the irritation may affect the behaviour of the animals confounding the interpretation. Therefore, testing of corrosive or highly irritating substances at dose levels causing corrosivity or irritation should be avoided as far as possible (see also Annex VII-X preamble).

Dose level selection (and vehicle used) must be justified and documented to allow independent evaluation of the choice made.

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[17] CLP, Annex I, Sections 3.7.2.5.7 – 3.7.2.5.9 state on the limit dose and very high dose levels the following: "There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model." Section 3.7.2.5.8: "In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, extensive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area." And section 3.7.2.5.9 continues: "However, specification of an actual 'limit dose' will depend upon test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by oral route, an upper dose of 1000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level."

[18] See the CLP guidance, i.e. the intro to section 3.7.2.2.1.1 "*Effects to be considered in the presence of marked systemic effects*"

Stage 4.2 Registrations of 1 to 10 tonnes per year (Annex VII)

For substances manufactured or imported at tonnage levels ≥1-<10 t/y (Annex VII) there are no specific standard information requirements for reproductive toxicity. However, the available relevant information needs to be evaluated and classification for reproductive toxicity should be considered and applied if the classification criteria are met. If no information on reproductive toxicity is available, relevant non-animal approaches like validated *in vitro* tests, (Q)SAR predictions, or other available *in vivo* studies with the substance or with structurally related substances may be used to evaluate if there are triggers for reproductive toxicity. In case the available information indicates a concern (trigger) for reproductive toxicity and relevant human exposure occurs, an animal study like the reproduction/developmental toxicity screening test (OECD TG 421 or 422) should be considered to be performed to address the concern as an option. If an Annex IX or X level study, such as prenatal development toxicity study (EU B.31, OECD TG 414) or extended-one-generation reproductive toxicity study (EU B.56, OECD TG 443) is considered necessary to address the concern, a testing proposal should be submitted to ECHA. A thorough scientific justification on how the concern has been addressed should be adequately documented.

Stage 4.3 Registrations of 10 to 100 tonnes per year (REACH Annexes VII and VIII)

At this tonnage level, progression beyond Stages 1-3, will trigger the reproduction/ developmental toxicity screening test (OECD TG 421) or a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422).

(i) Reproduction/developmental toxicity screening test

If a 28-day study (EU B.7, OECD TG 407) is not already available, the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to the reproduction/developmental toxicity screening test (OECD TG 421). This approach offers the possibility to avoid also carrying out a 28-day study, because the OECD TG 422 can at the same time fulfil the information requirement of Annex VIII, 8.7.1 and that of Annex VIII, 8.6.1. Furthermore, the combined OECD 422 screening study should provide more robust information on repeated dose toxicity because it has a higher statistical power and a comparable or even longer exposure duration compared to the 28-day study (see Section XX).

If available information indicates serious concerns[19] (trigger) about the potential of a substance for adverse effects on fertility or development, a screening test (OECD TG 421 or 422; REACH Annex VIII, Section 8.7.1) may not need to be performed. Instead, a testing proposal for either a prenatal developmental toxicity study (EU B.31, OECD TG 414; REACH Annex IX, Section 8.7.2) or an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443; REACH Annex IX, Section 8.7.3) may be submitted to ECHA depending on the type of trigger. A concern (trigger) that the substance may be toxic to reproduction could stem from non-animal approaches[20] or *in vivo* information with the substance under consideration or from structurally related substances. Concerns (triggers, for discussion on triggers see Appendix 5) for fertility could stem also e.g., from existing repeated dose toxicity studies showing histopathological changes in gonads, and/or effects in sperm parameters. The proper study to be proposed depends on the concern. In case there is a concern for hazardous

[19] Serious concern reflects a high likelihood for adverse effects on reproductive health.

[20] In order to be considered providing "*serious concern*", information from non-animal approaches should be reliable, relevant and from validated studies with appropriate applicability domain. Generally several information sources may be needed.

effects on fertility and/or development leading to developmental toxicity effects manifested after birth, an extended one-generation study should be proposed. In case there is a concern for hazardous effects on embryonic or foetal development, a prenatal developmental toxicity study should be proposed. However, because the fertility and reproductive performance and developmental toxicity manifested shortly after birth are not assessed in prenatal developmental toxicity study, it is strongly recommended to conduct also a screening study (testing proposal is not needed for a screening study) as already discussed earlier. An extended one-generation reproductive toxicity study (all various study designs) covers all the same parameters, exposure duration and statistical power of the screening study and, thus, an additional screening study is not required.

If a reproduction/developmental toxicity screening test (OECD TG 421 or 422) for an Annex VIII substance provides no triggers for reproductive and developmental toxicity, then further testing for reproductive toxicity is not required at this tonnage level. Similarly, if a clear and unequivocal reproductive and/or developmental toxicity effect is observed in a screening test which is deemed sufficient to enable a scientifically robust decision on classification and categorisation to 1B for reproductive toxicity and risk assessment, then no further testing beyond the screening test is recommended at this tonnage level.

However, if a screening test (OECD TG 421 or 422) shows effects which are deemed not sufficient to enable a scientifically robust decision on classification and risk assessment, further studies may be considered. Based on the type of trigger, a testing for either a prenatal developmental toxicity study (Annex IX, Section 8.7.2) or an extended one-generation study (Annex IX, Section 8.7.3) may be proposed. Specifically, if a clear and unequivocal reproductive and/or developmental toxicity effect is observed in a screening test which is deemed sufficient for classification in Category 2 for reproductive toxicity, , then this is a serious concern and either a prenatal developmental toxicity study (Annex IX, Section 8.7.2) or an extended one-generation study (Annex IX, Section 8.7.3) may be proposed.

Stage 4.4 Registrations of 100 to 1000 tonnes per year (REACH Annexes VII to IX)

At this tonnage level, progression beyond Stages 1-3 will trigger a prenatal developmental toxicity study in a first species (EU B.31, OECD TG 414) and, if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity, also an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443). For further information on triggers for extended one-generation toxicity study at Annex XI level, see point (iii) below.

If the results from existing studies (prenatal developmental toxicity test or repeated-dose studies) are sufficient to support classification to Category 1B for effects on developmental toxicity and/or sexual function and fertility and the risk assessment, the Column 2 adaptation rules for REACH Annex IX, point 8.7 should be followed. In case the classification criteria for sexual function and fertility are met, then further testing for developmental toxicity must be considered and vice versa. For details, see Stage 1.

(i) Reproduction/developmental toxicity screening test

A reproduction/developmental toxicity screening test (OECD TG 421 or 422) is a standard information requirement at Annex VIII level. Since the Column 1 requirements in the REACH Annexes are cumulative, a screening test should also be available at Annex IX and X level. However, if a prenatal developmental toxicity study, a two-generation reproductive toxicity study or an extended one-generation study is available, the screening study can be omitted based on Annex VIII, Section 8.7.1., Column 2 adaptation rules (at REACH Annex VIII).

Where a screening test is omitted based on a prenatal developmental toxicity study and an extended one-generation reproduction toxicity study will not be triggered at REACH Annex IX level, then information on fertility would be limited to evaluation of the reproductive organs after repeated dosing, if those studies are available. Where information from a reproductive toxicity study addressing a fertility endpoint is not available, it is strongly recommended that a screening study is considered to fulfil this endpoint.

(ii) Prenatal developmental toxicity study

A prenatal developmental toxicity study (EU B.31, OECD TG 414), conducted in one species, is a standard data requirement at Annex IX level.

Consideration of existing information and the testing approach is required to select the appropriate species for the prenatal developmental toxicity study (see especially Stage 4.1(v) above). According to the test methods (EU B.31, OECD TG 414), the rat is the preferred rodent species and the rabbit the preferred non-rodent species. Since most of the toxicity studies (e.g., acute, repeated-dose, and toxicokinetic studies) are conducted in the rat, it may be considered that the first prenatal developmental toxicity study should also be conducted in this species. Findings from previous studies may be useful in dose selection, or the identification of additional endpoints for evaluation. In addition, the outcome of the prenatal developmental toxicity study may be helpful in the interpretation of other reproductive toxicity studies, for which the rat is generally the preferred species.

In certain cases the rabbit might be selected as the species for the first prenatal developmental toxicity study. This may be done e.g. if the rabbit is considered to be a more sensitive species than the rat for that specific substance. The selection of the species for the prenatal developmental toxicity study should be made taking into account substance-specific aspects. If a species other than the rat and the rabbit is selected as the first or second species, the selection should be justified.

A decision on the need to perform a study on a second species at Annex IX level should be based on the outcome of the first study and all other relevant available data. A study on a second species might be necessary in case the available data contain triggers for prenatal developmental toxicity. For example, performance of a prenatal developmental toxicity study in a second species may be justified in case developmental effects that are not sufficient to meet classification criteria to Category 1B reproductive toxicant (but maybe sufficient to Category 2 reproductive toxicant) were observed in the prenatal developmental toxicity study with the first species. Further triggers may stem from non-animal approaches, structurally similar substances, mechanisms/modes of action or results from a screening study. However, in case there are no triggers and no indication of prenatal developmental toxicity in the first prenatal developmental toxicity study, no study on a second species is necessary at Annex IX level.

If a study on a second species is found to be necessary by the registrant, a testing proposal needs to be submitted. Testing in a second species should be performed in a non-rodent species (rabbit) if the first species was a rodent species (rat) and vice versa. Further considerations on the species selection are provided in Section R.7.6.4.2.2.

(iii) Extended one-generation reproductive toxicity study

An extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) is required at Annex IX level if the available repeated dose toxicity studies (e.g. 28- or 90-days studies or OECD TG 421/422 screening tests) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Information from non-animal approaches are thus not listed as triggers for this study at Annex IX level in the REACH Annex text. However, if there is a serious concern based on available information from non-animal approaches or structurally analogous substances, the study may be triggered.

Triggers for the study at Annex IX level

A detailed review of the available data is required to identify any reproductive toxicity triggers (see also Appendix 5 for evaluation and determination of triggers; examples of triggers for EOGRTS at Annex IX level are provided below).

The legal text does not especially specify that the adverse effects should be seen in intact animals, however, it is considered that findings observed in non-intact animals should generally be used as triggers unless there is evidence that the findings would not be relevant for intact animals and/or humans. Experiments with non-intact animals may include animals with removal of an endocrine organ, such as ovary (ovariectomy). Another possibility is hormonal manipulation, e.g. causing decrease or increase of organ weight. These animal models may be very sensitive to detect a change in e.g. hormonal response; however, it should be considered whether the same applies in intact animals.

Examples (not an exhaustive list) of triggers to conduct an extended one-generation reproductive toxicity study at REACH Annex IX level (considered as adverse, in line with other data, and not considered secondary to systemic toxicity) are as follows:

From a screening study or equivalent:

- Changes in reproductive or other endocrine organ weight in intact animals unrelated to body size;
- Effects in spermatogenesis or folliculogenesis *in vivo* and/or histopathological findings in reproductive organs and/or accessory sex organs;
- Effects in histopathology of the thyroid;
- Effects on sperm parameters analysis or oestrous cycle;
- Statistically significant changes in hormone levels *in vivo* (related to reproductive toxicity)*;*
- Reduced mating, fertility or litter size;
- Increased incidence of abortions compared to controls;
- Changes in gestation length (not secondary to maternal toxicity);
- Reduced survival of offspring;
- Reduced body weight of offspring independent of litter size or maternal toxicity;
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- Reduced maternal care;
- Changes in anogenital distance unrelated to body weight/size;
- Changes in nipple retention;
- Indication of other endocrine disrupting modes of action related to reproductive toxicity.

From a repeated dose toxicity study:

- Changes in reproductive or other endocrine organ weight in intact animals unrelated to body weight;
- Effects in spermatogenesis or folliculogenesis *in vivo* and/or histopathological findings in reproductive organs and/or accessory sex organs;
- Effects on sperm parameters analysis or oestrous cycle
- Statistically significant changes in hormone levels (related to reproductive toxicity)*;*
- Indication of other endocrine disrupting modes of action related to reproductive toxicity.

From in vivo studies from non-intact animals (if the findings are considered relevant for intact animals/humans):

- Changes in reproductive or other endocrine organ weight.
- Indication of other endocrine disrupting modes of action related to reproductive toxicity

Study design for extended one-generation reproductive toxicity study

In cases where triggers are identified that requires performance of an extended one-generation reproductive toxicity study, the appropriate study design as described in Column 1 and 2 and in Recital (7) of Commission Regulation (EU) 2015/282 amending REACH needs to be defined, justified, and documented. Specification is required for 1) length of the premating exposure duration and dose level selection, 2) the need to extend Cohort 1B and termination time for F2 generation, 3) the need to include Cohorts 2A and 2B, and 4) the need to include Cohort 3. The study design is described in Appendix 2 and evaluation related aspects and further considerations in section R.7.6.4.2.3. Appendix 1 lists the information that should be presented in the dossier in order to establish the existence or the nonexistence of the conditions specifying the study design. Appendix 3 is describing the premating exposure duration considerations.

The study design of the extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) specified in REACH in Column 1 as a standard information requirement is the so called "basic" study design of a one-generation reproductive study that includes Cohorts 1A and 1B. Recital (7) of Commission Regulation (EC) 2015/282 amending REACH states that the extended one-generation reproductive toxicity study should allow adequate assessment of fertility and that premating exposure duration and dose levels should be appropriate to meet the risk assessment and classification and labelling purposes [including categorisation][21]. The focus of the study in the REACH Annexes is on fertility, which should be considered in the study design of the EOGRTS, thus, as a starting point, a 10-week premating exposure duration and a highest dose level with the aim to induce some toxicity for all variant study designs of EOGRTS should be proposed, see Appendix 3 for details regarding to premating exposure duration. Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked.

The basic study design – including the premating exposure duration according to Appendix 3 – should be proposed by registrants unless the conditions specified in Column 2 are met. The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) must be proposed by the registrant if the conditions specified in Column 2 are met.

Based on specific triggers for neurotoxicity defined in Column 2, developmental neurotoxicity cohorts (Cohorts 2A and 2B) must be proposed by the registrant. Respectively, based on specific triggers for immunotoxicity defined in Column 2, developmental immunotoxicity cohort (Cohort 3) must be proposed by the registrant. The registrant may also propose a separate developmental neurotoxicity and/or developmental immunotoxicity study instead of the cohorts for developmental neurotoxicity and/or developmental immunotoxicity.

[21] Recital (7) of Commission Regulation (EU) 2015/282 of 20 February 2015 … amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European parliament and of the Council on the Registration, evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended one-generation reproductive toxicity study: "*It should be ensured that the reproductive toxicity study carried-out under point 8.7.3 of Annexes IX and X to Regulation (EC) No 1907/2007 will allow adequate assessment of possible effects on fertility. The premating exposure duration and dose selection should be appropriate to meet risk assessment and classification and labelling purposes as required by Regulation (EC) No 1907/2006 and Regulation (EC) No 1272/2008 of the European parliament and of the Council.*"

The conditions specifying the study design are listed in Annex IX, 8.7.3, Column 2 and explained in more detail in Appendix 2 and discussed in Section R.7.6.4.2.3 under "*extended one-generation reproductive toxicity study*". It is the registrant's responsibility to evaluate all the available information and to propose an adaptation of the standard information requirement following conditions described in Column 2 of Annex IX/X, 8.7.3.

The justification of the study design that is most appropriate for evaluation of the reproductive toxicity of a substance must be adequately documented. This documentation must include justifications why the registrant holds the conditions of deviations from the basic study design not to be fulfilled taking into account all the available information.

A study on a second species or strain

REACH Annex IX specific rules for adaptation states that the need to perform an EU B.56 (OECD TG 443) study in a second strain or a second species, either at this tonnage level or the next, may be considered, and a decision should be based on the outcome of the first test and any other relevant available data.

It is recognised that extended one-generation reproductive toxicity study is designed to be conducted in rats and it may be challenging to use other species. Thus, it has been made possible to conduct a second study using another rat strain instead of a second species. The need to conduct the study using a second species or strain will be in exceptional cases only.

A study on a second strain or species might be necessary if the available data contain triggers which have not been addressed in the study on the first species. For example, performance of a study in a second strain or species may be justified if effects were observed in the study with the first species cause further serious concern but are not sufficient to meet classification criteria to Category 1B reproductive toxicant. Further triggers may stem from validated non-animal approaches, structurally similar substances, modes of action or results from a screening study. However, if there are no triggers and no indication of effects on fertility in the first study and other available data, no study on a second species or strain is necessary at Annex IX level.

If a study on a second species or strain is found to be necessary by the registrant, a testing proposal would need to be submitted.

Stage 4.5 Registrations of 1000 tonnes or more per year (REACH Annexes VII to X)

Progression beyond Stage 1-3 will trigger a prenatal developmental toxicity study (EU B.31, OECD TG 414) on a second species, if not conducted at the previous tonnage level, and an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443), if not already conducted at the previous tonnage level.

(i) Prenatal developmental toxicity study

At Annex X level, a prenatal developmental toxicity study (EU B.31, OECD TG 414), conducted on a second species is a standard information requirement, in addition to a prenatal developmental toxicity study in a first species that is required at Annex IX level. Availability of information on two species allows a more comprehensive evaluation of prenatal developmental toxicity. The prenatal developmental toxicity study in a second species can be omitted, if – taking into account the outcome of the first test and all other relevant available data – an adaptation pursuant to Annex X, Section 8.7., Column 2 or pursuant to Annex XI can be justified.

According to the test methods (EU B.31, OECD TG 414), the rat is the preferred rodent species and the rabbit the preferred non-rodent species. Depending on whether the rat or the rabbit is selected as a first species, and/or is already available, the other should be the preferred second species. In certain cases the rabbit might be selected as the species for the first prenatal developmental toxicity study. This may be done e.g. if the rabbit is

considered to be the more sensitive species than the rat for that specific substance. The selection of the species for the prenatal developmental toxicity study should be made taking into account substance-specific aspects. If a species other than the rat and the rabbit is selected as the first or second species, the selection must be justified.

(ii) Extended one-generation reproductive toxicity study

The extended one-generation reproductive toxicity study (EU B.56; OECD TG 443) is a standard information requirement at Annex X level.

Study design for extended one-generation reproductive toxicity study

The appropriate study design as described in Column 1 and 2 and in Recital (7) of Commission Regulation (EU) 2015/282 amending REACH needs to be defined, justified, and documented. Specification is required for 1) length of the premating exposure duration and dose level selection, 2) the need to extend Cohort 1B and termination time for F2 generation, 3) the need to include Cohorts 2A and 2B, and 4) the need to include Cohort 3. The study design is described in Appendix 2 and evaluation related aspects and further considerations in section R.7.6.4.2.3

The study design of the extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) specified in REACH in Column 1 as a standard information requirement is the so called "basic" study design of a one-generation reproductive study that includes Cohorts 1A and 1B. Recital (7) of Commission Regulation (EC) 2015/282 amending REACH states that the extended one-generation reproductive toxicity study should allow adequate assessment of fertility and that premating exposure duration and dose levels should be appropriate to meet the risk assessment and classification and labelling purposes[22]. The focus of the study in the REACH Annexes is on fertility, which should be considered in the study design of the EOGRTS. Thus, as a starting point, a 10-week premating exposure duration and a highest dose level with the aim to induce some toxicity for all variant study designs of EOGRTS should be proposed, see Appendix 3 for details. Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked.

The basic study design – including the premating exposure duration according to Appendix 3 – should be proposed by registrants unless the conditions specified in Column 2 are met.

The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) must be proposed by the registrant if the conditions specified in Column 2 are met.

