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GUIDANCE

Guidance on information requirements and Chemical Safety Assessment

Chapter R.7a: Endpoint specific guidance

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

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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 inclorporate 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 independendtly 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 toxicty. The section has been fully revised as follows: • xx •	

Comment [SJ1]: This will be elaborated at the end of the update process.

1

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

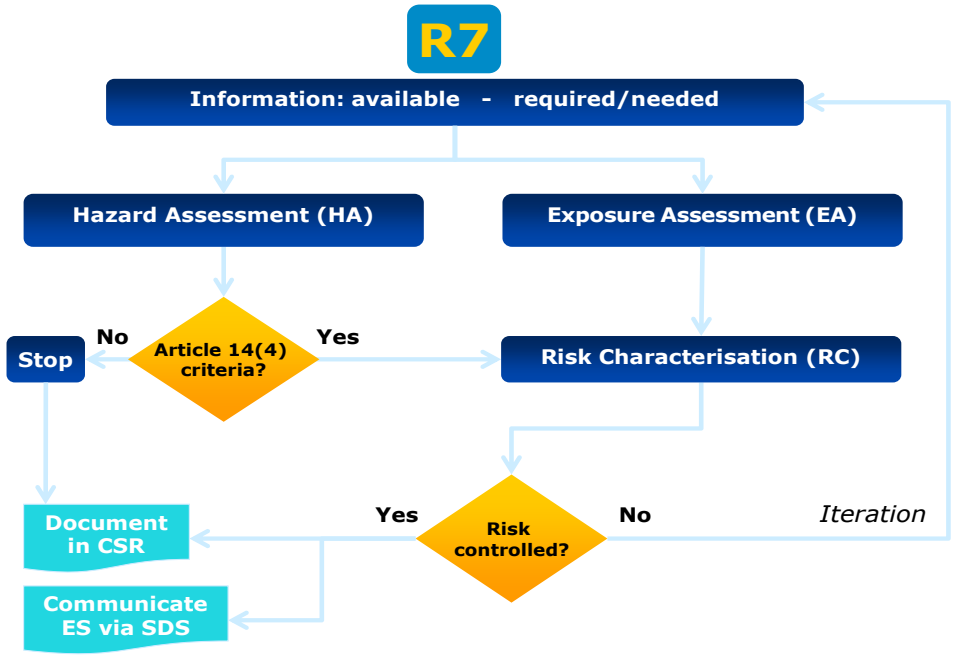

Table of Contents

Comment [SJ2]: PLEASE DO NOT UPDATE the lists of Table of Contents/Tables/Figures/Appendicies because all the section numbering will be lost/changed. This will be done at the end of the update. Thank you

Tables

Figures

Comment [SJ3]: To be addressed at the end of the update proces during formatting and publication

Appendices

Comment [SJ4]: To be addressed at the end of the update proces during formatting and publication.

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 refered 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;

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

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

PEG Members you are not required to comment on this section, except for editorial errors and incoreect facts.

- the third lists the preferred test method(s);
- 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 adapation 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;

[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.

- 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 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 alerts 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 fulfill 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 findings (see Section R.7.12 in Chapter R.7(c): Endpoint specific guidance). Therefore, in

[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].

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 alert 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.7.1 Physicochemical properties

R.7.2 Skin- and eye irritation/corrosion and respiratory irritation

R.7.3 Skin and respiratory sensitisation

R.7.4 Acute toxicity

R.7.5 Repeated dose toxicity

R.7.6 Reproductive toxicity

R.7.6.1 Introduction

At the population level the hazardous property of substances with regard to reproduction is of obvious high concern because the continuance of the human species is dependent on the reproductive health of humans. 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 legislations and context related to reproductive toxicity differs. In this guidance "*reproductive toxicity*" is used to cover both the effects on fertility and development. The terms used will be "*fertility*" and "*developmental toxicity*" under reproductive toxicity. Fertility is seen as a broad consept covering all the effects on reproductive cycle except for developmental toxicity as defined in the text below.

In REACH, the Chemical Safety Report format includes terms "*effects on fertility*" and "*developmental toxicity*" under main heading of "*toxicity to reproduction*". Also in other text in REACH, such as in Annexes, reproductive toxicity is devided to fertility and developmental toxicity[7]. 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), 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, on development of the offspring and effects on or mediated via lactation, as defined in Annex I of the CLP Regulation.

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. A term "*fertility*" is used in the present guidance document instead of "*sexual function and fertility*" as explained above to follow terminology 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 in this guidance as a hazard class in the same meaning as "*fertility*" alone. It is to be noted that fertility (as a REACH endpoint) covers functional fertility, morphological and histological changes related to fertility as well as ability to produce healthy offspring and nurse them.

In following, endpoints of fertility and developmental toxicity are explained based on description provided in 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*) and the induction of non-heritable harmful effects on the progeny (*developmental toxicity*) where effects on or via lactation are considered separately. Effects on male or female fertility include any effect of a substance that has the potential to interfere with sexual function and fertility. This

[7] in Column 2. Column 1 specifies the studies for the endpoints except and the main Section 8.7 is "*Reproductive toxicity*"

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 cycle normality, sexual behaviour, fertility, 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 postnatally, to the time of sexual maturation. 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. These effects can be manifested at any point in the life span of the organism. 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 requirements for the lowest tonnage level are given in Annex VII. Whenever a higher tonnage level is reached, the minimum requirements of the corresponding Annex have to be fulfilled in addition (see Annex VI).

For reproductive toxicity 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 (alerts and waivers), as well as its use for the purpose of classification, risk assessment and risk management measures.

R.7.6.2.1 REACH standard information requirements for reproductive toxicity

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 Annexes.

These information requirements are minimum information requirements. If there are concerns ("alerts" 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). 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.

[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).

REACH information requirements can also be fulfilled by adaptations that reduce the requirement for testing. Adaptations 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 are 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) 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[11];*

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

- *Pre-natal 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);*
- *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*. The 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/2B and/or Cohort 3 are specified in Column 2;

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

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9 Later referred also as a screening study

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

11 This needs to be read in combination with the adaptation rule in Column 2. The screening study is a standard information requirement. In case a prenatal developmental toxicity study is proposed due to a concern on developmental toxicity, it is strongly recommended that the registrant considers conducting a screening study in addition to the prenatal developmental toxicity study to cover the fertility endpoint.

12 See Stage 4.1 (iv) for discussion on route of administration under R.7.6.2.3.2.

requirements. The 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 are specified in Column 2.

A simplified summary of the information requirements for reproductive toxicity is presented in Table R.7.6-1 below. Only standard information requirements without adaptations (column 2) are described.

Table R.7.6-1. Summary of standard information requirements for reproductive toxicity in REACH.

Study	Annex VII	Annex VIII	Annex IX	Annex X
Screening test for reproductive /developmental toxicity (OECD TG 421 or 422)		Required	Strongly recommended if no higher tier fertility study (such as OECD 443) is/will be available	
Prenatal developmental toxicity study (EU B.31, OECD TG 414)		May be proposed in case of (serious) concern[1] for prenatal developmental toxicity. 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[2]	Required in two species
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)[3]		Recommended instead of the screening study in case of serious concern[1] for fertility	Required if triggered[4]	Required

[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 repoductive health.
[2] For discussion on triggers see Stage 4.4, prenatal developmental toxicity study under Chapter R.7.6.3.2.
[3] Basic study design addressing fertility with Cohort 1A and Cohort 1B without extension of Cohort 1B, see Chapter R.7.6.4.2.3 (extended one-generation reproductive toxicity study) for details
[4] For description of triggers see Stage 4.4, extended one-generation reproductive toxicity study under Chapter R.7.6.3.2.

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.7.4.6.2.

Annex IX and X level studies and potential other studies not considered as 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 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

The extended one-generation reproductive toxicity study (EOGRTS, EU B.56, OECD 443) allows evaluation of the test substance on the integrity and performance of the adult male and female reproductive system and offspring viability, health and physical and development of some functions until adulthood. The focus of the study in REACH is on fertility[13], thus, requiring a 10-week premating exposure duration and a highest dose showing systemic or reproductive toxicity for all variant study designs of EOGRTS. The basic study design[14] focuses on evaluation of the fertility of parental animals (only exposed as adults) and of some parameters on postnatal development until adulthood including sexual maturity and histopathology of gonads. The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) also provides information on the fertility of the offspring, i.e. the F1 generation, which has been exposed already during germ cell formation, preimplantation, *in utero* and postnatal periods. Cohorts 2A and 2B provide information on developmental neurotoxicity and Cohort 3 on developmental immunotoxicity.

