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GUIDANCE

Guidance on Information Requirements and Chemical Safety Assessment

Chapter R.7a: Endpoint specific guidance

Version 3.0

August 2014

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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 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 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

Further guidance documents will be published on this website when they are finalised or updated.

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

[1] Corrigendum to 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, 30.12.2006); amended by: Council Regulation (EC) No 1354/2007 of 15 November 2007 adapting Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), by reason of the accession of Bulgaria and Romania, Commission Regulation (EC) No 987/2008 of 8 October 2008 as regards Annexes IV and V; Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures; Commission regulation No 453/2010 of 20 May 2010 as regards Annex II; Commission Regulation No 252/2011 of 15 March 2011 as regards Annex I; Commission Regulation No 366/2011 of 14 April as regards Annex XVII (Acrylamide), Commission Regulation No 494/2011 of 20 May 2011, as regards Annex XVII (Cadmium).

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

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

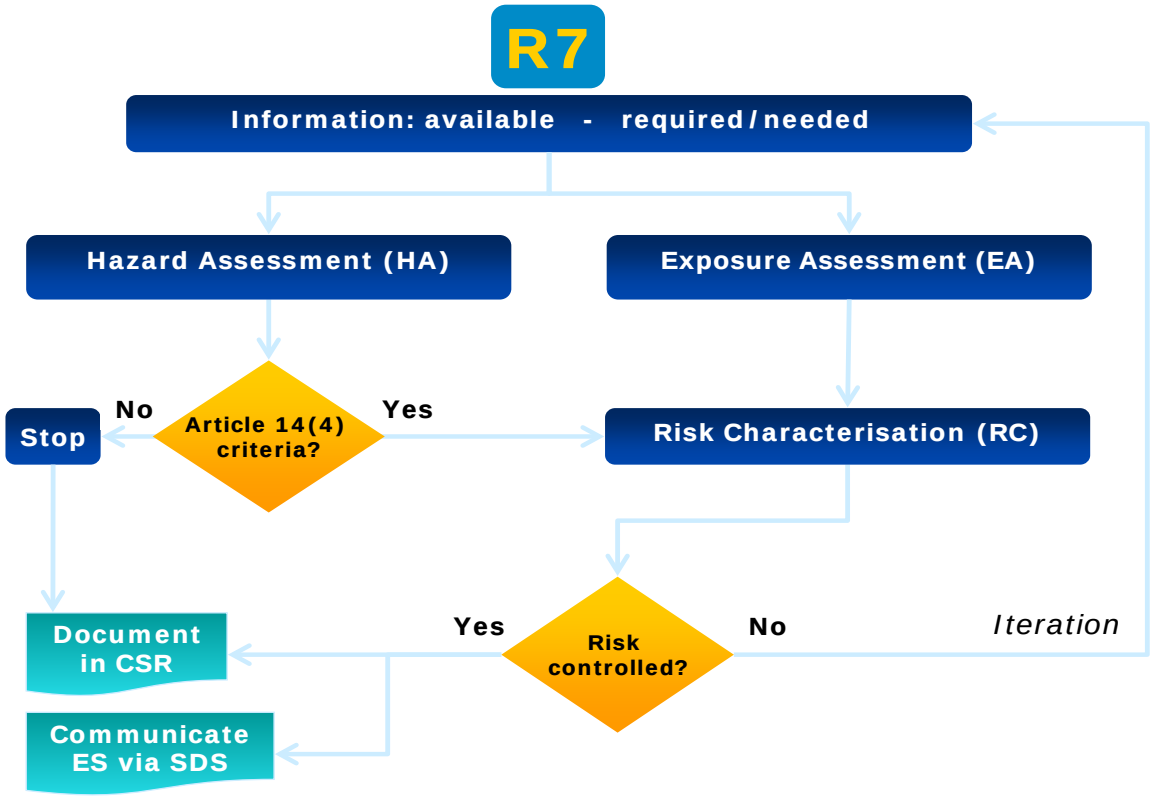

Table of Contents

Tables

Figures

Appendices

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 a *weight of evidence* (WoE) approach to allow a conclusion on whether or not the available information is sufficient for regulatory purposes, i.e. hazard assessment and risk assessment.

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 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. The first chapter, namely the R.7.1 Physicochemical properties, underwent a guidance revision process between 2011 and 2012 and therefore follows a revised chapter structure. The R.7.1 chapter covers both classification and non-classification related properties, where the sub-chapters covering the physicochemical properties have each six or seven sections, depending on the need for information on references and the sub-chapters covering the physical hazards have seven sections. In the physicochemical properties sub-chapters the first section details the type of property, the second section provides the definition of the property, the third lists the preferred test method(s). 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 sub-chapters 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 and 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.

Chapters tackling human health properties or hazards in R.7a remain unchanged. In those chapters there are six main sections to the guidance on each property or hazard; the introduction section 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 first section 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 the four logical steps that should be taken to assemble the information that is detailed under the second section; thus, the second section 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.

Guidance is given in the third section 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 also provides an indication of the remaining uncertainty inherent in the different types of data for the given endpoint.

The fourth section describes how conclusions may be drawn for a given substance on the suitability of the available information for regulatory purposes. Guidance is given on how to develop and apply a WoE approach for the endpoint in order to establish whether there is a need for further information and if so, what test should be performed. 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 fifth section 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 to 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. In those cases where the available information is judged to be sufficient to meet the regulatory needs even though the standard information requirements are not met, under certain circumstances, in particular for Annexes IX and X to REACH, this might be part of a justification for waiving a certain test that is requested in the standard information requirements.

The final section lists all used references on the given endpoints.

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

[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.

Information requirements in the light of the applicable classification regime

The main regulatory purpose of the information requirements set out in Annexes VI to XI to the REACH Regulation is to assess risks related to substances and to develop and recommend appropriate risk management measures, as highlighted in Recital 19 the REACH Regulation. According to Recital 26: '*in order to undertake chemical safety assessment of substances effectively, manufacturer 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 to the REACH Regulation. According to Section 0.6 of Annex I, the first three steps of the CSA require the carrying out of human health hazard assessment, human health hazard assessment of physicochemical properties and 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 I of Annex VII to 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 of 59 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 an 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.

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 (A) 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 alternative methods and all other options must 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 to perform the testing, such as:

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[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.

- testing requirements;
- exposure/use pattern (emissions, yes or no, consumer use etc);
- occurrence (monitoring data);
- indications of the effect/ property based on animal or human data, *in vitro* data and non-testing information;
- any concern e.g. based on toxicokinetics, read-across and (Q)SAR considerations,
- weight of evidence;
- seriousness of the effect;
- other effects of relevance for the endpoint.

All these issues should be considered, not only to design fit for purpose *in vivo* tests, but also for providing evidence for not performing *in vivo* testing 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 some cases it may be possible to use grouping approaches for

[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].

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 exposure 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 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 all available information including physicochemical properties of the substance, structure-activity relationships (SAR) or the data from available toxicity tests on the substance itself. Fundamentally, the administration of the substance by inhalation in animal tests should be considered only when human exposure via inhalation is relevant.

Route-to-route extrapolation can be used to assess potential health effects and their threshold in a route other than the one tested. It should be stressed that toxicity data obtained using the appropriate route of exposure are preferred. Route-to-route extrapolation should be considered on a case-by-case basis and may introduce additional uncertainties, especially if the toxicity data were obtained using an administration route that does not correspond to the most relevant route of human exposure. 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

Advice to registrants with regard to nanomaterials characterisation can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2 Recommendation for physicochemical properties arising from RIP-oN 2 for nanomaterials.

R.7.1.1 Introduction on physicochemical properties

According to Article 12 of the REACH Regulation, for registration purposes all physicochemical information that is relevant and available to the registrant must be included in the technical dossier, i.e. information such as:

- data on intrinsic properties of the substance (e. g. melting point/freezing point, boiling point, vapour pressure, density);
- data necessary to assess the physical hazards of a substance (e. g. flammability), with the view to determine its classification and labelling according to CLP (and until 1 June 2015, according to DPD, see Article 61 of CLP);
- supplementary data for hazard assessment and health and environmental classification (e. g. viscosity, n-octanol/water partition coefficient).

Some physicochemical properties - notably explosive, flammable and oxidising properties - are intimately linked to physical hazards. The most straight-forward way of assessing these properties is through the classification of the substance for the corresponding physical hazards. There is thus a link between the physical hazards classification and the information requirements on explosive, flammable and oxidising properties. This is further elaborated below (see table R.7.1-1) and in the various chapters addressing these endpoints. For substances manufactured or imported in quantities of 100 tonnes or more per annum, some additional physicochemical data are required; in accordance with Annex IX to REACH (see also Table R.7.1-1).

Further details are given in the sections dedicated to specific endpoints.

R.7.1.1.1 Information requirements on physicochemical properties

Commission Regulation (EU) No 252/2011[7] has amended Annex I to REACH in order to adapt the chemical safety assessment provisions to the criteria for classification laid down in the CLP Regulation. The relevant amendments have been applied since 5 May 2011; however, for registrations submitted prior to this date, the chemical safety report shall be updated in accordance with Regulation No 252/2011 by 30 November 2012 at the latest.

The information needed under Article 12, REACH on one hand and according to section 4 of Annex VI to REACH on the other (namely hazard classification according to Title I and II CLP) is often complementary but in some cases may be different. The reason is that the classification criteria and/or test methods under DSD and CLP regimes are different. This is also expressed by the fact that CLP classifications are distributed over a different grid of hazard classes and categories compared to the DSD regime, e.g. substances and mixtures classified as explosive under DSD may be classified as explosives or self-reactives or organic peroxides under CLP, or they may even be classified as flammable solids, oxidizing solids or

[7] Comission Regulation (EU) No 252/2011 of 15 March 2011 amending 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 Annex I.

not at all. A translation table from DSD to CLP classification is provided in Annex VII, CLP and an indication of potential classification outcomes under CLP compared to DSD classifications is provided by Table 1.7.2.1(a) in the 'Guidance on the Application of the CLP Criteria' which can be found on the following ECHA page: http://echa.europa.eu/web/guest/guidance-documents/guidance-on-the-different-methods-under-reach.

The CLP classification regime is not explicitly considered in Annex VII to REACH and therefore has to be understood as part of the information requirements under REACH. In particular, certain headlines set out in column 1 of Annex VII to REACH, namely 'explosive properties', 'flammability' and 'oxidizing properties', must be interpreted as covering the CLP hazard classes that are referred to in Article 58(11) of CLP.

The physical hazard classes according to CLP are structured differently from the corresponding classifications according to DSD. Despite this, most of the CLP physical hazard classes can unambigously be assigned to specific heading of the information requirements according to Annexes VI to IX to REACH. However, for some CLP physical hazard classes - notably the hazard class 'self-reactive substances and mixtures' and the hazard class 'organic peroxides' – the assignment to a specific heading is not straight-forward, since they may have both explosive and/or flammable properties. Therefore, some of the hazard classes are listed twice in Table R.7.1-1 below. It should be noted that this assignment is provided only as example and is done for the purposes of structuring this guidance document according to CLP but nevertheless also allowing the assignment to the respective information requirements according to Annexes VII to IX to REACH.

According to Article 1(6) CLP, CLP Regulation does not apply to the transport of dangerous goods by air, sea, road, rail or inland waterways (save where the specific rules for labelling of packaging applies under Article 33 of CLP). The transport of dangerous goods is, covered by the UN Model Regulations for Transport of Dangerous Goods (UN-RTDG) and related legal instruments (ADR, RID, ADN, IMDG Code and ICAO TI); the criteria listed in these instruments and in CLP Regulation for classification purposes are intended to be the same. Thus, a substance (or mixture) classified in a hazard class which is common to both CLP and the transport legislation will normally be classified the same according to both systems. Therefore the transport classification of a substance could be a source of information for the classification and labelling of substance (or a mixture) under CLP for physical hazards. However it should be kept in mind that the transport classifications do not cover all hazard categories which are relevant for CLP and it may be based on other considerations than just the test data and criteria (e.g classifications which are based on experience rather than testing or which apply only in connections with certain special provisions). As a result, the transport classifications may be different for the classification according to CLP. Similarly, the absence of a transport classification does not necessarily mean the substance (or mixture) should not be classified under CLP. Consequently in the case of a substance which has been tested for the purposes of the UN-RTDG and for which the same procedure was followed as required by the CLP Regulation, the same information could be used to comply with the REACH Regulation on a case-by-case basis. The limitations to the approach described above are described in detail in the 'Guidance on the Application of the CLP Criteria', Section 1.7.2.1.

For the preparation of the registration dossier, registrants are required to submit all the information listed in Article 10 of REACH. Article 14(1) in conjunction with Annex I and Article 10(a)(vii) of the REACH Regulation, require the provision of a Robust Study Summary (RSS) for information derived from the application of Annexes VI to XI to REACH. In order to facilitate the evaluation conducted by the European Chemicals Agency and the Member States, as well as to save registrant's resources in case of a tonnage update, it **is recommended** that registrants also use the RSS for covering physicochemical endpoints under section 4 of the IUCLID file. This guidance includes under each physicochemical property chapter a list of detailed information to be given for each respective endpoint. Note that no further guidance is provided on the general aspects related to information common for all endpoints in IUCLID. For

these aspects, further guidance can be found in 'Practical guide 3: How to report robust study summaries' available on the ECHA Website at: http://echa.europa.eu/web/guest/support/documents-library and in the 'IUCLID 5 End User Manual' available on the IUCLID Website at: http://iuclid.eu/index.php?fuseaction=home.documentation#usermanual.

Those endpoints, such as explosive properties and oxidising properties, which are intimately linked to classification, should be assessed according to CLP. For these endpoints, the test methods of CLP should preferably be used, in order to avoid double testing. For endpoints not linked to classification the preferred test methods are those found in the Test Method Regulation. For some endpoints (for example flammability), more than one test procedure is referred to in the standard test method reported in the Test Method Regulation. The one chosen should be suitable for the substance in question and be operating within its validity range.

Note that in the table below in order to distinguish the physicochemical properties that are directly linked to physical hazard classifications from those that are not, the former have been shaded in gray and that in addition the preferred test methods for the different endpoints have been put in bold text.

Table R.7.1-1 Information requirements as specified in Annexes VII to IX to REACH and corresponding tests methods according to the Test Method Regulation and CLP

Information requirement according to Art. 10 (a) (vi) of the REACH Regulation (EC) No. 1907/2006 (the no. in brackets is the respective no. in the table in Annexes VII to IX to REACH)	**Corresponding test method according to The Test Method Regulation 440/2008**	**Chapter in revised R.7(a) guidance**	**CLP Regulation (EC) No. 1272/2008** (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Corresponding test method according to CLP Regulation**
Melting/ Freezing point (7.2)	**A.1 Melting/Freezing temperature**	7.1.2	n.a.	n.a.
Boiling point (7.3)	**A.2 Boiling temperature**	7.1.3	n.a.	n.a.
Relative density (7.4)	**A.3 Relative density**	7.1.4	n.a.	n.a.
Vapour pressure (7.5)	**A.4 Vapour pressure**	7.1.5	n.a.	n.a.
Surface tension (7.6)	**A.5 Surface tension**	7.1.6	n.a.	n.a.
Water solubility (7.7)	**A.6 Water solubility**	7.1.7	n.a.	for metals - Transformation/Dissolution Protocol (Annex 10 to UN GHS)
Partition coefficient n-octanol/water (7.8)	**A.8 Partition coefficient**	7.1.8	n.a.	n.a.

Flash point (7.9)	A.9 Flash-point	7.1.9	n.a.	**CLP Annex I chapter 2.6.4.4**
Flammability (7.10)	A.11 Flammability (gases)	7.1.10.1	Flammable gases[8] (2.2)*	**ISO 10156** **EN 1839**
	for liquids: see Flash point	7.1.10.2	Flammable liquids (2.6)*	**see CLP, Annex I, Chapter 2.6.4.4, Table 2.6.3**
	A.10 Flammability (solids)	7.1.10.3	Flammable solids (2.7)*	**UN Test N.1**
	n.a.	7.1.10.4	Self-reactive substances and mixtures (2.8)*	**UN Test series A to H**
	A.13 Pyrophoric properties of solids and liquids	7.1.10.5	Pyrophoric liquids (2.9)*	**UN Test N.3**
		7.1.10.6	Pyrophoric solids (2.10)*	**UN Test N.2**
	n.a.	7.1.10.7	Self-heating substances and mixtures (2.11)*	**UN Test N.4**
	A.12 Flammability (Contact with water)	7.1.10.8	Substances and mixtures which in contact with water emit flammable gases (2.12)*	**UN Test N.5**
	n.a.	7.1.10.9	Organic peroxides (2.15)*	**UN Test series A to H**
Explosive properties (7.11)	A.14 Explosive properties	7.1.11.1	Explosives (2.1)*	**UN Test series 1 to 3 (further test series 4 to 6 are necessary for classification)**
	n.a.	R.7.1.11.2 see R.7.1.10.4	Self-reactive substances and mixtures (2.8)*	**A.14 (existing data only)**

[8] The Commission Regulation (EU) No 487/2013 of 8 May 2013 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (hereinafter referred to as '4th Adaptation to Technical Progress (ATP) to the CLP Regulation') amends the criteria in the CLP Annex I, Section 2.2 Flammable gases by including subclassifications for chemically unstable gases. The 4th ATP to the CLP Regulation will apply in respect of substances from 1 December 2014 and in respect of mixtures from 1 June 2015.

	n.a.	R.7.1.11.3 See R.7.1.10.9	Organic peroxides (2.15)*	**A.14 (existing data only)**
Self ignition temperature (7.12)	A.15 Auto-ignition temperature (liquids and gases)	7.1.12.1	For gases and liquids*	n.a.
	A.16 Relative self-ignition temperature for solids	7.1.12.2, 7.1.10.7	For solids* Note: the UN Test N.4 is preferable to generate the information for this endpoint. Refer to R.7.1.10.7.	n.a.
Oxidising properties (7.13)	n.a.	7.1.13.1	Oxidising gases (2.4) *	**ISO 10156**
	A.21 Oxidising properties (liquids)	7.1.13.2	Oxidising liquids (2.13) *	**UN Test O.2**
	A.17 Oxidising properties (solids)	7.1.13.3	Oxidising solids (2.14) *	**UN Test O.1**
Granulometry (7.14)	n.a.	7.1.14	n.a.	n.a.
Adsorption/Desorption (7.15)	n.a.	7.1.15	n.a.	n.a.
Stability in organic solvent and degradation products (7.16)	n.a.	7.1.16	n.a.	n.a.
Dissociation constant (7.17)	n.a.	7.1.17	n.a.	n.a.
Viscosity (7.18)	n.a.	7.1.18	n.a.	n.a.

* Note that regardless of whether the hazard class or category is listed in Article 14 (4) (a) of REACH, the chemical safety assessment (when required) must be performed in accordance with Article 14 (3) of REACH. Furthermore, according to Article 10 (a) (iv) of REACH the technical dossier of a registration for a substance under the REACH Regulation must include information on classification and labelling of the substance as specified in section 4 of Annex VI to the REACH Regulation.