Based on specific triggers for neurotoxicity defined in Column 2, developmental neurotoxicity cohorts (Cohorts 2A and 2B) must be proposed by the registrant. Respectively, based on specific triggers for immunotoxicity defined in Column 2, developmental immunotoxicity cohort (Cohort 3) must be proposed by the registrant. The registrant may also propose a separate developmental neurotoxicity and/or

[22] Recital (7) of Commission Regulation (EU) 2015/282 of 20 February 2015 amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European parliament and of the Council on the Registration, evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended one-generation reproductive toxicity study: "*It should be ensured that the reproductive toxicity study carried-out under point 8.7.3 of Annexes IX and X to Regulation (EC) No 1907/2007 will allow adequate assessment of possible effects on fertility. The premating exposure duration and dose selection should be appropriate to meet risk assessment and classification and labelling purposes as required by Regulation (EC) No 1907/2006 and Regulation (EC) No 1272/2008 of the European parliament and of the Council.*"

developmental immunotoxicity study instead of the cohorts for developmental neurotoxicity and/or developmental immunotoxicity.

The conditions specifying the study design are listed in Annex X, 8.7.3, Column 2 and explained in more detail in Appendix 2 and discussed in Section R.7.6.4.2.3 "*extended one-generation reproductive toxicity study*". It is the registrant's responsibility to evaluate all the available information and to propose an adaptation of the standard information requirement following conditions described in Column 2 of Annex IX/X, 8.7.3.

The justification of the study design that is most appropriate for evaluation of the reproductive toxicity of a substance must be adequately documented. This documentation must include justifications why the registrant holds the conditions of deviations from the basic study design not to be fulfilled taking into account all the existing information.

A study on a second species or strain

REACH Annex IX specific rules for adaptation states that the need to perform an EU B.56 (OECD TG 443) study in a second strain or a second species, either at Annex IX tonnage level or at Annex X tonnage level, may be considered, and a decision should be based on the outcome of the first test and any other relevant available data. It is recognised that extended one-generation reproductive toxicity study is designed to be conducted in rats and it may be challenging to use other species. Thus, it has been made possible to conduct a second study using another rat strain instead of a second species. The study on a second species or strain is needed in exceptional cases only.

A study on a second strain or species might be necessary in case the available data contain triggers which have not been addressed in the study on first species. For example, performance of a study in a second strain or species may be justified in case effects were observed in the study with the first species cause further serious concern but are not sufficient to meet classification criteria to Category 1B reproductive toxicant. Further triggers may stem from validated non-animal approaches, structurally similar substances, modes of action or results from a screening study. However, in case there are no triggers and no indication of effects on fertility in the first study and other available data, no study on a second species or strain is necessary at Annex X level.

If a study on a second species or strain is found to be necessary by the registrant, a testing proposal would need to be submitted.

R.7.6.3 Information Sources On Reproductive Toxicity

Information on reproductive toxicity can be obtained from various source categories, which are indicated below as headings. Examples from each source categories are provided. Evaluation of this information is described in R.7.6.4. Where *in vivo* testing is required, registrants must follow the EU Directive 2010/63 in selecting the test(s) requiring fewest animals and the least suffering.

R.7.6.3.1 Information on reproductive toxicity from non-animal approaches

Limited information of supportive nature may be inferred from numerous non-animal approaches (tests not using whole animals including embryos and foetuses after a certain developmental stage). For evaluation of the quality of the information, see section R.7.6.4 where reference to ECHA guidance on evaluation of available information is given (Chapter R.4).

- physico-chemical characteristics of a substance (distribution, accumulation);
- information on structurally analogue substances and (Q)SAR models;

- *in silico* and *in chemico* models (with adequate applicability domain);
- *in vitro* tests (with relevant concentrations) in reproductive toxicity or relevant modes on action; e.g.,:
  - Performance-based test guideline for stably transfected transactivation *in vitro* assays to detect estrogen receptor agonists (OECD TG 455, updated 2012);
  - BG1Luc Estrogen receptor transactivation test method for identifying estrogen receptor agonists and antagonists (OECD TG 457);
  - H295R steroidogenesis assay (EU B.57, OECD TG 456);
  - *In vitro* embryotoxicity tests;
  - *In vitro* organ and cell cultures;
- Where possible, well developed and justified reverse toxicokinetics models may be used to support results from *in vitro* tests to estimate exposures needed to achieve bioactive blood concentrations. Approaches combining various methodologies, e.g., from adverse outcome pathway (AOP) concept (OECD GD 184).

R.7.6.3.2 Information on reproductive toxicity in humans

If human information is available, it must – if possible – be presented in the form of a table as stated in Annex I, 1.2. of REACH.

Information may stem from epidemiological and/or occupational studies, medical records, case studies and accidents. For evaluation of the quality of the information, see section R.7.6.4 where reference to ECHA guidance on evaluation of available information is given (Chapter R.4).

R.7.6.3.3 Information on reproductive toxicity from *in vivo* animal studies

Data may be available from a wide variety of animal studies, with standard or non-standard study design, which give different amounts of direct or indirect information on the potential reproductive toxicity of a substance. For evaluation of the quality of the information, see section R.7.6.4 where reference to ECHA guidance on evaluation of available information is given (Chapter R.4).

In vivo studies referred to in REACH and providing information on reproductive toxicity:

- Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443);
- Two-generation reproductive toxicity study (EU B.35, OECD TG 416);[23]
- Prenatal developmental toxicity study (EU B.31, OECD TG 414).

In vivo studies referred to in REACH and providing preliminary information on reproductive toxicity:

- A reproduction/developmental toxicity screening test (OECD TG 421);[24]
- Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test (OECD TG 422)[25].

[23] Existing two-generation reproductive toxicity studies (EU B.35, OECD TG 416) fulfil the standard information requirement for Annex IX/X, 8.7.3 but new studies for REACH must be proposed according to extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) as described in Annex IX/X, 8.7.3.

[24] To date there are no corresponding EU testing methods available.

Other *in vivo* study on reproductive toxicity with EU and OECD test guidelines:

- One-generation reproductive toxicity study (EU B.34, OECD TG 415)

Repeated dose toxicity studies which may include parameters relevant for reproductive toxicity:

- 28- and 90-day repeated-dose toxicity studies (EU B.7; EU B.10), where relevant parameters are included, for example semen analysis, oestrous cyclicity, organ weights of reproductive organs and accessory sex organs, and/or reproductive organ histopathology;

Short-term *in vivo* tests on endocrine disrupting modes of action in intact or non-intact animals, e.g.:

- Uterotrophic bioassay in rodents: a short-term screening test for oestrogenic properties (EU B.54, OECD TG 440; OECD GD 71 for antioestronicity);
- Hershberger bioassay in rats: a short-term screening assay for (anti)androgenic properties (EU B.55, OECD TG 441 and GD 115);
- Studies on juvenile/peripubertal animals;

Other studies which may provide relevant information:

- Chernoff/Kavlock tests (see Hardin et al. 1987);
- a modified one-generation study by NTP (National Toxicology Program, U.S. Department of Health and Human Services; http://ntp.niehs.nih.gov/testing/types/mog/index.html)
- Reproductive Assessment by Continuous Breeding (RACB) protocol (e.g. Chapin and Sloane 1997)
- peri-postnatal studies;
- male or female fertility studies of non-standard design;

[25] To date there are no corresponding EU testing methods available.

- dominant lethal assay (EU B.22, OECD TG 478);
- mechanistic studies;
- toxicokinetic studies (EU B.36, OECD TG 417);
- studies in non-mammalian species.

Studies with focus on developmental neurotoxicity and developmental immunotoxicity:

- developmental neurotoxicity studies (such as EU B.53, OECD TG 426);
- developmental immunotoxicity studies (see section R.7.6.4.2.7 for references).

R.7.6.4 Evaluation of available information for reproductive toxicity

This section provides information on evaluation of the available data including aspects which influence the study designs. Both non-human (non-animal approaches and *in vivo* animal studies) and human data are considered. Under this section the studies required as standard information requirement are described as well as how to evaluate the conditions described in Column 2 to trigger a study or to adapt the study design. In addition, the evaluation of information from other internationally accepted *in vivo* studies are shortly described.

The generic guidance on the evaluation of available information gathered in the context of REACH Annexes VI-XI is provided in *Guidance on information requirements and chemical safety assessment, Chapter R4: "Evaluation of available information"*. The information should be evaluated for its completeness and quality for the purpose of REACH to assess whether (see the detailed wording in Chapter R.4):

- It fulfils the information requirements;
- It is appropriate for hazard classification and risk assessment.

The evaluation process of data quality by judging and ranking the available data for its relevance, reliability and adequacy is provided in Chapter R.4. Chapter R.4 applies for all kind of information; human, animal and non-animal sources and it is applicable also for information for reproductive toxicity endpoint. OECD guidance document 43 may be consulted for aid in the interpretation of reproductive and neurotoxicity results (see also e.g. OECD GD 106 for histologic evaluation, OECD GD 57 and 207 for thyroid hormone modulation assays, and OECD retrospective performance assay for developmental neurotoxicity, No 89).

In the present document some additional scientific aspects relevant for reproductive toxicity have been highlighted in context of the relevant information sources.

The main principles for evaluation of non-human information (information from animal studies and non-animal approaches) is presented in Annex I, 1.1 of REACH and it must be comprised of:

- Hazard identification for the effect based on all available non-human information;
- Establishment of the quantitative dose (concentration) response (effect) relationship.

Robust study summaries are necessary for key data on reproductive toxicity. If possible the information must be provided in the form of table(s) (see further details in Annex I, 1.1.3. of REACH).

R.7.6.4.1 Non-animal data

For reproductive toxicity, a grouping and category approach and weight of evidence approaches are the best fit-for-purpose tools for non-animal approaches for the time

being to adapt the standard information requirements for reproductive toxicity. However, appropriate justification and documentation must be provided. In addition, non-animal approaches may be used for prioritisation and screening chemical inventories.

Information on the current developments of *in vitro* tests and methodology can be found on the ECVAM website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam) and other international centres for validation of alternative methods. ECHA's website is also updated with new internationally accepted non-animal approaches (http://www.echa.europa.eu/support/oecd-eu-test-guidelines). However, the regulatory acceptance of these studies and approaches to replace the animal testing for reproductive toxicity has not been achieved as they do not provide equivalent information and cannot be used alone for classification and labelling and/or risk assessment. In spite of this, they may serve as elements in categories/read across and weight of evidence approaches. They may also provide important information on mechanisms and modes of action, or preliminary screening information which can be used in planning further testing. As these studies are not standard information requirements, the results from these studies are not required in dossier evaluation processes. However, when the results from these studies are used e.g. to support read across or to trigger additional studies, information from these studies must be included.

R.7.6.4.1.1 Physico-chemical properties

It may be possible to infer from the physico-chemical characteristics of a substance whether it is likely to be absorbed following exposure by a particular route and, furthermore, whether it (or an active metabolite) is likely to cross the placental, blood-brain or blood-testes barriers, or be secreted in milk. Information on the physico-chemical properties may contribute to a Column 2 adaptation (e.g., indicate concern on prolonged phase before reaching a steady state which is part of condition triggering extension of Cohort 1B in the extended one-generation reproductive toxicity study) or weight of evidence adaptation according to Annex XI, 1.2. of REACH.

R.7.6.4.1.2 (Q)SAR

There are a large number of potential targets/mechanisms associated with reproductive toxicity which, on the basis of current knowledge, cannot normally be adequately covered by a battery of QSAR models. In principle QSAR models are potential adaptation possibilities according to Annex XI, 1.3, but they should adequately cover the endpoint in question – all the key parameters should be covered.

QSAR models are usually trained (developed) to give binary results; the substance is predicted to have or not have a particular property, e.g., developmental toxicity. In case the substance is predicted to have that property, the result of a QSAR prediction is considered as positive. Similarly, if the substance is predicted not to have a particular property, the result of the QSAR prediction is considered negative. QSAR approaches are currently not well validated for reproductive toxicity and consequently no firm recommendations can be made concerning their routine use in a testing strategy in this area. A particular challenge for this endpoint is the complexity and amount of information needed from various functions and parameters to evaluate the effects on reproduction. Not all necessary aspects can be covered by a QSAR prediction. Therefore, a negative result from current QSAR models predicting that the substance has not a particular property, cannot be interpreted as demonstrating the absence of a reproductive hazard unless there is other supporting evidence. Another limitation of QSAR modelling is that dose response information, for example the N(L)OAEL, required for risk assessment is not provided.

However, a positive result from a validated QSAR model predicting that the substance has a particular property could provide a trigger for further testing beyond the standard information requirement (e.g., one element to trigger the extension of Cohort 1B in extended one-generation reproductive toxicity study). For evaluation of the triggers see

Appendix 5. Due to the limited confidence in this approach such a result would not normally be adequate for making a decision on classification on its own. It may, although not normally used, provide supportive information that can be used when concluding on the appropriate classification (see 3.7.2.5.4, Annex I, CLP).

Provided the applicability domain is appropriate, the results from using QSAR models may be used in a weight of evidence analysis where such data are considered alongside other relevant data (for classification and labelling and as one element for weight of evidence adaptation approach according to Annex XI, 1.2). Also, the results from using QSAR models can be used as supporting evidence when assessing the toxicological properties by read-across in a grouping approach, providing the applicability domain is appropriate. Both positive and negative QSAR modelling prediction results concerning the existence or non-existence of a particular property, respectively, may be of value in supporting a read-across assessment.

R.7.6.4.1.3 *In vitro* data and Adverse Outcome Pathways (AOPs)

The design of alternatives to *in vivo* testing for reproductive toxicity is especially challenging in view of the complexity of the reproductive process and large number of potential targets/mechanisms associated with this broad area of toxicity. In addition, many *in vitro* approaches do not include elements of biotransformation, which, in addition, may differ depending on organ.

Currently there are only three officially adopted EU test methods or OECD test guidelines for *in vitro* tests of relevance to modes of action for reproductive toxicity; two measuring estrogenicity (OECD TG 455 and OECD TG 457) and the other measuring steroidogenesis (EU B.57, OECD TG 456). Most assays under development and international validation are focusing on agonist/antagonistic properties measured by binding and activating or blocking a steroid (or a thyroid) hormone receptor.

Three *in vitro* embryotoxicity tests to predict developmental toxicity have been validated but have not been accepted for regulatory use (Genschow et al. 2002, Piersma et al. 2006, Spielmann et al. 2006). These tests, the embryonic stem cell test, the limb bud micromass culture and the whole embryo culture showed high predictivity for certain strongly embryotoxic chemicals. However, due to the nature of the methods and limitations in their predictivity, they may be used only as supporting information along with other more reliable data to predict the developmental toxicity. The value of these validated methods could be increased by incorporating molecular based markers through the application of proteomic and toxicogenomic approaches (Piersma, 2006; van Dartel et al.2010). The embryonic stem cell method may be combined with Physiologically Based Biokinetic modelling in order to derive quantitative points of departure *in vitro*, which are then extrapolated to *in vivo* points of departure for use in risk assessment (Worth et al. 2014).

The combination of assays in a tiered and/or battery approach may improve predictivity, but the *in vivo* situation remains more than the sum of the areas modelled by a series of *in vitro* assays (see Piersma 2006 for review). Therefore, a negative result predicting absence of a particular property for a substance with no supporting information cannot be interpreted as demonstrating the absence of a reproductive hazard with the same confidence as an animal study. Another limitation of *in vitro* tests is that a N(L)OAEL and other dose-response information required for a risk assessment is not provided.

However, a positive result predicting a particular reproductive hazard in a validated *in vitro* test could provide a justification for the need of further testing beyond the standard information requirement, dependent on the effective concentration and taking account of what is known about the toxicokinetic profile of the substance. However, because of limited confidence in this approach at this time, such a result in isolation would not be adequate to support hazard classification.

Additionally, validated and non-validated *in vitro* tests, provided the applicability domain is appropriate, could be used with other data in a weight of evidence approach according to Annex XI, 1.2 of REACH to gather information on hazardous properties. *In vitro* techniques can be used in mechanistic investigations, which can also provide support for regulatory decisions. Also, *in vitro* tests can be used as supporting evidence when assessing the toxicological properties by read-across within a substance grouping approach, providing the applicability domain is appropriate. Positive and negative *in vitro* test results may be of value in a read-across assessment and in category approach as one element.

Current developments on adverse outcome pathways (AOPs) to build a combination of studies and investigations to cover key events from initiating molecular event to adverse outcome may provide information on certain pathways, especially in developmental toxicity for certain malformations. Approaches may combine various different methods (e.g., *in vitro* tests, QSARs, *in chemico* assays etc). As these pathways do not cover all potential mechanisms/modes of action, negative results predicting absence of a particular property from those approaches do not provide enough confidence for regulatory decision making to demonstrate absence of a reproductive hazard. In addition, currently they do not provide N(L)OAEL value or other dose-response information for risk assessment. However, they may provide necessary support for read across justification and categories and contribute to a weight of evidence adaptation according to Annex XI, 1.2 of REACH.

R.7.6.4.2 Animal data

For evaluation of the results of a reproductive toxicity study, it is important, where possible, to distinguish between a specific effect on reproduction (fertility and/or pre- and postnatal development) as a consequence of an intrinsic property of the substance and an adverse reproductive effect which is a secondary non-specific consequence to the general toxicity. According to the criteria for classification, reproductive toxic effects should be considered if they occur in the absence of other (systemic) toxic effects or if they occur together with other toxic effects, are considered not to be a secondary non-specific consequence of the other toxic effects (see 3.7.2, Annex I CLP).

R.7.6.4.2.1 Reproduction/Developmental Toxicity Screening Test

The screening studies provide initial information of the effects on male and female reproductive performance as well as on developmental toxicity during, and shortly after, birth. These screening tests are not meant to provide complete information on all aspects of reproduction and development. However, the screening test (OECD TG 421/422) is standard information requirement for reproductive toxicity at Annex VIII level. Thus, a negative study result at Annex VIII is considered adequate although the screening study does not provide similar confidence than more comprehensive studies on reproduction toxicity. An evaluation of the screening tests (OECD TG 421 or TG 422) has confirmed that these tests are useful for initial hazard assessment and can contribute to decisions on further test requirements (Reuter et al 2003, Gelbke et al 2004, Beekhuisen et al 2009).

With regard to male and female fertility, the number of parameters investigated are less than in the more comprehensive generational study designs such as the extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) or the two-generation reproductive toxicity study (EU B.35, OECD 416) and the statistical power is much lower due to lower number of animals per dose group. Furthermore, the pre-mating exposure duration in these screening studies may not be sufficient to detect all effects on the spermatogenic cycle or folliculogenesis. The two weeks premating exposure duration used in this study is equivalent to the time for epididymal transit of maturing

spermatozoa, and thus, allows the detection of post-testicular effects on sperm at mating (during the final stages of spermiation and epididymal sperm maturation). For females, two weeks premating exposure duration covers 2-3 oestrous cycles and effects on cyclicity may be detected. Thus, the full spermatogenesis and folliculogenesis are not covered at the time of mating, or together before and after the mating, as they take 70 and 62 days in rats, respectively.

Because exposure during the full spermatogenic period and folliculogenesis are not covered at the time of mating, effects at earlier stages of spermatogenesis and folliculogeneiss cannot be reflected in the functional fertility examination. For instance, earlier stages of the spermatogenesis (spermatogonia) and/or specific cell types (Sertoli cell and Leydig cells) are sensitive to many chemicals (see e.g review by Bonde 2010). With a two-week premating exposure, the effects on functional fertility of exposure to these early stages of developing spermatozoa will not be covered. In addition, steady state may not be reached in all organs (see also discussion in Appendix 3). Because the duration of the study itself does not cover the full spermatogenesis or folliculogenesis, also histopathological data will be limited. Depending on the tonnage level, results from the 90-day study may be available with investigations of histopathology of gonads, however, sperm parameters or oestrous cycle are usually not investigated. ~~H~~istopathology of gonads may be among the most sensitive parameters to detect adverse effects on male fertility, and the most sensitive parameter may be used to derive the NOAEL. However, the clarity rather than the sensitivity of the effects observed are important for classification and labelling and will affect the category into which the substance is classified. Thus, to address the fertility also for the classification and labelling purposes, including the categorisation, it is necessary to consider how well all the available parameters address the fertility endpoint.