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 conditions described in Column 2 are met.

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

Comment [SJ6]: To Note: The Table number will be checked at the end of the process during formatting/publication (and any cross references corrected)

[13] Recital (7) of Commission Regulation (EU) No …. of.. 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-gneration 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."*

[14] Recital (6) of Commission Regulation (EU) No …. of.. 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-gneration reproductive toxicity study: *"The standard inofmration 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
Reproduction/ developmental toxicity screening test (OECD TG 421 and 422)	Exposure from 2 weeks prior to mating (P) until at least post-natal day 4 (F1) 3 dose levels plus control Preferred species rat Preferred route oral[1] N = 10 mating pairs per dose group	*Parental (P) generation:* Growth, survival, fertility (limited) Pregnancy length and litter size Histopathology and weight of reproductive organs Histopathology and weight of major non-reproductive organs (OECD TG 422 only) *Offspring (F1):* Growth and survival until post-natal day 4
Pre-natal 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	Exposure at least 10 weeks prior to mating (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)[2]. 3 dose levels plus control; highest dose level must be chosen with the aim to produce systemic or reproductive 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) N = 10 males and females per dose group (Cohorts 2A, 2B and 3)	*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) under certain conditions Developmental immunotoxicity (Cohort 3 or a separate study) under certain conditions

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

[2] 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.7.6.4.2.3.7, 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

The aim of this section is to provide advice on 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. 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 conditions or "alerts" 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, Stage 0, all existing available information relevant to reproductive toxicity must be collected for substances manufactured or imported at tonnage levels ≥1 t/y (Annexes VII-X)(see Annex VI, Step 1). Information from literature may assist indentifying 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 if it allows adequate assessment for the purpose 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. At higher production volumes, standard information requirements are staggered according to tonnage levels of the registrations (≥10 t/y, ≥100 t/y or ≥1000 t/y). 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 all the available toxicological data, classification (EU harmonised or self-classification) for reproductive toxicity, carcinogenicity and germ cell mutagenicity, human exposure characteristics and current risk management procedures 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, e.g., due to alerts, data gaps which cannot be adapted (for the purpose of classification and/or risk assessment), Annex upgrade, or any other reason, then a series of decision points are defined to help shape the scope of an appropriate testing programme, as described below. The REACH approach provides, after gathering and sharing existing information, a four-stage process for clear decision-making, relevant for all tonnage levels.

Stage 1: To 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 8.7.3 in REACH Annexes further information on reproductive toxicity may be omitted in certain conditions desribed 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: To clarify the standard information requirements relevant for manufactured/imported tonnage level of a single registrant or a SIEF[15].

Stage 3: Evaluation of the available toxicology database and consideration of reproductive toxicity alerts and conditions that may serve as triggers or allow omitting of further studies. This evaluation should also consider information from substances with a similar structure or causing toxicity via similar modes of action. The aim of this stage is to satisfy the REACH information requirements and to determine the need for reproductive toxicity testing necessary to adequately clarify the properties reproductive toxicity. It is possible that, following this review coupled to an analysis in Stage 1 or if sufficient data for risk assessment/risk management and classification purposes already are available allowing adaption based on column 2 or Annex XI adaptation rules, no further testing may be necessary.

Stage 4: Planning and conducting (a screening study) or planning and proposing (a prenatal developmental toxicity study or an extended one-generation reproductive toxicity study or specific other studies in exceptional cases) The reproductive and developmental toxicity tests upon which classification and labelling (including categorisation within a hazard class) and risk assessment decisions will be based for chemicals progressing beyond Stages 1-3.

R.7.6.2.3.2 Procedure for testing approaches and adaptations

Stage 0: **Collection of data**

At all Annex levels, the available relevant 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: Carcinogenic, germ cell mutagenic and reproductive toxic (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, no further testing for reproductive toxicity may be needed if the conditions are fulfilled and approprite risk management measures are in place.

Stage 1.1: Has the substance already been classified[16] 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 and proceed to Stage 2 via the Stage 1.2. If the available data are not adequate to support a robust risk assessment then proceed to Stage 2.

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[15] SIEF is a substance information exchange forum

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

Stage 1.2: Is the substance known to be[17] as 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: To 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, Column 2 adaptation rules may require further studies or certain study design if the conditions described in Column 2 are met.

Stage 3. Conduct a detailed review of all existing toxicological data to identify conditions to adapt standard information requirements for reproductive toxicity

At Stage 3, the available data is examined to verify if any of the adaptations rules are met.

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

REACH information requirements are minimum information requirements and alerts 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 (Annexes VII and VIII) or a testing approach must be proposed (Annexes IX and X). 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).

Following the adaptation based on CMR properties 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 Annexes and should be explored. These adaptation rules are described in Stages 3.1.1-3.1.8 below. These adaptation rules apply to substances for which standard information requirements apply because they passed the Stage 1.

If sufficient data are available to permit an adapation 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.

Alerts for standard information requirements are described in Column 1. At Annex IX, alert(s) for reproductive toxicity (fertility and postnatal development) will trigger an extended one-generation reproductive toxicity study. For definition of an alert, see Stage 3.2 below. The examples for alerts 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.

Alerts (conditions, triggers) for further information needs, beyond the standard information requirements, general and those referred to in Column 2 adaptation rules, are discussed in Stage 3.2.1 below.

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

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

Stage 3.1 Substances for which the standard information requirement apply after Stage 1

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. The data from these studies (one or several together) is 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) adequacy 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.

As examples, old studies conducted in other than preferrerd species, an NTP[18] modified one-generation study, non-GLP studies, or non-guideline investigations such as the NTP continuous breeding study (Chapin and Sloane, 1997) may be available and may, evaluated case by case, fulfil the criteria in Annex XI, Section 1.1.2. to conclude that the information provided by this study is equivalent to that forseen to be 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). The existing studies are evaluated according to Annex XI, 1.1.2, and key parameters should have been assessed. However, new testing proposals will not be accepted only for EU B.35 (see Chapter R.7.6.4.2.6 on two-generation reproductive toxicity study).

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. a shorter exposure duration than the current test method, the registrant should justify using substance-specific arguments why the study with a shorter exposure duration does not cause concern. As an example, there may be an existing prenatal developmental toxicity study conducted according to the old test method with a shorter exposure duration than required in the current test method. In case there is no concern based on the available information that a longer exposure duration could change the outcome of the study, the study will be acceptable. Similarly, if all the key parameters are not measured, but there are adequate substances-specific 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

[18] National Toxicology Program of NIEHS

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.)

Annex XI, Section 1.2. "weight of evidence", provides the possibility to adapt standard information requirements in the case when there is sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property. Alternatively, there may be sufficient evidence from several newly developed test methods not yet internationally accepted leading to a conclusion that a substance has or has not a particular hazardous property. In all cases adequate and reliable documentation need to be provided.

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 dangerous 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).

Elements of a weight of evidence approach for reproductive toxicity could be available 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 may not be currently sufficient to address the complex endpoints on reproductive toxicity to replace an animal test. However, they 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.

Comment [SJ7]: Links, references and EU legislation refrence number /dates highlighted in yellow, will be chekced, revised and added during the consultation process. This applies throughout the document.

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, with the conditions for using grouping and read-across approaches to fulfil information requirements set in Annex XI, 1.5. If the read-across approach is adequate, unnecessary testing could be avoided. A read-across approach can also support a conclusion for a REACH endpoint using a weightofevidence approach.

The application of the grouping concept described above 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 to other substances in the group, referred to as target substance(s), and this is called read-across.

Thus, read-across is regarded as a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s)). Consequently, 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 for which trends are not apparent: i.e. the simplest case is read-across from a single source substance to a target substance. Alternatively, with a higher number of substances in a group the term category approach is used.