In addition the CLP Regulation has the following hazard classes for which the REACH Regulation does not require the generation of information (Article 10(a)(vi) and (vii) REACH):

Table R.7.1-2 CLP Regulation hazard classes for which the REACH Regulation does not require the generation of information

CLP Regulation (EC) No. 1272/2008 (the no. in brackets is the respective chapter no. in Annex I to CLP)	Corresponding test method according to the Test Method Regulation	Chapter in revised R.7(a) guidance	Information requirement according to Art. 10(a)(vi) of the REACH Regulation	Corresponding test method according to CLP Regulation
Flammable aerosols[9] (2.3)	n.a.	7.1.21.1	n.a.	Test methods according to 75/324/EC amended by 2008/47/EC (harmonised with **UN-MTC Section 31)**
Gases under pressure (2.5)	n.a.	7.1.21.2	n.a.	n.a.
Corrosive to metals (2.16)	n.a.	7.1.21.3	n.a.	**UN Test C.1 (UN-MTC Section 37.4)**

In order to comply with the REACH information requirements, registrants have to take due account of specific rules for adaptation according to column 2 of the tables in Annexes VII to XI to REACH, including the provisions given within the individual test methods of the Test Method Regulation, which have to be interpreted and applied in relation to the appropriate CLP hazard class. Further adaptations according to Annex XI to REACH must then be read together with the adaptation possibilities provided for by Article 8(2) of CLP and the CLP criteria themselves, namely those in Part 2 of Annex I to CLP.

Physicochemical data are mostly numeric and should be provided in SI units. Normally a numeric value or range is required. Where relevant, additional information should be provided on test conditions, such as temperature and/or pressure and/or concentration level or range etc., and estimated uncertainty in the numerical value. Furthermore details of any observations made during testing should be reported, e.g. decomposition during melting or boiling, emulsion formation during partitioning.

R.7.1.1.2 Available information on physicochemical properties

There are many published sources of data for basic substance characterisation and of supplementary information for hazard assessment. The relevant references are listed under respective endpoint.

R.7.1.1.3 Evaluation of available information on physicochemical properties

Advice to registrants with regard to nanomaterials characterisation can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.1.3 Evaluation of available information.

[9] The 4th ATP to the CLP Regulation amends the criteria in the CLP Annex I, Section 2.3 Flammable aerosols by changing the scope and title to Section 2.3 Aerosols.

Experimental data

Further, according to Article 13 (3) of the REACH Regulation, tests to generate information on intrinsic properties of substances must be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the Agency as appropriate, such as european standards (www.cen.eu) or OECD guidelines (www.oecd.org). Data obtained from the tests in accordance with section 1.1.1 of Annex XI to REACH can be considered to be equivalent to data generated by the corresponding test methods referred in Article 13 (3) of REACH. Data for the purpose of physical hazard classification can be obtained using the test methods specified in the Articles 5 (1) and 8 (3) CLP. The test methods for the physicochemical properties are described in Regulation (EC) No 440/2008, whereas preferred tests for the purposes of physical hazard classification are referred to in Part 2 of Annex I to CLP, via references to the UN-MTC and to applicable standards. In Table R.7.1-1, the preferred test method for each endpoint is highlighted in bold. The test methods referred to in the CLP Regulation are also used for the transport of dangerous goods. Therefore, available information on physicochemical properties and physical hazards may also originate from tests that were carried out for the purposes of classification for transport. Such test data may be used for the information requirements according to the REACH Regulation. It should, however, be kept in mind that the classification for transport does not cover all hazard categories which are relevant for CLP and it may be based on other considerations than just the test data and criteria (e.g. classifications which are based on experience rather than testing or which apply only in connection with certain special provisions). As a result transport classifications may be different from the classification according to CLP. Such limitations are described in detail in the 'Guidance on the Application of the CLP Criteria', Section 1.7.2.1.

Where relevant recognised standards for testing are applicable, the use of the most recent updates is advised; they are accessible via numerous websites, for example:

1. EN standards;
2. ISO standards;
3. IEC standards.

The national editions of the EN or ISO standards are available via the national standardization organizations accessible via the CEN Website.

Measured values which are evaluated in reviews and assigned recommended values are given precedence over calculated values. The major criteria that characterise the analysis of the available information are:

- **Experimental data**. When assessing physicochemical properties, priority is given to first hand experimental results (primary references) provided that the methods are suitable for the substance under investigation and that they operate within their validity range. Proper documentation on the methods and the inherent uncertainty of the measurements should also be provided.
- **Non-testing information**. If the information described in point (a) is not available, QSPRs, read-across or secondary data sources (e.g. handbook data) can be used in accordance with the limitations described in the individual endpoint chapters (7.2 to 7.19 in this guidance) instead, and within the constraints of Annex XI to REACH.

Measurement uncertainty

Test data have an uncertainty of measurement. Some test methods include information about their uncertainty, which then may be referred to for test data generated using these test

methods. Where the uncertainty of measurement is not specified by the test method, it is recommended to determine uncertainty by generally accepted processes of measurement uncertainty estimation (e.g. according to ISO/IEC Guide 98-3:2008).

Quality assurance for the determination of physicochemical properties

Test data on physicochemical properties should be of sufficient quality i.e. they must be reliable. Normally this can only be achieved by testing that is carried out in compliance with a relevant recognised quality system (e.g. GLP) or by laboratories complying with a relevant recognised standard (e.g. EN ISO/IEC 17025). Under Article 8 (5) of CLP, where new tests for physical hazards are required for the purposes of CLP they have to be carried out in compliance with a relevant recognised quality system or by laboratories complying with a relevant recognised standard at the latest from 1 January, 2014.

Non-experimental data

Quantitative Structure Property Relationships (QSPR) models exist for some of the physicochemical endpoints[10]. Where applicable, the details of any specific QSPR models are given under each endpoint.

The majority of QSPR models have been built using training sets of substances. The model will have been optimised to calculate values for the training substances that most closely match measured ones. Therefore, the use of QSPR estimation techniques requires expert judgment. The calculated values need to be checked to ensure that they are reasonable and that the model used is appropriate.

A valid model will give values that are in reasonably close agreement with the measured ones for your chosen analogue substances (i.e. the substance with a data gap should have similar substances in the training set of the model). The models may not predict very well the properties of substances which are too dissimilar to the reference set for the model. Thus, the model can be used to provide a predicted value for your substance without the need for testing. Another check is that the values are realistic. This can be done by cross-referencing the calculated value to measured values for similar substances and related endpoints. If a QSAR method is used as a stand alone method to determine a value to meet the endpoint data requirements, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

Assessing the quality of QSPR models

The European Commission and the OECD member countries adopted five principles for the validation of (Q)SAR/(Q)SPR models in 2004 (OECD[a], 2004). According to these principles, a valid (Q)SPR model should have 1) a defined endpoint whose experimental conditions are clearly specified; 2) an unambiguous algorithm; 3) a defined domain of applicability that defines for what kind of substances predictions can be made; 4) appropriate measures of goodness-of-fit, robustness and predictivity; and 5) a mechanistic interpretation if possible. These principles are outlined on the ECB website and more extensively covered in IR/CSA R.6: QSAR and grouping of substances, section R.6.1.3. Moreover, a practical overview of these principles is given in the report from the expert group on (Q)SARs (OECD[b] 2004).

[10] A comprehensive review paper with a title: “QSPR prediction of physico-chemical properties for REACH” was published in the SAR and QSAR in Environment Research in 2013 (Dearden, J.C., Rotureau, P., Fayet G. (2013). QSPR prediction of physico-chemical properties for REACH, SAR and QSAR in Environmental Research, Vol. 24, No.4, 279-318).

Assessing the quality of read-across predictions

This paragraph reports the basic concepts of a read-across approach. Thorough information on this topic can be found in the guidance on the grouping of substances (see IR/CSA R.6: QSAR and grouping of chemicals, Section R.6.2).

A read-across/analogue approach assesses the relevance of a given property on one or more chemical structures and then makes some assessment (qualitative or quantitative) on the relevance of this information for another substance (see Annex XI, REACH). Since a read-across may involve two substances[11] it is of paramount importance to detail the reasoning behind the inference on the substance whose property is unknown. An analogue must:

- contain the same major structural features and the same functional groups as the substance under investigation;
- have a physicochemical profile comparable to that of the substance under examination as far as the known physicochemical properties are concerned;
- have comparable values for the relevant molecular descriptors (i.e. excess molar refractivity and hydrogen bond donor and acceptor abilities for water solubility predictions) generally used for the quantification of the property of interest;
- have approximately the same molecular weight.

The interpretative analysis of a read-across is usually the result of an expert judgement evaluation and detailed documentation should therefore always be provided to support the conclusions. It is important to point out that, in practice, read-across for physicochemical properties is not generally recommended, since reliable data should normally be available or easily obtainable. This is particularly true for physical hazard related physicochemical properties for which reliable test data must be available according to Article 8 (2) of CLP. Therefore, if read-across is used as a stand alone method to generate a value to meet the endpoint data requirements, the criteria given in section 1.5 of Annex XI to REACH must be met.

Use of secondary and historical data sources for physicochemical properties

The reliability of data must be demonstrated by providing information on the identity and purity of the test substance, the methodology used to make the measurement, and whether or not this was performed in compliance with a relevant recognised quality system (e.g. GLP) (Annex VI, REACH).

Numerical physicochemical data is particularly prone to data recycling (transfer from one database to another, often with loss of the original source and contextual information). Data from secondary and historical sources must be adequate and is especially important where the endpoint is relevant for classification, PBT/vPvB assessment, is the basis of waiving arguments for other endpoints, or has a large influence on the outcome of the risk assessment. The criteria in section 1.1.1 of Annex XI or section 1.2 of Annex XI to REACH must be met.

R.7.1.1.4 Overall consistency of the physicochemical information

[11] A read-across can also involve more than two substance: one-to-one (one analogue used to make an estimation for a single substance) b) many-to-one (two or more analogues used to make an estimation for a single substance c) one-to-many (one analogue used to make estimations for two or more substances) d) many-to-many (two or more analogues used to make estimations for two or more substances).

The physicochemical data for a given substance cannot contain incompatible values for two or more properties (i.e. high boiling point and high vapour pressure at normal temperature). This consistency check should be always done and it can turn out to be particularly useful when measured values are significantly at odds with predictions from QSPR models. Indeed, in this case a wider assessment of the known physicochemical properties should be performed in order to determine the possible cause of the inconsistencies.

Concluding on classification and labelling and chemical safety assessment

Data on physicochemical properties not only determine the presence or absence of a physical hazard but also have also an impact on the sections of the chemical safety assessment concerning the environment and human health. The assessment determines the risk posed to humans and the environment from all stages of the substance's lifecycle. This includes its manufacture, transfer, use and disposal. Firstly, the physicochemical data set provides the input parameters for the purpose of the human and environmental exposure estimation. For example, the vapour pressure and particle size information are required to estimate the likely exposure of humans, both in the workplace and in consumer use as well as to estimate the likelihood of forming flammable/explosive vapour/dust-air mixtures. The volatility (vapour pressure) or the size and nature of particles are indicators of the potential for inhalation exposure. Particle size is also important for determining the likely dermal exposure and the presence of a dust explosion hazard. Viscosity is a key parameter in determining aspiration hazards. The physical state of a substance at the process temperature is important for determining possible hazards. Further, physico-chemical data are essential for the correct planning of (eco)toxicological studies and for the optimisation of the test conditions.

R.7.1.1.5 References for introduction of Physicochemical properties

Recommendations on the transport of dangerous goods, Manual of Test and Criteria, United Nations. http://www.unece.org/trans/danger/publi/manual/manual_e.html

Guidance on the Application of the CLP Criteria, Version 4.0 - 2013, ECHA. http://echa.europa.eu/web/guest/support/guidance-on-reach-and-clp-implementation

OECD[a] (2004) Principles for the Validation of (Q)SARs http://ecb.jrc.it/QSAR/home.php?CONTENU=/QSAR/background/background_oecd_principles.php

OECD[b] (2004) series on testing and assessment Number 49 The report from the expert group on (quantitative) structure activity relationships [(Q)SARs] on the principles for the validation of (Q)SARs. 2nd Meeting of the ad hoc Expert Group on QSARs http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2004)24&doclanguage=en

R.7.1.2 Melting point/freezing point

R.7.1.2.1 Type of property

The melting point contributes to the indentification of a substance and to the designation of its physical state (liquid or solid[12]) of a substance. A number of physical hazard classes are distinguished based on the physical state. Therefore the melting point of a substance and the consequent designation as liquid or solid has also consequences for the assignment of the correct hazard class. Furthermore, the melting/freezing point together with vapour pressure serves as an indicator for the physical state (liquid or solid) of a substance under specific conditions (e.g environmental conditions, manufacturing process conditions). As a result, with regard to environmental relevance the melting point can give an indication of the distribution of the substance within and between the environmental media (water, soil and air).

R.7.1.2.2 Definition

The melting temperature is defined as the temperature at which the phase transition from the solid to the liquid state occurs at atmospheric pressure and this temperature ideally corresponds to the freezing temperature. As the phase transition of many substances takes place over a temperature range, it is often described as the melting range. For some substances, the determination of the freezing or solidification point is more appropriate. Where, due to the particular properties of the substance, none of the above parameters can be conveniently measured, a pour point may be appropriate.

R.7.1.2.3 Test method(s)

Method A.1 of Regulation (EC) 440/2008 or OECD Test Guideline 102 should be generally used for testing. Any procedure given in A.1 may be used within the scope and applicability specifications. However, it is advisable to use the Differential Scanning Calorimetry (DSC) or Differential Thermo-Analysis (DTA) method since they give additional information about the thermal stability of the substance like decomposition onset and energy. If decomposition occurs during the melting point study, determination of the boiling point need not be carried out. In this case, if DSC has been used, conducting the experiment under inert gas should be considered.

R.7.1.2.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for melting/freezing point:

'The study does not need to be conducted below a lower limit of - 20 °C.'

Therefore, Annex VII to REACH does not require determination of the melting point below a lower limit of -20 ° C. The lower limit should be confirmed through testing, except where a (Q)SAR indicates a melting point of -50 ° C or lower.

[12] Definitions of physical states can be found in Section 1.0. of Annex I to the CLP Regulation.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria of Annex XI, section 1.1.1, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in Annex XI, section 1.3.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria of Annex XI, section 1.1.1 or where several sources of similar reliability with deviating results exist, a weight-of-evidence approach may be used. The criteria of Annex XI, section 1.2 must then be met[13].

(Q)SAR

For the determination of the melting point, (Q)SAR approaches are discouraged, because the accuracy is not sufficient (± 25 °C or more) for the purposes of classification / risk assessment.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the melting point read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Some substances will decompose or sublime before the melting point is reached.

Further adaptation possibilities

Not foreseen.

R.7.1.2.5 Impurities; uncertainties

Impurities can have a significant influence on the melting point, as they will generally lower the melting point noticeably. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.2.6 Endpoint specific information in the registration dossier / IUCLID

Materials and methods

- type of method or reference to the standard or the test method applied.

Results and discussion

[13] National Institute of Standards and Technology (NIST) have a useful statistical approach which has been used for the evaluation of literature melting point data (ref.: http://webbook.nist.gov/chemistry/site-cal.html#AVG).

- melting point value (°C) as measured;
- rate of temperature increase if available;
- decomposition or sublimation temperature (if applicable);
- measurement uncertainty if available;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on melting point/freezing point can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.2	VII 7.2	Melting point/freezing point	E.4.3	3.2

R.7.1.3 Boiling point

R.7.1.3.1 Type of property

The boiling point is a property:

- which contributes to the characterisation of a substance and to the designation of its physical state (gas or liquid);
- which is the basis for the assignment of the correct hazard class because a number of physical hazard classes are distinguished based on the physical state;
- which is needed for the classification of flammable liquids into categories;
- which gives an indication of the distribution of the substance within and between the environmental compartments (air, soil and water);
- which have correlations with vapour pressure and therefore gives indications whether a substance may be available for inhalation as a vapour or may form flammable/explosive vapour-air mixtures, too;
- which is important for physical hazard assessment.

R.7.1.3.2 Definition

The normal boiling point is the temperature at which the vapour pressure of a liquid equals 101.3 kPa.

Note: If the vapour pressure equals 101.3 kPa or more at a given temperature this means the substance is completely gaseous at that temperature. If this is the case at temperatures ≤20°C the substance is a gas also according to the CLP Regulation.

R.7.1.3.3 Test method(s)

Method A.2 of Regulation (EC) 440/2008 or OECD Test Guideline 103 should be used for testing. Any determination method may be used within the scope and applicability specifications. DSC allows the determination of the melting and boiling point in a single test. Likewise, for some substances a single test can be used to determine both 'boiling point' and 'vapour pressure', as when the dynamic method is applied.

For high-boiling liquids or liquids which may decompose, auto-oxidize etc. before the boiling point at 101.3 kPa or more is reached, it is recommended to determine the boiling point either under inert gas or at reduced pressures, in order to derive the boiling point at reduced pressures from the vapour pressure curve.

If explosive substances, pyrophoric substances or self-reactive substances are to be characterized, determination of the boiling point is in general not practicable. For pyrophoric substances testing under inert gas or reduced pressures should be considered.

Where standards are applicable, the use of the most recent updates is advised; they are accessible via numerous websites, see above in section R.7.1.1.3.

R.7.1.3.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for boiling point:

'The study does not need to be conducted:

- *for gases; or*
- *for solids which either melt above 300 °C or decompose before boiling. In such cases the boiling point under reduced pressure may be estimated or measured; or*
- *for substances which decompose before boiling (e.g. auto-oxidation, rearrangement, degradation, decomposition, etc.).'*

Therefore the Annex VII to REACH does not require determination of the boiling point if:

- the substance is a gas;

However, for some gases the boiling point may be relevant. In the CLP Regulation, the boiling point is the main criterion to distinguish gases from liquids (see Annex I, section 1.0: Gas means a substance which (i) at 50 °C has a vapour pressure greater than 300 kPa (absolute); or (ii) is completely gaseous at 20 °C at a standard pressure of 101.3 kPa). Therefore it is important to report the boiling point in borderline cases where the transition from liquid to gas occurs close to 20 °C.

- the melting point of the substance is above 300 °C or when any chemical change occurs during the melting point study;
- the substance decomposes before boiling at ambient pressure.

In such cases the boiling point under reduced pressure (down to 0.2 kPa) should be determined if possible without decomposition.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria of Annex XI, section 1.1.1, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in Annex XI, section 1.3.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria of Annex XI, section 1.1.1 or where several sources of similar reliability with deviating results exist, a weight-of-evidence approach may be used. The criteria of Annex XI, section 1.2 must then be met[14].

(Q)SAR

For the determination of the boiling point, (Q)SAR approaches are discouraged for the purpose of classification / risk assessment, except when the mean absolute error of the method is lower than 2 K.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the boiling point read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing is not possible if:

- the substance is an explosive;
- the substance is self-reactive;
- any chemical change occurs during the melting point study;
- the liquid decomposes before the boiling point is reached even at reduced pressures below 0.2 kPa.

In such cases the decomposition temperature in relation to the (reduced) pressure should be reported, in order to allow determination of whether it is the substance itself or its decomposition products that should be considered under environmental conditions for the purpose of risk assessment. The details of the determination method should also be reported.

Further adaptation possibilities

Data generated with the same tests and classification principles as specified in the CLP Regulation on boiling point generated in conjunction with transport classification can be

[14] The NIST have a useful statistical approach which has been used for the evaluation of literature boiling point data (ref.: http://webbook.nist.gov/chemistry/site-cal.html#AVG).

deemed to satisfy the REACH requirements on a case-by-case basis. As stated in Annex IX of the REACH Regulation, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

R.7.1.3.5 Impurities; uncertainties

Impurities can have a significant influence on the boiling point. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.3.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- type of method or reference to the standard or the test method applied.