Due to its limitations, a screening study cannot be used to fulfil the information requirement of an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443). It should also be noted that those screening studies do not provide relevant information on post-natal developmental toxicity like a one- or two-generation reproductive toxicity studies (EU B.34/OECD 415 or EU B.56/OECD TG 443 or EU B.35/OECD 416) because the screening studies are terminated already at an earlier developmental stage than those more comprehensive studies.

With regard to developmental toxicity, these screening tests do not provide sufficient information on prenatal developmental toxicity because the pups are not examined for external, skeletal and visceral anomalies like in the prenatal developmental toxicity study (EU B.31, OECD TG 414). In addition, the pups in the screening studies are delivered naturally and the dams may cannibalise malformed pups. In the prenatal developmental toxicity study caesarean section is performed to avoid any cannibalism and to allow an appropriate evaluation of the foetuses. In addition, the statistical power of the screening study is lower than that of the prenatal developmental toxicity study. Therefore, a screening study cannot be used to fulfil the standard information requirement of a prenatal developmental toxicity study (EU B.31, OECD TG 414).

Depending on the tonnage level or based on adaptations, a screening study might be the only available reproductive toxicity study. However, the screening studies were not designed as an alternative or a replacement of the higher tier reproductive toxicity studies (EU B.31, OECD TG 414 and EU B.56, OECD TG 443). Therefore, the results of a screening study should be interpreted with caution and even statistically not significant effects may be an indicator for an impairment of reproduction. A result showing no effects in a OECD 421/422 screening test does not provide reassurance of the absence of any hazardous property for reproductive toxicity. Further information on reproduction toxicity may be available to assist the interpretation of the results.

The observation of clear evidence of adverse effects on reproduction or on reproductive organs in these tests may be sufficient to meet the information needs for classification

and labelling and risk assessment (using an appropriate assessment factor), and providing a N(L)OAEL from which a DNEL can be identified (by adding an additional assessment factor due to higher uncertainty involved than in more comprehensive studies).

Effects observed in the screening study may serve as triggers, leading to more comprehensive reproductive toxicity studies or they may constitute conditions which specify the study design of an extended one-generation reproductive toxicity study. For instance EU B.56 (OECD TG 443) may be triggered based on evidence indicating concern on reproductive toxicity, see Stage 4.4 Annex IX, extended one-generation reproductive toxicity study. For more detailed information on the extended one-generation reproductive toxicity study, see the section R.7.6.4.2.3 below and Appendix 2. Screening study may provide useful information when considering dose level selection for extended one-generation reproductive toxicity study.

R.7.6.4.2.2 Prenatal developmental toxicity study

The prenatal developmental toxicity study (EU B.31, OECD TG 414) provides a focused evaluation of potential effects on prenatal development, although only effects that are manifested before birth can be detected. Detailed information on external, skeletal and visceral malformations and variations and other developmental effects are provided. Cesarean section allows precise evaluation of number of foetuses affected.

For a comprehensive assessment of prenatal developmental toxicity, information from two species, one rodent (usually the rat) and one non-rodent (usually the rabbit) is assessed. However, depending on the REACH tonnage level, there might only be a standard information requirement for a prenatal developmental toxicity in one species (Annex IX) or even for none (Annex VII and VIII). Under such circumstances, it needs to be evaluated if testing beyond the standard information requirements is triggered. In case both or one of the default species (the rat and the rabbit) are not suitable species for prenatal developmental toxicity testing, a more suitable species considering the human relevancy should be selected for testing. An adequate justification for other species than the rat and the rabbit must be provided. The results from prenatal developmental toxicity studies are considered relevant to humans unless there is substance-specific toxicokinetic or toxicodynamic evidence showing otherwise.

For evaluation, developmental effects should be considered in relation to adverse effects occurring in the parents, for further information see the *Guidance on the Application of the CLP Criteria* (Chapter 3.7).

It has to be noted that a prenatal developmental toxicity study (EU B.31, OECD TG 414) does not provide information on postnatal development or sufficient information on female fertility. However, some findings might raise concerns. In case exposure started on gestation day 0, effects on preimplantation or implantation could indicate effects on female fertility. Also effects on maintenance of pregnancy and potentially on gestation length may be identified if significantly affected.

In case a study is conducted according to an old test method and, thus, uses a shorter administration period than current test method, it is important that there is no indication challenging the exposure period used. Thus, if there is a concern suggesting that a longer exposure period would have revealed developmental toxicity undiscovered using shorter exposure duration, this should be addressed e.g. by using an additional assessment factor, or in case of serious concern, a new study with longer exposure duration should be proposed. These indications challenging the exposure duration used may stem from fertility studies such as screening studies (OECD TGs 421/422) or from extended one-generation reproductive toxicity study or also from information on mechanisms/modes of action or structurally similar substances. It is to be noted that screening studies (OECD TG 421/422) or extended one-generation reproductive toxicity study do not provide equivalent information on prenatal developmental toxicity to that from the prenatal

developmental toxicity study. Thus, if the indication of challenging the exposure duration rises from other available data, the results from these fertility studies may not always, depending on the case, provide sufficient confidence to conclude that there is no prenatal developmental toxicity.

Prenatal developmental toxicity studies may provide triggers for further reproductive toxicity studies, e.g., in the form of foetotoxicity or foetal findings. In addition, some findings, such as increased foetal weight or placental weight, considered in light of litter size, may indicate an endocrine disrupting mode of action. Although there is no toxicological need to differentiate endocrine disrupting modes of action from other modes of action for developmental toxicity, in REACH the reproductive effects may trigger the extended one-generation reproductive toxicity study at Annex IX and the indication of endocrine disrupting modes of action are one element in triggering the extension of Cohort 1B in the extended one-generation reproductive toxicity study.

R.7.6.4.2.3 Extended one-generation reproductive toxicity study

R.7.6.4.2.3.1 Introduction

The test method of the extended one-generation reproductive toxicity study (EOGRTS, EU B.56, OECD TG 443) describes a flexible modular study design with several investigational options allowing each jurisdiction to decide on the study design required for the respective regulatory context. The study design for REACH is described in detail in Appendix 2 to this document.

The extended one-generation reproductive toxicity study allows evaluation of the effects of the test substance on the integrity and performance of the *adult* male and female reproductive system and offspring viability, health and some aspects of physical and functional development until adulthood. The extension of the cohort 1B (to mate the F1 animals to produce the F2 generation) also provides information on the fertility of the offspring (F1 generation), thus addressing the potential effects after exposure of the most sensitive life stages (i.e *in utero* and early postnatal period). Therefore, mating of the Cohort 1B animals will cover information on the complete reproductive cycle.

In REACH the standard information requirement includes cohorts 1A and 1B for reproductive toxicity (without extension to produce the F2 generation). Thus, the basic study design is a one-generation study providing information on the fertility of the parental animals (P0 or F0 animals) and extended postnatal development of F1 animals. In addition, for REACH purposes it is necessary that the study design allows the adequate assessment of possible effects on fertility for risk assessment and classification and labelling purposes, including categorisation. To ensure that the study design adequately addresses the fertility endpoint, the duration of premating exposure period and the selection of the highest dose level are key aspects to be considered, see Appendix 3 for further details. Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked.

In case the Column 2 conditions at Annex IX/X are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the mating, fertility and reproductive performance of the F1 animals. F1 animals are exposed already *in utero* and early postnatal period allowing a comprehensive assessment of effects induced during these sensitive life stages. Similarly developmental neurotoxicity (Cohorts 2A and 2B) and/or developmental immunotoxicity (Cohort 3) cohorts need to be conducted if the triggers (conditions) for such extensions of the basic study design which are provided in Column 2 of Annex IX/X, 8.7.3. are fulfilled. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information. Considerations for evaluation of developmental neurotoxicity and developmental immunotoxicity are provided later in this section.

It is recommended that results from a range-finding study (or range-finding studies) for the extended one-generation reproductive toxicity study are reported with the main study. This will support the justifications of the dose level selections and interpretation of the study results.

If a range-finding study indicates adverse effects on fertility but the effects do not meet the criteria for Reproductive toxicity Category 1B, it is recommended that the main study should be designed to confirm the findings from the range-finding study. However, if the results from the range-finding study already meet the criteria for Reproductive toxicity Category 1B reproductive toxicants, the adaptation of Column 2 may apply and further studies (including the main study) may not be needed.

R.7.6.4.2.3.3 General considerations related to investigation of (developmental) neurotoxicity and/or immunotoxicity

In case triggers for neurotoxicity or immunotoxicity are identified already at Annex VIII or IX level but the extended one-generation reproductive toxicity study is not triggered, a separate neurotoxicity or immunotoxicity study in developing organism or in adults must be proposed in line with Column 2 adaptation to Section 8.6.1 of Annex VIII or Section 8.6.2 of Annex IX[26]. Depending on the cases, also inclusion of additional parameters to the repeated dose toxicity study (including screening study), if not yet conducted, may be considered, to further characterise the effect.

Whether the neurotoxic and/or immunotoxic properties should be investigated in adults or in developing organisms at Annex VIII or Annex IX level, if an extended one-generation reproductive toxicity study is not triggered, should be considered case by case taking into account the various aspects affecting the decision, e.g., the target population, toxicokinetics and mode of action. Generally, a study in developing organisms is recommended as a more conservative approach.

At Annex X, extended one-generation reproductive toxicity study is a standard information requirement, and if there are triggers for the (developmental) neurotoxicity and/or (developmental) immunotoxicity meeting the triggers described in Column 2, Section 8.7.3, the registrant must propose Cohorts 2A and 2B to address the concern for developmental neurotoxicity or Cohort 3 to address the concern for developmental immunotoxicity. The general evaluation of triggers is presented in Appendix 5. Instead of these cohorts, the registrant may also propose separate developmental toxicity studies to address these concerns, as explained below in Section R.7.6.4.2.3.4. Likewise at Annex IX, if the extended one-generation reproductive toxicity study is triggered, these cohorts, or separate studies, must be proposed by the registrant to address the concern in question.

It is to be noted that neurotoxicity and/or immunotoxicity observed in adult animals may trigger developmental neurotoxicity and/or developmental immunotoxicity cohorts in extended one-generation reproductive toxicity study or separate studies unless substance specific information is provided why these effects or mode of action would not be relevant in a developing organism (for evaluation of triggers see Stage 3.2.1.) . In addition, in case of classification criteria for STOT are met based on studies in adults, this is not an adaptation rule allowing the omission of investigations on developmental neurotoxicity and/or developmental immunotoxicity. This is due to expected higher sensitivity of the developing organisms (see e.g. Dietert 2014), which may lead to a more severe classification and/or lower DNEL.

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[26] Column 2 at Annex VIII, 8.6.1 and Annex IX, 8.6.2: "Further studies shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41 in case of: …- indications of an effect for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity), …")

R.7.6.4.2.3.4 Proposals for developmental neurotoxicity or immunotoxicity studies

REACH specifies that "*Other studies on developmental neurotoxicity and/or developmental immunotoxicity instead of cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) of the Extended One-Generation Reproductive Toxicity Study may be proposed by the registrant in order to clarify the concern on developmental toxicity.*"

The cohorts for developmental neurotoxicity and developmental immunotoxicity included in the extended one-generation reproductive toxicity study provide information on these endpoints. Information on developmental neurotoxicity and developmental immunotoxicity are not standard information requirements in REACH but they must be proposed when particular concerns as specified in Column 2 are met. An advantage of this approach is that fewer animals are needed compared to running three separate studies (reproductive toxicity study, developmental neurotoxicity and developmental immunotoxicity study).

Other studies on developmental neurotoxicity

The registrant has a choice to propose a separate developmental neurotoxicity study if the conditions for a particular concern for developmental neurotoxicity are met instead of Cohorts 2A and 2B. The concern should be related to *developmental* neurotoxicity specifically. The study design for development neurotoxicity should follow the EU B.53 (OECD TG 426) protocol. The selection between the choices should be based on scientific and substance specific considerations taking into account which method adequately addresses the scientific concern with least amount of animals and investigations. However, practical limitations in testing laboratories can be a reason too, to propose separate studies. Some examples of aspects of these considerations are presented below.

The developmental neurotoxicity cohort integrated in an extended one-generation reproductive toxicity study contains no endpoints for social or cognitive dysfunctions (e.g. autism, attention deficient hyperactivity disorders, attenuated learning and/or memory), thus, if there are signs of behavioural disturbances from adult animal studies, the design of the developmental neurotoxicity cohort in extended one-generation reproductive toxicity study might have to be adjusted. Optionally EU B.53 (OECD TG 426) may be the preferred study design.

It should be borne in mind that – when it comes to developmental neurotoxicity– the outcome of a developmental neurotoxicity study (OECD 426) may differ from that of the developmental neurotoxicity Cohorts 2A and 2B in an extended one-generation reproductive study, considering the different exposure scenarios. For example, recent publications point at the importance of a healthy immune system of the mother during pregnancy for brain development of her offspring (e.g. Smith et al. 2007). In other words, the maternal impact in the cohort study on nervous system development may be larger than in the OECD 426 study (exposure from gestation day 6 to PND 21) due to an longer exposure period and the extent of effect often is unknown.

In case extended one-generation reproductive toxicity study is not triggered or a standard information requirement but there are triggers for neurotoxicity , separate studies must be proposed according to Annex VIII, 8.6.1, Annex IX, 8.6.2, or Annex X, 8.6.4.

Other studies on developmental immunotoxicity

The registrant has a choice to propose a separate developmental immunotoxicity study if the conditions for a particular concern for developmental immunotoxicity are met instead of Cohorts 3. The concern should be related to *developmental* immunotoxicity specifically. For developmental immunotoxicity there is currently no available internationally accepted protocol, and thus, the registrant must include the proposed protocol in his testing proposal until internationally accepted methods are available. The

selection between the choices should be based on scientific and substance specific considerations taking into account which method adequately addresses the scientific concern with least amount of animals and investigations. Some examples of aspects of these considerations are presented below.

The nature and/or severity of the triggers may provide guidance to select between a separate study or a cohort. Other aspects to consider may include statistical power and the investigations included. It should be considered whether the cohorts or a separate study best address the particular concern identified (see also Appendix 5).

The outcome of a separate developmental immunotoxicity study may differ from that of the developmental immunotoxicity Cohort 3 in an extended one-generation reproductive study, if the exposure scenarios and set ups are different.

In case extended one-generation reproductive toxicity study is not triggered or a standard information requirement but there are trigger(s) for immunotoxicity, separate studies must be proposed according to Annex VIII, 8.6.1, Annex IX, 8.6.2, or Annex X, 8.6.4.

Common to both developmental neurotoxicity and immunotoxicity studies

Conflicts may arise to decide on the dose levels and premating exposure duration. The adequacy of the study design to assess the effects on fertility should be ensured. Thus, the dose level selection should be based upon the fertility endpoint with the developmental neurotoxicity/immunotoxicity being tested at the same dose levels. The fertility endpoint is the only endpoint where *in vivo* data is typically available to make decisions on selecting dose levels for the extended one-generation reproductive toxicity study.

Even if there are trigger(s) for developmental neurotoxicity/immunotoxicity, the dose level setting must not compromise an appropriate investigation of the fertility endpoint. The challenge in deciding the dose levels and length for the premating exposure duration is that there may be a risk that in reducing fertility not enough pups will be produced e.g., at the highest dose level for the evaluation of the potential developmental neurotoxicity/immunotoxicity at all dose levels. However, results from lower dose levels can still be used. Another possibility is to add an additional dose level or to address the developmental neurotoxicity/immunotoxicity in (a) separate stud(y)ies.

R.7.6.4.2.3.5 Evaluation of findings from developmental neurotoxicity and developmental immunotoxicity cohorts

Currently there is not much experience on interpretation of the results of developmental neurotoxicity (see some considerations under R.7.6.4.2.6) and developmental immunotoxicity cohorts included in the extended one-generation reproductive toxicity studies. Guidance will be developed after gathering more experience. Until further experience on these cohorts, experiences from existing protocols on developmental neurotoxicity and developmental immunotoxicity can be used although all of them may not be standardised and internationally acceptable protocols yet. For evaluation of the results from separate developmental neurotoxicity and immunotoxicity studies, see Sections R.7.6.4.2.6 for developmental neurotoxicity and R7.6.4.2.7 for developmental immunotoxicity. ,.

R.7.6.4.2.3.6 Further aspects

The OECD GD 151 provides guidance for conducting the extended one-generation reproductive toxicity study as agreed at OECD level (OECD 2013) but does not e.g. define the study design or criteria for the extension of Cohort 1B or the inclusion of cohorts. Thus, the study design should be defined to meet the REACH requirements. OECD GD 117 includes the internal triggers for extension of the Cohort 1B, however, these triggers are not used in REACH as such. The registrant may expand the study

based on new information indicating a concern which needs to be addressed. The justification for the expansion must be documented.

For REACH purposes, the focus of the study should be on assessment of the effects on fertility and, thus, a 10-week premating exposure duration and dose level setting based on toxicity are required as a starting point as explained above. In addition, for REACH the conditions which specify the extension of the Cohort 1B and the inclusion of Cohorts 2A, 2B and 3 are listed in Column 2 of Annex IX/X, 8.7.3. EU B.56 (OECD TG 443) and OECD GD 151 should be followed only in conducting the study modules. It is recommended that results from a range-finding study (or range-finding studies) for the extended one-generation reproductive toxicity study are reported with the main study. This should support the justifications of the dose level selections, duration of the premating exposure and interpretation of the study results.

The study design of EU B.56 (OECD TG 443) selected must be adequately justified and documented in all cases.[27]

In general, all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of concurrent parental toxicity, see *Guidance on the Application of the CLP Criteria* (Chapter 3.7).

Most of the parameters investigated in the 90-day study are also included in the extended one-generation reproductive toxicity study. However, the results obtained may not be equivalent for several reasons and it may not be adequate to adapt the information requirement of a 90-day study by information from extended one-generation reproductive toxicity study. This is because the 90-day study and the extended one-generation study have different aims. A 90-day study is meant to provide relevant information on systemic and organ-specific toxicity after a sub chronic exposure and relevant route especially considering exposure conditions, and non-pregnant animals are to be used. Usually the dose level selection for a 90-day study is higher when based on toxicity than the dose levels which can be used in an extended one-generation reproductive toxicity study. This is because the exposure is longer and pregnant animals (and offspring) may be more sensitive than non-pregnant animals. In addition, haematological, clinical chemistry, urinary and histological samples may be collected after a shorter exposure period in an extended one-generation reproductive toxicity study (8-10 weeks) than in a 90-day study (13 weeks), and at different exposure history and developmental stages in F1 animals. A very careful evaluation is needed when considering whether the information from extended one-generation reproductive toxicity study could be used to adapt the information requirement of a 90-day study. In certain cases with adequate exposure levels and durations the results from an extended one-generation reproductive toxicity study may support e.g. an older somewhat limited results from a 90-day study.

Information from a 90-day study may be valuable in deciding the dose levels of an extended one-generation reproductive toxicity study.

Extended one-generation reproductive toxicity study provides information on peri-postnatal development but does not address the same parameters than the prenatal developmental toxicity study and, thus, does not provide equivalent information.

R.7.6.4.2.4 Two-generation reproductive toxicity study

Two-generation reproductive toxicity studies are no longer standard information requirements (EU B.35, OECD TG 416) in REACH but those studies initiated before 13

[27] REACH Art 3(28): "robust study summary: means a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report;"

March 2015 [Commission Regulation (EU) 2015/282, Recital (11) and Article 2] are considered appropriate to address the standard information requirement for Annex IX/X, 8.7.3. The two-generation reproductive toxicity study was the standard information requirement for REACH until the amendment of REACH Annexes IX and X. Because the two-generation reproductive toxicity studies initiated before the date indicated above are considered appropriate to address the standard information requirement, it means that they fulfil the Column 1 requirements but it does not automatically meet the adaptation criteria described in Column 2. If the available information shows triggers for developmental neurotoxicity and/or developmental immunotoxicity according to Column 2, these particular concerns must be addressed by proposing a separate developmental neurotoxicity and/or a separate developmental immunotoxicity study, respectively).