Read-across must be, in all cases, 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). 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 properties

(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 pre-natal 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 the prenatal developmental toxicity study is available, it provides information on embryonic and foetal development 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[19].

(b) 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.

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.

Stage 3.2 Substances for which there are alerts for further information needs beyond the standard information requirements

Stage 3.2.1 Alerts or conditions for further information needs

Alerts are findings which challenge the existing toxicity database from a reproductive toxicity perspective. This means that due to existing alerts it is not possible to

[19] 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 developmentl toxicity study.*"

conclude on the potential adverse health effects on reproduction for a substance, and to address the concern, further information may be needed. Before the concern can be addressed with adequate information, or the concern should be covered by applying adequate risk management methods.

Certain alerts (or conditions) for further testing are specified by Column 2 adaptation rules.

Use of the term *alert* in this Guidance*:* An *alert* (condition, trigger; indication of concern) is any factor present in the existing toxicological database, whether based on theoretical considerations or from experimental or observational data, that raises concerns that a substance may be a reproductive toxicant 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 alerts.

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

Adverse effects meeting the classification criteria for Category 1B reproductive toxicant are not alerts because they meet the classification criteria and trigger the self-classification or harmonised classification. However, effects meeting classification criteria for Caterogy 2 reproductive toxicant may be triggers because there may be a concern that classification to Category 1B may be met in case information from studies referred at the relevant tonnage level are missing or the reliability of the results may be questioned.

Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443) 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, the registrant must address the remaining concern by proposing further studies to clarify the issue.

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

- Are the alerts or conditions met for further studies/investigations specified in Column 2?
- Are there further alerts for reproductive toxicity? (Considering also structurally analogues 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 alerts 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 alerts is not needed. This means e.g., that in case a substance meets the classification criteria for Category 1 for any of the CMR properties and fulfills the adaptation criteria described in Column 2, then evaluation of alerts for further reproductive toxicity studies is not needed.

From a scientific perspective, it is not possible to generate an exhaustive and rigid list of alerts 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.

An alert (or alerts) may trigger:

- a standard study, which would fulfil an information requirement, which is only standard at a higher Annex, which is not directly applicable to the tonnage of the registration; or
- a certain study design (or a particular independent study) when specified conditions are met (e.g., extension of Cohort 1B to include F2 in the extended one-generation reproductive toxicity study or inclusion of Cohort 2 and/or 3 in the extended one-generation reproductive toxicity study); or
- or inclusion of certain selected additional investigational parameters to a standard study (e.g., selected parameters for immunotoxicity in a conditions where the alert(s) need(s) to be confirmed before requesting further information); or
- special investigational studies/tests, e.g., studies on mechanisms/modes of action.

The nature and severity of an alert should be considered when deciding the way of addressing the concern and the study type. In addition, other aspects such as statistical power and tonnage level need to be considered.

The following alerts are referred to in Column 2 adaptation rules:

- At Annex VIII level, based on alert(s) for reproductive toxicity, either for developmental toxicity or for fertility, causing serious concern[20], 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, postnatal developmental toxicity or on fertility[21]. The alerts may stem for example from relevant non-animal approaches[22] 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, alert(s) for prenatal developmental toxicity may trigger a prenatal developmental toxicity study on a second species as a Column 2 requirement. Examples of alerts for this study are shown under Stage 4.4, Annex IX, prenatal developmental toxicity study. At the same Annex level, extended one-generation reproductive toxicity study on a second species or strain may be triggered at this Annex or the next Annex level (Annex X). Examples of alerts are presented under Stage 4.4, Annex IX, extended one-generation reproductive toxicity study.
- At Annex X level, the standard information requirements include information from various *in vivo* studies on prenatal developmental toxicity in two species

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

[21] 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.

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

and a study addressing fertility (extended one-generation reproductive toxicity study). However, if the information available does not allow a conclusion on reproductive properties of a substance, the registrant must address the remaining concern by proposing further studies while applying interim risk management measures or in very exceptional cases by applying adequate risk management measures.

Exposure alerts/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 alerts/conditions

- If the alerts 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.

Stage 4. Reproductive toxicity tests triggered by tonnage level or alerts identified in Stages 1-3

Stage 4.1 Preliminary considerations

(i) Introduction

It has to be noted that if studies listed in 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, interim risk management measures have to be put in place, recorded in the chemical safety report and recommeded to downstream users according ot Annex I, 0.5.

A brief description of the study protocols 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 regarding to e.g., 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 the nutritional status of the animals.

(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. If another route of administration is used, the tester 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 adminstration 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 (irritation) but not death or severe suffering.

(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 pre-natal developmental toxicity studies, testing in two species (one rodent and one non-rodent) is a standard information requirement for registrations at 1000 or more tonnes per year (and might be triggered by alerts 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. Extended one-generation reproductive toxicity study may need to be conducted using a second

strain or species in certain cases. For details see Stage 4.5 (ii) under Section R.7.6.2.3.2.

In case it is known which species and/or strain is the most sensitive and relevant to human, that species/strain should be used already as a first species. Studies should be performed on the most sensitive animal species and these studies should be selected as the significant ones, unless toxicokinetic and toxicodynamic data show that this species is less relevant for human risk assessment. 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 especially 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 toxicity but not death or severe suffering unless limited by physical or chemical properties of the substance. 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 as 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[23]. 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 regimen. 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 irrespective of the level of parental toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be

[23] 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 differencies 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.*"

performed[24]. 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 information to allow evaluation of the potency for reproductive toxicity of a substance. Therefore, the highest dose level must produce systemic toxicity to allow evaluation of 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.

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 alerts for reproductive toxicity. In case the available information indicates a concern (alert) for reproductive toxicity and relevant human exposure occurs, an animal study like the reproduction/developmental toxicity screening test (OECD TG 421 or 422) might be considered to be performed. 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 neccessary to address the concern, a testing proposal should be made. 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 (Annexes VII and VIII)

At this tonnage level, progression beyond Stages 1-3 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) is the standard information requirement.

(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 can lead to 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.

[24] 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*"

Furthermore, the combined OECD 422 screening study might 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 R.7.5).

In case available information indicates serious concerns[25] (alert) about the potential of a substance for adverse effects on fertility or development, a screening test (OECD TG 421 or 422; Annex VIII, Section 8.7.1) may not need to be performed. Instead, a testing proposal for either a pre-natal developmental toxicity study (EU B.31, OECD TG 414; Annex IX, Section 8.7.2) or a extended one-generation reproductive toxicity study (EU B.56, OECD TG 443; Annex IX, Section 8.7.3) should be submitted to ECHA depending on the type of alert. A concern (alert) that the substance may be toxic to reproduction could stem from non-animal approaches[26] or *in vivo* information with the substance under consideration or from stucturally related substances. Concerns (alerts) 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 effects on embryonic or foetal development, a prenatal developmental toxicity study should be proposed. However, because the fertility and reproductive performance is not assessed in prenatal developmental toxicity study, it is strongly recommended to conduct a screening study (testing proposal is not needed for a screening study). 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, can replace the screening study.

If an existing or a newly performed reproduction/developmental toxicity screening test (OECD TG 421 or 422) for an Annex VIII substance provides no alerts 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, in case a screening test (OECD TG 421 or 422) provides alerts 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 alert, 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 to enable a scientifically robust decision on classification and categorisation to 2 for reproductive toxicity and risk assessment, then this is a serious concern and 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.

Stage 4.4 Registrations of 100 to 1000 tonnes per year (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

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

[26] 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.

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).

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 Annex IX, Section 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.

(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 Annexes are cumulative, a screening test should also be available at Annex IX and X level. However, if a pre-natal 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.

In case the screening test will be omitted based on a pre-natal developmental toxicity study but an extended one-generation study will not be triggered at Annex IX level, then no information on fertility would however be available. In case information from reproductive toxicity study addressing a fertility endpoint is not available, it is strongly recommended to consider whether a screening study should be available to address fertility 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 acute, repeated-dose, and toxicokinetic studies are conventionally conducted in the rat, it may be considered that the first prenatal developmental toxicity study chould 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 pre-natal 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 decison 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 alerts 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 alerts may stem from non-animal approaches, structurally similar substances, modes of action or results from a screening study. However, in case there are no alerts and no indication of prenatal developmental toxicity in the first prenatal

developmental toxicity study, no study on a second species is nececssary at Annex IX level.