Results and discussion

- boiling point value (°C) as measured;
- pressure value and unit;
- rate of temperature increase if available;
- decomposition (if applicable);
- measurement uncertainty if available;
- boiling point value in ºC (corrected to standard pressure, except where the boiling point has been determined at specified reduced pressures) (as above, but in a separate block of fields);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Note: In cases where the boiling point is determined at reduced pressure a determination at ambient pressure is obviously not possible. A boiling point at standard pressure could then only be derived by extrapolation of the vapour pressure curve in cases where a vapour pressure curve is known. Even in such cases this corrected/extrapolated boiling point could only be nominal one and would be potentially misleading because it is not possible to determine it at ambient pressure.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on boiling point can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.3	VII 7.3	Boiling point	E.4.4	3.3

R.7.1.4 Relative density

R.7.1.4.1 Type of property

For gaseous materials, relative density is of value in determining the tendency to settle or to disperse when discharged at high concentrations into the atmosphere. The relative density of gaseous substances can be calculated from molecular weight using the Ideal Gas Law.

For insoluble liquids and solids, (absolute) density will be a determining factor in the settling of the substance.

R.7.1.4.2 Definition

Density (ρ) of a substance is the quotient of the mass m and its volume V:

$$\rho = m/V \qquad \text{SI units } (kg/m^3)$$

The relative density is related to a standard, the density of which is set to 1. It has no dimension. For gases air is used as standard so that gases with a relative density of less than 1 are lighter than air (and and those with a value above 1 heavier).

The relative density, D_4^{20}, of solids or liquids is the ratio between the mass of a volume of substance to be examined, determined at 20 ºC, and the mass of the same volume of water, determined at 4 ºC (at which temperature, water has its maximum density, i.e. 999.975 $kg/m^{3)}$.

R.7.1.4.3 Test method(s)

Test methods for determining (absolute) density are applicable to solids and liquids. Table R.7.1-3 lists the respective test methods.

Table R.7.1-3 Test methods for determining density

Method	Applicability	Maximum Dynamic Viscosity (Liquids only) / Pa.S
Hydrometer	Liquids	5
Hydrostatic balance	Solids and Liquids	5
Immersion ball	Liquids	20
Pycnometer	Solids and Liquids	500
Air comparison pycnometer	Solids	-
Oscillating densitimeter	Liquids	5

EU Test guideline A.3 for relative density Regulation (EC) No 440/2008 includes a list of standards with technical information about the different methods and actual measuring of different types of substances.

R.7.1.4.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for relative density:

'The study does not need to be conducted if:

- *the substance is only stable in solution in a particular solvent and the solution density is similar to that of the solvent. In such cases, an indication of whether the solution density is higher or lower than the solvent density is sufficient; or*
- *the substance is gaseous at room temperature. In this case, an estimation based on calculation can be made from its molecular weight and the Ideal Gas Laws.'*

For liquids, it is useful to have some indication of the dynamic viscosity as this can affect the choice of method. The physical state of test substances should always be homogeneous, this is particularly relevant for highly viscous substances where internal bubbles can be formed; in these cases, the test substance should be allowed to rest until all internal bubbles have disappeared.

The summary should include the numerical value for density and temperature at which it was measured, test material identity, purity of the sample used, physical state, method and guideline used and reference substance (if any).

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met[15].

(Q)SAR

(Q)SAR is generally not applicable for determination of relative density.

For this endpoint there are often experimental measurements and therefore QSPR models for this property have not received special attention in the environmental literature. Several software programs can be used to calculate the density of a given substance but the documentation and validation of the methods is limited.

[15] The NIST have a useful statistical approach which has been used for the evaluation of literature data (ref.: http://webbook.nist.gov/chemistry/site-cal.html#AVG.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the relative density read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies. Waiving relative density testing on the basis of not being technically possible is not applicable.

Further adaptation possibilities

Not foreseen.

R.7.1.4.5 Impurities; uncertainties

Impurities can have a significant influence on the density. This influence depends on the amount and density of the impurity; thus, the higher the amount of impurity and the higher the difference between the densities of the main component and the impurity, the higher the influence. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

Density is temperature dependant. Whenever possible, determinations should be performed at 20°C.

R.7.1.4.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- type of method or reference to the standard or the test method applied.

Results and discussion

- temperature (°C);
- relative (for gases)/ absolute (for liquids and solids) density value (dimensionless);
- measurement uncertainty if available;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on relative density can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.4	VII 7.4	Relative density	E.4.5	3.4

R.7.1.5 Vapour pressure

R.7.1.5.1 Type of property

Vapour pressure is a property:

- for substance characterisation;
- which serves as a key parameter for assessing some toxicological and environmental hazards;
- which gives indications whether a substance may be available for inhalation as a vapour or may form flammable/explosive vapour-air mixtures;
- which allows determination of the volatility of a substance from an aqueous medium or soil, in terms of the Henry's Law constant (Appendix R.7.1-1) and partition coefficient air/soil, respectively;
- which allows determination of the right container/vessel to ensure safety during storage, transport and use;
- which is importiant for physical hazard assessment.

R.7.1.5.2 Definition

The vapour pressure of a substance is defined as the saturation pressure above a solid or a liquid substance at constant temperature. At the thermodynamic equilibrium, the vapour pressure of a pure substance is a function of temperature only.

R.7.1.5.3 Test method(s)

Method A.4 of Regulation (EC) 440/2008 or OECD Test Guideline 104 (Vapour pressure) should be used for testing. It is useful to have preliminary information on the structure, the melting point and the boiling point of the substance to perform this test.

There is no single measurement procedure applicable to the entire range of vapour pressure values. Therefore, several methods are recommended to be used for the measurement of vapour pressure from < 10^{-10} to 10^{5} Pa. For the selection of the test method the scope and applicability specifications have to be taken into account. The results should be checked for consistency with other physical data like boiling point, flash point etc.

It is recommended to determine the vapour pressure at least for two temperatures, for volatile substances (boiling point up to 150 °C) preferably at 20 °C and at 50 °C.

Where standards are applicable, the use of the most recent updates is advised, please check section R.7.1.1.3 for further information.

R.7.1.5.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for vapour pressure:

'The study does not need to be conducted if the melting point is above 300 °C.

If the melting point is between 200°C and 300°C, a limit value based on measurement or a recognised calculation method is sufficient.'

Vapour pressure testing is also not required for substances with a standard boiling point of < 30 ºC, as these substances will have vapour pressures above the limit of measurement (i.e. 10^5 Pa).

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

For the determination of the vapour pressure, (Q)SAR approaches may be used if determination by experiment is not possible.

The vapour pressure depends on the temperature. This dependence was modelled by Grain (Grain, 1982), based on thermodynamic principles. The estimation methods differ for vapour pressure that can be applied for compounds that are liquid or gaseous at the temperature of interest, and for solid and liquid compounds. The former can be estimated by the Antoine equation, while the latter could be predicted by the Watson correlation, which accounts also for the heat of vaporisation. Another method, described by Mackay *et al*. (1982), is applicable only for hydrocarbons and halogenated hydrocarbons. Further, the Grain model was modified to be applicable for all solids, liquids, and gases. These methods are still in practical use today.

The OECD guideline 104 reports that the Watson correlation is applicable over the pressure range from 10^5 Pa to 10^{-5} Pa. It should in any case be pointed out that estimated values for vapour pressure can be subjected to great uncertainty if the computed pressure is lower than 1 Pa, especially when the boiling point has not been experimentally determined (OECD monograph 67). The uncertainty is even greater if the estimated value is used together with water solubility in order to estimate the Henry's Law constant.

The environment monograph 67 of the OECD describes all of the above mentioned methods and the OECD guideline 104 supports the use of the Watson correlation for the calculation of vapour pressure, but does not specifically reject other calculation methods.

The handbook for estimating the physico-chemical properties of organic compounds (Reinhard and Drefahl, 1999) reports another method based on thermodynamic properties and elaborated by Mishra and Yalkowsky that discussed the application of the method of Mackay (Mackay *et al*., 1982).

The equation by Mishra and Yalkowsky gave significantly better estimates than the method of Mackay on the same data set (Mishra and Yalkowsky, 1991).

Another methodology that proved to be effective in estimating vapour pressure relies on group contribution approaches. Several models using this strategy have been proposed (Reinhard and Drefahl, 1999; see Table R.7.1-4).

Table R.7.1-4 Group contribution approach and vapour pressure

Compounds	Authors	Methodology	Statistics
Alkyl aromatic compounds	Amidon and Anik	Group contribution approach	Standard error ¼ 1.1 kJ on the estimation for the free energy of vaporisation
Mono-, di-, tri- and tetra substituted	Hoshino *et al.*	Group contribution approach	Average error 3.7% Max. Error 30.9%
Perfluorinated saturated hydrocarbons	Kelly *et al.*	Group contribution approach	Arithmetic mean deviation <0.5%

Numerous other models are available for the estimation of vapour pressure, and Schwarzenbach *et al.* (1993), Delle Site (1996), Sage and Sage (2000) and Dearden (2003) have reviewed many of these. The descriptors used in vapour pressure QSPRs include physico-chemical, structural and topological descriptors, and group contributions. Katritzky *et al.* (1998) used 4 CODESSA descriptors to model the vapour pressure (in atmospheres at 25 °C) of 411 diverse organic chemicals, with r^2 = 0.949 standard error = 0.331 log unit. A number of studies (Andreev *et al.* 1994, Kühne *et al.* 1997, Yaffe & Cohen 2001) allow of the estimation of vapour pressures over a range of temperatures.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of vapour pressure read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Vapour pressure testing is not required for substances with a standard boiling point of < 30 ºC, as these substances will have a vapour pressure value above the limit of measurement (i.e. 10^5 Pa).

For substances which decompose during measurement or which are unstable or explosive, determination of the vapour pressure may not be technically possible. This also applies to self-reactive substances and organic peroxides.

Pyrophoric substances may be difficult to handle experimentally. If fully inert conditions cannot be maintained during sample preparation and measurement, use of an appropriate calculation method is recommended.

A calculation method should also be applied in the case of some corrosive substances which would destroy essential metallic parts of the measurement apparatus.

Further adaptation possibilities

Not foreseen.

R.7.1.5.5 Impurities; uncertainties

Impurities can have a large influence on vapour pressure. The influence depends on the amount of the impurity and the vapour pressure of that impurity. Small amounts of volatile impurities may increase the vapour pressure by several orders of magnitude. This has to be kept in mind when performing the measurements and for the interpretation of results. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

Where there are volatile impurities in the sample which could affect the result, the substance may be purified. Test method A.4 states that it may also be appropriate to quote the vapour pressure for the technical material. However, in consideration of the large effect that impurities may have (see above), doing so is strongly discouraged.

R.7.1.5.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- type of method or description of the apparatus or reference to the standard or the test method applied.

Results and discussion

- if testing is waived, the reasons for waiving must be documented in the dossier;
- measured value of the vapour pressure for at least two temperatures;
- estimate of the vapour pressure at 20 or 25 ºC (if not measured at these temperatures);
- if a transition (change of state, decomposition) is observed, the following should be noted:
    - nature of change;
    - temperature at which change occurs.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on vapour pressure can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.6	VII 7.5	Vapour pressure	E.4.7	3.6

R.7.1.5.7 References on vapour pressure

OECD Guidelines for the Testing of Chemicals / Section 1: Physical-Chemical properties, Test No. 104: Vapour Pressure, OECD Code: 979910401E1, July 2006.

Andreev N.N, Kuznetsov S.E, Storozhenko S.Y. (1994) Prediction of vapour pressure and boiling points of aliphatic compounds. Mendeleev Commun. 173-174.

Grain C.F., (1982) Handbook of chemical property estimation methods. New York, Mc Graw-Hill

Delle Site A. (1996) The vapour pressure of environmentally significant organic chemicals: a review of methods and data at ambient temperature. J. Phys. Chem. Ref. Data 26:157-93.

Dearden JC. (2003) Quantitative structure-property relationships for prediction of boiling point, vapour pressure, and melting point. Environ Toxicol Chem 22(8):1696-709.

Katritzky AR, Y. W, Sild S, Tamm T, Karelson M. (1998) QSPR studies on vapour pressure, aqueous solubility, and the prediction of water-air partition coefficients. J. Chem. Inf. Comput. Sci. 38:720-5.

Kühne R, Ebert RU, Schüürmann G. (1997) Estimation of vapour pressures for hydrocarbons and halogenated hydrocarbons from chemical structure by a neural network. Chemosphere 34:671-86.

Mackay D, Bobra A, Chan W, Shiu WY. (1982) Vapour pressure correlation for Low-Volatility Environmental Chemicals. Environ. Sci. Technol. 16:645-9.

Mishra DS, Yalkowsky SH. Estimation of vapour pressure of some organic compounds. Ind. Eng. Chem. Res. 1991;30:1609-12.

OECD Guidelines for Testing of Chemicals, Method 104 “Vapour Pressure Curve”

Reinhard M, Drefahl (1999).A. Handbook for Estimating Physico-Chemical Properties of Organic Compounds. New York: Wiley.

Sage M.L, Sage G.W.(2000) Handbook of Property Estimation Methods for Chemicals. Boca Raton, FL: Lewis.

Schwartzenbach, R.P., Gswend, P.M., Imboden, D.M. (1993). Environmental Organic Chemistry. John Wiley and Sons.

Yaffe D, Cohen, Y (2001) Neural network based temperature-dependent quantitative structure property relationships (QSPRs) for predicting vapour pressure of hydrocarbons. J. Chem. Inf. Comput. Sci. 41:463-477.

R.7.1.6 Surface tension

R.7.1.6.1 Type of property

Surface tension measurements of aqueous solutions are significant since decreasing the surface tension of water may impact on the properties of the solution and other physicochemical measurements.

R.7.1.6.2 Definition

- Surface tension:

'The free surface enthalpy per unit of surface area is referred to as surface tension' (Council Regulation (EC) No 440/2008).

The surface tension is given as: N/m (SI unit) or mN/m (SI sub-unit). 1 N/m = 10^3 dyne/cm or 1mN/m = 1 dyne/cm in the obsolete cgs system.

The surface tension of an aqueous solution of a substance can be used to determine whether the substance is surface active.

- Surface active substance (surfactant):

''Surfactant' means any organic substance and/or preparation [mixture] *used in detergents, which has surface-active properties and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that it is capable of reducing the surface tension of water, and of forming spreading or adsorption monolayers at the water-air interface, and of forming emulsions and/or microemulsions and/or micelles, and of adsorption at water-solid interfaces'* (see Article 2(6) of Council Regulation (EC) No 648/2004).

R.7.1.6.3 Test method(s)

Testing should be done in accordance with one of the methods specified under section A.5 of Regulation (EC) No 440/2008. These methods are applicable to most chemical substances.

It is useful to have preliminary information on the water solubility, the structure, the hydrolysis properties and the critical concentration for micelles formation of the substance before performing the test.

Surface tension measurements require a test material that is stable against hydrolysis during the test period and soluble in water at concentrations of > 1 mg/l. Measurements should be performed on a solution at either 90 % of the solubility limit or 1 g/l (where viscosity permits), whichever is smaller.

R.7.1.6.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for surface tension:

'The study need only be conducted if:

- *based on structure, surface activity is expected or can be predicted; or*
- *surface activity is a desired property of the material.*

If the water solubility is below 1mg/l at 20 °C the test does not need to be conducted.'

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At the time of writing, no reliable (Q)SAR methods exist for sufficiently accurate predictions of surface tension.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the surface tension read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies. Testing may not be possible for reactive substances which react with water or air (hydrolyse, are pyrophoric, evolve gas, etc).

Further adaptation possibilities

Not foreseen.

R.7.1.6.5 Impurities; uncertainties

For the measurement of surface tension the ring or plate tensiometer methods are preferred. The error on the measurement is in the order of 0.1–0.3 mN/m. Use of the standard protocols and GLP procedures are recommended. Surface active impurities in substances may in some cases lead to false-positive surface tension measurements.

R.7.1.6.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;

- test material identity: apart from general issues, if surface tension of active impurities affects results, it should be noted.

Results and discussion

- surface tension value and unit (preferably mN/m or N/m but other units are also acceptable);
- concentration of the solution*[16];
- age of solution*;
- type of water or solution used*;
- results from repeated measurements with varied equilibrium time (of the solution);
- several measurement results should be provided to assess the possible time-dependency of the measurement. Equilibration times may vary from minutes to hours. Measurements should be sufficient to prove that a constant surface tension was reached;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on surface tension can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.10	VII 7.6	Surface tension	E.4.11	3.9

[16] *As indicated in test A.5. Surface tension described in Council Regulation (EC) No 440/2008).

R.7.1.7 Water solubility

Advice to registrants with regard to nanomaterials characterisation of water solubility can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.1 Water solubility.

R.7.1.7.1 Type of property

Water solubility is a significant parameter for a number of reasons:

- the mobility of a test substance is largely determined by its solubility in water. In general, highly soluble substances are more likely to be distributed by the hydrological cycle;
- water soluble substances gain access to humans and other living organisms;
- knowledge of the water solubility is a prerequisite for setting up test conditions for a range of fate (e.g. biodegradation, bioaccumulation) and effects studies;
- it is also used to derive other environmental parameters, such as K_{ow}, K_{oc} and Henry's Law Constant (Appendix R.7.1-1). It is also used as input for some QSAR models;
- water solubility is used as a regulatory trigger for waiving certain physicochemical and ecotoxicological endpoints.

R.7.1.7.2 Definition

'The solubility of a substance in water is specified by the saturation mass concentration of the substance in water at a given temperature. The solubility in water is specified in units of mass per volume of solution. The SI unit is kg/m^3 (grams per litre may also be used)' (see Regulation (EC) No 440/2008, A.6, section 1.2).

Mixtures of organic compounds, e.g. petroleum substances, behave differently from their single constituent compounds when brought into contact with water. Petroleum substances are typically hydrophobic and exhibit low solubility in water. However, reflecting the range of structures, constituent hydrocarbons will exhibit a wide range of water solubility. Therefore, water solubility measurements for these substances are loading rate dependent due to their complex composition. This water solubility behaviour impacts on both the conduct and interpretation of aquatic toxicity tests for these complex substances. The complex composition, and generally low water solubility, impact also on the choice and conduct of biodegradation studies.

Consequently, the above definition for solubility of a single substance in water is not applicable to substances which are multi-component, such as multi-constituent or UVCB substances, i.e. complex substances. The usually accepted meaning of 'solubility' in such cases is 'the composition of the aqueous solution formed at equilibrium under a defined set of conditions'. Temperature and the amount of substance added per unit volume of water (i.e. the 'loading') are the main factors to consider. It may not always be possible to establish that equilibrium of all components has been achieved; in these cases, time and type of agitation of the test vessels must also be described.