Although the two-generation reproductive toxicity study may lack information on some parameters which are part of EU B.56 (OECD TG 443), it addresses the fertility endpoint in two-generations and is adequate for risk assessment and classification and labelling, including categorisation, when conducted according to the EU B.35 (OECD TG 416).

From the legal text it is clear that two-generation reproductive toxicity studies initiated after the date indicated in the legislation are not considered appropriate to address the standard information requirement at Annex IX/X, 8.7.3 and including the study design adaptation described in Column 2. This means that testing proposals of two-generation reproduction toxicity studies to fulfil the (standard) information requirement at Annex IX/X, 8.7.3 cannot be accepted. However, the registrant may explore the possibilities to adapt the information requirement by substance specific justifications according to Annex XI adaptation rules if the study already exists and was started after March 13, 2015. Similar to testing proposals for read across approaches, in certain cases, it may be possible to accept a testing proposal where the registrant aims to use an adaptation rule according Annex XI if the adaptation justification presented in conjunction with the testing proposal seems to be scientifically plausible although ECHA can evaluate and finally accept the adaptation approach only when the results of the study are available for evaluation.

When considering the relevance of an old non-guideline compliant two(multi)-generation reproductive toxicity studies to address the fertility endpoint (Annex IX/X, 8.7.3), these studies will be assessed in line with Annex XI, 1.1.2 adaptation rules for existing information. Thus, old existing non-guideline studies may fulfil the Column 1 standard information requirement or may serve as elements in a weight of evidence approach according to Annex XI, 1.2 of REACH to identify hazardous properties or support a category approach.

If a two-generation reproductive toxicity study is available and there are triggers for (developmental) neurotoxicity and/or (developmental) immunotoxicity, the registrant may propose a separate study as indicated above under heading “Selecting a separate study for developmental neurotoxicity and/or developmental immunotoxicity”.

R.7.6.4.2.5 One-generation reproductive toxicity study

The one-generation reproductive toxicity study (EU B.34, OECD TG 415) is not an appropriate study to fulfil the information requirement for an extended one-generation reproductive toxicity study because of limited postnatal exposure duration and inadequate coverage of key parameters (Annex XI, 1.1.2 of REACH).

This study does not correspond to any REACH standard information requirement but could potentially be enhanced with certain parameters to fulfil the information requirement of the screening study. Compared to the screening study it has a higher statistical power, it addresses the functional fertility by covering the spermatogenesis and folliculogenesis before the mating and reproductive performance until weaning. However, the test method lacks requirements of various important parameters as compared with the extended one-generation reproductive toxicity study. Existing studies

may be used as one element in a weight of evidence approach according to Annex XI, 1.2 of REACH to adapt the standard information requirement of Annex IX/X, 8.7.3. together with other information, or to support a category approach.

R.7.6.4.2.6 Developmental neurotoxicity studies

Developmental neurotoxicity studies are not standard information requirements but may be triggered by Annex VIII point 8.6.1 or Annex XI point 8.6.2 or Annex X point 8.6.4 based on Column 2 adaptation rules[28]. There the Column 2 adaptation requires the registrant to propose further studies in case there are indications of an effect for which the available evidence is inadequate for toxicological evaluation and/or risk characterisation. A separate developmental neurotoxicity study may also be proposed by the registrant instead of the developmental neurotoxicity cohorts (Cohorts 2A and 2B) in an extended one-generation reproductive toxicity study, in case these cohorts are triggered.

Developmental neurotoxicity studies (e.g. EU B.53, OECD TG 426) are designed to provide information on the potential functional and morphological hazards of the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation. These studies investigate changes in structure and function of the central nervous system (CNS) and the peripheral nervous system (PNS) using extensive neuropathology (structure) and behavioural (function) surveys. Advanced neuropathology may be assessed including quantitative structural measures, as changes in cell structures related to e.g. delayed development may be of quantitative rather than qualitative nature. Such quantitative changes may be significant, but may still go unrecognised without quantification (De Groot et al., 2005). To investigate behaviour, a range of parameters, such as a behavioural test battery addressing different functions (domains) of the nervous system, motor activity, and more advanced tests addressing cognitive behaviour are performed. As behaviour may also be affected by the function of other organs such as liver, kidneys and the endocrine system, toxic effects on these organs in offspring may also be reflected in general changes in behaviour. No single behaviour is able to reflect the entire complex and intricate function of behaviour and so, integration of findings of different tests is deemed relevant to evaluate the relevance of the results on substance exposure. Likewise, it may be helpful for the interpretation to review behavioural (functional) changes in light of the neuropathology (structural) findings.

The severity and nature of the effect should be considered. Generally, a pattern of effects (e.g. impaired learning during several consecutive trials) is more persuasive evidence of developmental neurotoxicity than one or a few unrelated changes. The reversibility of effects should be considered, too. Important to mention in this context is that 'development' of an organism *a priori* goes with 'normal' structural and functional changes. Under toxic or pathologic circumstances, a substance or disease may disturb 'normal' development, and 'toxic' changes are built on top of 'normal' developmental changes. The nervous and immune systems are still under development up to (far) and after birth. Moreover, different time-windows have been recognised for speed of developmental growth which, in turn, may differ for different parts and structures of the developing nervous and immune systems. As a consequence, also the vulnerability of these organ-systems differs during different time-windows of exposure. The nervous

[28] Column 2 at Annex VIII, 8.6.1, Annex IX, 8.6.2, and Annex X, 8.6.4: "Further studies shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41 in case of: …- indications of an effect for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity), …")

system possesses reserve capacity for repairing. We may e.g. find the nervous system impaired during puberty, whereas the adult nervous system seems intact. In such a case, however, one should still realise that not only the trajectory from birth to puberty differed between control and substance-exposed individuals; but the trajectory from puberty to adulthood as well. So, even when a developmental neurotoxicant may not show adverse effects in the adult, the trajectories towards adulthood have been affected and the consequences of this are so far unknown. The nervous system may compensate for damage, but the resulting reduction in reserve capacity is of concern and neurotoxicity occurring during development should be regarded as an adverse effect. If developmental neurotoxicity is observed only during some time of the lifespan then compensation should be suspected. Also, effects observed for example during the beginning of a learning task but not at the end should not be interpreted as reversible effects. Rather the results may indicate that the speed of learning is decreased.

The experience of offspring especially during infancy may affect their later behaviour. For example, frequent handling of rats during infancy may alter the physiological response to stress and the behaviour in tests for emotionality and learning. In order to control for environmental experiences, the conditions under which the offspring are reared should be standardised within experiments with respect to variables such as noise level, handling and cage cleaning. The performance of the animals during the behavioural testing may be influenced by e.g. the time of day, and the stress level of the animals. Therefore, the most reliable data are obtained in studies where control and treated animals are tested alternatively and environmental conditions are standardised.

In interpreting the results, maternal toxicity should be taken into account as the development of pups may be affected by maternal toxicity. During early postnatal period pups are dependent of maternal care and maternal toxicity, e.g. in way of CNS depression, may compromise the survival and development of pups. In addition, dams and pups should not be separated other than for very short periods of time during the first five postnatal days (e.g. for dose administration) and also later dams should not be moved from cages more than necessary (e.g. for inhalation exposure). In practise this would mean than for inhalation exposure, a whole-body exposure may be considered instead of nose-only exposure.

Adverse effects observed in a development neurotoxicity study will be relevant to hazard classification and the human health risk assessment, providing a N(L)OAEL, unless there is information to show that effects seen in these studies could not occur in humans. Due to a complexity of the endpoint, adversity should be based on a holistic analysis of data by grouping similar parameters rather than a change in a single parameter.

For more detailed reviews of how to interpret the developmental neurotoxicity results see OECD TG 426, OECD GD 43 and Tyl et al., 2008.

R.7.6.4.2.7 Developmental immunotoxicity studies

Developmental immunotoxicity studies are not standard information requirements but may be triggered by Annex VIII point 8.6.1 or Annex IX point 8.6.2 or Annex X, point 8.6.4 based on Column 2 adaptation rules[29]. There the Column 2 adaptation requires the registrant to propose further studies in case there are indications of an effect for which the available evidence is inadequate for toxicological evaluation and/or risk

[29] Column 2 at Annex VIII, 8.6.1, Annex IX, 8.6.2, and Annex X, 8.6.4: "Further studies shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41 in case of: …- indications of an effect for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity), …")

characterisation. A separate developmental immunotoxicity study may be proposed by the registrant instead of the developmental immunotoxicity cohort (Cohort 3) in an extended one-generation reproductive toxicity study, in case these cohorts are triggered.

Developmental immunotoxicity studies are designed to provide information on the potential functional and morphological hazards to the immune system arising in the offspring from exposure of the mother during pregnancy and lactation. Currently there is no OECD test guideline for developmental immunotoxicity testing. Recent reviews provide information on the available approaches and considerations (Gupta RC (ed) 2011, page 219-225; WHO 2012; De Jong & Van Loveren (2007); DeWitt et al 2012a and b) Dietert and DeWitt 2010; Dietert and Holsapple 2007; Holsapple et al 2005; Rooney et al 2009; Boverhof et al 2013).

These studies investigate changes in immune response due to effects on the innate or acquired immune system. As immune response may also be affected by the function of other organs such as liver, kidneys and the endocrine system, toxic effects on these organs in offspring may also be reflected in changes in immune response. No single immune parameter is able to reflect the entire complex and intricate function of immune system and so, integration of findings of different tests is relevant to evaluate the relevance of the results on substance exposure.

Effects considered as adverse will be relevant to hazard classification and the human health risk assessment, providing a N(L)OAEL, unless there is information to show that effects seen in these studies could not occur in humans. Due to a complexity of the endpoint, adverse should be based on a holistic analysis of data by grouping similar parameters rather than a change in a single parameter.

R.7.6.4.2.8 Repeated-dose toxicity studies

Although not aimed directly at investigating reproductive toxicity, repeated-dose toxicity studies are standard information requirements (e.g. the 28-day study EU B.7, OECD TG 407 or the 90-day study EU B.26, OECD TG 408) and may reveal clear effects on reproductive organs in adult animals. In addition to histopathology of reproductive organs and changes in organ weights, parameters evaluated, such as sperm analysis and measurements of oestrous cycle, may provide relevant information for reproductive toxicity or indicate a concern (trigger(s)). However, no observed effects in measured parameters predicting fertility in repeated dose toxicity studies do not rule out the possibility that the substance may have the capacity to affect fertility. At Annex IX level, triggers for reproductive toxicity from repeated dose toxicity studies trigger an extended one generation reproductive toxicity study (EU B.56, OECD TG 443). At Annex VIII level the registrant may consider proposing an extended one-generation reproductive toxicity study instead of a screening study, based on triggers from 28-day study.

The observation of effects on reproductive organs in repeated-dose toxicity studies may also be sufficient to be used for classification and labelling and for identifying a N(L)OAEL for use in the risk assessment. It should, however, be noted that the sensitivity of repeated-dose toxicity studies for detecting effects on reproductive organs may be less than reproductive toxicity studies because of the lower number of animals per group (lower statistical power). In addition, a number of cases have demonstrated that effects on the reproductive system may occur at lower doses when animals are exposed during the development or as young animals rather than as adults. Consequently, in cases where there are adverse effects on the reproductive organs in adult animals in the absence of reproductive toxicity studies, an increased assessment factor may be considered in the risk assessment process at Annex VII-VIII levels. An extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) may be triggered based on findings from a repeated dose toxicity study at lower REACH Annexes, and must be proposed at Annex IX.

The adversity of some effects seen in repeated dose toxicity studies may be difficult to interpret, for example changes in sex hormone levels, and may need to be investigated further as part of studies that may be required to meet standard REACH information requirements (for example EU B.26 (OECD TG 408) or other repeated-dose toxicity studies), rather than serve as a trigger for the immediate conduct of an extended one-generation reproductive toxicity study. Whether or not a finding will serve as a triggers depends on the reliability of the finding and if it can be considered as adverse (see discussions in Appendix 5). It may be considered that statistically significant changes from relevant studies can be considered as triggers, however, sometimes a statistically non-significant change can be also considered as biologically relevant if not contradicting to other available information.

Repeated-dose toxicity studies may also provide indications of a particular concern to evaluate the need to investigate developmental neurotoxicity or developmental immunotoxicity endpoints. The potential triggers for these cohorts in the extended one-generation reproductive toxicity study or separate studies are described in the context of the extended one-generation reproductive toxicity study (section R.7.6.4.2.3).

R.7.6.4.2.9 *In vivo* assays for endocrine disruption mode of action

The endocrine system has a critical role in the control of all aspects of the reproductive cycle and therefore endocrine disruption is a potential mechanism for reproductive toxicity. None of the available *in vivo* assays focusing only on identification of endocrine disrupting potency, such as Uterotrophic assay (EU B.54, OECD TG 440) and Herschberger assay (EU B.55, OECD TG 441) correspond to standard REACH information requirements. These studies involve dosing of immature or ovarectomised/castrated animals, and the weighing of oestrogen/ androgen dependent tissues (e.g. uterus or prostate). The methods can be used to identify (anti)oestrogenic or (anti)androgenic modes of action and the results may serve as triggers for further studies in certain cases. These animal models are sensitive to detect the hormonal mode of action. However, only investigation in intact animals proves if the mode of action is relevant in non-manipulated conditions. A comprehensive collection of screening tests and tests for endocrine disrupting chemicals are presented in OECD GD 150 and are included within the "*OECD Conceptual Framework for the Screening and Testing of Endocrine Disrupting Chemicals*".

A result in the uterotrophic assay in a well conducted dose-response study showing no effect indicates that the test substance is not an oestrogen receptor (ER)-ligand in those *in vivo* conditions. Equally, a result in the Hershberger assay showing no effect indicates that the test substance is neither an androgen receptor (AR)-ligand nor a 5-alpha reductase inhibitor in those *in vivo* conditions. A test compound not causing effect in these assays may, however, still have endocrine disrupting properties as well as a potential for reproductive toxicity mediated through other mechanisms. The uterotrophic and Hershberger assays may be used to provide NOEL/LOELs for these endocrine disruption modes of action only in case immature (intact) animals are used. The results may also support findings from other studies or serve as triggers for further studies and examinations.

A number of assays in experimental animals may provide information on the ability of a substance to act on the production of steroids, and the pubertal assays and the intact male assay may provide information about the endocrine disruption potency of the compound *in vivo* (US-EPA 2002). Effects on the various endpoints included in these assays may be considered adverse and/or as representing an effect on a mechanism relevant for humans and serve as triggers for further studies and examinations.

In summary, while these *in vivo* assays in intact animals may be considered predictive for adverse effects on reproduction, they do not provide adequate information on reproductive toxicity for risk assessment and classification and labelling. The repeated dose 28-day oral toxicity study (EU B.7, OECD TG 407) has been updated (2008) to include parameters aiming to identify substances acting through (anti)estrogenic,

(anti)androgenic and (anti)thyroid mechanisms. Validation studies indicate that enhanced design can reliably identify substances with strong potential to act through endocrine modes of action on the gonads and thyroid. A result suggesting no effect in such a study up to the highest dose tested provides some evidence of the absence of potent endocrine activity. However, effects induced by a lower endocrine disrupting potency cannot be ruled out and therefore a result showing no effects does not provide reassurance of the absence of the capability to cause reproductive toxicity via the mechanism of endocrine disruption. Notably in this context, prolongation of exposure from 28 days up to 90 days is unlikely to improve the detectability of endocrine effects (Gelbke *et al*. 2006). Evidence of effects on reproductive organs potentially via endocrine disrupting mode of action seen in a repeated-dose toxicity study provides a trigger for the conduct of a more comprehensive study, i.e., the extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) at Annex IX.

The potential triggers related to endocrine disrupting modes of action to be used to define the study design of the extended one-generation reproductive toxicity study are presented along with other triggers in Appendix 2 of this Guidance.

The screening studies (OECD TGs 421/422) may be updated with additional parameters for endocrine disrupting modes of action, such as measurement of anogenital distance, nipple/areolae retention and thyroid hormone (T4 and TSH) levels. These parameters indicate endocrine disrupting mode of action and may be predictive for adverse effects on reproduction. A statistically significant change in anogenital distance that cannot be explained by the body weight/size of the animal indicates an anti androgenic mode of action and should be used for setting the NOAEL. To support the adversity of this parameter an association with reduced human reproduction has been reported (Jain et al. 2013; Eisenberg et al. 2011 and 2012; Mendiola et al. 2011). A statistically significant change in nipple/areolae retention indicates also an anti androgenic mode of action but likely via other spectrum of mechanisms than that of anogenital distance. Due to the difference in biology in controlling the final number of nipples between male rats and human, there is not likely a similar association between nipple/areolae retention findings in rats and adversity in human than for anogenital distance. However, as the assumed mode of action (antiandrogenicity) and potential underlining mechanisms affecting nipple/areolae retention in rats are also relevant to humans, although not causing similar effects, this finding can be considered likely to predict an adverse effect and used to set the NOAEL. Nipple/areolae retention measures the same mode of action (antiandrogenicity) than anogenital distance but due to different tissue specific underlining mechanisms niplle/areolae retention may be more or less sensitive than anogenital distance. It is recommended that these endpoints are evaluated together.

As the extended one-generation reproductive toxicity study is a more comprehensive reproductive toxicity study which includes certain parameters to detect endocrine disrupting modes of action, it may be possible a) to identify an endocrine disrupting mode of action, b) to identify an adverse effect on reproduction, c) both of these not necessarily indicating a causal relationship. In case an endocrine disrupting mode of action is identified without an adverse effect on reproduction (e.g. reduced thyroid hormone level in pups), further studies or actions may be considered. In case the findings on reproduction meet the classification criteria to Category 1B reproductive toxicant, irrespective indications of an endocrine disrupting mode of action, the substance should be classified accordingly.

R.7.6.4.3 Human data on reproductive toxicity

Epidemiological data require a detailed critical appraisal that includes an assessment of the adequacy of controls, the quality of the health effects and exposure assessments, and of the influence of bias and confounding factors. Epidemiological studies can

generally only provide associations, no causality because it may be possible to show the link and estimate the likelihood of the causality but not give a final proof.

Epidemiological studies, case reports and clinical data may provide sufficient hazard and dose-response evidence for classification of chemicals as reproductive toxicants in Category 1A and for risk assessment, including the identification of a NAEL or LAEL. In such cases, there will not normally be a need to test the chemical. However, convincing human evidence of reproductive toxicity for a specific chemical is rarely available because it is often impossible to identify a population suitable to study that is/was exposed only to the chemical of interest. Human data may provide limited evidence of reproductive toxicity that indicates a need for further studies of the chemical; the test method selected should be based on the potential effect suspected.

When evidence of a reproductive hazard has been derived from animal studies it is unlikely that the absence of evidence of this hazard in an exposed human population will negate the concerns raised by the animal model. This is because there will usually be methodological and statistical limitations to the human data. For example, statistical power calculations indicate that a prospective study with well-defined exposure during the first trimester with 300 pregnancies could identify only those developmental toxins that caused at least a 10-fold increase in the overall frequency of malformations; a study with around 1000 pregnancies would have power to identify only those developmental toxins that caused at least a 2-fold increase (EMEA/CHMP Guideline, 2006). Extensive, high quality and preferable prospective, data are necessary to support a conclusion that there is no risk from exposure to the chemical. Thus, the absence of effects in humans at dose level below the dose levels inducing reproductive toxicity in animals will not negate the concerns raised by the animal model.