If a study on a second species is found to be necessary by the registrant and the test has not been required by ECHA in a compliance check decision, a testing proposal would need 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 is 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 valid triggers.

A detailed review of the available data is required to identify any reproductive toxicity alerts (see also Stage 3.2.1 for determination of an alert; examples of alerts 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 not be used as triggers unless there is evidence that the findings would 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.

Examples of alerts for conduction an extended one-generation reproductive toxicity study at Annex IX level (considered as adverse):

From a screening study or equivalent:

- Changes in reproductive or other endocrine organ weight in intact animals;
- Effects in spermatogenesis or folliculogenesis *in vivo* and/or histopathological findings in reproductive organs and/or assessory sex organs;
- Effects in sperm analysis or oestrous cycle
- Statistically significant changes in hormone levels *in vivo;*
- Reduced mating, fertility or litter size;
- Abortions;
- Changes in gestation length;
- Reduced survival of offspring;
- Reduced body weight of offspring;
- Effects on lactation;
- Reduced maternal care;
- Changes in anogenital distance;
- 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;
- Effects in spermatogenesis or folliculogenesis *in vivo* and/or histopathological findings in reproductive organs and/or assessory sex organs;
- Effects in sperm analysis or oestrous cycle
- Statistically significant changes in hormone levels *in vivo;*
- Indication of other endocrine disrupting modes of action related to reproductive toxicity.

From in vivo studies from non-intact animals:

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

In case alerts are identified that requires performance of this study, the appropriate study design as required in Column 1 and 2 and in Recital (7) of Commission Regulation (EU) No…. amending REACH[27] needs to be defined, justified, and documented. Specification is required for 1) length of the premating exposure duration and dose level selection, 2) extension of Cohort 1B and termination time for F2 generation, 3) inclusion of Cohorts 2A and 2B, and 4) inclusion of Cohort 3.

The study design of the extended one-generation reproductive toxicity study (EU B.56, OECD TG443) 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 (EU) No …. amending REACH statesthat the study design should ensure an adequate assessment of potential hazardous properties of substances on fertility. The premating exposure duration and dose selection should be approapriate to meet risk assessment and classification and labelling purposes, including categorisation[25]. Thus, a 10 weeks premating exposure duration and a highest dose level producing systemic or reproductive toxicity are required (for further discussion see Chapter R.7.6.4.2.3). The selection of dose levels and the duration of the pre-mating perid of the F0 generation, if deviated from 10-weeks, must be justified. The basic study design – including the premating exposure as just described – should be proposed by registrants unless the conditions specified in Column 2 are met.

The extension of Cohort 1B to produce a second filial generation (F2 animals) must be proposed based on conditions potentially presenting the highest risk to consumers and professional users[28]. The conditions include two elements: (a) the substance has uses

[27] Recital (7) of Commission Regulation (EU) No …. of.. 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-gneration 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.*"

[28] Recital (8) of Commission Regulation (EU) No …. of.. 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-gneration reproductive toxicity study: "*Considering that the remaining scientific concerns as regards the value of the F2 generation should be clarified on the basis of empirical data, and that substances potentially presenting the highest risk to consumers and professional users should be assessed on the basis of a conservative approach, the production and assessment of the F2 generation should be triggered for certain substances on case-by case basis. The Expert Group recommended that an exposure based trigger, associated with uses leading to exposures of consumers and professional users should be implemented in the relevant points of Annexes IX and X to Regulation (EC) No 1907/2006. Additional criteria based on evidence indicating that a substance is of concern as a function of the*

leading to significant exposure of consumers or professionals and (b) at the same time indications of certain toxicity- and toxicokinetic-related properties of concern (see Column 2 of Annex IX, Section 8.7.3). In that case, it must be decided at which time point the F2 generation will be terminated (see SectionR.7.6.4.2.3, further aspects to consider related to extension of the Cohort 1B).

In cases where the available information on a substance indicates a particular concern on neurotoxicity and/or immunotoxicity, the inclusion of the developmental neurotoxicity (Cohorts 2A and 2B) and/or developmental immunotoxicity (Cohort 3) must be proposed based on specific conditions[29]. Evidence supporting these concerns could originate from existing information derived from *in vivo* or non-animal approaches, from the knowledge of relevant mechanisms/modes of action of the substance itself, or from existing *in vivo* information on structurally related substances. REACH Annexes IX and X, Section 8.7.3, column 2 specify the conditions meeting the particular concern, that trigger performance of those cohort(s). Based on specific alerts for neurotoxicity defined in Column 2, developmental neurotoxicity cohorts (Cohorts 2A and 2B) must be proposed by the registrant. Respectively, based on specific alerts for immunotoxicity defined in Column 2, developmental

available toxicity and toxicokinetic information, should be included to further optimise the selection of substances for which the F2 generation should be produced and subject to testing."

[29] Recital (9) of Commission Regulation (EU) No …. of.. 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: "*Developmental neurotoxicity and developmental immunotoxicity are important and relevant developmental toxicity endpoints, which could be further investigated. However, analysing the DNT and DIT cohorts entails significant additional costs as well as technical and practical difficulties for testing laboratories. Therefore, it is considered appropriate to subject the analysis of the DIT and DNT cohorts, or only one of them, to specific concern-driven scientific triggers. Specific rules for the adaptation of the information requirement defined in point 8.7.3. of Annexes IX and X to Regulation (EC) No 1907/2006 should be introduced, so as to trigger the immunotoxicity and neurotoxicity testing. In cases where the available information on a substance indicates a particular concern on neurotoxicity or immunotoxicity, the inclusion of the DNT and the DIT cohorts, or only one of them, justified on a case-by-case basis, should be possible. Evidence supporting these concerns could originate from existing information derived from in vivo or non-animal approaches, from the knowledge of relevant mechanisms/modes of action of the substance itself, or from existing information on structurally related substances. Therefore, if any such particular concerns are justified, the registrant should be required to propose, and ECHA should be able to request the performance of the DNT and DIT cohorts, or only one of them.*"

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 neurotoxocity and/or developmental immunotoxicity. (see R.7.6.4.1.2 for details)

The conditions specifying the study design are listed in Annex X, 8.7.3, column 2 and explained in more detail in Section R.7.6.4.2.3 under "*extended one-generation reproductive toxicity study*". It is the registrant's responsibility to evaluate 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.

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, based on the outcome of the first test and any other relevant data.

A study on a second strain or species might be necessary in case the available data contain alerts 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 concern but are not sufficient to meet classification criteria to Category 1B reproductive toxicant. Further alerts may stem from non-animal approaches, structurally similar substances, modes of action or results from a screening study. However, in case there are no alerts and no indication of effects on fertility in the first study and other available data, no study on a second species is nececssary at Annex IX level.

If a study on a second species is found to be necessary by the registrant and the test has not been required by ECHA in a compliance check decision, a testing proposal would need to be submitted.

Stage 4.5 Registrations of 1000 tonnes or more per year (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 pre-natal 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 pre-natal developmental toxicity study. This may be done e.g. if the rabbit is considered for 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. The appropriate study design as required in Column 1 and 2 and in Recital (7) of Commision Regulation (EU) No…. amending REACH[30] needs to be defined, justified, and documented. Specification is required for 1) length of the premating exposure duration and dose level selection, 2) extension of Cohort 1B and termination time for F2 generation, 3) inclusion of Cohorts 2A and 2B, and 4) inclusion of Cohort 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 (EU) No …. amending REACH states that the study design should ensure an adequate assessment of potential hazardous properties of substances on fertility. The premating exposure duration and dose selection should be approapriate to meet risk assessment and classification and labelling purposes (including categorisation). Thus, a 10 weeks premating exposure duration and a highest dose level producing systemic or reproductive toxicity are necessary. For further discussion see Chapter R.7.6.4.2.3). The selection of dose levels and the duration of the pre-mating perid of the F0 generation, if deviated from 10-weeks, must be justified. The basic study design – including the premating exposure as just described – should be proposed by registrants unless the conditions specified in column 2 are met.