Similar testing issues also apply to inorganic compounds. Water solubility among compounds of the same metal may differ by several orders of magnitude. Differences in the solubility of metal compounds are related to the metal species and the characteristics of the aqueous medium. Highly soluble inorganic metal compounds can be assessed through the normal procedures. For sparingly soluble metal compounds, a solubility product can be calculated

thermodynamically (e.g. by using the Facility for Analysis of Chemical Thermodynamics ('F*A*C*T', FACT-Win version 3.05). Although metals are generally insoluble, metals in the elemental state may react with water or a dilute aqueous electrolyte to form soluble or sparingly soluble cationic or anionic products. During this process the metal will oxidise, or transform, from the neutral or zero oxidation state to a higher oxidation state. The OECD Test Guidance on transformation/dissolution of metals and sparingly soluble metal compounds (OECD, 2001) can be used to determine the rate and extent to which metals and sparingly soluble metal compounds can produce soluble bioavailable ionic and other metal-bearing species in aqueous media under a set of standard laboratory conditions representative of those generally occurring in the environment. The outcomes of the transformation/dissolution tests are to be used for aquatic environmental hazard classification purposes.

R.7.1.7.3 Test method(s)

No single method is available to cover the whole range of solubility values in water, from relatively soluble to very low soluble substances. General test guidelines (OECD Method 105; EU Method A.6, Regulation (EC) No 440/2008) include two test methods which cover the whole range of solubility values but are not applicable to volatile substances. Water solubility determinations are normally run at 20 ºC in distilled water according to standard test guidelines (OECD Method 105; EU Method A.6). Solubility data determined using these standard physico-chemical guidelines may differ if the test material is solubilised in either aqueous solutions containing salts or at different test temperatures (or both) (e.g. ecotoxicological test media).

The methods should be applied to essentially pure substances that are stable in water. Details of suitable methods are shown in Table R.7.1-5.

A number of standardised methods are available for the determination of single substances and complex mixtures of liquids and solids. For metals and sparingly soluble inorganic metal compounds a specific water solubility approach was designed to measure transformation to the dissolved fraction under standard conditions. The test methods are not applicable to volatile substances. Care should be taken to ensure that the test substances examined are as pure as possible and their solubility levels are determined analytically using a specific analytical method wherever possible. Precautions should be taken to minimise degradation of the test substance, in particular if long periods of equilibration are required (e.g. 'slow stir' methods).

Measurement of water solubility does not usually impose excessive demands on chemical techniques. However, measurement of the solubility of sparingly soluble compounds requires extreme care to generate saturated solutions of the material without the introduction of dispersed material; invariably specific methods of analysis are able to determine the low levels (sub ppb-ppm) in solution. Reported water solubility data for such compounds can often contain appreciable errors.

Table R.7.1-5 Test methods for the determination of water solubility

Method details	Applications and requirements	Repeatability and sensitivity
Column elution method Based on elution of the test substance with water from a micro-column which is charged with an inert carrier material such as glass beads, silica gel or sand and an excess of test substance. The water solubility is determined when the mass concentration of the eluate is constant. The mass concentration of the test substance is determined analytically	Applicable to essentially pure substances only Used for low solubilities (< 10^{-2} g/l) Organic substances, but not mobile oils or liquids	< 30% ; down to 1 µg/l
Flask method The test substance is dissolved in water at a temperature somewhat above the test temperature. When saturation is achieved the mixture is cooled and kept at the test temperature, stirring as long as necessary to reach equilibrium The mass concentration of the test substance is determined analytically	Applicable to essentially pure substances and also complex substances. Use of fast stirring techniques (300-400 rpm) appropriate for higher solubility (> 10^{-2} g/l) test substances. Use of slow-stirring techniques (<100 rpm) appropriate for low solubility (< 10^{-2} g/l) test substances (Letinski et al, 2002) Requires equilibration study to determine the time taken to equilibrate the test substance and water	< 15%; down to 1 µg/l
OECD series on Testing and Assessment Number 29 - Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous media.	Applicable to all metals and sparingly soluble inorganic metal compounds	/

R.7.1.7.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for water solubility:

'The study does not need to be conducted if:

- *the substance is hydrolytically unstable at pH 4, 7 and 9 (half-life less than 12 hours); or*
- *the substance is readily oxidisable in water.*

If the substance appears 'insoluble' in water, a limit test up to the detection limit of the analytical method shall be performed.'

For ionising substances, the pH-dependence of the water solubility should be known. At least the pH of the test water needs to be identified. In the context of marine risk assessment, when the pK_a is close to 8 it may be necessary to obtain realistic measurements using seawater.

For volatile compounds, it can be useful to have information on the vapour pressure.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Most physical properties, such as molecular weight, melting point, boiling point, density and water solubility can be obtained from commonly used environmental Handbooks, such as Verschueren's Handbook of Environmental Data on Organic Chemicals (1983), Howard's Handbook of Environmental Fate and Exposure Data, Vol. I and II (1990), Lide's CRC Handbook of Physics and Chemistry, Lange's Handbook of Chemistry, the Merck Index, the Aldrich Catalog, Kirk-Othmer Encyclopaedia of Chemical Technology and other handbook compilations such as Riddick *et al*. (1986).

Alternatively, searching on various environmental databases, such as HSDB (http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB), will provide summaries of chemical and physical properties of substances.

It is not unusual to find in the literature a wide range of solubilities for the same product. The oldest literature generally yields the highest solubility values: this is due to the fact that products were originally not as pure as they are nowadays and also non-specific methods were used which would not differentiate between the dissolved product and any impurities. Reported water solubility data for such compounds can often contain appreciable errors. Therefore, the reliability of data must be demonstrated by providing information on the identity and purity of the test substance, the methodology used to make the measurement, and whether or not this was performed to GLP standards.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Secondary data sources can be used in a WoE approach and they can collectively support the choice of a specific value for the water solubility. These secondary sources have to be based on a critical evaluation of peer-reviewed data and a consequent selection of a reliable and representative value for the water solubility. The use of Klimisch codes, can be extended to these secondary sources and a reliability code of (2) valid with restrictions should be assigned when using an authoritative secondary source.

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

For an organic solute to dissolve in water, firstly, the solute molecules must be separated from one another. Secondly, the solvent molecules must become partially separated from one another to create a cavity large enough to accommodate the solute. Once the solute occupies the cavity, there will be new attractive forces between solute and solvent. Finally, the water molecules in the solvation shell will form extra H-bonds to neighbouring water molecules. Thus, the water solubility depends not only on the affinity of a solute for water, but also on its affinity for its own structure. Molecules that are strongly bound to each other require considerable energy to separate them. This also means that such compounds have high melting points (for solids). Generally, solids with a high-melting temperature have poor solubility in any solvent.

Removal of a molecule from its crystal lattice means an increase in entropy, and this can be difficult to model accurately. For this reason, as well as the fact that the experimental error on solubility measurements can be quite high (generally reckoned to be about 0.5 log unit), the prediction of aqueous solubility is not as accurate as is the prediction of octanol/water partitioning. Nevertheless, many papers (Dearden 2006) and a book (Yalkowsky & Banerjee 1992) have been published on the prediction of aqueous solubility, as well as a number of reviews (Lyman 1990, ECETOC 1998, Reinhard & Drefahl 1999, Mackay 2000, Schwarzenbach *et al.* 2003, Dearden 2006). There are also a number of software programs available for that purpose (ECETOC 2003, Dearden 2006). Livingstone (2003) has discussed the reliability of aqueous solubility predictions from both QSPRs and commercial software.

It should be noted that there are various ways that water solubilities can be reported: in pure water, at a specified pH, at a specified ionic strength, as the undissociated species (intrinsic solubility), or in the presence of other solvents or solutes. Solubilities are also reported in different units, for example g/100 ml, mole/litre, mole fraction. The use of mole/litre is recommended, as this provides a good basis for comparison.

For solids, work has to be done to remove molecules from their crystal lattice, and the simplest way to account for this is to use what Yalkowsky and co-workers have termed the general solubility equation (GSE), which incorporates a melting point term to account for the behaviour of solids (Sanghvi *et al* 2003):

$\log S_{aq} = 0.5 - \log K_{ow} - 0.01(MP - 25)$

where MP is the melting point (^{o}C). The melting point term is taken as zero for compounds melting at or below 25^{o}C. Calculated log K_{ow} and MP values can be used in the GSE, although measured values are preferred. Aqueous solubilities of 1026 non-electrolytes, with a log S_{aq} range of – 13 to + 1 (S in mole L^{-1}), calculated with the GSE had a standard error of 0.38 log unit.

Good predictions for a large diverse data set have been obtained by the use of linear solvation energy descriptors (Abraham & Le 1999). These included two terms for polarity/polarisability, the sums of hydrogen bond donor and acceptor abilities of the solute molecule, and an expression of molecular volume

According to the Abraham and Le equation, the main factors controlling aqueous solubility seem to be hydrogen bond acceptor ability and molecular size, both of which are important elements in the molecular mechanisms of solubility.

Solubility can vary considerably with temperature, and it is important that solubility data are reported at a given temperature.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the water solubility read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

For this endpoint, testing should almost always be possible and water solubility should usually be determined experimentally. Nonetheless, testing by the flask method might be precluded when the high viscosity of the saturated test solutions prevent from normal stirring. If it is technically not possible to conduct the study as a consequence of the properties of the substance (e.g. substances flammable in contact with water or substances readily oxidisable in water), testing may be omitted according to general rules for adaptation of the standard testing regime described in REACH Annex XI, Section 2.

Further adaptation possibilities

Not foreseen. However, for complex substances the information obtained from such testing is not relevant or of practical use, and therefore conducting the test may be waived where the data is irrelevant for subsequent assessments.

R.7.1.7.5 Impurities; uncertainties

The water solubility of the test substance can be considerably affected by the presence of impurities.

For a complex substance, the measured solubility is dependent on the amount of test substance added. In practical terms, solubility data are generated using at least two loading rates (e.g. 100 mg/l and 1000 mg/l). Accuracy in determining water solubility decreases as the water solubility of a test substance is reduced (e.g. as shown for reference substance data in OECD Method 105). When dealing with test substances with water solubilities of the order of < 10 µg/l, precautions need to be taken to avoid the introduction of dispersed material into the final extract.

Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.7.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- results from preliminary test (if any);
- chemical identity and impurities (preliminary purification step, if any);
- water temperature during saturation process;
- analytical method employed;
- any evidence of chemical instability;
- all information relevant for the interpretation of the results.

If Column Elution method:

- concentrations, flow rates and pH for each sample;
- mean and standard deviation of five samples at least;
- average for each of two successive runs at least;

- nature and loading of support material;
- solvent used.

If Flask method:

- pH of each sample;
- individual analytical determinations and the average;
- average of the values for different flasks.

Results and discussion & Applicant's summary and conclusion

- water solubility in (mg/l) at temperature (°C);
- pH value and concentration of test substance;
- description of solubility (if relevant);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on water solubility can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.8	VII 7.7	Water solubility	E.4.9	3.8

R.7.1.7.7 References on water solubility

Abraham M.H. and Le J. (1999) The correlation and prediction of the solubility of compounds in water using an amended solvation energy relationship. *J. Pharm. Sci.* 88, 868-880.

Dearden J.C. (2006) *In silico* prediction of aqueous solubility. *Expert Opinion on Drug Discovery* 1, 31-52.

EC Method A6. "Water Solubility", Dir 92/69/EEC, Official Journal of the European Communities, O.J. L383 A)

EC Method A7. "Hydrolysis ", Dir 92/69/EEC, Official Journal of the European Communities, O.J. L383 A)

ECETOC Technical Report No. 74: *QSARs in the Assessment of the Environmental Fate and Effects of Chemicals*. ECETOC, Brussels, 1998.

ECETOC Technical Report No. 89: (Q)SARs: *Evaluation of the Commercially Available Software for Human Health and Environmental Endpoints with Respect to Chemical Management Applications.* ECETOC, Brussels, 2003.

Livingstone D.J. (2003) Theoretical property predictions. *Current Topics in Med. Chem.* 3, 1171-1192.

Letinski, D.J., Connolly, M.J., Peterson, D.R. and Parkerton, T.F. (2002) "Slow-stir water solubility measurements of selected alcohols and diesters", *Chemosphere*, 48, 257 – 265).

Mackay D. Solubility in water. In Boethling R.S. and Mackay D. (Eds.), *Handbook of Property Estimation Methods for Chemicals: Environmental and Health Sciences.* Lewis, Boca Raton, FL, 2000, pp. 125-139.

OECD Environmental Health and Safety Publications, 2000. Number 23. Guidance document on aquatic toxicity testing of difficult substances and mixtures.

OECD Environment, Health and Safety Publications, 2001. Series on Testing and Assessment, No. 29, Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media

OECD Guidelines for Testing of Chemicals – Method 105 "Water Solubility"

OECD Guidelines for Testing of Chemicals – Method 111 "Hydrolysis as a Function of pH"

Reinhard M. and Drefahl A. Handbook for Estimating Physico-chemical Properties of Organic Compounds. Wiley, New York, 1999.

Sanghvi T., Jain N., Yang G. and Yalkowsky S.H. (2003) Estimation of aqueous solubility by the general solubility equation (GSE) the easy way. *QSAR Comb. Sci.* 22, 258-262.

Schwarzenbach R.P., Gschwend P.M. and Imboden D.M. (2003) *Environmental Organic Chemistry*, 2nd edition, Wiley, Hoboken, NJ.

Yalkowsky S.H. and Banerjee S. (1992). Aqueous Solubility: Methods of Estimation for Organic Compounds. Marcel Dekker, New York.

R.7.1.8 Partition coefficient n-octanol / water

Advice to registrants with regard to nanomaterials characterisation of water solubility can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.2 Partition coefficient n-octanol/water.

R.7.1.8.1 Type of property

The n-octanol/water partition coefficient (K_{ow}) is one of the key physicochemical parameters, and it is used in numerous estimation models and algorithms for environmental partitioning, sorption, bioavailability, bioconcentration, bioaccumulation and also human toxicity and ecotoxicity. As such K_{ow} is a critical parameter for chemical safety assessment, classification and labelling, and PBT assessment/screening (where required).

The generation of a K_{ow} value is required at all tonnage bands (i.e. > 1 t/y; information requirements according to REACH Annexes VII-X).

R.7.1.8.2 Definition

The n-octanol/water partition coefficient (K_{ow}) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of the largely immiscible solvents n-octanol and water. The property is moderately temperature-dependent and typically measured at 25 °C. For further information on definition and units please see the Test Methods Regulation ((EC) No 440/2008), test method A.8, section 1.2.

R.7.1.8.3 Test method(s)

EU test method A.8 of the Test Methods Regulation ((EC) No 440/2008) describes two test procedures; a direct measurement via the Shake Flask method (OECD Test Guideline 107) and a correlation approach using the HPLC method (OECD Test Guideline 117). The Shake Flask method falls within the logKow range -2 to 4 and the HPLC method within the range 0 to 6. The applicability of the methods differ depending on the substance type and the amount of impurities in the test substance. Neither of the methods is applicable to surface active materials, for which an estimated value based on individual solubilities, or a calculated value along with calculation details should be provided. As with any endpoint and predictive method, the documentation and training set of the predictive method should be examined carefully to decide whether it is applicable to special categories of substances, such as zwitterionic or surface active substances.

Regardless of the method used, highly accurate measurements of log K_{ow} > ~5 are complicated by the fact that small amounts of octanol are entrained in the aqueous phase, leading to a potential underestimation of the measured log K_{ow} values. All of the direct methods for measuring log K_{ow} require quantifying the test material in either octanol or water and preferably in both matrices.

In addition, the OECD test guideline 123, Slow-stirring method, can be used to generate data for this endpoint.

Table R.7.1-6 Methods for determination of partition coefficient n-octanol/water

Method details	REPEATABILITY	APPLICABILITY RANGE
Shake Flask Method (EU A.8, OECD TG 107) The Shake Flask method is the default procedure. It is considered to give accurate results for low to medium hydrophobic substances. For substances with a high expected log K_{ow}, alternative methods are recommended. A suitable analytical method is needed to determine the concentration of the test material in the octanol and water phases. By applying mass balance considerations, it may be possible to measure the test material in only the less-soluble phase. However, this approach significantly decreases the reliability in the reported value. This technique is not suitable for surface active compounds (surfactants), or compounds that hydrolyse rapidly.	Three replicates should fall within +/- 0.3 log K_{ow}	-2 < log K_{ow} < 4
HPLC Method (EU A.8, OECD TG 117) This is a relatively quick way of estimating log K_{ow}. It is not measured directly, but from a correlation between log k (capacity factor) and log K_{ow} for a series of reference substances. It therefore depends on the quality of the log K_{ow} measurement of reference substances (often measured by the shake flask method). A series of reference compounds with similar chemical functionality to the test material should be used to generate the log k: log K_{ow} correlation. In general, the HPLC method is less sensitive to impurities than the shake flask method. The RP-HPLC is not recommended for strong acids and bases, metal complexes or surface active agents, or for measurements across very different classes of substances. The HPLC method is also very suitable for measuring the K_{ow} of mixtures of chemical homologues.	Three replicates should fall within +/- 0.1 log K_{ow}	0 < log K_{ow} < 6
Slow-Stirring Method (OECD TG 123) This is a more recent method developed as an alternative to the shake flask procedure (OECD TG 107, EU A.8). The advantage of slow stirring versus shaking is that emulsion formation will be reduced. The method requires a few days to reach equilibrium. The method may be difficult to adapt to a high throughput approach. As with the other direct methods, a suitable analytical method is needed to measure the concentration of the test material in the octanol and water phases. NB: Radiolabelled substances – which may be synthesised for use in other tests – can be very useful for accurate log K_{ow} determination.	Intralaboratory median standard deviation from 0.15 – 0.3 Log K_{ow} (Tolls et al, 2003).	Validation has shown that this method can also be used for very hydrophobic substances, up to Log K_{ow} 8.3 (OECD 2003, Tolls *et al*, 2003).
Estimation method based on individual solubilities in EU A.8 This method enables partition coefficients to be estimated based on the ratio of the solubility of the material in octanol and water. For some substances (e.g. some surfactants and pigments) it is technically not feasible (or good practice) to measure an octanol-water partition coefficient by OECD 107. For such substances it		

may be possible to obtain a ratio of the saturated water solubility (OECD 105) and saturated octanol solubility (no guideline currently available but based on the principles of OECD 105). This method however has the drawback that it does not include the interaction between the water and solvent phase (i.e. a substance with high Kow is rather 'pushed out of the water' than 'pulled into octanol'). This explains the poor correlation typically observed between octanol solubility and K_{ow} (Dearden, 1990, Sijm *et al*., 1999). The ratio was found to be somewhat more representative if one uses octanol/saturated water and water/saturated octanol. As such, a ratio estimation would be a less preferred yet acceptable alternative for the octanol/water partition coefficient (Kow), but must be treated with caution as it would not have been derived in the same manner as other K_{ow}s (OECD TG 107).		

R.7.1.8.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for n-octanol water partition coefficient:

'The study does not need to be conducted if the substance is inorganic. If the test cannot be performed (e.g. the substance decomposes, has a high surface activity, reacts violently during the performance of the test or does not dissolve in water or in octanol, or it is not possible to obtain a sufficiently pure substance), a calculated value for log P as well as details of the calculation method shall be provided.'

If experimental testing including estimation from the individual solubilities is not possible, log K_{ow} must normally be calculated by an appropriate numeric method based on the molecule's structure.

In case of rapid hydrolysis the registrant needs to provide evidence in the form of a hydrolysis endpoint study record (study summary) and should consider testing for the hydrolysis products instead, as information on the properties of (environmentally and toxicologically) relevant degradation products are needed for conducting the risk assessment of the substance to be registered.