R.7.6.4.4 Derivation of DNELs and DMELs

Identification of DNEL(s) are referred to in Annex I, 1.4. Depending on the available information and the exposure scenario(s), it may be necessary to identify different DNELs for each relevant human population (consumers, professional, workers, humans exposed indirectly via environment and certain vulnerable subpopulations (children, pregnant woman) and for different routes of exposure and all routes combined. In certain cases exposure from various sources may need to be considered. For reproductive toxicity endpoints it is especially relevant to consider deriving the different DNELs for vulnerable subpopulations.

Generally, effects on reproduction have been considered as effects having a threshold and, thus, allowing derivation of a DNEL. However, in certain cases, the possibility for a non-threshold mode of action may need to be considered (e.g. in cases a substance has(anti)hormonal activity similar to a hormone having a primary biological control role and there is a concern of lack of body's regulation capacity). For these cases derivation of DMEL may need to be considered.

In order to be suitable for CSA appropriate DNELs (DNEL for fertility and DNEL for development) have to be established for each exposure scenario. Typically, the derivation of the DNEL takes into account a dose descriptor, modification of the starting point and application of assessment factors - see *Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response for human health* (Chapter R.8 and Appendix R.8-12 and R.7.6.4.3).

Appendix R.8.12 Reproductive toxicity provides specific advice for reproductive toxicity studies.

R.7.6.5 Classification and labelling

Guidance on classification and labelling is given in the *Guidance on the Application of the CLP Criteria* (Chapter 3.7).

R.7.6.6 Conclusions on reproductive toxicity

Reproductive toxicity endpoints should be considered separately for establishing the relevant endpoint(s) and NOAEL(s) to be used in risk assessment (for fertility and developmental toxicity endpoints) and for classification (for sexual function and fertility; developmental toxicity; and lactation). The study or studies giving rise to the highest concern must normally be used to establish the DNEL(s) (see Annex I, 1.2.4 of REACH). If another study / other studies are used an acceptable justification for this exception needs to be provided. Derivation of DMEL needs to be considered in cases where adverse effects are likely to be induced via a non-threshold mode of action.

Risk assessment and determination of classification involves the consideration of all data that is available and may be relevant to reproductive toxicity (see Section 0 for different data sources). There can be no firm rules on how to the conduct the risk assessment and determination of classification for hazards as these process involves expert judgment and also because the mix and reliability of information available for a particular substance will probably be unique. Also data resulting from studies on other hazards, e.g. repeated dose toxicity, can be relevant to consider in the risk assessment and determination of classification of reproductive toxicity.

In order to conclude on a proper hazard classification and category, all the available information needs to be taken into account, and compared with the criteria in Annex I of the CLP Regulation (see also Guidance on the Application of the CLP criteria). If the information is not adequate to decide on classification and labelling, the registrant must indicate and justify the action or decision he has taken as a result (Annex VI, 4.1 and Annex VI, 1.3.2 of REACH).

In case the substance has an EU harmonised classification for Reproductive toxicity (included in Annex I, CLP) or meets the classification criteria and is subject to self-classification, exposure scenarios should be established and the risk characterisation ratio (RCR) calculated to indicate the safe use of the substance.

R.7.6.7 Integrated Testing Strategy (ITS) for reproductive toxicity

Section R.7.6.2 of this Guidance, includes guidance on how to define and generate relevant information on substances in order to meet the information requirements and address the concerns related to intrinsic properties of substances related to reproductive health.

An integrated testing strategy (ITS) may be defined as an approach which combines one or more non-animal methods with animal studies to fulfil the information requirements or only with several non-animal methods covering all key aspects of reproductive toxicity. Thus, Annex XI adaptations (with the exception of section 3.2.a – substance tailored exposure-driven testing) play an important role in ITSs for reproductive toxicity. An ITS must produce information usable for a robust risk assessment and/or for classification and labelling. The definition for ITS is given e.g. by Blaauboer et al., (1999)[30]. The ITS

[30] "An Integrated Testing Strategy is any approach to the evaluation of the hazard which serves to reduce, refine or replace an existing animal procedure, and which is based on the use of two or more of the following: physicochemical data, in vitro data, human data (for example, epidemiological, clinical case reports), animal data (where unavoidable), computational methods (such as quantitative structure activity relationships (QSARs) and biokinetic models" (Blaauboer et al., 1999).
http://echa.europa.eu/documents/10162/13632/information_requirements_r7_a_en.pdf

concept is similar to that of IATA, Integrated Approaches to Testing and Assessment. In principle, ITS and IATA are approaches where information is collected, evaluated and weighed aiming to provide a sufficient amount of information by development of the weight of evidence. ITS and IATA could be used with a view to generate information in a step-wise approach, allowing for justifying an adaptation of one or more standard information requirements according to Annex XI, 1.2. (weight of evidence) taking into account that Annex XI, 1.2 is a hazard-based approach and exposure and risk-based consideration cannot be used.

A comprehensive use of ITS for reproductive toxicity endpoint requires knowledge on all different mechanistic steps and processes involved in the outcome of a possible adverse effect. Reproductive toxicity relates to a number of potential target tissues and comprises a huge number of interacting processes, which are not even known in their entirety and which at present are far from being fully understood in their complexity. Another particular challenge in the identification of reproductive toxicity effects relates to the potential impact of systemic toxicity on the fertility and maternal toxicity on the development of the offspring. The existence of windows of particular sensitivity during the development of the embryo is another characteristic feature of reproductive toxicity. However, currently adverse outcome pathways (AOPs) are under development each covering one specific effect e.g. vasculogenesis and cleft palates. It is to be noted that also the specific effects like clefts can be formed via several different mechanisms and AOPs increasing the complexity. AOP may form a basis for ITS/IATA in describing the key events in a toxicity pathways that need to be addressed by and ITS/IATA.

Combined approaches including various methods may be used as preliminary steps only because they do not provide equivalent information on the standard information requirements. In addition they may be elements in WoE approach according to Annex XI, 1.2 approach or supporting categories and read across according to Annex XI, 1.5 approach. However, as these combined approaches include more uncertainty due to

missing parts of information, this should be addressed when such approaches are proposed. As all the potential molecular mechanisms and regulatory mechanisms are not covered these approaches may not be appropriate to prove the absence of an effect. Currently derivation of a NOAEL is not possible with these methods.

R.7.6.8 References

Comment [SJ7]: ECHA will review and update the References further during consultation.

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Comment [SJ8]: ECHA will review and update the format for listing ECHA Guidance references during the consultation.

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Appendix 1: A check list for information requirements for EOGRTS

This is a "check list" of the information requirements for EOGRTS that should be presented in the dossier in order to establish the existence or the nonexistence of the triggers and conditions specifying the study design proposed for the extended one-generation reproductive toxicity study. More details are provided in Appendix 2 (EOGRTS study design) and length of the premating exposure duration is discussed in Appendix 3.

Comment [SJ9]:
NOTE for the Consultation Procedure
Appendix 1 is meant to be a check list to guide the registrant which studies/information should be checked and presented in dossiers when evaluating the existence or absence of the triggers.

Condition/trigger	Where to find the information to decide on the existence or nonexistence of the triggers and conditions
E1: Uses leading to significant exposure of consumers or professional, taking into account inter alia consumer exposure from articles	Consumer and/or professional uses (one very wide uses or several limited uses): • Substance is used neat or in a chemical mixture • Substance is in an article and it is intended to be released from the article Substance is in consumer articles exhibiting significant migration from the matrix and dermal absorption is relevant. The registrant must record and justify the existence or nonexistence of the condition and if existing together with any of the other three conditions below (E2, E3 or E4), then the extension fo the Cohort 1 B must be proposed.
E2: Genotoxicity potentially meeting classification criteria to Mutagen Category 2	Results from *in vivo* mutagenicity studies (if one of the *in vitro* tests is positive, then an *in vivo* somatic cell mutagenicity test must have been conducted). The registrant must record the findings and justify the existence or nonexistence of the condition.
E3: Extended exposure is needed to reach the steady state kinetics.	Information from toxicokinetic studies in animals or human data, e.g., blood or organ level measurements. Generally, time longer than a week to reach the steady state may be considered extended. Human biomonitoring data indicating high level of substance or metabolites. Indications from existing *in vivo* studies that after a longer exposure duration effects are more severe/occurring at lower dose levels than would be expected based on assessment factors generally used to extrapolate the dose descriptor between studies with different exposure duration. Any other indication of potential to accumulate, such as prediction from log Pow, non-animal approaches (QSAR predictions). Information from ecotoxicity: elevated levels in biota, high levels at the top of food chain, very slow depuration, irreversibility of exposure, bioaccumulation potency (B or vB, or similar concern), biomagnifications. All the components and metabolites of the multicomponent substance must be considered and justified. The registrant must record the findings and justify the existence or nonexistence of the condition.
E4: Indications of modes of action related to endocrine disruption from *in vivo* or non-animal approaches	Repeated dose toxicity studies, especially the 28-day repeated dose toxicity study (EU B.7, OECD TG 407) updated in the year 2008, may provide indication of endocrine disrupting mode of action. Check the parameters related to endocrine mode of action. Reproductive toxicity studies may provide indication of endocrine mode of

	action. Check the parameters related to endocrine mode of action. Check *in vivo* assays for endocrine disrupting modes of action. Check the non-animal approaches for prediction to endocrine disrupting modes of action. Check data from eco-toxicity testing for predicting endocrine disrupting modes of action The registrant must record the findings and justify the existence or nonexistence of the condition.
N1: Information on neurotoxicity from *in vivo* studies or non-animal approaches.	*In vivo* toxicity studies may provide information on neurotoxicity. Check all the parameters related to nervous system. Check the non-animal approaches for prediction of (developmental)neurotoxicity. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental neurotoxicity.
N2: Specific mechanism/modes of action with association to (developmental) neurotoxicity.	Some studies may include measurements which reveal the mechanism, or there may be specific mechanistical studies (*in vivo* or *in vitro*) available. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental neurotoxicity.
N3: Existing information on (developmental) neurotoxicity from structurally analogous substances	Structurally analogous substances should be identified and effects indicating (developmental) neurotoxicity must be checked from available studies. In principle all *in vivo* studies may provide information on neurotoxicity. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental neurotoxicity.
I1: Information on immunotoxicity from *in vivo* studies or non-animal approaches.	*In vivo* toxicity studies may provide information on immunotoxicity. Check all the parameters related to immune system. Check the non-animal approaches for prediction of (developmental) immunotoxicity. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental neurotoxicity.
I2: Specific mechanism/modes of action with association to (developmental) immunotoxicity.	Some studies may include measurements which reveal the mechanism or there may be specific mechanistical studies (*in vivo* or *in vitro*) available. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental immunotoxicity.
I3: Existing information on (developmental) immunotoxicity from structurally analogous substances	Structurally analogous substances should be identified and effects indicating (developmental) immunotoxicity must be checked from available studies. In principle all *in vivo* studies may provide information on immunotoxicity. The registrant must record the findings and justify the existence or nonexistence of the triggers and particular concern for developmental immunotoxicity.

Appendix 2: EOGRTS Study Design

The registrant must propose the study design of the extended one-generation reproduction toxicity study with the following specifications. Relevant justifications are needed including the existence or nonexistence of the conditions for extension of the Cohort 1B and trigger(s) for the Cohorts 2A and 2B, and Cohort 3.

The specifications for study designs in REACH are needed for the following aspects:

1) Premating exposure duration and dose level selection;
2) The need to extend the reproduction toxicity Cohort 1B and to define the termination time for F2;
3) The need to include the developmental neurotoxicity Cohorts 2A and 2B;
4) The need to include the developmental immunotoxicity Cohort 3.

In the following text the specifications and triggers (conditions) are presented for each study design. The Table in Appendix 1 provides a check list for the registrants in order to assess which studies/tests could provide information on triggers which specify the study design of the extended one-generation reproductive toxicity study. The existence or the nonexistence of triggers (conditions) must be recorded in order to allow an independent evaluation.

The study design should be decided before the study is started. For REACH the in-study triggers are not recommended. However, the registrant may expand the study based on new information indicating a concern which needs to be addressed. The justification for the expansion must be documented.

The OECD guidance document GD 151 provides guidance for conduction of cohorts of extended one-generation reproductive toxicity study (OECD 2013) but the study design applicable for REACH and CLP is outlined in REACH Annexes IX and X and Recital (7) of Commission Regulation (EC) 2015/282 amending REACH and described in more detailed in this guidance.

Specifications needed in testing proposals:

1) Premating exposure duration and dose level selection

Recital (7) of Commission Regulation (EC) No 2015/282 of 20 February 2015 amending REACH states that the extended one-generation reproductive toxicity study should allow adequate assessment of fertility and that premating exposure duration and dose levels should be appropriate to meet the risk assessment and classification and labelling purposes[31].

Both the length of premating exposure duration and dose level setting are aspects which influence the possibility to adequately assess potential adverse effects on fertility. To adequately address the assessment of the fertility endpoint, the starting point for deciding on the length of premating exposure period should be 10 weeks to cover the full

[31] Recital (7) of Commission Regulation (EU) No 2015/282 Of 20 February 2015 amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended one-generation reproductive toxicity study: "*It should be ensured that the reproductive toxicity study carried-out under point 8.7.3 of Annexes IX and X to Regulation (EC) No 1907/2007 will allow adequate assessment of possible effects on fertility. The premating exposure duration and dose selection should be appropriate to meet risk assessment and classification and labelling purposes as required by Regulation (EC) No 1907/2006 and Regulation (EC) No 1272/2008 of the European parliament and of the Council.*"

spermatogenesis and folliculogenesis before the mating allowing meaningful assessment of the effects on fertility. The exposure can be started when the animals are around 5 weeks old and mate them around 15 weeks of age. However, based on substance specific justifications a shorter premating exposure duration may be proposed, but it should not be shorter than two weeks. Discussion on premating exposure duration is provided in Appendix 3.

In case the registrant prefers another length of premating exposure duration, an acceptable substance-specific scientific justification must be provided. The highest dose for the extended one-generation reproductive toxicity study should be selected with the aim to induce some toxicity (or to use the limit dose of 1000 mg/kg bw/day), , in order to allow conclusion on whether effects on reproduction are considered to be secondary non-specific consequence of other toxic effects seen (see also the dose level selection under Section R.7.6.2.3.2, Stage 4.1(6) of this Guidance). Only in this way is it possible to assess if the substance is a reproductive toxicant and/or if the effects on reproduction are potentially associated with systemic toxicity and to which extent.

The possibility to select the highest dose level based on the toxicokinetic data, as mentioned in EU B.56 (OECD TG 443) and in the OECD GD 151, may not allow comparison of adverse effects on fertility with systemic toxicity and, thus, does not support production of data for classification and labelling purposes, including categorisation. Regarding the highest dose level, it is important to ensure that toxicity in both female and male animals is considered to ensure that reproductive toxicity in either gender is not overlooked.

Both the 10 weeks premating exposure duration and the highest dose level meeting the requirement of inducing toxicity, should allow conclusion on classification and labelling, including categorisation, for the hazard endpoint for sexual function and for fertility according to CLP.

2) Extension of Cohort 1B and termination time for F2

REACH specifies that the extension of cohort 1B to include the F2 generation shall be proposed by the registrant or may be required by the Agency if:

a) *"the substance has uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles, and*

b) any of the following conditions are met:

- *the substance displays genotoxic effects in somatic cell mutagenicity tests in vivo which could lead to classifying it as Mutagen Category 2, or*
- *there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, or*
- *there are indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches."*

In the following, examples are provided for the criteria when the registrant shall propose the extension of Cohort 1B to mate the Cohort 1B animals to produce a F2 generation:

Guidance for uses leading to significant exposure:

- If the substance is intended to be used[32] in the EU by consumers (i.e. members of the public) or professionals (i.e. workers in trades), either neat or in a

[32] Registrant to provide data to support his registration

chemical mixture and there is one very wide use or several limited uses potentially affecting major part of consumers and/or professionals, then this is considered as meeting the criterion.

- If the substance is in an article used by consumers or professionals in the EU the criterion would be met if the substance is intended to be released from the article during use of the article by the consumers or professionals and there is one very wide use or several limited uses potentially affecting major part of consumers and/or professionals.
- Use of a substance in consumer articles exhibiting significant migration from the matrix and for which dermal absorption is relevant.

Guidance for toxicity conditions to be used together with criteria for uses leading to significant exposure:

(i) "*The substance displays genotoxic effects in somatic cell mutagenicity tests in vivo which could lead to classifying it as Mutagen Category 2*":

- Genotoxicity/mutagenicity observed *in vivo* potentially meeting the classification criteria to Mutagen Category 2.
  - Note: If the substance meets the criteria to Mutagen Category 1A/1B and the adequate risk management measures are in place then the reproductive toxicity studies need not to be conducted (according to adaptation possibility in Annex IX/X, point 8.7, Column 2).
  - *An in vivo* mutagenicity study should be available if one of the *in vitro* mutagenicity studies is positive (predicts mutagenicity). In case one of the *in vitro* mutagenicity studies is positive, an *in vivo* mutagenicity study should be conducted before deciding on the study design of the extended one-generation reproductive toxicity study, if the other criteria for extending the Cohort 1B are not met.

(ii) "*There are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure*":

Extended time to reach the steady state may be indicated by available toxicokinetic information, physico-chemical properties and information from (eco)toxicological data. The effect of sex and life stages could be also considered[33]. Information can be obtained from:

- Assessment of toxicokinetic behaviour of the substance
  - Generally, duration of longer than a week to reach the steady state may be considered as extended (in practise a steady state can be considered to be achieved after 4 to 6 half-lives)[34].
  - Attention need to also be given on indications of very slow clearance (e.g. PFOA and APFO which are Category 1B reproductive toxicants).

[33] See e.g. Blagojević, J et al., Age Differences in Bioaccumulation of Heavy Metals in Populations of the Black-Striped Field Mouse, *Apodemusagrarius* (Rodentia, Mammalia) *Int. J. Environ. Res., 6(4):1045-1052, Autumn 2012)*

[34] Steady state is achieved when the rate of elimination equals the rate of administration. Accumulation factor is 2 for a substance given once every half-live. Accumulation can be expected for a substance with slow elimination; e.g., with high octanol-water coefficient and no predicted hydrophilic metabolites. For lipophilic substances excretion may be impossible if there is no metabolism.

- Physico-chemical properties of the substance
  - An octanol-water partition coefficient (log Kow) value e.g above 4.5 indicates (bio)accumulative potential (determined experimentally or estimated by QSAR models) of the substance and/or its metabolites unless the substance is fully metabolised to hydrophilic metabolites
- Indications on (bio)accumulation in animals or from human biomonitoring data
  - High level of substance/metabolites in human body fluids or tissues, such as blood, milk, or fat which are indicative of a concern on accumulation and persistence. Substances of purely endogenous origin and high levels due to high exposure only are excluded.
  - Bioaccumulation potency, for example if the substance properties meet the bioaccumulation screening criteria described in Table C.4-1 of Guidance on Information Requirements and Chemical Safety Assessment Part C: PBT/vPvB assessment Version 2.0, November 2014. The assessment approach is described further in Section R.11.4.1.2 of Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment Version 2.0, November 2014.
  - If the substance fulfils the bioaccumulation criterion (B or vB) described in Annex XIII of REACH.
  - Indications of biomagnifications (high levels of the substance in biota or terrestrial animals in the top of food chains, resulting from the effective accumulation of the substance in organisms and the slow elimination (not from high releases). This is further discussed under 'Field data and biomagnification', page 52, Section R.11.4.1.2 of Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11 PBT/vPvB assessment Version 2.0, November 2014.
- Indications from existing *in vivo* studies that after longer exposure duration the effects are more severe/occurring at lower dose than would be expected based on assessment factors generally used to extrapolate the dose descriptor between studies with different exposure duration.
  - E.g., if the NOAEL of a subchronic study (90-day) is more than 3 times lower than the NOAEL from a subacute study (28-day), taking the dose level selection into account.
  - Effects observed only at later time point in chronic studies, thus indicating a need to have a longer exposure time to cause the toxicity likely due to accumulation of a substance or its metabolites.