The extension of Cohort 1B to produce a second filial generation (F2 animals) must be proposed based on conditions potentially presenting the highest risk to consumers and professional users[31]. The conditions include two elements: (a) the substance has uses leading to significant exposure of consumers or professionals and (b) at the same time indications of certain toxicity- and toxicokinetic-related properties of concern (see Column 2 of Annex IX, Section 8.7.3). In that case, it must be decided at which time point the F2 generation will be terminated (see Chapter R.7.6.4.2.3, further aspects to consider related to extension of the Cohort 1B).

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[30] Recital 7 of Commission Regulation (EU) No …. of.. 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-gneration 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.*"

[31] Recital (8) of Commission Regulation (EU) No …. of.. 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-gneration reproductive toxicity study: "*Considering that the remaining scientific concerns as regards the value of the F2 generation should be clarified on the basis of empirical data, and that substances potentially presenting the highest risk to consumers and professiona users should be assessed on the basis of a conservative approach, the production and assessment of the F2 generation should be triggered for certain substances on case-by case basis. The Expert Group recommended that an exposure based trigger, associated with uses leading to exposures of consumenrs and professional users should be implemented in the relevant points of Annexes IX and X to Regulation (EC) No 1907/2006. Additional criteria based on evidence indicating that a substance is of concern as a function of the available toxicity and toxicokinetic information, should be included to further optimise the selection of substances for which the F2 generation should be produced and subject to testing.*"

In cases where the available information on a substance indicates a particular concern on neurotoxicity and/or immunotoxicity, the inclusion of the developmental neurotoxicity (Cohorts 2A and 2B) and/or developmental immunotoxicity (Cohort 3) must be proposed based on specific conditions[32]. Evidence supporting these concerns could originate from existing information derived from in vivo or non-animal approaches, from the knowledge of relevant mechanisms/modes of action of the substance itself, or from existing *in vivo* information on structurally related substances. REACH Annexes IX and X, Section 8.7.3, column 2 specifise the conditions meeting the particular concern, that trigger performance of those cohort(s). Based on specific alerts for neurotoxicity defined in Column 2, developmental neurotoxicity cohorts (Cohorts 2A and 2B) must be proposed by the registrant. Respectively, based on specific alerts 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. (see R.7.6.4.2.3 for details)

The conditions specifying the study design are listed in Annex X, 8.7.3, column 2 and explained in more detail in Section R.7.6.4.2.3 "*extended one-generation reproductive toxicity study*". It is the registrant's responsibility to evaluate 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.

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, based on the outcome of the first test and any other relevant data.

If a study on a second species is found to be necessary by the registrant and the test has not been required by ECHA in a compliance check decision, 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

[32] Recital (9) of Commission Regulation (EU) No .... of.. 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-gneration reproductive toxicity study: "*Developmental neurotoxicity and developmentl immunotoxicity are importnatn and relevant developmental toxicity endpoints, which could be further investigated. However, analysing the DNT and DIT cohorts entails significant additional costs as well as technical and practical difficulties for testing laboratories. Therefore, it is considered appropriate to subject the analysis of the DIT and DNT cohorts, or only one of them, to specific concern-driven scientific triggers. Specific rules for the adaptation of the information requirement defined in point 8.7.3. of Annexes IX and X to Regulation (EC) No 1907/2006 should be introduced, so as to trigger the immunotoxicity and neurotoxicity testing. In cases where the available information on a substance indicates a particular concern on neurotoxicity or immunotoxicity, the inclusion of the DNT and the DIT cohorts, or only one of them, justified on a case-by-case basis, should be possible. Evidence supporting these concerns could originate from existing information derived from in vivo or non-animal approaches, from the knowledge of relevant mechanisms/modes of action of the substance itself, or from existing information on structurally related substances. Therefore, if any such particular concerns are justified, the registrant should be required to propose, and ECHA should be able to request the performance of the DNT and DIT cohorts, or only one of them.*"

provided. Evaluation of this information is described in R.7.6.4. of this Guidance as well as based on which triggers various study designs of extended one-generation reproductive toxicity study must be proposed.

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.

- physico-chemical characteristics of a substance;
- information on structurally analogue substances and (Q)SAR models;
- *in silico* and *in chemico* models;
- *in vitro* tests 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 antogonists (OECD TG 457);
  - H295R steroidogenesis assay (EU B.57, OECD TG 456);
  - *In vitro* embryotoxicity tests;
  - *In vitro* organ and cell cultures;
- approaches combining various methodologies, e.g., from adverse outcome pathway (AOP) concept.

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.

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.

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);[33]
- 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);[34]

[33] 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).

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

- Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test (OECD TG 422)[35].

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 and/or reproductive organ histopathology;

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

- Uterotorphic bioassay in rodents: a short-term screening test for oestrogenic properties (EU B.54, OECD TG 440);
- Hershberger bioassay in rats: a short-term screening assay for (anti)androgenic properties (EU B.55, OECD TG 441);
- 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;
- peri-postnatal studies;
- male or female fertility studies of non-standard design;
- 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.

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

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

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 also 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.

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

R.7.6.4.1 Non-animal data on reproductive toxicity

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;
- Establishement 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).

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.

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 (add link). 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.

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: for reproductive toxicity all the key parameters.

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 usually not provided.

However, a positive result from a validated QSAR model predicting that the substance has a particular property could provide a trigger (alert) 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) but because of limited confidence in this approach such a result would not normally be adequate for making a decision on classification on its own. It may however 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 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/antiagonist 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 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 with confidence as demonstrating the absence of a reproductive hazard. 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 initial 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 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 on reproductive toxicity and aspects to define the study design

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. 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 epidymal transit of maturing spermatozoa, and thus, allows the detection of posttesticular effects on sperm at mating (during the final stages of spermiation and epidymal 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, as they take 70 and 62 days in rats, respectively. Because exposure during full spermatogenic period and follicologenesis are note covered at the time of mating, effects at earlier stages of spermatogenesis and folliculogeneiss can not be reflected in the functional fertility examination. 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 pre-natal developmental toxicity because the pups are not examined for external, skeletal and visceral anomalies like in the pre-natal 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 developmentl toxicity study. Therefore, a screening study cannot be used to fulfil the information requirement of a pre-natal 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 TGs 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 hazardous property for reproductive toxicity.

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 alerts, triggering 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 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 Chapter R.7.6.4.2.3 below. Screening study may be used as a range-finding study 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 focussed 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 pre-natal 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 pre-natal 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 by an alert. 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, see discussion under Section R.7.6.5.

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 information on maintenance of pregnancy and potentially on gestation length may be identified.

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 uncovered using a shorter exposure duration, this should be addressed e.g. by using an additional assessment factor, or in case of serious concern, a new study should be proposed.

Prenatal developmental toxicity studies may provide alerts 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 may indicate an endocrine disrupting mode of action.

R.7.6.4.2.3 Extended one-generation reproductive toxicity study

R.7.6.4.2.3.1 Introduction and overview

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 extended one-generation reproductive toxicity study allows evaluation 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. More information on reproductive toxicity may be needed in cases if the result does allow an adequate assessment as a basis for a regulatory decision on classification (including categorisation) and risk assessment.

In REACH the standard information requirement includes cohorts 1A and 1B (without extension to produce the F2 generation). Thus, the basic study design is a one-

generation study. 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 (Recital 7 of the Commission Regulation (EU) No .... amending REACH). 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.

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. Similarly developmental neurotoxicity and/or developmental immunotoxicity cohorts need to be conducted if the conditions for such extensions of the basic study design which are provided in column 2 of Annex IX/X, 8.7.3. are fulfilled.

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 defined by Annexes IX and X of REACH and Recital (7) of Commission Regulation (EC) No .. amending REACH.

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.

The study design of EU B.56 selected must be adequately justified and documented in all cases.

R.7.6.4.2.3.2 The specifications for study designs in REACH are needed for the following aspects:

1) Premating exposure duration and dose level selection;
2) Extension of Cohort 1B and termination time for F2;
3) Inclusion of Cohorts 2A and 2B;
4) Inclusion of Cohort 3.