Adaptation possibilities according to Annex XI to REACH

The reporting of the K_{ow} information cannot usually be waived (except for inorganic substances), because it is essential for CSA, classification and labelling and PBT assessments.

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Log K_{ow} is a commonly documented property in substance databases, such as IUCLID (http://ecb.jrc.it). Additional sources are the Canadian National Committee for CODATA (CNC/CODATA) database with evaluated log K_{ow} values for over 20000 substances (http://logkow.cisti.nrc.ca/logkow/) and the QSAR Toolbox (http://www.qsartoolbox.org).

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Measured values are given precedence over calculated values. For organic substances experimentally derived high-quality K_{ow} values, or values which are evaluated in reviews and assigned *recommended values*, are preferred over other determinations of K_{ow}. Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

When no experimental data of high quality are available, or if experimental methods are known to be unreliable, valid (Q)SARs for log K_{ow} may be used e.g. in a weight-of-evidence approach. Due to the availability of large number of measured log K_{ow} values and robust QSAR models for this property, the QSARs can, in some cases, predict the partition coefficient of a molecule with higher accuracy compared to a single test. Such valid QSAR models may be used if they are restricted to substances for which their applicability is well characterised. In order to be used as a stand alone source of values to meet the data requirements of Annex VII, 7.8, the QSARs must meet the criteria set out in Annex XI, 1.3.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the partition coefficient n-octanol/water read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies.

Further adaptation possibilities

Not foreseen.

R.7.1.8.5 Impurities; uncertainties

The effect of impurities in the test substance are discussed in the referenced test guidelines.

Difficult to test substances:

There are certain structural or physico-chemical properties that can make the accurate determination of Kow or its measurement difficult. Difficult to test substances include poorly soluble, volatile, surface active, ionisable substances, mixtures of substances, as well as substances subject to rapid degradation due to such processes as phototransformation, hydrolysis, oxidation, or biotic degradation.

Guidance on regulatory compliant Kow determination for ionisable substances and salts:

The Kow is typically defined as the partition coefficient of the neutral, undissociated form of a substance. However, the relative extent to which an ionisable substance is likely to be dissociated in the environment (with pH usually in the range 5-9) can have a marked effect on

its physicochemical properties, especially the octanol-water partition coefficient and water solubility, which in turn affect fate and behaviour. As log Kow is routinely used to predict bioconcentration/bioaccumulation potential, this aspect is especially important in a PBT context. For substances which dissociate within an environmentally relevant pH range (pK_a 5-9), values for Kow must be derived for the neutral form, and preferably also for the dissociated form. In some cases a factor 4-5 has been recorded between the log Kow of both species. The value for the dissociated molecule determined around a pH of 7 (sometimes referred to as Dow) is considered more realistic for PBT and chemical safety assessment.

Based on practical experience the following guidance is provided:

Simple acids and bases in the normal pH range:

- The HPLC method is to be applied to acids and bases in their non-ionised forms, although the pH should be kept in the range 2 to 9 (however pH 5 to 9 is preferred).
- For the shake-flask method, the approach must be followed in which the study is conducted at a pH where the substance is not ionised, if possible, or at a pH where the extent of ionisation is minimised.
- Validated QSAR estimations may be useful for acids and bases.

Zwitterionic substances:

- For zwitterions, the shake-flask method should be used to develop a valid Kow value. Even if the ionic charge pattern of the compound in octanol is not known, the value represents a practical and useful parameter. It is not justifiable to expect a full description of all the equilibria in both water and octanol. The pH of such a study should be 7 or the iso-electric point (pH value at which the molecule has no net electrical charge), as long as that point is in the range pH 5 to 9, so as to maximise the possibility of partition into octanol. There is no need to give both pH values.
- The HPLC method must not be used. The usual estimation methods should be valid, but particular care should be exercised.
- QSAR estimations may be useful provided that they are validated.

Salts of organic compounds:

- The shake-flask method should be used, usually at pH 7, or at any pH in the range 5 to 9 which maximises the potential for partition into octanol. For salts, the nature of the analytical method compared to the chemical composition will have to be considered. The ideal is to monitor cation and anion** individually in both phases. When only one half can be analysed, then the result must be understood as partial, even if it is the best that is achievable.
- Estimation by HPLC is not valid for the whole salt.
- QSAR methods will be valuable in assessing the properties of each half of the salt. Current estimation methods cannot estimate the Kow of the ion pair.

Guidance on regulatory compliant Kow determination for surfactants:

In many cases a calculated Kow value based on the octanol and water solubilities will be the first choice for surfactants. It is also useful to compare a calculated with a measured value. For the calculation approaches, one needs to consider the pH of the system (which determines the

ionisation of the surfactant – see Section R.7.1.17). None of the experimental methods is very well suited for determining the Kow of surface active substances. The shake flask method is the least suitable experimental method for surfactants. HPLC methodology may fail due to secondary interactions, and is sensitive to fluctuations of ionic strength. The slow stirring method in theory is the best, but still not demonstrated to be perfect. If using slow stirring, one needs to demonstrate a consistent result when starting with the surfactant in either phase, not just in the octanol. A working approach for surfactants might be the comparison of measured solubilities in octanol and water. However, it would then be prudent to take the critical micelle concentration in water (CMC) as a solubility limit, in order to avoid the artefact of unrealistically low Kow values.

Guidance on regulatory compliant Kow determination for mixtures:

It is possible that different components of mixtures have significantly different behaviour in the physico-chemical tests and therefore also *in vivo* and in the environment. It is therefore important to ensure that the results presented for the physico-chemical tests represent each component rather than the mixture being treated as a single component. For simple mixtures where the components are known and easily identifiable, this may mean presenting individual values for Kow. For complex mixtures, the HPLC method is ideal for determination of Kow, and a defined range of values should be presented, with an indication of the proportion of substance within a given range (e.g. > 90 % of components have log Kow in the range 4-5), to allow the significance of these results to be reflected in the risk assessment. The HPLC method is also recommended for petroleum products, which are typically mixtures.

R.7.1.8.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

Shake-flask method (EU A.8 / OECD TG 107):

- equilibrium concentrations of the test substance in both phases;
- relative volumes of the two phases;
- analytical method(s).

Calculation method (EU A.8):

- identification of the method;
- working principle of the method;
- reference to the method;
- information on source chosen to justify K_{ow} values of fragments being manipulated;
- applicability of the method.

HPLC method (EU A.8 / OECD TG 117):

- column(s) used;
- mobile phase (composition, buffer, pH);
- reference substances with respective Kow values from the literature;
- concentrations measured.

Slow-stirring method (OECD TG 123):

- label purity of labelled substances and molar activity (where appropriate);
- sampling times;
- description of the test vessels and stirring conditions;
- number of replicates;
- temperature during the experiment;

- volumes of 1-octanol and water at the beginning, during and remaining after the test;
- determined concentrations of the test substance in 1-octanol and water as a function of time;
- description of the test vessels and stirring conditions (geometry of the stirring bar and of the test vessel, vortex height in mm, and when available: stirring rate) used;
- analytical methods used to determine the test substance (its repeatability and sensitivity) and the method limit of quantification;
- sampling times;
- pH of the aqueous phase and of the buffers used, when pH is adjusted for ionisable molecules;
- number of replicates;
- demonstration of mass balance;
- temperature and standard deviation or the range of temperature during the experiment;
- the regression of concentration ratio against time.

Results and discussion

- final value for log Kow;
- Kow values and their mean;
- standard deviation of individual Kow values;
- theoretical value when it has been calculated;
- temperature of the test solutions (°C);
- pH value(s) of the aqueous solution(s);
- composition and concentration of buffers;
- concentration of the stock solution;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used and reasons for it or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on partitition coefficient can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.7	VII 7.8	Partitition coefficient	E.4.8	3.7

R.7.1.9 Flash point

R.7.1.9.1 Type of property

The flash point is a property:

- for substance characterization;
- for the classification of flammable liquids;
- which is importiant for physical hazard assessment.

R.7.1.9.2 Definition

The flash point is the lowest temperature of the liquid (as measured in a prescribed manner) at a pressure corrected to 101.325 kPa, at which application of an ignition source causes the vapour of the liquid to ignite momentarily and the flame to propagate across the surface of the liquid under the specified conditions of test (see section 1.2, Test Method A.9).

R.7.1.9.3 Test method(s)

The EU test method A.9 – Flash point from the Regulation (EC) 440/2008 can be used. Suitable methods are listed in the CLP Regulation Annex I, 2.6.4.4, Table 2.6.3.

The method to be used has to be chosen taking into account the properties of the liquid (viscosity, halogenated compounds present) and the scope of the standard.

For substances with a high decomposition potential, a method using small amounts of liquid (e.g. EN ISO 3679: Determination of flash point - Rapid equilibrium closed cup method) is recommended to reduce the amount of substance under test.

For classification purposes it is recommended to use the mean of at least two test runs. If the experimentally determined flashpoint is found to be within ± 2 °C of the limiting criterion for classification or assigning a category when using a non-equilibrium method, it is recommended to repeat the determination with an equilibrium method.

R.7.1.9.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for flash point:

'The study does not need to be conducted if:

- *the substance is inorganic;*
- *the substance only contains volatile organic components with flash-points above 100 °C for aqueous solutions; or*
- *the estimated flash-point is above 200°C; or*
- *the flash-point can be accurately predicted by interpolation from existing characterised materials.'*

The first point has to be further specified as:

- The substance is inorganic except where there are covalent bonds;

because some inorganic liquids with covalent bonds are flammable e.g. CS_2, N_2H_2, HCN.

The third point should only be applied when a well validated estimation model was used.

The fourth point should only be applied when there are enough reliable experimental data from existing characterised materials to be able to accurately interpolate to estimate the flash point.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) which meet the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

For the determination of the flash point, weight of evidence is not possible.

(Q)SAR

For the determination of the flash point, QSAR approaches are discouraged for the purpose of classification / risk assessment, except where the mean absolute error of the QSAR is less than 2 °C.
For non-halogenated liquids calculation based on the vapour pressure curve and lower explosion limit of the substance can be used as a screening test and a flashpoint need not be determined experimentally if the calculated value is at least 5 °C higher than the relevant classification criterion.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the flash-point read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

This applies if:

- the liquid is an explosive;
- the liquid is pyrophoric or self-reactive;
- decomposition occurs during the melting point study;
- some impurities have an inpact on the ignition source in such a way as to distort/invalidate the results.

Testing should always be considered, if none of the waiving possibilities applies.

Further adaptation possibilities

The flash point does not need to be determined experimentally if conclusive and consistent literature data are available.

Data generated with the same tests and classification principles as specified in the CLP Regulation for flash point generated in conjunction with transport classification can satisfy the REACH requirements, but this needs to be checked on a case by case basis.

R.7.1.9.5 Impurities; uncertainties

Impurities can have a significant influence on the flash point. The influence depends on the amount and the vapour pressure of the impurity. Even if their concentration is below 0.5 %, especially if their boiling point is substantially lower, they may have a strong effect on the flash point. Impurities with a higher boiling point will normally have no effect on the flashpoint. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.9.6 Endpoint specific information in the registration dossier / in IUCLID

Materials and methods

- reference to the standard or the test method applied;
- open cup or closed cup (for classification purposes only the closed cup methods are allowed);
- equilibrium or non-equilibrium method.

Results and discussion

- corrected flashpoint and unit;
- data on repeatability and reproducibility as given in the method;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flash point can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.11	VII 7.9	Flash point	E.4.12	3.10

R.7.1.10 Flammability

Some of the information requirements according to REACH Annex VII were phrased in a way that they correspond to 'indications of danger' as given in Annex II of the DSD. For substances, classification and labelling according to CLP Regulation has been mandatory since 1 December 2010 (and will become mandatory for mixtures (preparations) from 1 June 2015, when the DPD will be repealed). Consequently properties associated with flammability are covered by classification of the substance according to the CLP Regulation. However, the physical hazards according to the CLP Regulation are structured completely differently from the physicochemical properties according to the DSD (and therefore also REACH, Annex VII). This means that for some of the CLP hazard classes an unambiguous assignment to one of the headlines (information requirements) in Annex VII to REACH is not possible. The assignment of hazard classes to the headline 'Flammability' as shown in the table below must therefore only be understood as a means to structure this document in accordance with Annex VII to REACH. It has to be noted that self-reactive substances and organic peroxides are assigned to the headline 'Flammability' and only a cross reference is added under the headline 'Explosive properties' because these two hazard classses can have explosive and/or flammable properties.

Table R.7.1-7 Assignment of CLP hazard classes to the information requirement 'Flammability' according to REACH, Annex VII and correlation between the Test Method Regulation and the test method according to CLP and supporting link with the Guidance on the Application of the CLP Criteria.

Information requirement according to Art. 10 (a) (vi) of the REACH Regulation (EC) No. 1907/2006 (the no. in brackets is the respective no. in the table in Annexes VII to IX to REACH)	**CLP Regulation (EC) No. 1272/2008** (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Chapter in revised R.7(a) guidance**	**Corresponding test method according to The Test Method Regulation Regulation (EC) No 440/2008**	**Corresponding test method according to CLP Regulation**	**Chapter in the Guidance on the application of the CLP Criteria (ex RIP 3.6)**
Flammability (7.10)	Flammable gases[17] (2.2)*	7.1.10.1	A.11 Flammability (gases)	**ISO 10156 EN 1839**	2.2
	Flammable liquids (2.6)*	7.1.10.2	for liquids: see Flash point	**see CLP, Annex I, Chapter 2.6.4.4, Table 2.6.3**	2.6
	Flammable solids (2.7)*	7.1.10.3	A.10 Flammability (solids)	**UN Test N.1**	2.7
	Self-reactive substances and mixtures	7.1.10.4	n.a.	**UN Test series A to H**	2.8

[17] The 4th ATP to the CLP Regulation amends the criteria in the CLP Annex I, Section 2.2 Flammable gases by including subclassifications for chemically unstable gases.

	(2.8)*				
	Pyrophoric liquids (2.9)*	7.1.10.5	A.13 Pyrophoric properties of solids and liquids	**UN Test N.3**	2.9
	Pyrophoric solids (2.10)*	7.1.10.6		**UN Test N.2**	2.10
	Self-heating substances and mixtures (2.11)*	7.1.10.7	n.a.	**UN Test N.4**	2.11
	Substances and mixtures which in contact with water emit flammable gases (2.12)*	7.1.10.8	A.12 Flammability (Contact with water)	**UN Test N.5**	2.12
	Organic peroxides (2.15)*	7.1.10.9	n.a.	**UN Test series A to H**	2.15

* Note that regardless of whether the hazard class or category is listed in Article 14(4)(a) REACH the chemical safety assessment (where required) must be performed in accordance with Article 14(3) REACH. Furthermore, according to Article 10(a)(iv) of REACH the technical dossier of a registration of a substance under the REACH Regulation must include information on classification and labelling of the substance as specified in section 4 of Annex VI to the REACH Regulation.

In addition, it has to be noted that some substances have flammable properties which do not result in classification. Examples are the following:

- gases that do not have a flammable range at 20 °C and standard pressure (and therefore are not classified as flammable gases) might have a flammable range at higher temperatures and/or pressure (e.g. ammonia);
- liquids that do not have a flash point (and therefore are not classified as flammable liquids) might have an explosion range (especially halogenated hydrocarbons).

Information about such properties should also be indicated in the dossier.

R.7.1.10.1 Flammable gases

Definition

'Flammable gas means a gas or gas mixture having a flammable range with air at 20 °C and a standard pressure of 101.3 kPa' (Annex I to CLP, Section 2.2.1).

Classification criteria and relevant information

Flammable gases are classified into two categories depending on their flammability range (Annex I to CLP, Section 2.2.2. Table 2.2.1).

Detailed guidance on the classification criteria and the test method(s) can be found in the *Guidance on the application of the CLP Criteria*, section 2.2[18].

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for flammability:

'The study does not need to be conducted:

- *if the substance is a solid which possesses explosive or pyrophoric properties. These properties should always be considered before considering flammability;or*
- *for gases, if the concentration of the flammable gas in a mixture with inert gases is so low that, when mixed with air, the concentration is all time below the lower limit; or*
- *for substances which spontaneously ignite when in contact with air.'*

The relevant points can be paraphrased (first point is not relevant for this chapter), namely the study does not need to be conducted:

- if the concentration of the flammable gas in a mixture when mixed with air is below the lower limit;
- if the gas spontaneously ignites when in contact with air.

Gases that spontaneously ignite in contact with air are pyrophoric and are therefore flammable gases.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

Many gases are classified in Annex VI to CLP either as Flam. Gas 1 or Flam. Gas 2, and additional flammable gases are listed in the UN-RTDG whose classifications correspond to Flam. Gas 1 according to CLP.

[18] The 4th ATP to the CLP Regulation amends the criteria in the CLP Annex I, Section 2.2 Flammable gases by including subclassifications for chemically unstable gases. Consequently the *Guidance on the Application of the CLP Criteria*, Part 2: Physical hazards has been restructured to take account of the 4th ATP, which applies to substances from 1 December 2014 and to mixtures from 1 June 2015. When the 4th ATP is applied a Guidance corrigendum will be made to delete the outdated sub-chapter 2.2.1 Flammable gases in the *Guidance on the Application of the CLP criteria*.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

For gases that are not classified in Annex VI to the CLP Regulation nor in the UN-RTDG, there is ample scientific literature giving the flammability range for most gases (e.g. IEC 60079-20-1 *Data for flammable gases and vapours, relating to the use of electrical apparatus* – (under revision).

(Q)SAR

At present (Q)SAR is generally not applicable for determination of explosion (/flammability) limits of gases.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the flammable gases read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies.

FURTHER ADAPTATION POSSIBILITIES

Further adaptation is possible for gases that are known to be non-flammable. Examples are nitrogen, the noble gases (helium, neon, argon, krypton, xenon), carbon dioxide and sulphur hexafluoride. As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

Impurities; uncertainties

Tests should be performed with the lowest concentration of impurities in the gas encountered in the normal manufacturing process and the moisture content should be less than or equal to 0.01 mol %. Utmost care should be taken in the selection of the key study(s) and/or use of weight-of-evidence approaches that the data selected is representative of the substance being registered by the respective companies.

How to conclude on the DSD classification

All gases with a flammability range in air are classified ‘Extremely flammable F+ ; R12’ according to DSD, unless classified differently according to Annex VI, Table 3.2 of the CLP Regulation. This means that all gases classified as flammable gases according to CLP (either Category 1 or 2) are classified as ‘Extremely flammable F+; R12’.

Endpoint specific information in the registration dosser/in IUCLID

Material and methods:

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- test temperature;

- tested concentrations.

Results and discussion & Applicant's Summary and conclusion (interpretation of results)

- indicate lower and upper explosion limits in % volume;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

For the testing of flammable gases according to CLP classification requirements, refer also to the *Guidance on the Application of the CLP Criteria*, section 2.2, and in Directive 2008/47/CE.

R.7.1.10.2 Flammable liquids

Definition

Flammable liquid means a liquid which may form flammable/explosive vapour-air mixtures. Within the CLP Regulation 'Flammable liquid' means a liquid having a flashpoint of not more than 60 °C (see CLP Annex I, section 2.6.1).