(iii) "*There are indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches*".

Evidence from endocrine disrupting mode(s) of action[35] such as (anti)estrogenicity, (anti)androgenicity or influence on thyroid hormone activity or other modes of action related to endocrine disrupting properties relevant to reproductive toxicity. These modes of action have been associated with adverse effects on fertility, reproductive performance or development of offspring. See Appendix 5 for evaluation of triggers.

[35] A comprehensive collection of screens and tests for endocrine disrupting chemicals are presented in OECD GD 150, covering the oestrogen receptor, androgen receptor and thyroid hormone mediated and steroidogenesis interference modalities. Both the test results for toxicity and ecotoxicity may be relevant.

- Endocrine disrupting modes of action may be indicated from *in vivo* studies by 1) changes in organ weight sensitive to endocrine disrupting activity (intact and/non-intact animals), 2) (increased) body weight, 3) measurements of hormone levels, or 4) effects on reproduction associated to endocrine disrupting modes of action.
- Repeated dose toxicity studies, especially the 28-day repeated dose toxicity study (EU B.7, OECD TG 407) updated in 2008, may provide indication of endocrine disrupting mode of action. Check the parameters related to endocrine mode of action; e.g.:
  - Changes in reproductive organs and other endocrine organs (e.g., ovaries, testes, uterus, cervix, epididymides, seminal vesicles, coagulating glands, prostate, vagina, pituitary, mammary gland, thyroid and adrenal gland)
  - Changes in body weight (increase)
  - Changes is oestrus cycle
  - Changes in relevant hormone levels
- Reproductive toxicity studies (e.g. screening study) may provide indication of endocrine mode of action. Check the parameters related to endocrine mode of action; e.g.:
  - Changes in reproductive organs and other endocrine organs (see above)
  - Changes in indicators of hormonal mode of action, such as anogenital distance, nipple retention, mammary gland histopathology
  - Changes is oestrus cycle
  - Changes in gestation length
  - Other effects showing a likely endocrine disrupting mode of action
- Endocrine effects from ecotoxicology studies and tests predicting endocrine disrupting modes of action (especially thyroid, see OECD GD 150)
- Non-animal approaches and specific animal studies may provide mechanistic data, information on receptor binding, epigenetics or other regulatory mechanism for endocrine disruption, e.g.:
  - Uterotrophic assay (EU B.54, OECD TG 440)
  - Hershberger assay (EU B.55, OECD TG 441).
  - Performance-based test guideline for stably transfected transactivation *in vitro* assays to detect estrogen receptor agonists (OECD TG 455)
  - H295R steroidogenesis assay (OECD 456)
  - BG1Luc Estrogen receptor transactivation test method for identifying estrogen receptor agonists and antagonists
  - Yeast Estrogen Screening (YES) and Yeast Androgen Screening (YAS) Tests
  - Androgen receptor binding study
  - Aromatase assay
  - Endocrine organ cultures
  - QSAR and computational predictions considered adequately reliable to serve as trigger(s)

The relevance and quality of triggers from the *in vivo* studies and non-animal approaches used must be adequately documented and justified. Case by case considerations are needed in evaluating trigger(s), evaluation is discussed in Appendix 5.

Further aspects to consider related to extension of the Cohort 1B:

Extension of Cohort 1B and termination time for F2

An extension of Cohort 1B to F2 is considered relevant in the context for classification and labelling and categorisation especially if the effect in P0 parental/F1 offspring is significant but not meeting classification criteria to Repr 1Band more severe effect is seen in the F1 mating pairs/F2 offspring, thus affecting both P0 parental/F1 offspring and F1 mating pairs/F2 generations but being more prominent or with a broader/different spectrum in F1 mating pairs/F2 offspring This could lead to a change in the classification from Repr. 2 to Repr. 1B.

Substances meeting the classification to Mutagen Category 2 are considered to have properties which increase the concern for reproductive toxicity and especially to the vitality and health of the second generation. The substance may have adverse effects on primordial germ cell development, proliferation and migration during *in utero* development, which may then be observed as reduced fertility in the F1 animals. Many genotoxic compounds are also reproductive toxicants.

The test method for extended one-generation reproductive toxicity study provides the possibility to terminate the F2 generation on postnatal day (PND) 4 based on a weight of evidence based approach (integrated evaluation of the existing data). A weight of evidence adaptation approach according to Annex XI, 1.2 of REACH could be used e.g., if the results already meet the classification criteria to Repr 1B and it is highly likely that results from the rest of the lactation period (PND 5-21) would not lead to a lower NOAEL value. To cover the remaining uncertainty, an additional assessment factor may be applied.

The decision on whether or not to extend the Cohort 1B to F2 generation is/should be done before starting the study when the specified conditions are met. The testing proposal must include the study design proposed with justifications. The registrant is responsible for the overall design, conduct and interpretation of the study in order to meet the regulatory requirements and to insure the scientific integrity of the study in line with the test method.

So called internal triggers or in-study triggers for mating the Cohort 1B animals to produce the F2 generation (as those described in OECD TG 117) are not recommended to be used as such in REACH. However, the registrant may expand the study based on new information indicating a concern which needs to be addressed. The justification for the expansion must be documented.

3) *Inclusion of Cohorts 2A and 2B*

The main concepts of the triggers (conditions) for Cohort 2 (developmental neurotoxicity, DNT) are based on a particular concern for (developmental)[36] neurotoxicity[37]. A

[36] Both particular concerns for neurotoxicity as well as for developmental neurotoxicity may be addressed. See discussion in section R.7.6.4.2.3.3 and R.7.6.4.2.3.4

[37] (Nielsen et al. 2008) "Signs of neurotoxicity in standard acute or repeated dose toxicity tests may be secondary to other systemic toxicity or to discomfort from physical effects such as a distended or blocked gastrointestinal tract. Nervous system effects seen at dose levels near or above those causing lethality should not be considered, in isolation, to be evidence of neurotoxicity. In acute toxicity studies where high doses are administered, clinical signs are often observed which are suggestive of effects on the nervous system (e.g. observations of lethargy, postural or behavioural changes), and a distinction should be made between specific and non-specific signs of neurotoxicity." "A consistent pattern of neurotoxic findings rather than a single or a few unrelated effects should be taken as persuasive evidence of neurotoxicity."

particular concern means that the concern should be specific to (developmental) neurotoxicity but also that the concern needs to reach a certain level of severity. Based on text in e.g. Annex VIII, 8.6.1, it can be understood that a particular concern may be indicated e.g. by serious or severe effects[38]. There should be sufficient evidence, weighing all the information, to raise a reasonable expectation that the substance could be a developmental neurotoxicant.

REACH specifies that an extended one-generation reproductive toxicity study including Cohorts 2A and 2B (developmental neurotoxicity cohorts) shall be proposed by the registrant or may be required by ECHA in case of a particular concern on (developmental) neurotoxicity.

Conditions for a particular concern for developmental neurotoxicity:

- existing information on the substance itself derived from relevant available *in vivo* or non-animal approaches, or
- specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity, or
- existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action.

For the precise legal text see REACH regulation Annexes IX and X, 8.7.3. The registrant must record the findings and justify the existence or nonexistence of the trigger(s) for the need to include the Cohorts 2A and 2B.

Examples for findings which may indicate a particular concern justifying inclusion of the developmental neurotoxicity cohort:

- abnormalities observed in the central nervous system or nerves
  - changes in brain weight not secondary to body weight or changes specific neural areas
  - changes in brain volume or specific neural areas, obtained e.g. from morphometry/stereology measurements
    - (histo)pathological findings in brain, spinal cord and/or nerves (e.g. sciatic nerve)
- any signs of behavioural or functional adverse effects on the nervous system in adult studies e.g. repeated-dose and acute toxicity studies and neurotoxicity studies, not likely to be secondary to general toxicity.
  - clinical and/or behavioural signs (such as abnormal gait, narcosis, seizures or any other altered activity) if seen in absence of general toxicity
- specific mechanism/mode of action that has been closely linked to (developmental) neurotoxic effects (see e.g. Gupta RC (ed) 2011, pages 835-862),
  - (adult) brain cholinesterase inhibition (by 20%);
  - relevant changes in thyroid hormone levels or signs of thyroid toxicity indicating such changes,

[38] A serious or severe effect is an effect which has regulatory consequences, i.e leads to a NOAEL values and/or contributes to hazard classification. Thus, a particular concern is an expectation that the substance has (developmental) neurotoxic properties contributing to the regulatory decision making. This also means that they are not secondary to other systemic toxicity.

- evidence of hormonal modes of action with clear association with the developing nervous system, such as oestrogenicity (Fryer et al. 2012) and antiandrogenicity (Pallarés et al. 2014)

- Information from non-animal approaches, such as from an *in vitro* developmental neurotoxicity test (see e.g. de Groot 2013 and Westerink 2013), predicting developmental neurotoxicity, e.g.,
  - Any sign of adverse neuronal differentiation *in vitro*
    - Neurite outgrowth
    - Neural stem cell proliferation
    - Gene expression (mRNA and protein) biomarkers that are linked to neuronal differentiation, synaptogenesis and other neurodevelopmental differentiation
  - Functional endpoints, e.g. cell membrane potential, excitability, electrical activity
  - Specific modes of action that are linked to neurotoxic effects in vivo can be indicated in vitro by non-validated assays, eg. cholinesterase inhibition, neuropathy target (neurotoxic) esterase inhibition.
- structurally analogue substances show (developmental) neurotoxic effects in *in vivo* or *in vitro* studies suggesting similar effects or similar mechanisms/modes of action
  - adequacy of the read cross of the trigger(s) must be justified

The identified triggers should not be contradicted by other findings in the available data. The relevance and quality of triggers from the *in vivo* studies and non-animal approaches used must be adequately documented and justified. Evaluation of triggers is described in Appendix 5

Further consideration related to adults vs developmental neurotoxicity is provided under Chapter R.7.6.4.2.3.4.

4) *Inclusion of Cohort 3*

The main concepts of the triggers (conditions) for Cohort 3 (developmental immunotoxicity, DIT) are based on a particular concern for (developmental) immunotoxicity[39]. A particular concern means that the concern should be specific to (developmental) immunotoxicity but also that the concern needs to reach a certain level of severity. Based on text in e.g. Annex VIII, 8.6.1, it can be understood that a particular concern is indicated e.g. by serious or severe effects[40]. There should be sufficient evidence, weighing all the information, to raise a reasonable expectation that the substance could be a developmental immunotoxicant.

REACH specifies that an extended one-generation reproductive toxicity study including Cohort 3 (developmental immunotoxicity cohort) shall be proposed by the registrant or may be required by ECHA in case of a particular concern on (developmental) immunotoxicity.

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[39] Both particular concerns for immunotoxicity as well as for developmental immunotoxicity may be addressed. See discussion in Section R.7.6.4.2.3.3 and R.7.6.4.2.3.4.

[40] A serious or severe effect is an effect which has regulatory consequences, i.e leads to a NOAEL values and/or contributes to hazard classification. Thus, a particular concern is an expectation that the substance has (developmental) immunotoxic properties contributing to the regulatory decision making. This also means that they are not secondary to other systemic toxicity.

Conditions for particular concern for developmental immunotoxicity:

- existing information on the substance itself derived from relevant available *in vivo* or non-animal approaches, or
- specific mechanisms/modes of action of the substance with an association to (developmental) immunotoxicity, or
- existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action.

For the precise legal text see REACH regulation Annexes IX and X, 8.7.3. The registrant must record the findings and justify the existence or nonexistence of the trigger(s) for the need to include the Cohort 3.Examples for findings which may indicate a particular concern justifying inclusion of the potential triggers for developmental immunotoxicity cohort:

- Combination of at least two statistically significant and biologically meaningful changes in clinical chemistry and/or organ weight associated with immunotoxicity, e.g., reduced leucocyte count in combination with reduced spleen weight.
- One severe (see footnote 43) statistically and/or biologically significant organ weight or histopathological finding related to an immunology organ, e.g., thymus atrophy.
- (respiratory) sensitisation
- Evidence of changes in immune function involving innate (e.g. NK-cell function, phagocytosis and oxidative burst) or acquired immunity (e.g. generation of immunological memory, cytotoxic T-cells and antibody production)
- Evidence of hormonal modes of action with clear association with the immune system, such as oestrogenicity and effects on thyroid.
- Structural similarity with a substance causing structural or functional immunotoxicity or suggesting a similar mechanism/mode of action
    - adequacy of the read-cross of the trigger(s) must be justified

WHO Guidance document for immunotoxicity provides further examples of potential triggers for immunotoxicity testing (WHO 2012). In summary, all effects on any immune-parameters found either *in vivo* (adult animals), *in vitro* or *in silico* may have impact on the developing immune system. These effects could be defined as quantitative or qualitative changes in cell counts or histopathology studying immune-specific organs or cell-populations in peripheral blood but may also include functional end-points such as antibody-production, delayed-type hypersensitivity test (to investigate cytotoxic T-cell activity), cytokine production, lymphocyte proliferation, NK-cell-function, phagocytosis, and oxidative burst.

The identified triggers should not be contradicted by other findings in the available data. The relevance and quality of triggers from the in vivo studies and non-animal approaches used must be adequately documented and justified. Evaluation of triggers is described in Appendix 5.

Appendix 3 Premating exposure duration in extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)

1. Importance of the premating exposure duration

The two main aspects in a reproductive toxicity study influencing how well fertility parameters and, thus, the potential adverse effects on fertility can be evaluated are the length of the premating exposure duration and dose level setting.

The fertility part of the reproductive toxicity study should be capable of providing information on fertility that is adequate for both risk assessment and classification, including categorisation. For the classification purpose, it is important to produce and evaluate the full spectrum of effects on fertility. Just to detect a most sensitive effect may not be enough for deciding on classification categorisation because full information on magnitudes, incidences, severity and types of all effects (MIST information) should be evaluated together to assist the decision.

If the registrant applies 10 weeks premating exposure duration in an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) no justification for premating exposure duration is needed. Substance specific justifications should be provided substantiated with data if shorter than 10 weeks premating exposure duration is proposed.

1.1 Main parameters for evaluating effects on fertility

Mating/fertility

Mating and fertility are functional parameters which include effects on mating behaviour and fertility outcome. Parameters such as precoital interval, mating index, fertility index, preimplantation loss, post-implantation loss, number of corpora luteae, number of implantations, number of resorptions, dead foetuses, abortions, gestation length, litters size, and number of live pups are measuring effects on fertility (some of these parameters may also reflect developmental toxicity).

The length of the premating exposure may influence the mating and fertility parameters if the substance 1) causes adverse effects on primordial germ cell development, their migration and/or proliferation, 2) causes adverse effects on sperm development and maturation, 3) causes adverse effects on follicle development and/or development of ovum, 4) causes adverse effects on brain sexual development, 5) causes effects on hypothalamus-pituitary-gonad axis or other effects on hormonal control mechanisms.

The primordial germ cells develop, migrate and proliferate already during embryonic development. In addition to histopathological analysis of gonads, organ weight measurements and sperm parameter analysis, adverse effects on germ cell development/migration/proliferation during these early stages, as well as the other effects listed above, can be fully evaluated only by exposing the animals already *in utero* and then until adulthood and mating them. This full evaluation is possible in cases where the mating and littering of the Cohort 1B animals is triggered in an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443).

An effect on fertility may be due to exposure *in utero,* postnatal period or during adulthood. In some cases it may be possible to conclude that effects on fertility are of developmental origin. For instance if there is information on fertility in both the parental animals and their offspring and effects on fertility are only seen in the mature offspring.

Sperm parameter analysis

Sperm parameter analysis includes e.g., total cauda epididymal sperm number, percent progressively motile sperm, percent morphologically normal sperm and potentially percent of sperm with each identified abnormality for animals. In extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) these parameters are to

be reported for both the P and F1 males at termination. Other studies required in REACH as standard information requirements do not normally report results from sperm parameter analysis.

Sperm parameter analysis inform on the number of cauda epididymal sperm and their quality in terms of motility and morphological normality. The results from sperm parameter analysis reflect the effects during the spermatogenic cycle in testes and during the epididymal maturation, if the exposure is long enough to cover both of these periods. The ability of sperm to fertilise eggs and produce alive and healthy offspring is examined in the reproductive toxicity studies by mating the animals and let them litter. If the measurement of sperm parameters coincides close to mating, it assists and supports the evaluation of effects on fertility with the same exposure history through the same life stages. Sperm parameters may provide important information because in humans even a slight reduction in sperm quality/count may be critical for fertility.

Oestrous Cycle

Oestrous cycle measurement reflect the normality of the hormonal level changes affecting the responsiveness of females. Direct measurements on function of hypothalamus-pituitary-gonad axis is not generally done in reproductive toxicity studies. It is important to measure oestrous cycle before mating and also after sexual maturity.

Organs Weights Of Gonads And Accessory Sex Organs

Organ weights of gonads and accessory sex organs, together with other parameters, can predict effects on fertility. These measurements can be done only at termination. Thus, this information can be obtained from the P males soon after the mating but from the P females only after weaning. However, the measurements should be done as close as possible with the other information because information from various sources after the same exposure history allow combined and meaningful evaluation of effects on fertility based on all the data.

Histopathology Of Gonads And Accessory Sex Organs

Histopathology of testes, ovaries and accessory sex organs can be done only at termination. Histopathological evaluation of testes allows to assess the structural normality of testes including Leydig cells, Sertoli cells and seminiferous tubules with various developmental stages of sperm (e.g. Russell et al. (eds) 1990). The information is generally qualitative and quantitative measurements are not made and not required in test methods. Thus, it may not be possible to judge on the amount of various cell types including the amount of various developmental stages of sperm. There may be a reduction of sperm at one developmental stage but it may be difficult to evaluate. Histopathological evaluation should reveal if multinuclear cells are present or another effect on sperm development if a significant reduction in amount of certain cell types or their developmental stages is present. The information obtained is related to the morphological normality of testes but does not inform on the functional fertility and ability of the sperm to fertilise the eggs.

The sperm count is measured by counting the number of sperm in cauda epididymis (sometimes also from testis as homogenisation resistant spermatid counts).

Histopathological evaluation of ovaries is complicated. The structure of an ovary is not organised and follicles at various developmental stages are distributed throughout the organ without a clear system. Thus, to count the number of follicles at different developmental stages requires several slices for histopathological examination. Quantitative evaluation of various cell types (e.g. granulosa cells and theca cells), indicative of toxicity is not generally done or required in the test methods. The number of primordial follicles (which can be combined with small growing follicles) is counted in extended one-generation reproductive toxicity study in F1 animals which reflects the number of potential ova for future ovulations. The number of primordial and small

growing follicles does not inform on actual functional fertility of the females but if the follicle number is reduced, it is a clear indication of gonad toxicity and should be taken into account in assessing effects on fertility.

Histopathology of accessory sex organs provides valuable information on how these organs have been developed and their morphological normality. The information should be evaluated together with other fertility findings.

It is important to be able to analyse the histopathological findings after the same exposure length and history as the other effects, including the mating, to be able to understand a full picture of the spectrum of effects. Information in morphology is one important parameter in evaluation but as an alone measurement focus on morphology is too limited to provide a comprehensive picture of all the relevant aspects of fertility but may be sufficient for classification (e.g. findings in histopathology alone from a repeated dose toxicity study may meet the classification criteria to category 1B for reproductive toxicity).