In the following text the specifications and conditions are presented for each study design. The Table R.7.2-2 in Appendix 1 provides a check list for the registrants in order to assess which studies/tests could provide information on conditions which specify the study design of the extended one-generation reproductive toxicity study. The conditions must be recorded in order to allow an independent evaluation.

The study design should be decided before the study is started. For REACH study-in triggers are not recommended.

R.7.6.4.2.3.3 Specifications and conditions for study designs to be proposed:

1) Premating exposure duration and dose level selection

Recital (7) of Commission Regulation (EC) No ... 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[36].

[36] Recital (7) of Commission Regulation (EU) No ....of... amending Annexes VIII, IX and X to Regulation (EC) No 1907/2006 of the European parliament and of the Council on the Registration, evalution, 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*

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 endpoint. To adequately address the assessment of the fertility endpoint, the premating exposure duration should be at least 10 weeks to cover the full period of spermatogenesis and folliculogenesis before the mating allowing meaningful assessment of the effects on fertility. A shorter premating exposure duration, such as two weeks referred to in EU B.56 is equivalent to only 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 epidymal sperm maturation). 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. However, two weeks before mating may be too short and not adequate for classification and labelling purposes and allowing production of data to conclude whether the results are meeting the criteria for Category 1B reproductive toxicant. Ten weeks premating exposure duration covers the full spermatogenesis meaning that the full cycle of development of sperm from spermatogonia is covered. Thus, the toxic effect potentially affecting the functional fertility can be assessed at the time of mating in addition to the histopathological findings on spermatogeneisis of the testes and assessory sex organs. Similarly the folliculogenesis lasts around 62 days and are fully covered only after a longer exposure period. Thus, for a more comprehensive assessment of effects on fertility, evaluation of effects on fertility caused by an exposure covering one full spermatogenic cycle and folliculogenesis is needed. In case the registrant prefers another length of premating exposure duration, an acceptable substance-specific scientific justification must be provided. Such a reasoning could be that effects on fertility are already adequately addressed and extended one-generation repoductive toxicity study is used to address developmental toxicity.

The highest dose for the extended one-generation reproductive toxicity study should be selected with the aim to induce systemic toxicity, but not death or severe suffering of the animals, or effects on reproduction (specifically on fertility) in order to allow conclusion

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labelling purposes as required by Regulation (EC) No 1907/2006 and Regulation (EC) No 1272/2008 of the European parliament and of the Council."

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 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.

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.

In case a range-finding study indicates adverse effects on fertility but where the effects does 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, in case the results from the range-finding study already meets the criteria for Reproductive toxicity Category 1B reproductive toxicants, the adaptation of column 2 may apply and no further studies (including the main study) may be needed.

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 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:

Criteria for uses leading to significant exposure:

- If the substance is used in the EU by consumers (i.e. members of the public) or professionals (i.e. workers in trades), either neat or in a chemical mixture (above the criteria for Art. 57(f) of 0.1% for endocrine disrupters), 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 (e.g. ink jet printers or photocopy toners) and there is one very wide use or several limited uses potentially affecting major part of consumers and/or professionals.

Criteria 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 (column 2 adaptation rule).
    - *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 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 the toxicokinetic information, physico-chemical properties and information from (eco)toxicological data. The effect of gender and life stages could be also consided[37]
- Information can be obtained from

    1) 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)[38].
    - Attention need to also be spent on indications of very slow depuration (e.g PFOA and APFO which are Category 2 reproductive toxicants).

    2) Physico-chemical properties of the substance
    - An octanol-water partition coefficient (log Kow) value indicates (bio)accumulative potential (determined experimentally or estimated by QSAR models)
    - When substance is highly sorptive to the "food" of the test animal.

    3) Human biomonitoring
    - High level of substance/metabolites in human body fluids or tissues, such as blood, milk, or fat.

    4) Indications from existing *in vivo* studies that after a 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.

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[37] 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)*

[38] 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.

- E.g., if the NOAEL of a subschronic 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.
- Severe effects in chronic studies

5) Indications of substance being perisitent in the environment based on Annex XIII of REACH

6) Indications of substance bioaccumulating in the test species

- Bioaccumulation potency (assessment described in Section R.11.4.1.2)
- If the substance fulfils the bioaccumulation criterion (B or vB).

7) 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)

8) Irreversibility of exposure

9) Any other relevant information

(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[39] 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.
  - 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, testesuterus, cervix, epididymides, seminal vesicles, coagulating glands, prostate, vagina, pituitary, mammary gland, thyroid and adrenal gland)
    - Changes in body weight (increase)
    - Changes in thyroid hormone levels
    - Changes is oestrus cycle
    - Changes in 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 nedocrine organs (see above)

[39] A comprehensive collection of screens and test for endocrine disrupting chemicals are presented in OECD GD 150. Both the test results for toxicity and ecotoxicity may be relevant.

- Changes in indicators of hormonal mode of action, such as anogenital distance, nipple retention, mammary gland histopathology
- Changes is oestrus cycle
- Prolonged gestation
- Other effects showing a likely endocrine disrupting mode of action

- Endocrine effects from ecotoxicology studies and tests
- 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)
  - Herschberger assay (EU B.55, OECD TG 441).
  - HeLa Test on estrogenic effects (OECD TG 455)
  - Steroidogenesis assay (OECD 456)
  - Yeast Estrogen Screening (YES) and Yeast Androgen Screening (YAS) Tests
  - Estrogen or androgen receptor binding study
  - Aromatase assay
  - Endorine organ cultures
  - QSAR and computational predictions considered adequately reliable to serve as an alert

The relevance and quality of alerts/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 an alert/trigger. In case the non-animal approach is not reliable or the results are observed at extreme conditions (e.g. over 100x higher concetrations than the biologically plausible concentration), the validity and relevance of such single test result should be confirmed. In best conditions results from two ro more non-animal approaches are available supporting each others or in case of a single study, it is not contradicting other data base available.

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

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 weighed evidence based approach (integrated evaluation of the exisiting 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 weaning period (PND 5-21) would not lead to a lower NOAEL value. To cover the remaing uncertainty, and additional assessment factor may be applied.

The decision on whether or not to extend the Cohort 1B to F2 generation is recommended to be done before starting the study when the specified conditions are met.

So called internal triggers or study-in 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 in REACH.

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

The main concepts of the conditions for cohorts 2 (developmental neurotoxicity, DNT) are based on a particular concern for (developmental)[40] neurotoxicity[41]. Based on text in e.g. Annex VIII, 8.6.1, it can be understood that a particular concern is indicated e.g. by a serious or severe effects[42].

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 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 from in vivo studies on adverse effects caused by substances structurally analogous to the substance being studied suggesting such effects or mechanisms/modes of action.*

Examples for findings which may indicate a particular concern justifying inclusion of the developmental neurotoxicity cohort (add ref?):

- abnormalities observed in the central nervous system or nerves
  - changes in brain weight or specific neural areas
  - (histo)pathological findings in nerves (spinal cord or sciatic nerve) and/or brain
- clear signs of behavioural or functional adverse effects of involvement of the nervous system in adult studies e.g. repeated-dose and acute toxicity studies
  - clinical and/or behavioural signs (such as abnormal gait, narcosis, reduced activity)
- specific mechanism/mode of action that has been closely linked to (developmental) neurotoxic effects,
  - cholinesterase inhibition;
  - relevant changes in thyroid hormone levels clearly associated to adverse effects,
- Information from non-animal approaches, such as from an *in vitro* developmental neurotoxicity test, predicting developmental neurotoxicity

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[40] Both particular concern for neurotoxicity as well as for developmental neurotoxicity may be addressed. See discussion in section R.7.6.4.2.3.4 and R.7.6.4.2.3.5

[41] (ref) *"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."*

[42] 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.

- structurally analogue substances show (developmental) neurotoxic effects in an *in vivo* study suggesting a similar mode of action

The relevance and quality of alerts/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 an alert/trigger.In case the non-animal approach is not reliable or the results are observed at extreme conditions (e.g. over 100x higher concetrations than the biologically plausible concentration), the validity and relevance of such single test result should be confirmed. In best conditions results from two ro more non-animal approaches are available supporting each others or in case of a single study, it is not contradicting other data base available.