Classification criteria and relevant information

Flammable liquids are classified in three categories according to the criteria of the CLP Regulation (see CLP Annex I, section 2.6, table 2.6.1) based on their boiling point and their flash point. Derogation is possible (see CLP Annex I, section 2.6.4.5) for Flam. Liquid Cat. 3 having a flashpoint above 35 °C based on the information on sustained combustibility. Furthermore, gas oils, diesel and light heating oils having a flash point between ≥ 55 °C and ≤ 75 °C may be regarded as Category 3 flammable liquids according to the CLP Regulation (CLP Annex I, section 2.6, footnote to table 2.6.1).

In addition EUH018 - *'In use may form flammable/explosive vapour-air mixture'* has to be assigned to substances classified under the CLP Regulation which may form flammable/explosive vapour-air mixtures although they do not have a flash point e. g. CH_2Cl_2, $C_2H_3Cl_3$. In such cases it is possible to make the decision on whether flammable/explosive vapour-air mixture may be formed based on either the determination of explosion limits according to EN 1839 or the determination of explosion points according to EN 15794. It is sufficient to determine either the lower explosion limit or the lower explosion point.

Detailed guidance on the classification criteria and the test method(s) can be found in the *Guidance on the application of the CLP Criteria*, section 2.6.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

The entries 'flammability' (7.10), 'boiling point' (7.3) and 'flashpoint' (7.9) are the relevant ones. For the latter two entries, see their respective relevant sections in this document.

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for flammability:

'The study does not need to be conducted:

- *if the substance is a solid which possesses explosive or pyrophoric properties. These properties should always be considered before considering flammability;or*
- *for gases, if the concentration of the flammable gas in a mixture with inert gases is so low that, when mixed with air, the concentration is all time* [i.e. 'always'] *below the lower limit; or*
- *for substances which spontaneously ignite when in contact with air.'*

The relevant points can be paraphrased (first two points are not relevant for this chapter), namely the 3rd point specifies that for flammability, Annex VII to REACH does not require testing for substances which spontaneously ignite when in contact with air.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

To be used as a stand alone value to meet the data requirements of Annex VII, 7.8, QSAR models must meet the criteria set out in Annex XI, 1.3. The entries 'boiling point' (7.3) and 'flashpoint' (7.9) are also the relevant ones, therefore please check under each respective QSAR sub-section for more information.

Sustained Combustibility:

No (Q)SAR exists currently.

For further reference see also *the Guidance on the Application of the CLP Criteria*, section 2.6.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

The entries 'boiling point' (7.3) and 'flashpoint' (7.9) are again the relevant ones. For both these entries, see their respective sections in this document.

Sustained Combustibility:

For the determination of the sustained combustibility read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing is not possible if:

- the liquid is an explosive;
- the liquid is pyrophoric or self-reactive.

Testing should always be considered if none of the waiving possibilities applies.

FURTHER ADAPTATION POSSIBILITIES

Use of data on boiling point, flashpoint when determined with a closed cup method, explosion limits or lower explosion point from validated literature (see below chapter Further information/ references) is possible. Data on boiling point generated in relation to transport classification may also satisfy the Annex XI requirements. Data on flashpoint generated in relation to with transport classification may satisfy the Annex XI requirements if closed cup methods have been used. However care has to be taken in cases where there is no transport classification as 'flammable liquid', because certain substances can form flammable/explosive vapour-air mixtures although they do not have a flash point.

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

Impurities; uncertainties

Boiling point:

Impurities will influence the boiling point of the main component. The influence depends on the amount and boiling point of the impurity. The higher the amount and the higher the difference between the boiling points of the main component and the impurity, the higher the influence.

Flashpoint:

Special care has to be taken when a sample contains impurities with a lower boiling point than the main component. Even if their concentration is below 0.5 %, especially if their boiling point is substantially lower, they may have a strong effect on the test result. Impurities with a higher boiling point will normally have no effect on the flashpoint.

Sustained combustibility:
Impurities with lower boiling point may influence the ability to sustain combustion. However it is not yet possible to quantify the influence of impurities.

How to conclude on the DSD classification

Based on the data on boiling point and flashpoint the DSD classification according to the respective DSD criteria is possible. Simplified direct translation between CLP classification and DSD classification is not possible, see figure below.

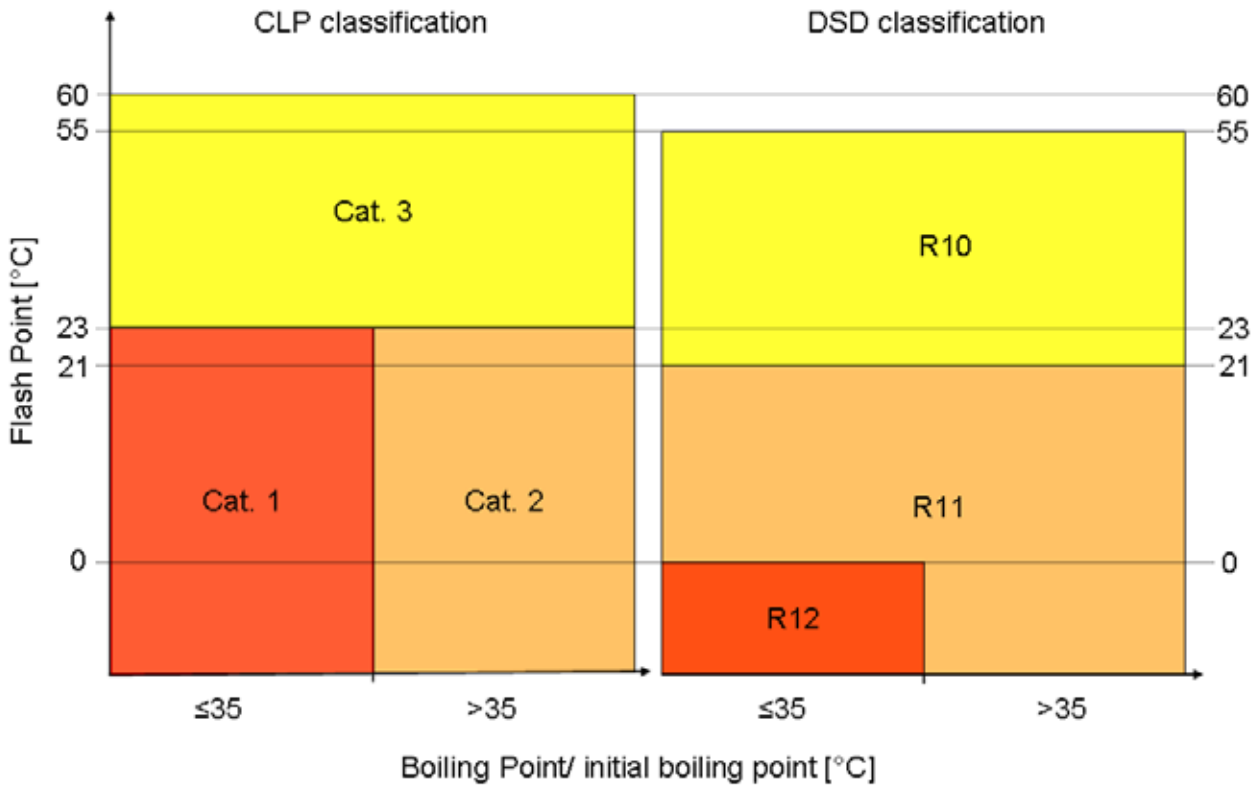

Figure R.7.1-1 Comparison of the DSD and the CLP classification

Substances exempted from classification in Cat. 3 because of their flashpoint and behaviour when tested for sustained combustibility can be exempted from being classified under DSD as R10, if they don't show additional dangerous properties relevant for classification.

Endpoint specific information in the registration dosser / in IUCLID

The physicochemical studies reporting data relevant for classification as a flammable liquid (flashpoint and boiling point) are to be reported in the relevant IUCLID endpoint records.

Material and methods

See chapter flash point 0 and boiling point R.7.1.3.

Results and discussion

- corrected flashpoint and unit;
- data on repeatability and reproducibility as given in the method;
- boiling point value (°C) as measured;
- pressure value and unit;
- rate of temperature increase;

- decomposition (if applicable);
- measurement uncertainty if available;
- boiling point value in ºC (corrected to standard pressure, except where the boiling point was determined at reduced pressures) (as above, but in a separate block of fields);
- if available explosion limits;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

See also R.7.1.3 Boiling point and 0 Flash point. For testing of flammable liquids according to CLP classification requirements refer also to the *Guidance on the Application of the CLP Criteria*, section 2.6.

R.7.1.10.3 Flammable solids

Definition

'A flammable solid means a solid which is readily combustible, or may cause or contribute to fire through friction. Readily combustible solids are powdered, granular, or pasty substances or mixtures which are dangerous if they can be easily ignited by brief contact with an ignition source, such as a burning match, and if the flame spreads rapidly' (see CLP Regulation, Annex I, section 2.7.1).

Classification criteria and relevant information

Solid substances and mixtures are classified as flammable in two categories according to their burning behaviour (see the CLP Regulation, Annex I, section 2.7) using UN Test N.1 as described in section 33.2.1 of the UN-MTC.

Chapter 2.7 of the *Guidance on the Application of the CLP Criteria* gives detailed information on the CLP classification of flammable solids, the UN Test N.1 and the relation to the DSD and the UN-RTDG regulations.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for flammable solids:

'The study does not need to be conducted:

- *if the substance is a solid which possesses explosive or pyrophoric properties. These properties should always be considered before considering flammability;or*
- *for gases, if the concentration of the flammable gas in a mixture with inert gases is so low that, when mixed with air, the concentration is all time* [i.e. always] *below the lower limit; or*
- *for substances which spontaneously ignite when in contact with air.'*

Concerning the first indent, testing for flammability of a solid is a part of classification in CLP Regulation. Refer also to the *Guidance on the Application of the CLP Criteria*, section 2.7 on classification requirements. For substances having explosive properties, testing for a classification as a flammable solid may be waived. This applies to substances and mixtures classified as explosives, organic peroxides and self-reactive substances and mixtures.

Second indent is not applicable for this endpoint.

With regards to the third indent, substances which spontaneously ignite when in contact with air are pyrophoric substances as defined by the CLP Regulation (see the *Guidance on the Application of the CLP Criteria*, section 2.10). Such substances are not classified as flammable solids but as pyrophoric solids under the CLP Regulation.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Literature data – even if available – should not be used since flammability strongly depends on particle size, surface treatment and other parameters.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

If available data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary. However, if the A.10 test method has come to the conclusion 'highly flammable', it will be necessary to also determine the influence of the wetted zone as described in the UN Test N.1.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At present (Q)SAR is generally not applicable for flammable solids. Application of (Q)SAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present, grouping and read across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies.

FURTHER ADAPTATION POSSIBILITIES

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

If a suitable screening test clearly shows that the substance is not flammable, further testing is not necessary (see also the *Guidance on the application of the CLP Criteria*, section 2.7.4.2). An example for a suitable screening test is the burning index as described in VDI guideline (VDI Guideline, 1990) if a burning index of 3 or less is found, the substance should not be classified as a flammable solid and no further testing is required.

Substances and mixtures classified according to the CLP Regulation as explosives, organic peroxides, self-reactive substances and mixtures as well as pyrophoric or oxidising solids should not be considered for classification as flammable solids (see the *Guidance on the Application of the CLP criteria*, section 2.7.3).

However, if a substance gives a positive result in UN Test Series 1 or 2 as described in the UN-MTC, but is exempted from classification as an explosive on the basis of UN Test Series 6, a test for classification as a flammable solid should be performed.

Impurities; uncertainties

Impurities do not tend to have a large effect on the flammability of a solid. However, if a solid which is not flammable in the pure state contains flammable organic liquids or organometallic impurities it may burn more rapidly and thus become flammable. Therefore utmost care should be taken in the selection of the key study(s) and during use of weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

How to conclude on the DSD classification

Any substance found to be a flammable solid according to CLP Regulation has to be classified as ‘F; R11’ according to the DSD.

Endpoint specific information in the registration dosser/in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied**.**

Solid flammability:

- indicate if preliminary and/or main test performed;
- moisture content;
- particle size and distribution (if available) (see R.7.1.14.1. Granulometry).

Results and discussion

- indicate burning time;
- pass/non pass of the wetted zone (in the case of the UN Test N.1);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

VDI guideline 2263, part 1, (1990): 'Test methods for the Determination of the Safety Characteristics of Dusts'.

For testing of flammable solids according to CLP classification requirements, refer also to the *Guidance on the Application of the CLP Criteria*, section 2.7.

R.7.1.10.4 Self-reactive substances and mixtures

In the CLP Regulation self-reactive substances are a distinct hazard class. Self-reactive substances are classified into one of the seven categories of 'Types A to G' according to the classification criteria given in section 2.8.2.3 of Annex I of CLP. In the Dangerous Substances Directive (67/548/EEC) no hazard class for 'self-reactive substances' is defined. Nevertheless, self-reactive substances were also classified as dangerous according to the DSD, e.g. as flammable or as substances with explosive properties.

As mentioned below under the sub-section 'Definition', self-reactive substances are excluded from testing as explosives according to Test Series 1 to 8 in Part I of the UN-MTC (see R.7.1.11.1 Explosives). In Test Series A to H however, no tests on sensitivity to impact (solids and liquids) and friction (solids only) are included. For the risk assessment and the safe use and handling, data according to the EU test method A.14 as described in Regulation (EC) No 440/2008, if available, or UN Test 3 (a) (ii) BAM Fallhammer and Test 3 (b) (i) BAM friction apparatus (see R.7.1.11) should be part of the hazard communication in the registration dossier (REACH Annex VII, 7.11) and the safety data sheet.

Definition

The definition of a self-reactive substance is given in section 2.8.1 of Annex I to CLP Regulation:

'Self-reactive substances or mixtures are thermally unstable liquid or solid substances or mixtures liable to undergo a strongly exothermic decomposition even without participation of oxygen (air). This definition excludes substances and mixtures classified according to this Part as explosives, organic peroxides or as oxidising. A self-reactive substance or mixture is

regarded as possessing explosive properties when in laboratory testing the formulation is liable to detonate, to deflagrate rapidly or to show a violent effect when heated under confinement.'

Background information and guidance on the definition is given in the *Guidance on the Application of the CLP Criteria*, sections 2.8.1 and 2.8.2.

Classification criteria and relevant information

Classification principles are given in CLP Regulation Annex I, sections 2.8.2 and 2.8.4. Background information and guidance on relevant aspects regarding the classification is given in the *Guidance on the Application of the CLP Criteria*, sections 2.8.4, 2.8.5 and 2.8.6.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Only self-reactive substances, as defined in the section definition, have to be tested according to the UN-MTC, Part II test series A - H.

CLP Annex I, section 2.8.2.1 provides the following specific rules for adaptation of the standard information requirement for self-reactive substances and mixtures.

'Any self-reactive substance or mixture shall be considered for classification in this class as a self-reactive substance or mixture unless:

a. *they are explosives, according to the criteria given in 2.1;*

b. *they are oxidising liquids or solids, according to the criteria given in 2.13 or 2.14, except that mixtures of oxidising substances, which contain 5 % or more of combustible organic substances shall be classified as self-reactive substances according to the procedure defined in 2.8.2.2;*

c. *they are organic peroxides, according to the criteria given in 2.15;*

d. *their heat of decomposition is less than 300 J/g; or*

e. *their self-accelerating decomposition temperature (SADT) is greater than 75 º C for a 50 kg package*[19]*.'*

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

A number of already tested and classified substances and mixtures are listed in UN-RTDG, section 2.4.2.3.2.3. Available information may originate from the classification for transport. More details are given in the *Guidance on the Application of the CLP Criteria*, sections 1.7.2.1 and 2.8.6.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column

[19] 'See UN RTDG, sub-sections 28.1, 28.2, 28.3 and Table 28.3.'

2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

For the determination of the self-reactive substances and mixtures, weight of evidence is not possible.

(Q)SAR

At present (Q)SAR is generally not applicable for determination of self-reactive substances. Application of (Q)SAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read-across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

A few of substances can, for safety reasons, only be handled and tested in diluted form, see the substances and mixtures listed in UN-RTDG, section 2.4.2.3.2.3.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

Minor impurities can have an influence on thermal stability. Background information and guidance on these aspects is given in the *Guidance on the Application of the CLP Criteria*, section 2.8.4.3.

How to conclude on the DSD classification

In the DSD self-reactive substances are not covered. They may be classified in other DSD classes (e.g. explosive substance, flammable solid or liquid). See also the *Guidance on the Application of the CLP Criteria*, section 2.8.6.1.

What information is required in the registration dossier in IUCLID

Material and methods

- see UN-MTC, Part II, classification procedures and test series A-H.

Results and discussion

The following data on self-reactive substances should be submitted:

- type of self-reactive substance;
- decomposition energy (value and method of determination);
- SADT (Self accelerating decomposition temperature) together with the volume the SADT relates to;
- detonation properties (Yes/Partial/No);
- deflagration properties (Yes rapidly/Yes slowly/No);
- effect of heating under confinement (Violent/Medium/Low/No);
- explosive power if applicable (Not low/Low/None).

For assigning the type of self-reactive substance, the list of currently assigned self-reactive substances according to the 2.4.2.3.2.3 of the UN-RTDG can be used, in cases where the assignment was based on test(s) according to the UN-MTC. The relevant underlying test data may be collected from the respective UN documents from the UN Committee of experts on the transport of dangerous goods, from test reports produced by competent authorities or industry, or from other reliable sources.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

The following example shows how the data mentioned above could be documented in the chemical safety report (CSR):

Figure R.7.1-2 Example 2,2'-Azodi (isobutyronitrile)

UN Test Series A to H	Test method	Results + Evaluation	Remarks
Propagation of detonation	A.5	"yes"	Apparent density (kg/m^3): 366 Fragmented length (cm): 40
Propagation of deflagration #1	C.1	"yes, slowly"	68 ms
Propagation of deflagration #2	C.2	"no"	
Effect of heating under defined confinement #1	Koenen E.1	"violent"	Limiting diameter 3.0 mm Type of fragmentation: F
Effect of heating under defined confinement #2	DPVT E.2	"medium"	Limiting diameter 5.5 mm
Explosive power	F.4	"not Low"	Average net expansion (cm^3): 18
SADT	H.4	50 °C	500 ml Dewar vessel
Competent Authority approval number	*Example from UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria*		

Reference to other ECHA Guidance Documents

A template data set does not currently exist in IUCLID for the hazard class 'self-reactive substances'. As long as there is no specific section available in IUCLID the test results in IUCLID section 4.23 'Additional physico-chemical information' under the endpoint title 'Self-reactive substances' should be inserted. In the CSR the information should be included under flammability.

Further information / references

Background information and guidance on classification testing, additional testing and available information is given in the *Guidance on the Application of the CLP Criteria*, section 2.8.

R.7.1.10.5 Pyrophoric liquids

Definition

The definition of a pyrophoric liquid is given in the section 2.9.1 of Annex I to CLP Regulation:

'Pyrophoric liquid means a liquid substance or mixture which, even in small quantities, is liable to ignite within five minutes after coming into contact with air.'

Background information and guidance on the definition is given in the *Guidance on the Application of the CLP Criteria*, sections 2.9.1 and 2.9.2.

Classification criteria and relevant information

Classification principles are given in CLP Regulation Annex I, section 2.9.2.