1.2 Ten Weeks Premating Exposure Duration

The full spermatogenesis, without sperm maturation, and folliculogenesis take 48-53 and 62 days in rats, respectively (e.g. Kerr et al 2006, McGee and Hsueh 2000). In addition to spermatogenesis, sperm maturation in rats takes around two weeks in epididymides. When the exposure is long enough, it covers both the sperm and follicle development through all the stages. Ten weeks premating exposure duration covers the full spermatogenesis and maturation meaning that the full cycle of development of sperm from spermatogonia into mature sperm is exposed. Thus, 10 weeks premating exposure duration allows an assessment of the adverse effects on fertility by combining the information from all possible parameters in males evaluated at the same time. Similarly, the folliculogenesis, which lasts around 62 days, is fully covered only after a long exposure period, such as 10 weeks. It is important to expose all the developmental stages of the sperm and follicles before the mating in order to be able to evaluate any potential adverse effect on fertility. Earlier stages of the spermatogenesis have been reported to be generally more sensitive than later stages to chemical and radiation exposure (Sjöblom et al. 1995) which also support that the exposure should cover all the stages before the mating

For a more comprehensive assessment of effects on fertility, which is often needed when deciding on classification for fertility effects, evaluation of the full spectrum of effects on fertility is necessary. Information from a limited number of parameters only allows to conclude on the absence of effects on fertility. Best outcome can be obtained when mating is allowed after an exposure covering one full spermatogenic cycle (including sperm maturation) and folliculogenesis, and an analysis of sperm parameters, organ weights and histopathology of gonads and accessory sex organs are conducted around the same time after the same exposure history.

In an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443), ten weeks premating exposure duration together with sperm parameter analysis, organ weights and histopathology of testis and accessory sex organs with the same exposure history is achievable for males. For females the histopathological analysis of gonads and accessory sex organs can be made only later and not near the mating. However, it is considered that the most important aspect is that the exposure duration for the female gonads covers the folliculogenesis before mating.

Organ weights (e.g. Bailey et al, 2004, Sellers et al. 2007, Hood et al. (ed) (2011)) and/or histopathology (e.g. Jacobson-Kram and Keller, 2006) of gonads may be among the most sensitive parameters for male fertility. For instance, testicular weight is quite stable parameter because generally it is not influenced by small or moderate changes in body weight. Several studies have not establish a correlation between testes-to-body weight and testes-to-brain weight (Bailey et al, 2004). Therefore, it could be concluded

that variations on testicular weight will be linked to direct effects within the testes.

The most sensitive parameter showing an adverse effect is used to derive the NOAEL. However, the findings from the most sensitive parameters may not be sufficient for deciding on classification, including categorisation because the value of the NOAEL is not predictive for classification and (other) effects may be more relevant for classification purposes than the effect leading to a NOAEL. It is the clarity and the spectrum of the effects observed which counts for the classification and labelling. Thus, to address the fertility also for the classification and labelling purposes, including the categorisation, it is necessary to consider how well all the available parameters address the fertility endpoint. Information on magnitude, incidence, severity and type of all effects (MIST) influence on the classification, including categorisation. Evaluation of various parameters after the exposure length covering the critical reproductive aspects and after the same exposure history improves the quality of the assessment.

Environmental factors, such as chemical substances, pesticides, high temperatures and radiation have been associated with a reduction of sperm DNA integrity in infertile men (Evgeni et al, 2014). It is to be noted that some effects on sperm, such as DNA fragmentation, may affect fertility and cannot be examined by routine gonadal histopathology (morphology) or sperm analysis. Several studies have attempted to investigate the possible correlation between human sperm DNA fragmentation and conventional sperm parameters. Most of them found an inverse correlation between DNA fragmentation rate and sperm quality (Evgeni et al, 2014). In contrast, several authors have failed on finding a correlation between DNA fragmentation and standard sperm parameters, such as sperm concentration, motility and morphology (Evgeni et al, 2014). Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) does not contemplate sperm DNA damage assessment and consequently would not identify those cases where a reduction of sperm DNA integrity is not manifested in routine histopathology or in sperm parameter analysis.

The blood-testis barrier prevents free exchange of large proteins and some xenobiotics between the blood and the fluid within the seminiferous tubules (see e.g. Gupta RC (ed) 2011, page 14). This may prolong time needed before the substance reach the developing sperm supporting a long premating exposure duration. All the different cell types representing various developmental stages of spermatogenesis are available in the testis at the same time and may allow detecting an adverse effect for a specific developmental stage or stages. However, a potential cumulative effect requiring exposure through several sequential stages cannot be detected with limited exposure duration. In summary, the 10 weeks premating exposure duration is one of the elements together with the appropriate dose level selection which allow production of data for an informed decision making for classification and labelling, including categorisation, for the hazard endpoint for sexual function and fertility according to CLP Regulation and for risk assessment.

1.3 Shorter Than Ten Weeks Premating Exposure Duration

Shorter than 10 weeks premating exposure duration may be also used based on substance specific justifications - but not shorter than 2 weeks. It is important to consider and document the reasoning why it is assumed that a longer premating exposure duration will not induce more or more severe effects.

A two weeks premating exposure duration is equivalent to the time for epidymal transit of maturing spermatozoa and, thus, allows only the detection of post-testicular effects on sperm at mating (during the final stages of spermiation and epididymal sperm maturation). With a two-week premating exposure, the effects on functional fertility of exposure to the early stages of developing spermatozoa will not be covered as described above under heading 1.2.

The two weeks premating exposure duration is considered adequate to detect most of

the male reproductive toxicants according to OECD GD 151. For females, two weeks premating exposure duration covers 2-3 oestrous cycles and effects on cyclicity may be detected. The detection of an effect may be adequate for NOAEL derivation but for classification and labelling purposes, including categorisation, information on magnitude, incidence, severity and all type of effects, i.e. full spectrum of effects is important (see text under heading 1.2).

Because exposure during the full spermatogenic period and ovarian folliculogenesis are not covered at the time of mating, if only two weeks premating exposure duration has been selected, effects at earlier stages of spermatogenesis and folliculogenesis cannot be reflected in the functional fertility examination. This is a disadvantage and limited information may not allow adequate evaluation, including categorisation for classification, of potential adverse effects on fertility. It is to be noted that for the screening study (OECD TG 421/422) the histopathological data will be limited also due to the limited duration of the whole study and limited statistical power as compared to the more comprehensive reproductive toxicity study such as extended one-generation reproductive toxicity study.

A two-week premating period may be too short to produce results appropriate to conclude whether the substance meets the criteria for a category 1B reproductive toxicant, and thus may not be sufficient for classification and labelling purposes. Under point 2 below some considerations are presented on when a shorter than 10 weeks premating exposure duration could be applied. In these cases substance specific justifications must be provided.

2. Considerations to be made in deciding if shorter than a ten weeks premating exposure duration could be adequate

2.1 Starting Point

To adequately assess the fertility endpoint, the best place to start considering the length of the premating exposure period should be 10 weeks. Ten weeks cover the full spermatogenesis, sperm maturation and folliculogenesis before the mating allowing a meaningful assessment with the full spectrum of the effects after the same exposure history.

Based on substance specific justifications a shorter premating exposure duration may be proposed, but it should not be shorter than two weeks and sufficiently long to reach a steady-state (in reproductive organs) if such kinetic information is available.

2.2 Examples Of Cases Where The Existing Information May Support Shorter Than Ten Weeks Premating Exposure Duration

In case the registrant prefers another length of premating exposure duration, an acceptable substance-specific scientific justification substantiated with adequate data should be provided.

Such a reasoning could be that *effects on fertility are already adequately addressed* and extended one-generation reproductive toxicity study is used to address developmental toxicity. (It is however, to be noted that extended one-generation reproductive toxicity study does not provide equivalent information to the prenatal developmental toxicity study and thus cannot replace a prenatal development toxicity study.)

There may be existing information from a good quality one-generation reproductive toxicity study (EU B.34, OECD TG 415) addressing the fertility parameters. If information on a good quality one-generation reproductive toxicity study is available, then the fertility parameters are normally covered with adequate statistical power and the premating exposure duration may be shorter in the planned extended one-generation reproductive toxicity study.

Extended one-generation reproductive toxicity study is normally still needed to address

the standard information requirement in Annex IX/X, 8.7.3 because a one-generation reproductive toxicity study (EU B.34, OECD TG 415) does not cover the extended exposure period of F1 animals and the same parameters (e.g. sexual maturity and hormonal activity). In addition, the column 2 provisions of Annex IX/X, 8.7.3 are not covered by one-generation reproductive toxicity study if triggered (for further details see Appendix 2 for extended one-generation reproductive toxicity study and section R.7.6.4.2.3.4 for separate developmental neurotoxicity and separate immunotoxicity studies).

There may be existing information from a good quality two-generation reproductive toxicity study (EU B.35, OECD TG 416) addressing the fertility parameters. If information on a good quality two-generation reproductive toxicity study is available, then the standard information requirement in Annex IX/X, 8.7.3 is covered and extended one-generation reproductive toxicity study may not be needed. However, the registrant must fulfil the column 2 provisions regarding to developmental neurotoxicity and/or developmental immunotoxicity if the triggers are met. In these cases the registrant may consider fulfilling the adaptation requirements by proposing separate developmental neurotoxicity and/or developmental immunotoxicity study rather than an extended one-generation reproductive toxicity study. Similarly, if there are concerns related to the endocrine disrupting modes of action/properties not assessed in an existing two-generation reproductive toxicity study but which would have been measured in an extended one-generation reproductive toxicity study, the registrant may consider addressing these concerns in separate studies or add relevant parameters to other studies to be conducted (for further details see Appendix 2 for extended one-generation reproductive toxicity study and section R.7.6.4.2.3.4 for separate developmental neurotoxicity and separate immunotoxicity studies).

There may be also cases where the fertility effects based on the existing information do meet the criteria for Reproductive toxicity Category 1B, but the column 2 adaptation (Annex IX/X, 8.7) is not applicable due to further concern on developmental toxicity. This information on fertility effects may stem e.g. from good quality repeated dose toxicity studies (sex organ weights, histopathology of gonads and/or accessory sex organs, sperm parameters analysis), screening studies (OECD 421/422; e.g. reduced fertility, litter size) or equivalent. In these cases, as the fertility is already addressed, a shorter premating exposure duration in an extended one-generation reproductive toxicity study, if conducted to address the developmental toxicity, may be considered. If the effects from these studies only meet the classification criteria for Reproductive toxicity Category 2 for fertility, those should not be used as an argumentation to reduce the premating exposure length as the findings should be confirmed in a more comprehensive reproductive toxicity study (EU B.56, OECD TG 443).

There may be good quality information from existing repeated dose toxicity 90-day studies showing no effects in organ weights or histopathology of reproductive organs, and covering also the spermatogenesis and folliculogenesis. However, this information alone, or with the results from a screening study (OECD TG 421/422) may not provide adequate confidence to shorten the premating exposure duration from 10 weeks. This is because the information on mating and fertility from a screening study as well as the data from the repeated dose toxicity study is limited. Mating and fertility data from screening studies (OECD TG 421/422) is after two weeks premating exposure duration not covering the full spermatogenesis and folliculogenesis and may not also be adequately long for detecting toxicity in hypothalamus-pituitary-gonad axis. In addition, the statistical power is low in these studies as they are not meant to provide definitive information on reproductive toxicity. Repeated dose toxicity 90-day studies may provide information on organ weights and histopathology but no mating data. The statistical power in 90-day study is lower than that in the extended one-generation reproductive toxicity study, also considering the data for histopathology. In addition, the exposure duration and exposure history are different in screening studies (OECD TG 421/422) and

90-day studies. Thus, it may be difficult to conclude based on this information that a two weeks premating exposure duration is sufficient for a substance in question. However, the registrant may have additional information that may provide elements which together may support the justification, such as very low general toxicity (no effects up to the limit does of 1000 mg/kg bw/day in any of the existing studies), fast elimination, no distribution to sex organs, accessory sex organs and brain, and no concern on germ cell toxicity/mutagenicity (no effect in germ cell mutagenicity test). The substance specific justifications should be substantiated with adequate data.

Results showing no effects or some effects in reproductive organ weights and histopathology from 28-day repeated dose toxicity study generally do not provide conclusive information to justify a shorter than 10 weeks premating exposure duration. First of all, the length of the study is only 28-day not covering the full spermatogenesis and folliculogenesis and the statistical power is low due to low number of animals.

Finally, if animals of Cohort 1B in the extended one-generation reproductive toxicity study are mated to produce the F2 generation, then the premating exposure duration will be 10 weeks for these Cohort 1B animals and the fertility parameters will be covered allowing an evaluation of the full spectrum of effects on fertility. In these cases, shorter premating exposure duration for parental (P) animals may be considered. The consideration should take into account whether the findings from P animals (such as clinical signs, clinical chemistry, haematology) after a longer premating exposure would provide important information for interpretation of the findings in F1 animals, e.g., when considering the potential developmental origin of such findings. It is to be noted that the results of the hazard class classification may differ depending on the interpretations of the origin of the results (differences in classification for specific target organ toxicity and developmental toxicity).

3. Summary

To fully evaluate effects on fertility, effects on all critical aspects and development stages should be covered; this can be done only by exposing the animals already in utero and then until adulthood and mating them. this full evaluation is possible in cases where the extension (mating) of the cohort 1b animals is triggered in an extended one-generation reproductive toxicity study (EU B.56, OECD TG 443). the premating exposure duration of 10 weeks is also covered in mated Cohort 1B animals.

In cases where the extension of the Cohort 1B animals is not triggered, a 10 weeks premating exposure duration should be the starting point. This allows for assessing the consequences of early effects on the sex organs (spermatogenesis and folliculogenesis) assessor sex organs, hypothalamus-pituitary-gonad axis, and e.g. prolonged distribution or any accumulation to relevant organs and tissues.

The registrant may prefer another length of premating exposure duration. Substance specific justifications are needed to support a shortened premating exposure duration.

Appendix 4 Procedure for testing approaches and adaptation; Stage 3 - Stages 3.1.1 – 3.1.8

General adaptation rules of Annex XI and certain specific adaptation rules in Column 2 provide possibilities for omitting the testing. These rules, except for those already passed at Stage 1 are presented here and the possibilities to omit the testing according to Stages 3.1.1 – 3.1.8 should be explored before conducting (Annex VIII level test) or proposing (REACH Annexes IX and X level tests) the test.

Stage 3.1.1 Adaptation based on existing information not carried out according to GLP or the test methods indicated in the test method regulation (Annex XI, 1.1.2.)

Although the REACH standard information requirements refer to a specific series of reproductive studies, it is recognised that there may be other studies already performed that could address some of the endpoints covered by these standard protocols, reducing the need for new animal testing (adaptation according to REACH, Annex XI 1.1.2). The available data should be evaluated to assess their suitability for use, taking account of the robustness of design, and quality as outlined in Chapter R4. The data from these studies (one or several together) are considered to be equivalent to data generated by the REACH standard test methods if the conditions of Annex XI, Section 1.1.2 are met. An illustrative summary of these conditions is given below:

1) adequate for classification and labelling and/or risk assessment;
2) adequate and reliable coverage of key parameters;
3) exposure duration comparable or longer, if exposure duration is a relevant parameter;
4) adequate and reliable documentation;
a. adequate and reliable reporting of study design including dose levels tested.

Examples of other studies include: old studies conducted in other than preferred species; an NTP[41] modified one-generation study; non-GLP studies; or non-guideline investigations such as the NTP continuous breeding study (Chapin and Sloane, 1997).Such studies may be available and should be evaluated for fulfilling the criteria in Annex XI, Section 1.1.2, in order to conclude that the information provided is equivalent to that foreseen to be the information provided by the EU test method. In addition, a study conducted according to a new test method not yet internationally acceptable may be valid and provide equivalent information.

It is to be noted that existing information on the two-generation reproductive toxicity study (EU B.35, OECD TG 416) is considered to fulfil the standard information requirement for Annex IX/X, 8.7.3 (EU B.56, OECD TG 443), because this was the previous standard information requirement before the revision of the REACH Annexes to require extended one-generation reproductive toxicity study. For further details see Section R.7.6.4.2.4 on the two-generation reproductive toxicity study (EU B.35, OECD TG 416).

Tests carried out according to old methods are evaluated case by case taking into account the toxicological properties of the substance. In case the old study has e.g. shorter exposure duration than the current test method, the registrant should justify using substance-specific arguments why the study with shorter exposure duration does not cause concern; for an example see section R.7.6.4.2.2 . Similarly, if not all the key parameters are measured, but there are adequate substances-specific

[41] National Toxicology Program of NIEHS

justifications to show that the missing information is of no concern, the old study may be acceptable. In case the conditions summarised above for Annex XI, 1.1.2 are not met, the study or test could be still usable e.g., under Annex XI, 1.2 as one element for weight of evidence adaptation.

Stage 3.1.2 Adaptation based on existing historical human data (Annex XI, 1.1.3.)

Epidemiological studies, conducted in the general population or in occupational cohorts, may provide information on possible associations between exposure to a chemical and adverse effects on reproduction. Clinical data and case reports (e.g. biomonitoring after accidental substance release) may also be available.

The criteria for assessing the adequacy of historical human data are listed in Annex XI, Section 1.1.3. In exceptional cases human data may meet the classification criteria to Reproductive toxicity Category 1A and provide adequate information for risk assessment.

Stage 3.1.3 Adaptation based on existing information in a weight of evidence approach (Annex XI, 1.2.)

There are two possibilities to use the weight of evidence adaptation;

1) sufficient evidence from several independent sources of information; or
2) sufficient evidence from the use of newly developed test methods

leading to the conclusion that a substance has or has not a particular hazardous property.

It is to be noted that the weight of evidence approach described in Annex XI, Section 1.2. needs to be substance and case specific and address the relevant standard information requirements of Annex VII to X. Furthermore, it is hazard-based: it has to be shown whether a substance has or has not a particular hazardous property. Because the weight of evidence approach is hazard-based, it means that exposure conditions or risk considerations are not part of the approach. To address the particular hazardous property of a substance, the key parameters of the study of the standard information requirement for which a weight of evidence approach is proposed, need to be addressed to a sufficient extent.

In any case, adequate and reliable documentation of the information need to be provided.

Adequate reporting of a weight of evidence approach is explained in the ECHA Practical Guide 2 (add link).

Comment [SJ10]:
ECHA will review and update the references and formatting further during consultation.

Elements of a weight of evidence adaptation approach according to this adaptation rule for reproductive toxicity could be available from experimental studies addressing reproductive toxicity endpoints, reproductive toxicity studies performed with structurally similar substances, and non-animal approaches, such as suitable validated *in vitro* methods, valid qualitative and quantitative structure-activity relationship models ((Q)SARs) or adverse outcome pathways (AOPs) (for further information on non-animal approaches see Stages 3.1.4 and 3.1.5).

Stage 3.1.4 Adaptation based on non-animal approaches such as QSAR approaches and in vitro methods (Annex XI, 1.3. and 1.4.)

Annex XI, Sections 1.3. "Qualitative or Quantitative structure-activity relationship (QSAR) and Section 1.4. "*in vitro* methods" are potential adaptation possibilities. However, the available methods are currently not sufficient to address the complex endpoints on reproductive toxicity to replace an animal test. QSAR and *in vitro* methods may be used to support grouping and read-across approaches and may have a role in weight of evidence approach. For further details see Chapter R.7.6.4.1.1.

Stage 3.1.5 Adaptation based on grouping and read-across (Annex XI, 1.5.)

The grouping of substances and read-across offer a possibility for adaptation of the standard information requirements of the REACH Regulation. If the read-across approach is adequate, unnecessary testing can be avoided. A read-across approach can also support a conclusion for a REACH endpoint using a weight of evidence approach.

The application of the grouping concept means that REACH information requirements for physicochemical properties, human health effects and/or environmental effects may be predicted from tests conducted on reference substance(s) within the group, referred to as source substance(s), by interpolation (extrapolation is generally not recommended for grouping) to other substances in the group, referred to as target substance(s), and this is called read-across.