In case there are only weak indications (e.g. from one non-animal approach; or less reliable source) of concern related to (developmental) neurotoxicity, not sufficient to meet the conditions specified in the REACH Regulation for a developmental neurotoxicity cohort or an individual study, inclusion of certain additional neurotoxicity parameters like thyroid hormone measurements, advanced neurohistopathology or behavioural assessments for repeated dose or reproductive toxicity studies may be considered to clarify if there is indeed a particular concern and conditions for a DNT investigations are met.

For further consideration related to adults vs developmental neurotoxicity is provided under Chapter R.7.6.4.2.3.4 below.

4) *Inclusion of Cohort 3*

The main concepts of the conditions for cohort 3 (developmental immunotoxicity, DIT) are based on a particular concern for (developmental) immunotoxicity[43]. Based on text in e.g. Annex VIII, 8.6.1, it can be understood that a particular concern is indicated e.g. by a serious or severe effects[44].

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.

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 from in vivo studies on adverse effects caused by substances structurally analogous to the substance being studied suggesting such effects or mechanisms/modes of action.*

Examples for findings which may indicate a particular concern justifying inclusion of the potential triggers for developmental immunotoxicity cohort[45]*:*

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[43] Both particular concern for immunotoxicity as well as for developmental immunotoxicity may be addressed. See discussion in Section R.7.6.4.2.3.4 and R.7.6.4.2.3.5.

[44] 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.

[45] WHO Guidance document for immunotoxicity provides further examples of potential triggers for immunotoxicity testing (WHO 2012).

- Combination of at least two statistically significant changes in clinical chemistry and/or organ weight associated with immunotoxicity, e.g., reduced leucocyte count in combination with reduced spleen weight.
- One severe statistically and/or biologically significant organ weight or histoplogical finding finding related to an immunology organ, e.g., thymus atrophy.
- Evidence of hormonal modes of action with clear association with the immune system, such as oestrogenicity.
- Structural similarity with a substance causing structural or functional immunotoxicity *in vivo*.

The relevance and quality of alerts/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 an alert/trigger. In case 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 concentration), the validity and relevance of such single test result should be confirmed. In best conditions results from two or more non-animal approaches are available supporting each others or in case of a single study, it is not contradicting other data base available.

In case there are only weak indications of concern related to (developmental) immunotoxicity, not sufficient to meet the conditions specified in the REACH Regulation for a developmental immunotoxicity cohort or an individual study, inclusion of certain additional immunotoxicity parameters e.g. a functional immune test such as T-cell dependent antigen response (TDAR) for repeated dose or reproductive toxicity studies may be considered to clarify if there is indeed a particular concern and conditions for a DIT investigations are met.

R.7.6.4.2.3.4 General considerations related to investigation of (developmental) neurotoxicity and/or immunotoxicity

It is considered that if a substance is a neurotoxicant or immunotoxicant in adults it is that also in developing organism. However, it is currently unclear if there are any substance which is a developmental neurotoxicant and/or developmental immunotoxicant but not neurotoxic or immunotoxic in adults (ref to be added). It is known, however, that developing organisms may be more sensitive to neurotoxic or immunotoxic insult of some subtances than adult organisms (ref to be added).

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[46]. 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.

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[46] 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), …*")

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 conditions 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. Instead of these cohorts, the registrant may also propose separate developmental toxicity studies to address these concerns, as explained below in Section R.7.6.4.2.3.5. Likewise at Annex IX, if the extended one-generation reproductive toxicity study is triggered, these cohorts must be proposed by the registrant to address the concern in question or separate studies may be proposed.

It is to be noted that neurotoxicity and/or immunotoxicity observed in adult animals are triggers for developmental neurotoxicity and/or developmental immunotoxicity cohorts in extended one-generation reproductive toxicity study or separate studies. In addition, in case of classification criteria for STOT are met based on studies in adults, this is not an adaptation rule allowing omitting the investigations on developmental neurotoxicity and/or developmental immuotoxicity. This is due to expected higher sensitivity of the developing organisms, which may lead to a more severe classification and/or lower DNEL.

R.7.6.4.2.3.5 Proposals for separate 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."*

Thus, 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. Likewise, the registrant may propose a separate developmental immunotoxicity study instead of Cohort 3, if the conditions for a particular concern for developmental immunotoxicity are met. The concern should be related to *developmental* neuro- or immunotoxicity specifically. The study design for development neurotoxicity should follow the EU B.53 (OECD TG 426) protocol. 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 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 Column 1 standard information requirements in REACH but they must be proposed when Column 2 conditions 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). However, the inclusion of cohorts in an extended one-generation reproductive toxicity study may have relevant shortcomings. For example, the developmental neurotoxicity cohort integrated in an extended one-generation reproductive toxicity study does not include investigations on learning and memory as compared to the OECD TG 426. Furthermore, 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 (Recital (7) of Commission Regulation No… amending REACH). Even if there are alerts for developmental neurotoxicity or developmental 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

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 and/or developmental immunotoxicity in (a) separate stud(y)ies.

The nature and/or severity of the alerts may provide guidance to select between a separate study or a cohort(s). 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 Section R.7.6.4.2.3.4).

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

R.7.6.4.2.3.6 Evaluation of findings from developmental neurotoxicity and developmental immunotoxicity cohorts

Special attention should be paid to interpreting the neurotoxicity and/or immunotoxicity findings observed in F1 animals in the EOGRTS. This is because the animals are exposed *in utero*, during postnatal and adult periods and it may be challenging to conclude whether the effects observed are due to exposure during the developmental period, which includes the lactational period and/or adulthood. In case the neurotoxicity or immunotoxicity findings are due to prenatal or early postnatal exposure, classification for Reproductive toxicity Category 1B or 2 may apply. However, effects on or via lactation may lead to classification in the category for lactation effects. However, in case the neurotoxicity or immunotoxicity findings support neurotoxicity or immunotoxicity induced also during adult exposure, classification to STOT RE may be appropriate. If effects may occur as a result of developmental, lactational and/or adult exposure, and the criteria are fulfilled, classification for all hazards may apply. Whether a neurological and/or an immunological change reflects a neurotoxic or immunotoxic effect, which is not a secondary non-specific consequence of other effects, should be carefuly evaluated.

Discrimination of adults and developmental neurotoxicity or immunotoxicity may be difficult, but may be facilitated by the findings from the F0 generation (non-prenant) compared with the findings in the non-pregnant F1 animals. The physiological changes in the pregnancy and lactational periods may have an impact on the effects in females and, thus, it is advisable to compare the effects before the pregnancy during a 10-week premating exposure duration. Related to the evaluation of the histopathological findings, the potential differences in non-pregnant and pregnant animals should be considered. If the Cohort 1B animals are mated, this would allow an improved possibility to interpret the potential differences histopathological findings in F0 and F1 animals because the histopathological examination of Cohort 1B animals will be conducted after an extended exposure duration.

In order to be able to justify the conditions which specify the study design of the extended one-generation reproductive toxicity study, the existence and/or the absence of the alert/condition must be recorded and justified. The justification and recording should allow an independent evaluation of the existence or absence of the condition. It is to be noted that the condition must be based on adequate and reliable studies. This means, e.g. that non-animal approaches and the results must be adequately described allowing assessment of their relevance.

R.7.6.4.2.3.7 Further aspects

The OECD GD 151 provides guidance for conducting the extended one-generation repoductive 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.

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 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 selected must be adequately justified and documented in all cases.[47]

For evaluation of the results, 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).

In general, all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of concurrent parental toxicity, see Chapter R.7.6.5.

R.7.6.4.2.4 Two-generation reproductive toxicity study

Two-generation reproductive toxicity studies are not standard information requirements (EU B.35, OECD TG 416) in REACH but those studies initiated before [date to be added] [add reference]…to the REACH Regulation are considered appropriate to address the standard information requirement for Annex IX/X, 8.7.3. Although the two-generation reproductive toxicity study may lack information on some parameters measuring endocrine disrupting mode of action which are requested in EU B.56, 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.

When considering the relevance of the old non-guideline compliant two(multi)-generation reproductive toxicity studies to address the fertility endpoint, 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.

In case a two-generation resproductive toxicity study is available and there are alerts 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 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).