The criterion for a pyrophoric liquid is as follows: '*The liquid ignites within 5 min when added to an inert carrier and exposed to air, or it ignites or chars a filter paper on contact with air within 5 min.'*

Background information and guidance on relevant aspects regarding the classification is given in the *Guidance on the Application of the CLP Criteria*, sections 2.9.1, 2.9.2, 2.9.3 and 2.9.4.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Other flammability tests do not have to be performed as well as the determination of the self-ignition temperature, if the substance is a pyrophoric substance. However, flammability in contact with water may be relevant.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

The UN Test N.3 of the UN-MTC is also used for classification according to the regulations on the transport of dangerous goods (ADR and RID). If the liquid in question has been classified as belonging to Class 4.2, packing group I of the ADR/RID on the basis of UN Test N.3 results, it is a pyrophoric liquid according to CLP criteria. Packing group I of the ADR/RID directly corresponds to Category 1 of the CLP.

According to the DSD, the A.13 method of Regulation (EC) 440/2008 is used for the assessment of pyrophoric properties for liquids and liquids. This method is identical to the UN Test N.3.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH,

or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

Application of (Q)SAR is not possible, however assessment of the chemical structure may be used to exclude pyrophoric properties of a substance. Such an assessment of chemical structure, in conjunction with experience in manufacture and handling, could also formally form part of a weight-of-evidence argument.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

Assessment of the chemical structure may be used to anticipate pyrophoric properties of a substance.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. Due to pyrophoric properties a number of other tests on physicochemical, toxicological and eco-toxicological endpoints cannot be conducted.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

More background information and guidance on this and other aspects is given in the *Guidance on the Application of the CLP Criteria*, section 2.9.

How to conclude on the DSD classification

Because the test methods of DSD and CLP are identical for this endpoint there is no difference in classification, see also the *Guidance on the Application of the CLP Criteria*, section 2.9.6.

Endpoint specific information in the registration dossier IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied.

Note that in this case the experience in handling may be sufficient.

Results and discussion

- whether ignition occurs when poured or whether the filter paper is charred;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

Background information and guidance on classification testing, additional testing and available information is given in the *Guidance on the Application of the CLP Criteria*, section 2.9.

R.7.1.10.6 Pyrophoric solids

Definition

The definition of a pyrophoric solid is given in CLP Regulation Annex I, section 2.10.1.

'Pyrophoric solid means a solid substance or mixture which, even in small quantities, is liable to ignite within five minutes after coming into contact with air.'

Background information and guidance on the definition is given in the *Guidance on the Application of the CLP Criteria*, sections 2.10.1 and 2.10.2.

Classification criteria and relevant information

Classification principles are given in CLP Regulation Annex I, section 2.10.2.

The criterion for a pyrophoric solid is as follows: '*The solid ignites within 5 minutes of coming into contact with air.'*

Background information and guidance on relevant aspects regarding the classification is given in the *Guidance on the Application of the CLP Criteria*, sections 2.10.1, 2.10.2, 2.10.3 and 2.10.4.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Other flammability tests do not have to be performed in addition to the determination of the self-ignition temperature, if the substance is a pyrophoric substance. However, flammability in contact with water may be relevant.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

The UN Test N.2 of the UN-MTC is also used for classification according to the regulations on the transport of dangerous goods (ADR and RID). If the solid in question has been classified as belonging to Class 4.2, packing group I of the ADR/RID on the basis of UN Test N.2 results, it is a pyrophoric solid according to CLP Regulation criteria. Packing group I of the ADR/RID directly corresponds to Category 1 of CLP.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

According to the DSD, the A.13 method of Regulation (EC) 440/2008 is used for the assessment of pyrophoric properties for solids and liquids. This method is identical to the N.2 test method.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

Application of (Q)SAR is not possible, however assessment of the chemical structure may be used to exclude pyrophoric properties of a substance. Such an assessment of chemical structure, in conjunction with experience in manufacture and handling, could also formally form part of a weight-of-evidence argument.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

Assessment of the chemical structure may be used to anticipate pyrophoric properties of a substance.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. Due to pyrophoric properties a number of other tests on physicochemical, toxicological and ecotoxicological endpoints cannot be conducted.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

Particle size may play an important role. More background information and guidance on this and other aspects is given in the *Guidance on the Application of the CLP Criteria*, section 2.10.

How to conclude on the DSD classification

Because the test methods of DSD and CLP Regulation are identical for this endpoint there is no difference in classification, see also the *Guidance on the Application of the CLP Criteria*, section 2.10.6.

Endpoint specific information in the registration dosser / in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;

- particle size and distribution (if practicable);

Note that in this case experience in handling may be sufficient.

Results and discussion

- whether ignition occurs when poured;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

Background information and guidance on classification testing, additional testing and available information is given in the *Guidance on the Application of the CLP Criteria*, section 2.10.

R.7.1.10.7 Self-heating substances and mixtures

Definition

For solids and liquids adsorbed onto a large surface, self-heating may occur by reaction with air with subsequent ignition. According to the section 2.11.1.1 of Annex I to CLP Regulation:

'A self-heating substance or mixture is a liquid or solid substance or mixture, other than a pyrophoric liquid or solid, which, by reaction with air and without energy supply, is liable to self-heat; this substance or mixture differs from a pyrophoric liquid or solid in that it will ignite only when in large amounts (kilograms) and after long periods of time (hours or days).'

Classification criteria and relevant information

Self-heating substances and mixtures are classified in two categories according to the criteria of the CLP Regulation (see section 2.11, table 2.11.1). In general, self-heating occurs only for solids in contact with air. The *Guidance on the Application of the CLP Criteria*, section 2.11 gives detailed background information about this phenomenon.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of the REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for self-ignition temperature.

'The study does not need to be conducted:

- *if the substance is explosive or ignites spontaneously with air at room temperature; or*
- *for liquids non flammable in air, e.g. no flash point up to 200 °C, or*
- *for gases having no flammable range, or*
- *for solids, if the substance has a melting point < 160 °C, or if preliminary results exclude self-heating of the substance up to 400 °C.'*

The first indent specifies that no data is required for substances which is explosive or ignites spontaneously with air at room temperature.

Second and third indent are not applicable for this endpoint.

With regards to fourth indent, for the purposes of REACH, no data are required for solids classified as:

- pyrophoric; or
- explosive, unstable or division 1.1 to 1.6; or
- organic peroxide; or
- self-reactive substance.

Further, no data are required for substances with a melting point below 160 °C. This means also that liquids do not have to be tested for this endpoint for the purposes of this regulation. Annex VII of REACH also allows waiving *'if preliminary results exclude self-heating of the substance up to 400 °C'.* This refers to Test Method Regulation 440/2008, method A.16. However, the criteria are not very clear, and therefore it is recommended to instead refer to the CLP Regulation classification criteria, if applicable, and to waive otherwise.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Literature data – even if available – should not be used since self-heating strongly depends on particle size, surface treatment and other parameters.

The use of existing data is possible provided that the test has been carried out by a qualified institution. If available data from a test according to method A.16 indicate that a classification as a self-heating substance does not apply, no more testing is necessary. However, the interpretation of the A.16 test method data in terms of the CLP criteria requires appropriate expert knowledge.

WEIGHT OF EVIDENCE

For the determination of the self-heating substances and mixtures, weight of evidence is not possible.

(Q)SAR

At present (Q)SAR is generally not applicable for self-heating substances and mixtures. Application of QSAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read-across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

In some cases, exothermic decomposition may occur when performing the test, and special care will be necessary with respect to performing the tests and interpreting the results; see the *Guidance on the Application of the CLP criteria*, section 2.11.4.4.3. In such cases, it may not be possible to determine these properties.

FURTHER ADAPTATION POSSIBILITIES

According to the UN-MTC, the classification procedure for self-heating substances or mixtures need not be applied if the results of a screening test can be adequately correlated with the classification test and an appropriate safety margin is applied. Examples of screening tests are:

a. the Grewer Oven test (VDI guideline, 1990) with an onset temperature 80 K above the reference temperature for a volume of 1 litre;

b. the Bulk Powder Screening Test (Gibson *et al.,* 1985) with an onset temperature 60 K above the reference temperature for a volume of 1 litre.

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used. The *Guidance on the Application of the CLP Criteria*, section 2.11.4.2 should be consulted for details about waiving and screening criteria.

Impurities; uncertainties

Particle size may play an important role. More background information and guidance on this and other aspects is given in the *Guidance on the Application of the CLP Criteria*, section 2.11.

How to conclude on the DSD classification

This hazard class is not defined in DSD, therefore translation is not possible.

Endpoint specific information in the registration dosser/in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- indicate if preliminary and/or main test performed;
- moisture content;
- particle size and distribution (if available).

Results and discussion

- indicate temperature rise obtained for the individual tests and classification result.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

ECHA guidance document *the Guidance on the Application of the CLP Criteria* gives in section 2.11 detailed information on the self-heating property, the CLP-classification, the relevant test method and the relation to the DSD and the UN-RTDG.

VDI guideline 2263, part 1 (1990): 'Test methods for the Determination of the Safety Characteristics of Dusts'.

Gibson, N. Harper, D.J. Rogers (1985): 'Evaluation of the fire and explosion risks in drying powders', Plant Operations Progress, 4 (3), 181-189.

R.7.1.10.8 Substances which in contact with water emit flammable gases

Definition

The CLP Regulation, Annex I, section 2.12.1 provides the following definition:

'Substances or mixtures which, in contact with water, emit flammable gases means solid or liquid substances or mixtures which, by interaction with water, are liable to become spontaneously flammable or to give off flammable gases in dangerous quantities.'

Classification criteria and relevant information

Classification according to the CLP Regulation is required if the gas produced upon contact with water ignites spontaneously and/or if the reaction rate with which the flammable gas is produced is ≥ 1 l/kgh.

If the gas produced ignites spontaneously, this does not necessarily imply that the gas produced is pyrophoric but this generally is the case if the heat of reaction is sufficient to result in ignition of the gas.

The test method for classification of substances and mixtures which in contact with water emit flammable gases is described in the UN-MTC (UN Test N.5, see Section 33.4). This method is referred to in Annex I, Part 2 of the CLP Regulation and it is strongly recommended to use this method and not to apply test method A.12 of the Test Methods Regulation if new testing is carried out. UN Test N.5 foresees dividing into three categories depending on the violence and rate of the reaction whereas test method A.12 does not allow any further dividing of the substances. Furthermore, the results of both methods might differ slightly due to some differences in the testing procedure (for these differences see the *Guidance on the Application of the CLP Criteria*, Section 2.12.6). Therefore unnecessary testing can be avoided by applying only UN Test N.5 because it leads to more detailed information (and has in any case to be applied for other purposes such as classification and transport).

Data which is based on the classification according to DSD may be available. There are, however, differences between the methods UN Test N.5 and A.12 which should be considered.

They are described in detail in the *Guidance on the Application of the CLP Criteria*, section 2.12.6.

Detailed guidance on the test method itself can be found in the *Guidance on the Application of the CLP Criteria*, section 2.12.4.4.1.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for flammability.

'The study does not need to be conducted:

- *if the substance is a solid which possesses explosive or pyrophoric properties. These properties should always be considered before considering flammability; or*
- *for gases, if the concentration of the flammable gas in a mixture with inert gases is so low that, when mixed with air, the concentration is all time below the lower limit; or*
- *for substances which spontaneously ignite when in contact with air.'*

The first point is valid with regard to explosive substances because they are not classified as substances which in contact with water emit flammable gases. In that case testing can be waived.

The other waiving possibilities are not applicable with regard to substances which in contact with water emit flammable gases.

The first point is not correct with regard to pyrophoric substances because pyrophoric substances can be classified as substances which in contact with water emit flammable gases based on UN Test N.5 which is referred to by CLP. UN Test N.5 explicitly requires testing of pyrophoric substances under nitrogen (see UN-MTC, section 33.4.1.3.1).

The second point is not applicable because gases do not fall under the hazard class of substances which in contact with water emit flammable gases.

For the same reasons, the last point (waiving would be possible for substances which spontaneously ignite when in contact with air) is also not valid in this case.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

There are currently no QSPR models for predicting whether a substance in contact with water emits flammable gases and if so what the gas evolution rate is.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read-across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be possible if none of the waiving possibilities applies. If the substance is known to be soluble in water to form a stable solution, or if it is clearly known that it does not react with water, e.g. because it is manufactured or washed with water, testing is not necessary.

FURTHER ADAPTATION POSSIBILITIES

Classification in certain hazard classes do not foresee the assignment of further physical hazard classes or at least normally do not match with classification in this hazard class:

Substances that are classified as explosives, self-reactives or organic peroxides are not classified in this hazard class (or any other physical hazard class). For explosives this is considered through the first point of the adaptation possibilities according to REACH Annex VII, column 2 (see above).

Oxidizing substances are generally not considered for flammability and therefore are also not classified in this hazard class (there may be some exceptions, however).

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

Impurities; uncertainties

The descriptions of the methods UN Test N.5 and A.12 are not very detailed and therefore allow for technical variations such as with regard to the apparatus used or the procedure. In particular, the testing protocol does not prescribe a specific method for measuring the gas evolution rate. An interlaboratory comparison for this test method has shown that laboratories - based on the freedom the description of the test methods gives - apply different approaches when performing this test. Furthermore, the interlaboratory comparison showed that the test results vary in a rather wide range. It therefore has to be kept in mind that this test method has a non-negligible uncertainty with regard to trueness and precision. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

Sea water may be a particular case of interest (in case of maritime transport).

How to conclude on the DSD classification

Substances which in contact with water emit flammable gases would be classified as ‘F; R15’ under DSD (the sum of categories 1 to 3 corresponds to ‘F; R15’).

Endpoint specific information in the registration dosser / in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- partice size and distribution.

Results and discussion

- indicate whether full test was performed or whether it was terminated at a particular step/stage;
- substance identity of evolved gas;
- indicate whether the gas evolved ignites spontaneously;
- rate of gas evolution (unless the test has been terminated);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on flammability is found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.13	VII 7.10	Flammability	E.4.14	3.12

Further information / references

The ECHA document *Guidance on the Application of the CLP Criteria* gives in its section 2.12 detailed information on substances and mixtures which, in contact with water, emit flammable gases, their CLP-classification, the relevant test method and the relation to the DSD and the transport of dangerous goods regulations.

Janès *et al.*, 'Towards the improvement of UN N.5 test method intended to the characterization of substances which in contact with water emit Flammable Gases', submitted in revised form to the Journal of Loss Prevention in the Process Industries.

Interlaboratory test on the method UN Test N.5 / EC A.12 'Substances which, in contact with water, emit flammable gases' 2007, Kunath, K., Lüth, P., Uhlig, S., ISBN 978-3-9814634-1-5, http://www.bam.de/de/service/publikationen/publikationen_medien/short__report_rv_un_n_5.pdf.

R.7.1.10.9 Organic peroxides

In the Dangerous Substances Directive (DSD) (67/548/EEC) organic peroxides were classified on the basis of their chemical structure either as explosive or as oxidising. In general, organic peroxides have only weak oxidising properties or do not show oxidizing properties at all. In the CLP Regulation organic peroxides are a distinct hazard class. Organic peroxides are classified in one of the seven categories of 'Types A to G' according to the classification criteria given in Section 2.15.2 of Annex I, of CLP.

As mentioned below under sub-section Definition, organic peroxides are excluded from testing as explosives according to Test Series 1 to 8 in Part I of the UN-MTC (see R.7.1.11.1 Explosives). In Test Series A to H however, no tests on sensitivity to impact (solids and liquids) and friction (solids only) are included. For the risk assessment and the safe use and handling, data according to the EU test method A.14 as described in Regulation (EC) No 440/2008, if available, or UN Test 3 (a) (ii) BAM Fallhammer and Test 3 (b) (i) BAM friction apparatus (see R.7.1.11) should be part of the hazard communication in the registration dossier (REACH Annex VII, 7.11) and in the safety data sheet.

Definition

The definition of an organic peroxide is given in CLP Annex I, section 2.15.1:

'*Organic peroxides means liquid or solid organic substances which contain the bivalent -O-O- structure and may be considered derivatives of hydrogen peroxide, where one or both of the hydrogen atoms have been replaced by organic radicals. The term organic peroxide includes organic peroxide mixtures (formulations) containing at least one organic peroxide. Organic peroxides are thermally unstable substances or mixtures, which can undergo exothermic self-accelerating decomposition. In addition, they can have one or more of the following properties:*

(i) be liable to explosive decomposition;

(ii) burn rapidly;

(iii) be sensitive to impact or friction;

(iv) react dangerously with other substances.

An organic peroxide is regarded as possessing explosive properties when in laboratory testing the mixture (formulation) is liable to detonate, to deflagrate rapidly or to show a violent effect when heated under confinement.'

Background information and guidance on the definition is given in *Guidance on the Application of the CLP Criteria*, sections 2.15.1 and 2.15.2.

Classification criteria and relevant information

The Classification principles are given in CLP Annex I, sections 2.15.2 and 2.15.4. Background information and guidance on relevant aspects regarding the classification is given in *Guidance on the Application of the CLP Criteria*, sections 2.15.3, 2.15.4, 2.15.5, 2.15.6 and 2.15.7.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Only organic peroxides, as defined in CLP, Annex I, section 2.15.1 definition, have to be tested according to the UN-MTC, Part II test series A - H.

CLP Annex I, section 2.15.2.1. provides the following specific rules for adaptation of the standard information requirement for organic peroxides.

'Any organic peroxide shall be considered for classification in this class, unless it contains:

(a) not more than 1.0% available oxygen from the organic peroxides when containing not more than 1.0% hydrogen peroxide; or

(b) *not more than 0.5% available oxygen from the organic peroxides when containing more than 1.0% but not more than 7.0% hydrogen peroxide.*

NOTE: The available oxygen content (%) of an organic peroxide mixture is given by the formula:

$$16 \times \sum_{i} \left(\frac{n_i \times c_i}{m_i} \right)$$

where:

n_i *= number of peroxygen groups per molecule of organic peroxide i;*

c_i *= concentration (mass %) of organic peroxide i;*

m_i *= molecular mass of organic peroxide i.'*

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

A number of already tested and classified substances and mixtures are listed in the UN-RTDG, 2.5.3.2.4.

Available information may especially originate from the classification for transport. In the DSD organic peroxides were classified as oxidizing substances, by definition. More details are described in the *Guidance on the Application of the CLP Criteria*, sections 1.7.2.1 and 2.15.6. If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

For the determination of the organic peroxides, weight of evidence is not possible. Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At present QSAR is generally not applicable for organic peroxides. Application of (Q)SAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

A number of substances can, for safety reasons, only be handled and tested in diluted form, see the substances and mixtures listed in UN TDG, 2.5.3.2.4. Testing should always be considered if none of the waiving possibilities applies.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

Minor impurities can have an influence on thermal stability. Background information and guidance on these aspects is given in *Guidance on the Application of the CLP Criteria*, section 2.15.4.

How to conclude on the DSD classification

In the DSD organic peroxides are classified as oxidizing substances and a few of them as having explosive properties.

Endpoint specific information in the registration dossier / in IUCLID

Material and methods

- See UN MTC, Part II, classification procedures and test series A-H.