The read-across approach has to be considered on an endpoint-by-endpoint basis due to the different complexities (e.g. key parameters, biological targets) of each endpoint. This means that read across (and category approach) is endpoint specific.

The term analogue approach is used when read-across is employed within a group of a very limited number of substances.

Read-across must, in all cases, be justified scientifically and documented thoroughly. There may be several lines of evidence used to justify the read-across, with the aim of strengthening the case.

Guidance on read-across is provided in *Guidance on information requirements and chemical safety assessment, Chapter R.6 "QSAR and grouping of chemicals"*. Further guidance can be found following this link: http://echa.europa.eu/support/grouping-of-substances-and-read-across.

Stage 3.1.6 Testing is technically not possible (Annex XI, Section 2.)

Tests do not need to be performed if it is not technically possible to do so. It may be that it is not possible to administer the substance for a particular reason. For example, the substance may be flammable in air, or degrades explosively. It may also be not possible to produce high enough exposure levels due to technical reasons. Justification for not performing tests is required and must be documented.

Stage 3.1.7 Substance-tailored exposure-driven testing (Annex XI, Section 3.)

The information requirements for reproductive toxicity at Annex VIII, IX, and X levels may be omitted *if relevant human exposure can be excluded*. This clause states that tests may be omitted based on exposure scenarios developed in the Chemical Safety Report. The criteria defines three alternative sets of conditions that can – when justified and demonstrated – lead to an adaptation of standard information requirements (Annex XI, 3.2.(a), (b) or (c)).

The adaptation according to Annex XI Section 3.2.(a) of the REACH Regulation is usually not applicable for Annex IX and X reproductive toxicity studies as a DNEL derived from a reproduction/developmental toxicity screening test must not be considered appropriate to omit prenatal developmental toxicity study or an extended one-generation reproductive toxicity study (see Annex XI, 3.2(a)(ii)footnote).

At Annex IX level, the prenatal developmental toxicity study on second species may be omitted based on case-by-case justifications if the triggers for the study on a second species are observed at very high exposure levels only compared with the identified human exposure and there are no indications that the second species would be more relevant to humans than the first species used.

However, for substances following strictly controlled conditions as described in Annex XI, 3.2(b) or for substances rigorously permanently incorporated in an article

according to Annex XI, 3.2(c), the use of substance-tailored exposure-driven waiving may be possible.

In all cases, adequate justification and documentation must be provided (see Annex XI, 3.2).

Stage 3.1.8 Adaptation based on column 2 rules others than CMR classification

(a) Annex VIII (applicable for any registration of 10 tonnes or more per year)

The screening test for reproductive/developmental toxicity does not need to be conducted if a prenatal developmental toxicity study (OECD TG 414), an extended one-generation reproductive toxicity study (B.56, OECD TG 443) or a two-generation reproductive toxicity study (B.35, OECD TG 416) is available.

The screening test for reproductive/developmental toxicity provides initial information on reproduction toxicity. An extended one-generation reproductive toxicity study or a two-generation reproductive toxicity study provides more comprehensive information on the same and further key parameters with a higher statistical power. Thus, it is clear that these studies can cover the key parameters of the screening study and are superior to the screening study. However, in case where the prenatal developmental toxicity study is available, it provides information on embryonic and foetal development and the ability of the dam to maintain pregnancy, but not on fertility (or postnatal development). Thus, even though a prenatal developmental toxicity study is available, it is strongly recommended that the conduct of the screening study should be considered to obtain preliminary information on the fertility endpoint[42] and peri/early postnatal development.

(b) REACH Annexes IX and X (applicable for any registration of 100 tonnes or more per year)

The reproductive toxicity studies (prenatal developmental toxicity study(ies) and the extended one-generation reproductive toxicity study) do not need to be conducted if the following criteria are met:

1. The substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available) *and*
2. It can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) *and*
3. There is no or no significant human exposure[43].

It is necessary that all three criteria are fulfilled. The starting assumption is that substances with low toxicological activity may be less likely to be reproductive toxicants. The likelihood of the lack of reproductive toxicity potency is further increased and strengthened by requiring information proving no systemic absorption. When the substance has in addition no significant human exposure, it is considered safe to waive the reproductive toxicity study at Annex IX and Annex X levels.

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[42] This position is supported by a relevant Ombudsman Case: "Hence it is strongly recommended in accordance with the endpoint specific REACH Guidance on information requirements and chemical safety assessment R.7, more specifically, paragraph 7.6.6.3 for reproductive toxicity that you consider conducting a screening reproductive/development toxicity study (OECD 421/422) in addition to the pre-natal developmental toxicity study."

[43] "No significant human exposure" must be considered in relation to the toxicity and amount and quality of available information.

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Appendix 5 Evaluation of triggers

Most of the triggers lead to information needs beyond the standard information requirements. For reproductive toxicity, the only standard information requirement which is triggered by toxicity and not only by a tonnage level is an extended one-generation reproductive toxicity which is triggered as indicated in Column 1 of Annex IX, 8.7.3.

What is a trigger?

Triggers are findings which challenge the existing toxicity database. This means that due to existing triggers it is not possible to conclude on the potential for adverse health effects for a substance, and to address the concern, further information may be needed or is needed, depending on the condition. Before the concern is addressed with adequate information, the concern should be covered by applying (adequate) risk management methods.

In this document a general term of trigger is used. It is used instead of all the various possible terms used in the REACH Regulation or other places, such as an alert, condition, indication, indication of concern, serious concern, a particular concern.

A trigger is any factor present in the existing toxicological database, whether based on theoretical scientific considerations or from experimental or observational data that raises concerns that a substance may cause toxicity but information is not comprehensive enough to allow a conclusion to be drawn. It helps identifying where testing may need to go beyond the applicable standard information requirements. Where a standard information requirement applies, testing is required, unless an adaptation can be justified, irrespective of triggers. Case by case considerations are needed in evaluating triggers.

What needs to be done if there are triggers?

The term triggers is used as a general term. It depends also if there is legal text specifying what are the following actions needed. E.g. if the legal text states "*if the conditions are met, the registrant shall…*" it means that in the existence of a trigger registrant must act accordingly. On the other hand, if the legal text states that the registrant may propose a test based on an indication or concern, then the registrant may act.

In the REACH Annex text for the information requirements the following terms are used as triggers:

1) **Condition**: if the conditions are met, the registrant **must** act. Condition may be e.g., **an (adverse)** effect, **an indication**, or **other relevant** existing information; thus, it may be e.g.:
    a. an effect which has (had) a regulatory consequence (NOAEL, classification; e.g., Muta 2), or
    b. a non-adverse effect (e.g., change in hormone level, *in vitro* results), other information (e.g., toxicokinetics), or
    c. indications of an effect inadequate for toxicological evaluation, or
    d. indications of modes of action from *in vivo* studies or non-animal approaches
    e. a combination of two or several indications (e.g. for a mode of action)
    f. a result of weighing all the relevant data for an endpoint (e.g., genotoxicity data)

2) **A particular concern**: if there is a particular concern, the registrant **must** act. A particular concern may be e.g., **serious/severe** effects, **adverse** effects, focused on a **specific type** of effects, or **other relevant** existing information; thus, it may be e.g.:

   a. an effect which has (had) a regulatory consequence (NOAEL, classification; e.g., STOT 1 or 2), or
   b. existing information on from non-animal approaches
   c. specific mechanisms/modes of action
   d. existing information on effects from various different data sources (in some cases also from structurally analogous substances)
   e. information from one source may be sufficient when severe
   f. a combination of two or several indications (e.g. for a mode of action)
   g. a result of weighing all the relevant data (e.g., (developmental) neurotoxicity)

An exception: At Annex VIII, 8.7.1, Column 2, based on a serious concern the registrant may act

3) **Indications**: may be

   a. A condition
   b. Adverse effects
   c. Non-adverse effects, e.g. hormonal change
   d. Mechanism/mode of action
   e. From animal studies
   f. From non-animal approaches
   g. Indications are not the same as a particular concern, but may still require an action from the registrant, depending on the context

Sources for triggers

Triggers may stem from various sources of information including non-animal approaches, mechanistic studies, structurally analogous substances and *in vivo* studies and information from humans.

Findings observed in non-intact animals should generally be used as triggers unless there is evidence that the findings would not be also relevant for intact animals and/or humans. Experiments with non-intact animals may include animals with removal of an endocrine organ, such as ovary (ovariectomy). Another possibility is hormonal manipulation, e.g. causing decrease or increase of organ weight. These animal models may be very sensitive to detect a change in e.g. hormonal response; however, it should be considered whether the same applies in intact animals.

Classification and triggers

Adverse effects meeting the classification criteria for Category 1A or 1B reproductive toxicant are not triggers for further studies because they trigger the self-classification or harmonised classification and may allow omitting further reproductive toxicity studies according to Annex VIII-X, point 8.7, Column 2 adaptation rules. However, effects meeting classification criteria for Category 2 reproductive toxicant may be triggers because they can raise concern that classification criteria for a higher category may be met.

Adverse effects not meeting classification criteria may be triggers. Whether findings which are considered non-adverse may serve as triggers depends on the parameter(s) in question and this is discussed below. The relevance and quality of triggers from the *in vivo* studies and non-animal approaches used should be adequately documented and justified.

Standard information requirements and triggers for further studies

The full standard information requirement in Annex X of REACH, i.e. the Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) (or an existing two-generation reproductive toxicity study; EU B.35, OECD TG 416), and prenatal development toxicity study (EU B.31, OECD TG 414) performed in two species, when adequately conducted, should normally provide reliable information for conclusion on reproductive toxicity properties. If no conclusion can be drawn from the standard information requirement at the respective Annex level, the registrant should address the remaining concern by proposing further studies to clarify the issue.

For certain studies (e.g. extended one-generation reproductive toxicity study, the study design is to be defined based on the existence/absence of the conditions/triggers.

Quality and relevance of the triggers

The generic guidance on the evaluation of available information gathered in the context of REACH Annexes VI-XI is provided in *Guidance on information requirements and chemical safety assessment, Chapter R4: "Evaluation of available information"*.

Chapter R.4 applies for all kind of information; human, animal and non-animal sources and it is applicable also for information for reproductive toxicity endpoint. Principles described in Chapter R.4 apply to some extent also to the evaluation of triggers, although it is to be noted that a trigger is an indication of concern which challenges the available data as indicated in the definition of a trigger above and does not necessarily allow for conclusion on the hazardous properties to reproductive health – conclusion on classification or NOAEL values.

Certain general important aspects to assist the evaluation of triggers are presented below.

Consistency

It is important that the identified triggers are not contradicted by other findings in the available data. If findings are inconsistent, consideration should be given to the statistical power and overall quality of the available data. Sometimes when the data is scarce it may not be possible to evaluate the consistency more than by noting that other data is not contradicting with the trigger(s).

When evaluating the consistency, differences in the existing studies must be taken into account. Apparent inconsistencies may be due to species/strain differences, different route and/or dose levels, different exposure duration, differences in methodology in measuring parameters, etc. Thus, whether the inconsistencies are likely due to methodological differences or differences in statistical power and not real inconsistencies in results, those must be analysed prior to weighing the results and deciding on the existence/absence of triggers.

Statistical significance and biological relevance

Dose responsiveness would provide more confidence and be more indicative of a chemically mediated effect rather than just a statistically significant finding in one dose group. The statistical power of the results from screening studies (OECD TG 421/422) or 28-day study is quite low and there it may be more important to look at

the ranges rather than statistical significances. It should also be remembered that statistical significance is not the same as biological relevance. There may be e.g., 20% change in a parameter with biological relevance but without statistical significance. On the other hand there may be a statistically significant finding without a biological relevance. If the statistical power is high and biological variation is low for a parameter, the biological relevance of a change is high. It is necessary to evaluate if the statistical power is adequate in respect to the biological variation of a parameter. Historical data may provide guide for normal ranges but the control group of the study should generally be the main source of information in deciding on normal values and variation.

It should be also considered, case-by-case, the possibility of a non-monotonic dose-response curve.

Deciding on biological relevance of information from non-animal approaches may be challenging. Generally these predictive methods provide indication(s) and triggers rather than conclusions on hazardous properties of substances. If the non-animal approach is not reliable or the results are observed at extreme conditions (e.g. over 100x higher concentrations than the biologically plausible maximal concentration), the validity and relevance of such a single test result should be confirmed before conclusion. In best conditions results from two or more non-animal approaches are available supporting each other.

Human relevance

In the absence of further knowledge and proof, it is assumed that biologically relevant findings in animals are also relevant to humans. To justify that findings/modes of action/mechanisms of action are not relevant to human, information on humans is needed. It is not enough to state that there are no indications of the same findings/modes of action/mechanisms of action in humans than in animals, if the issue has not been adequately investigated.

Relationship of triggers with systemic toxicity

Clear triggers occur at dose levels without (other) systemic toxicity. However, the triggers have to be considered case-by-case as the relationship with the systemic toxicity may not be always clear although they may occur at the same dose level as the triggers. Generally triggers should be considered relevant even if observed at the same dose level as the (other) systemic toxicity findings if it cannot be justified why the triggers are secondary to (other) systemic toxicity.

Quality of the studies and tests

The quality of the studies or the reliability of the information should be considered. E.g. triggers from *in vivo* and *in vitro* tests should have been tested with the biologically relevant material, in a robust system, and the data should be determined to be of adequate quality. Many non-animal approaches, e.g., *in vitro* tests are not validated yet, but the result from them may be used if considered to be reliable case by case. For example, no *in vitro* tests for neuronal differentiation are validated but as triggers for motivating evaluation of developmental neurotoxicity, results from scientifically evaluated (peer reviewed) publications and reports may be used as triggers when considered relevant. The same goes for *in vitro* tests for other triggers such as for developmental immunotoxicity and endocrine disrupting modes of action/mechanisms.

When evaluating the results from non-animal approaches the predictivity and applicability domain and potential other limitations of the approaches need to be considered. Triggers from non-animal approaches such as QSAR predictions may be challenging to interpret especially when various methods show diverging results.

Generally, consistent results from more than one non-animal approach are needed to increase the confidence of the existence or nonexistence of a trigger.

Triggers from structurally analogous substances

Triggers may also stem from structurally analogous substances. In that case, the adequacy to read-across the triggers should be considered and justified.

Evaluation of data for identification of triggers:

As part of the Stage 3.2.1 data review the following questions should be asked:

- Are there triggers for further studies/investigations specified in Column 2?
- Are there triggers for reproductive toxicity not specified in Column 2? (Considering also structurally analogous substances)
- Is there any knowledge of the substance, chemical groups or categories that would indicate special features related to reproductive toxicity to be included in the study design? If so, which?
- Are there triggers for mechanisms/modes of action relevant for reproductive toxicity? (Considering also structurally analogue substances)
- If Column 2 specific adaptation rules and Annex XI general adaptation rules apply and the data is adequate for assessing and concluding the classification and labelling and risk assessment, evaluation of triggers is not needed. This means e.g., that if a substance meets the classification criteria for Category 1 for any of the CMR properties as defined at Stage 1 in section R.7.6.2.3.2 and fulfils the adaptation criteria described in Column 2, then evaluation of triggers for further reproductive toxicity studies is not needed.

From a scientific perspective, it is not possible to generate an exhaustive and rigid list of triggers that would automatically trigger a particular study or have clearly defined implications for classification and risk assessment. However, certain conditions are specified in Annexes and, when met, require a particular study or study design to be proposed.

A trigger (or triggers) may trigger:

- a study, which would fulfil a standard information requirement, which otherwise only applies at a higher tonnage level,; or
- a certain study design (or a particular independent study) when specified conditions are met (e.g., extension of Cohort 1B to include F2 or inclusion of Cohort 2 and/or 3 in the extended one-generation reproductive toxicity study); or
- inclusion of certain selected additional investigational parameters to a range-finding study or a study required in the standard information requirement (e.g., selected parameters for immunotoxicity under conditions where the trigger(s) need(s) to be confirmed before considering the need for further studies to address the concern; or
- special investigational studies/tests, e.g., studies on mechanisms/modes of action.

The following triggers are referred to in REACH Annex IX 8.7.3 and trigger a standard information requirement:

- At Annex IX level, extended one-generation reproductive toxicity study may be triggered by triggers from repeated dose toxicity studies (including screening studies) according to description in Column 1 (see further details in this Guidance, section R.7.6.2.3.2, Stage 4.4 (iii).

The following triggers are referred to in Column 2 adaptation rules for reproductive toxicity/developmental neurotoxicity/developmental immunotoxicity:

- **At Annex VIII level**, based on trigger(s) for reproductive toxicity, either for developmental toxicity or for fertility, causing serious concern[44], the registrant **may** propose a prenatal developmental toxicity study or an extended one-generation reproductive toxicity study instead of a "screening for reproduction/developmental toxicity" test, as appropriate. The appropriate study depends on whether the concern is on prenatal developmental toxicity, prenatal developmental toxicity manifested postnatally, postnatal developmental toxicity or on fertility[45]. The triggers may stem for example from relevant non-animal approaches[46] or *in vivo* studies e.g., from 28-day repeated dose toxicity study which is required at this Annex level or respective other information. A testing proposal is required for Annex IX/X level studies.
- **At Annex IX level**, trigger(s) for prenatal developmental toxicity should trigger a prenatal developmental toxicity study on a second species as a Column 2 requirement. Examples of triggers for this study are shown under section R.7.6.2.3.2, Stage 4.4 (ii), prenatal developmental toxicity study.
- At Annex IX level, if the extended one-generation reproductive toxicity study is triggered, in Column 2 triggers for extending the Cohort 1B, including Cohorts 2 and/or 3 are given. The study design of extended one-generation reproductive toxicity study and triggers to expand the study are described in Appendix 2
- At the same Annex level, extended one-generation reproductive toxicity study on a second species or strain may be triggered at this Annex (Annex IX) or the next Annex level (Annex X). Examples of triggers are presented under section R.7.6.2.3.2, Stage 4.4 (iii), extended one-generation reproductive toxicity study.
- **At Annex X level**, the extended one-generation reproductive toxicity study is a standard study design. The triggers for extending the Cohort 1B, including Cohorts 2 and/or 3 are given in Column 2. The study design of extended one-generation reproductive toxicity study and triggers to expand the study are described in Appendix 2
- At Annex X level, the full standard information requirements i.e. the Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) (or an existing two-generation reproductive toxicity study; EU B.35, OECD TG 416), and prenatal development toxicity study (EU B.31, OECD TG 414) performed in two species, when adequately conducted, should normally provide reliable information for conclusion on reproductive toxicity properties as indicated above.

If no conclusion can be drawn from the standard information requirement, the registrant must address the remaining concern by proposing further studies to clarify the issue.

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[44] Serious concern reflects a high likelihood for adverse effects on reproductive health.

[45] However, in case of proposing a prenatal developmental toxicity study it is strongly recommended that the registrant should consider conducting a screening study because a prenatal developmental toxicity study does not address the effects on the fertility endpoint and developmental toxicity manifested shortly after birth.

[46] In order to be considered providing "*serious concern*", information from non-animal approaches should be reliable, relevant and from validated studies with appropriate applicability domain. Generally several information sources may be needed.

Exposure triggers/conditions upgrading testing requirements

- Guidance on exposure-based adaptation and triggering of information requirements is provided in Section R.5.1 in *Guidance on information requirements and chemical safety assessment*, Chapter R.5: *Adaptation of information requirements.*
- The use pattern and the exposure to a substance may indicate a concern with the need for additional information requirements, on a case-by-case basis. For example, there may be serious concerns that human exposure, particularly to consumers, are close to the levels at which human health effects might be expected. Such concerns for human health need to be addressed by producing additional information on hazard. In very exceptional cases such concerns may be satisfactorily addressed by improved risk management measures.

Documentation and addressing the triggers/conditions

If the triggers for reproductive toxicity or the conditions described in Column 1 or 2 are met for further investigations, they must be described in the dossier as well as how they are addressed at the respective endpoint section.

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