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[47] 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;*"

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 stastical 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 at Annex VIII under Section 8.6.1 or Annex XI Section 8.6.2 or at Annex X Section 8.6.4 based on Column 2 adaptation rules[48]. 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 evalution 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 behaviour due to effects on the central nervous system (CNS) and the peripheral nervous system. As behaviour also may 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 behavioural test is able to reflect the entire complex and intricate function of behaviour. For testing behaviour therefore, a range of parameters, such as a behavioural test battery, is used to identify changes in individual functions.

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. Irreversible effects are clearly serious, while reversible effects may be of less concern. However, it is often not possible to determine whether an effect is truly reversible. The nervous system possesses reserve capacity, which may compensate for damage, but the resulting reduction in reserve capacity 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

[48] 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), …*")

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.

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 test guidelines mentioned in this section, including a discussion of their strengths and limitations see the reports from Nordic Chemicals Group (2005), ECETOC (2002) and WHO (2001).

R.7.6.4.2.7 Developmental immunotoxicity studies

Developmental immunotoxicity studies are not standard information requirements but may be triggered at Annex VIII under Section 8.6.1 or Annex XI Section 8.6.2 based on Column 2 adaptation rules[49]. 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 evalution and/or risk 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 (WHO 2012; De Jong & Van Loveren (2007); DeWitt et al 2012a and b) Dietert and DeWitt 2010; Dietert and Holsapple 2007; Hosapple 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 an immune response may also be affected by the function of other organs such as the endocrine system, toxic effects on endocrine organs in offspring may also be reflected in changes in immune response.

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, adversity 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

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[49] 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), …*")

measurements of oestrous cycle, may provide relevant information for reproductive toxicity or indicate a concern (alert). 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, alerts 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 repoductive toxicity study based on alerts 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 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/alert 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. It may be considerd that statistically significant changes from relevant studies can be considered as triggers, however, sometimes a statitstically non-significant change can be also considered as biologically relevant.

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 very sensitive to detect the hormonal mode of action. However, only investigation in intact animals prove 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 thorough dose-response study showing no effect indicates that the test substance is not an 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 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 disruption-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 disruption-modes of action to be used to define the study design of the extended one-generation reproductive toxicity study are presented under the heading of that study in Section R.7.6.4.2.3 of this Guidance.

The screening study may be updated with additional parameters for endocrine disruptions, 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. However for N(L)OAEL derivation an association to adverse effects has to be demonstrated. For anogenital distance there is association with reduced human reproduction (ref to be added). Nipple retention measures the the same mode of action (antiandrogenicity) but may be more or less sensitive than anogenital distance. Thus, it is recommended that these endpoints are evaluated together with other parameters reflecting antiandrogenicity.

As the extended one-generation reproductive toxicity study is a more comprehensive reproductive toxicity study which includes parameters to detect endocrine disruption, 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. In case an endocrine 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 warranted. In case the findings on

reproduction meet the classification criteria to Category 1B reproductive toxicant, irrespective indications of an endocrine mode of action, the substance should be regulated 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, 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.

R.7.6.4.4 Derivation of DNELs

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, a non-threshold mode of action may need to be considered (e.g. exposure to a hormonally active substance during an early stage of development when it is biologically essential to manifest a certain hormonal activity, and when body's own hormonal control regulation is not yet active).

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).

All types of reproductive toxic effects may be considered as secondary to parental toxicity. With current knowledge it is not possible to identify specific effects indicating toxicity in parental animals which do not have any relevance to reproductive toxicity (e.g. peroxisome proliferation). However parental toxicity that is less than marked should not influence the classification for reproductive toxicity independent of the specific parental effects observed. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be performed.

R.7.6.5.1 Fertility effects

Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes.

There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification.

R.7.6.5.2 Developmental effects

Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother through non-specific mechanisms related to stress and the disruption of maternal homeostasis.

Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification. In general it is very difficult to prove a causal relationship between a parentally mediated mechanism and adverse effects in the offspring. Usually data are insufficient to conclude if an effect on the offspring is a direct effect or secondary to parental toxicity. In order to determine whether a reproductive toxic effect is independent or secondary to a parental effect, it would be most appropriate to correlate individual data for offspring and their parents. Nevertheless, associations between parental and offspring effects do not by default prove a causal relationship. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity.

In cases where a causal relationship is established between reproductive and parental toxicity and the effects on the offspring can be proved to be secondary to maternal toxicity, they may still be relevant for developmental classification, dependent on the severity of the effects.

A comparison between the severity of the maternal toxicity and the severity of the findings in the offspring must be performed. There are several examples showing that the developing organism can be more susceptible and the long-term consequences can be more severe than in the adult. The mother might recover while the offspring could be permanently affected.

For further information see section 3.7 in Annex I, CLP and in CLP Guidance on the Application of the CLP criteria (see also ECB 2004).

The nature, severity and dose-response of all effects observed in progeny and parental animals should be considered and compared together to achieve a balanced integrated assessment of available data on all endpoints relevant for reproductive toxicity.

Impairment of fertility and/or offspring development that results from relevant reproductive toxicity studies will be relevant to hazard classification and the human health risk assessment, unless the mode of action(s) or mechanism(s) for the effect(s) has been clearly identified and is conclusively demonstrated to be not relevant to humans.

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.

Risk assessment and determination of classification involves the consideration of all data that is available and may be relevant to reproductive toxicity (see Section R.7.6.3 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 I, 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 instrinsic 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 fulfill 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 labeling. The ECHA guidance R.7a cites the definition given by

Blaauboer et al., (1999)[50]. The ITS 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), which can be seen as similar approaches as ITS and IATA, 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.

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 approapriate to prove the absence of an effect. Currently derivation of a NOAEL is not possible with these methods.

R.7.6.8 References on reproductive toxicity

Comment [SJ8]: This section will further revised during consutlaiotn: it is propsoed to present the referenes in 2 parts (i) refernces cited int eh guidance doc and (ii) becakground references for application of REACH requirements.

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Comment [SJ9]: Links can be added: the format for listing ECHA Gidance references is being reviewed: this can be amended later.
Also applies to references listed below.

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Appendix 1:

Table R.7.6-2 A check list for information that should be presented in the dossier in order to establish the existence and/or the absence of the conditions specifying the study design proposed for the extended one-generation reproductive toxicity study.

Condition	Where to find the information to decide on the existence and/or absence of the condition, including some examples
Uses leading to significant exposure of consumers or professional, taking into account inter alia consumer exposure from articles	Consumer and/or professional uses: • Consumer and/or professional use • Substance is in an article used by consumers or professionals and it is intended to be released from the article The registrant must record and justify the existence and/absence of the condition.
Genotoxicity potentially meeting classification criteria to Mutagen Category 2	Results from *in vivo* mutagenicity studies (if one of the *in vitro* test is positive, then an *in vivo* somatic cell mutagenicity test must have been conducted). The registrant must record the findings and justify the existence and/or non-existence of the condition.
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), indications of persistency, 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 and/or non-existence of the condition.
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 endocine mode of action. Reproductive toxicity studies may provide indication of endocrine mode of action. Check the parameters related to endocine mode of action. Check *in vivo* assays for endocrine disrupting mode of action. Check the non-animal approaches for prediction to endocrine disrupting mode of action. The registrant must record the findings and justify the existence and/or

	non-existence of the condition.
Information on neurotoxicity from *in vivo* studies or non-animal approaches.	*In vivo* toxicity studies may provide information on neurotoxicity. Check the parameters related to nervous system. Check the non-animal approaches for prediction to (developmental)neurotoxicity. The registrant must record the findings and justify the existence and/or non-existence of the condition.
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 and/or non-existence of the condition.
In vivo information on (developmental) neurotoxicity from structurally analogues substance	Structurally analogue substances must 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 and/or non-existence of the condition.
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 effects to immune system. Check non-animal approaches for prediction to (developmental) immunotoxicity. The registrant must record the findings and justify the existence and/or non-existence of the condition.
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 available. The registrant should record the findings and justify the existence and/or non-existence of the condition.
In vivo Information on (developmental) immunotoxicity from structurally analogues substance	Structurally analogue substances must 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 and/or non-existence of the condition.

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R.7.7 Mutagenicity and carcinogenicity

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