Results and discussion

The following data on organic peroxides should be submitted:

- if testing is waived, the reasons for waiving must be documented in the dossier;
- type of organic peroxide;
- SADT (Self accelerating decomposition temperature) together with the volume the SADT related to;
- detonation properties (Yes/Partial/No);
- deflagration properties (Yes rapidly/Yes slowly/No);
- effect of heating under confinement (Violent/Medium/Low/No);
- explosive power, if applicable (Not low/Low/None).

The following example shows how data mentioned above could be documented in the CSR:

Figure R.7.1-3 Example: Di-tert-butyl peroxide

UN Test Series A to H	Test method	Results + Evaluation	Remarks
Propagation of detonation	A.1	“No”	Fragmented length (cm): 16
Propagation of deflagration #1	C.1	“Yes, slowly "	Maximum pressure (kPa): > 2070 Time for a pressure rise from 690 to 2070 kPa (ms): 100
Propagation of deflagration #2	C.2	“No"	deflagration rate (mm/s): 0.27

Effect of heating under defined confinement #1	Koenen E.1	"No"	Limiting diameter (mm): < 1.0 Type of fragmentation (and pieces): O
Effect of heating under defined confinement #2	DPVT E.2	"Medium"	Limiting diameter (mm): 3.5
Explosive power	F.3	"Not Low"	Expansion (cm^3/10 g test sample): 28
Explosive power	F.4	"Not Low"	Average net expansion (cm^3): 12
SADT	H.4	80 °C	500 ml Dewar vessel
Competent Authority approval number	*Example from UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria*		

For assigning the Type of organic peroxide, the list of currently assigned organic peroxides according 2.5.3.2.4 of the UN RTDG can be used, in case the assignment was based on a test according to the UN MTC. The relevant underlying test data may be collected from the respective UN documents from the UN Committee of experts on the transport of dangerous goods, from test reports produced by either competent authorities or industry, or from other reliable sources (such as e.g. the dedicated database 'DATATOP').

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

A Template data set in IUCLID does not exist for the hazard class 'organic peroxides'. As long as there is no specific section in IUCLID the test results in section 4.23 'Additional physico-chemical information' should be inserted under the endpoint title 'organic peroxides'. The information on organic peroxides should not be included in IUCLID section 4.15 'Oxidising properties'. In the registration dossier the information should be included under flammability.

Further information / references

Background information and guidance on classification testing, additional testing and available information is given in *Guidance on the Application of the CLP Criteria*, section 2.15.

Data from the 'DATATOP' database can be obtained from the gatekeeper of this database TNO, Department Energetic Materials, Lange Kleiweg 137, 2288GJ, Rijswijk The Netherlands.

Various national guidelines which provide guidance and outline safe standards for handling and storage of organic peroxides for the assignment of organic peroxides to storage groups are available e.g. Netherlands Directive: Publication Series on Dangerous Substances 8 (PGS 8) Storage of Organic Peroxides, UK HSE: The storage and handling of organic peroxides - Guidance Note CS21 or German guideline: BGV B4.

R.7.1.11 Explosive properties

Some of the information requirements according to the REACH Regulation, Annex VII were phrased such that they correspond to 'indications of danger' as given in Annex II of DSD. For

substances, classification and labelling according to the CLP Regulation has been mandatory since 1 December 2010 (and will become mandatory for mixtures (preparations) from 1 June 2015, when the DSD and DPD will be repealed). Consequently, explosive properties are covered by classification of the substance according to the CLP Regulation. However, the physical hazards according to CLP are structured completely differently from the physico-chemical properties according to the DSD (and therefore also REACH, Annex VII). This means that for some of the CLP hazard classes an unambiguous assignment to one of the headlines (information requirements) in Annex VII to REACH is not possible. The assignment of hazard classes to the headline 'Explosive properties' as shown in Table below must therefore only be understood as a means to structure this document in accordance with Annex VII to REACH. It has to be noted that self-reactive substances and organic peroxides are primariliy assigned to the headline 'Flammability' and only a cross reference to corresponding sub-chapter under headling 'Flammability' is included in the sub-chapters on 'Explosive properties' below because these two hazard classses can have explosive and/or flammable properties.

Table R.7.1-8 Assignment of CLP hazard classes to the information requirement 'Explosive properties' according to REACH, Annex VII and correlation between the Test method Regulation and the test method according to CLP and supporting link with the Guidance on the Application of the CLP Criteria.

Information requirement according to Art. 10 (a) (vi) of the REACH Regulation (EC) No. 1907/2006 (the no. in brackets is the respective no. in the table in Annexes VII to IX to REACH)	**CLP Regulation (EC) No. 1272/2008** (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Chapter in revised R.7(a) guidance**	**Corresponding test method according to the Test Method Regulation, Regulation (EC) 440/2008**	**Corresponding test method according to the CLP Regulation**	**Chapter in the Guidance on the Application of the CLP Criteria (ex RIP 3.6)**
Explosive properties (7.11)	Explosives (2.1)*	R.7.1.11.1	A.14 Explosive properties	**UN Test series 1 to 3 (further test series 4 to 6 are necessary for classification)**	2.1
	Self-reactive substances and mixtures (2.8)*	R.7.1.11.2 see R.7.1.10.4	n.a.	**A.14 (existing data only)**	2.8
	Organic peroxides (2.15)*	R.7.1.11.3 See R.7.1.10.9	n.a.	**A.14 (existing data only)**	2.15

* Note that regardless of whether the hazard class or category is listed in Article 14(4)(a) REACH the chemical safety assessment must be performed in accordance with Article 14 (3) of REACH. Furthermore, according to Article 10(a)(iv) of REACH the technical dossier of a registration of a substance under the REACH Regulation must include information on classification and labelling of the substance as specified in section 4 of Annex VI to the REACH Regulation.

In addition, it has to be noted that some substances have explosive properties which do not result in classification. Examples are the following:

- substances with a positive result in UN Test Series 1 or 2 but which are exempted from the classification as explosives based on their packaging in UN Test Series 6;
- substances which are mechanically sensitive only. These are substances with a sensitiveness to impact (determined by UN Test Series 3 (a) (ii)) of 40 J or less and/or a sensitiveness to friction (determined by Test Series 3 (b) (i)) of 360 N or less for substances and mixtures which may have explosive properties based on the screening procedure according to Appendix 6, Part 3 of the UN-MTC and which are not classified as explosives, self-reactive or organic peroxide.

Such substances may be classified in other hazard classes (e.g. as flammable solids, oxidizing solids, corrosive to metals) or even not at all. Information about such explosive properties should be indicated in the dossier as well.

R.7.1.11.1 Explosives

Please note that explosive atmospheres as, for example, created by flammable liquids and by powders are not the subject of this chapter.

Definition

The following definitions are provided in CLP Annex I, section 2.1.1:

'An explosive substance or mixture is a solid or liquid substance or mixture of substances which is in itself capable by chemical reaction of producing gas at such a temperature and pressure and at such a speed as to cause damage to the surroundings. Pyrotechnic substances are included even when they do not evolve gases.

A pyrotechnic substance or mixture is a substance or mixture of substances designed to produce an effect by heat, light, sound, gas or smoke or a combination of these as the result of non-detonative self-sustaining exothermic chemical reactions.

An unstable explosive is an explosive substance or mixture which is thermally unstable and/or too sensitive for normal handling, transport and use.

An explosive article is an article containing one or more explosive substances or mixtures.

A pyrotechnic article is an article containing one or more pyrotechnic substances or mixtures.

An intentional explosive is a substance, mixture or article which is manufactured with a view to producing a practical, explosive or pyrotechnic effect.'

Organic Peroxides and Self Reactive Substances may also have explosive properties and should be screened. See chapter R.7.1.11.3 for Organic peroxides and chapter R.7.1.11.2 for Self Reactive Substances and Mixtures.

Intentional explosive

Council Directive 93/15/EEC of 5 April 1993 lays down rules for the harmonisation of the provisions relating to the placing on the market and supervision of explosives for civil uses.

Directive 2007/23/ EC on the placing on the market of pyrotechnic articles establishes rules designed to achieve the free movement of pyrotechnic articles in the internal market while, at the same time, ensuring a high level of protection of human health and public security and the protection and safety of consumers and taking into account the relevant aspects related to environmental protection. Pyrotechnic articles (CLP, Annex I, Section 2.1.1.2) are classified as explosives for CLP and as class 1 for transport (see UN-RTDG). Accoding to Article 9 and Annex II of Directive 2007/23/EC the conformity assessment procedures are carried out by notified bodies, which have to issue an EC type-examination certificate to the applicant. All data included in the EC type-examination certificate are sufficient for the information requirements under the REACH Regulation.

Classification criteria and relevant information

Substances, mixtures and articles of this class are classified as an unstable explosive on the basis of the flowchart in Annex I to CLP Regulation, Figure 2.1.2. The test methods are described in Part I of the UN-MTC.

Explosives, which are not classified as an unstable explosive, must be classified in one of the six Divisions referred to in paragraph 2.1.2.2 of Annex 2.1 to the CLP Regulation, based on the

results of the tests laid down in Table 2.1.1 on Test Series 2 to 8 in Part I of the UN-MTC. If explosives are unpackaged or repacked in packaging other than the original or similar packaging, they must be retested. If a substance gives a positive result in any of the test series 1 or 2 this should be mentioned in the REACH registration dossier for the substance, even if it would not be classified as an 'Explosive' in Test Series 6.

The test methods used for deciding on provisional acceptance into the class of explosives are grouped into four series, numbered 1 to 4 (see CLP Annex I, Figure 2.1.2).

It may be important for the safety of testers that certain tests, using small amounts of material, be conducted first before proceeding to test with larger quantities. Therefore it is highly recommended to start the testing procedure with Test Series 3, because these tests involve relatively small sample sizes, which reduces the risk to personnel.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for explosive properties.

'The study does not need to be conducted if:

- *there are no chemical groups associated with explosive properties present in the molecule, or*
- *the substance contains chemical groups associated with explosive properties which include oxygen and the calculated oxygen balance is less than -200, or*
- *the organic substance or a homogenous mixture of organic substances contains chemical groups associated with explosive properties, but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 °C, or*
- *for mixtures of inorganic oxidising substances (UN Division 5.1) with organic materials, the concentration of the inorganic oxidising substance is:*
  - *less than 15 %, by mass, if assigned to UN Packaging Group I (high hazard) or II (medium hazard),*
  - *less than 30 %, by mass, if assigned to UN Packaging Group III (low hazard).*

Note: Neither a test for propagation of detonation nor a test for sensitivity to detonative shock is required if the exothermic decomposition energy of organic materials is less than 800 J/g.'

Note on the use of the Oxygen Balance:

The oxygen balance is calculated for the chemical reaction:

$$C_xH_yO_z + [x + (y/4) - (z/2)]\ O_2 \rightarrow x\ CO_2 + (y/2)\ H_2O$$

Using the formula:

Oxygen balance = -1600 [2x + (y/2)-z]/molecular weight;

The oxygen balance was developed for compounds containing only nitrate groups and it applies only to organic substances. Extending its use to molecules with other oxygen containing groups should be done with care. As an example the presence of hydroxyl-groups will strongly affect the oxygen balance towards higher values, whereas this group does not contribute to explosive properties. In addition the presence of for instance halogens tends to decrease the flammability and explosivity but this is not accounted for.

Please also check Appendix 6, Section 3 of the UN-MTC.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met. Application of weight of evidence is possible with substances where explosive properties can clearly be excluded. Weight of evidence should be accompanied with extensive and reliable literature references.

(Q)SAR

There is currently no QSPR/(Q)SAR software known with sufficient accuracy and reliability to assist in assessing (potential) explosive properties. DSC testing is cheap and fast and is strongly recommended to identify potential hazards connected with the substance.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

An assessment of chemical structure would formally form part of a column 2 waiver. For further information please refer to the *Guidance on the Application of the CLP Criteria*, Part 2: Physical Hazards, Section 2.1 Explosives.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. Testing for explosives may be omitted if it is technically not possible to conduct the study as a consequence of the properties of the substance.

FURTHER ADAPTATION POSSIBILITIES

Testing may be waived if there are no chemical groups associated with explosive properties present in the molecule. The potential generation of explosive atmospheres by flammable

gases/liquids or combustible solids is not considered an explosive property and should therefore not be reported under this heading.

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

Impurities; uncertainties

Small amounts of other compounds may enhance or suppress the chemical reaction that gives the explosive property to a substance. Therefore impurities may considerably influence the explosive properties of a substance. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

How to conclude on the DSD classification

For DSD explosives are substances and preparations which may explode under the effect of flame or which are more sensitive to shocks or friction than dinitrobenzene.

Reclassification of substances classified as explosive according to DSD:

Under the regime of the old DSD, testing of explosive properties was achieved by performing test method A.14. For classification purposes under the CLP Regulation this test is not adequate in the case of a negative result for thermal sensitivity. The test method A.14 stops with a limiting diameter of 2 mm, while UN Test E.1 proceeds to down to a 1 mm orifice. Testing according to the CLP Regulation is the same as that described in Part I of the UN-MTC. This is why the translation table of Annex VII of the CLP Regulation states that there is no direct translation possible for classification from (E, R2) and (E, R3) to CLP criteria.

Therefore, if the screening procedure of section 2.1.4.2 of the CLP Regulation identifies a substance or mixture to be a potential explosive, appropriate data are required for classification.

Moreover, if data from performing test method A.14 or the UN Test series 3 tests 3a or 3b indicate that a substance is sensitive to impact or friction such information should be provided in the REACH registration dossier.

Endpoint specific information in the registration dosser / in IUCLID

Material and methods

- reference to the standard and the test method applied;
- description of the substance that was tested.

Results and discussion

- if testing is waived, the reasons for waiving must be documented in the dossier;
- if testing is not waived then the tests done according to the UN Test Manual and the outcome (explosive or not explosive) must be documented in the dossier. The mechanical sensitivity test according to UN Test Series 3a and 3b must be done and documented if UN Test Series 1 or 2 give a positive result. If data according to test method A.14 are available, then the results can be used instead of UN Test series 3a and 3b.

An example is given below of how summarised results from the application of the class 1 procedure for the hypothetical substance 'New explosive substance' could be presented.

Figure R.7.1-4 Results from application of the class 1 acceptance procedure

1. Name of substance	New explosive substance
2. General data	2.1 Composition : technically pure 2.2 Physical form : Fine crystalline powder 2.3 Colour : Yellow
3. Box 2	Is the substance manufactured with the view to producing a practical explosive or pyrotechnic effect? 3.1 Answer : **No**
4. Box 3	4.1 Propagation of Detonation : UN-Test A.1 Result : "-", no propagation of detonation 4.2 Effect of heating under confinement: 4.2.1 Koenen test (test 1(b)) Result : "+", 4.2.2 Time/pressure test (test 1(c)(i)) Result : "-", no effect on ignition under confinement 4.5 Exit : Go to Box 4
5. Box 4	Is it an explosive substance? 5.1 Answer from Test Series 1 : **Yes** 5.2 Exit : Go to box 5
6. Box 5	6.1 Sensitivity to shock : based on the test result of UN-Test A.1 Result "-" 6.2 Effect of heating under confinement: Koenen test (test 2(b)): limiting diameter 2,5 mm Result: "+" 6.3 Exit : Go to Box 6
7. Box 6 :	Is the substance too insensitive for acceptance into Class 1? 7.1 Answer from Test Series 2 : **No** 7.2 Conclusion : Substance to be considered for Class 1 (box 8) 7.3 Exit : Go to Box 9
8. Box 9	Test Series 3 8.1 Thermal Stability: based on the DSC measurement data Result: thermally stable 8.2 Impact sensitivity : BAM fallhammer test (test 3(a)(ii)) Result : "-", not too dangerous to transport in form tested 8.3 Friction sensitivity : BAM friction test (test 3(b)(i))

	Result : "-", not too dangerous to transport in form tested 8.4 Exit : Go to box 10
9. Box 10	Is the substance thermally stable? 9.1 Answer from DSC data : **Yes** 9.2 Exit : Go to box 11
10. Box 11	Is the substance too dangerous for transport in the form in which it was tested? 10.1 Answer from Test Series 3 (a)(ii) and 3 (b)(i): **No** 10.2 Exit : Go to box 18
11. Conclusion	**PROVISIONALLY ACCEPT INTO CLASS 1** 11.1 Exit : Apply the Class 1 assignment procedure

Figure R.7.1-5 Results from the application of the class 1 assignment procedure

1. Box 19	Is the substance a candidate for Division 1.5? 1.1 Answer : **No** 1.2 Exit : Go to box 25
2. Box 25	2.1 UN-Tests 6(a) and 6(c) were not conducted because the substance showed no propagation of detonation in the UN-Test A.1 and also no propagation of deflagration in the UN-test 1(c)(ii). 2.2 UN-Test 6 (c) Sample conditions: 1 × 30 kg fibre drum Observations: Only slow burning with black smoke and soot occurred. 2.3 Exit : Go to box 26
3. Box 26	Is the result a mass explosion? 3.1 Answer from Test Series 6 : **No** 3.2 Exit : Go to box 28
4. Box 28	Is the major hazard that from dangerous projections? 4.1 Answer from Test Series 6 : **No** 4.2 Exit : Go to box 30
5. Box 30	Is the major hazard radiant heat and/or violent burning but with no dangerous blast or projection hazard? 5.1 Answer from Test Series 6 : **No** 5.2 Exit : Go to box 32
6. Box 32	Is there nevertheless a small hazard in the event of ignition or initiation? 6.1 Answer from Test Series 6 : **No** 6.2 Exit : Go to box 35
7. Box 35	Is the substance or article manufactured with the view to producing a practical explosive or pyrotechnic effect? 7.1 Answer : **No**

	7.2 Exit : Go to box 38
8. Conclusion	**NOT CLASS 1** 8.1 Exit : Consider for another class/division

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on explosiveness can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.14	VII 7.11	Explosiveness	E.4.15	3.13

Further information / references

Further information about classification and testing for explosives can be found in the *Guidance on the application of CLP criteria*, section 2.1.

Gharagheizi F. Quantitative structure-property relationship for prediction of the lower flammability limit of pure compounds. Energy & Fuels 22 (2008) 3037-3039.

Gharagheizi F. A new group contribution-based model for estimation of lower flammability limit of pure compounds. J. Haz. Mat. 170 (2009a) 595-604.

R.7.1.11.2 Self-reactive substances and mixtures

Self-reactive substances are primariliy assigned to the headline ‘Flammability’ therefore please also refer to chapter R.7.1.10.4.

The sensitivity of self-reactive substances to impact (solids and liquids) and friction (solids only) may be of importance for the safe handling of the substances, in the event that these substances have pronounced explosive properties. If data according to EU test method A.14 as described in Regulation (EC) No 440/ 2008 are available, then this information should be part of the hazard communication in the registration dossier (REACH Annex VII, 7.11).

R.7.1.11.3 Organic peroxides

Organic peroxides are primariliy assigned to the headline ‘Flammability’ therefore please also refer to chapter R.7.1.10.9.

The sensitivity of organic peroxides to impact (solids and liquids) and friction (solids only) may be of importance for the safe handling of the substances, in the event that these substances have pronounced explosive properties. If data according to EU test method A.14 as described in Regulation (EC) No 440/ 2008 are available, then this information should be part of the hazard communication in the registration dossier (REACH Annex VII, 7.11).

R.7.1.12 Self-ignition temperature

The terminology used in Annex VII of REACH is not very precise. Therefore, some guidance in interpretation appears necessary:

For liquids and gases, the term '**auto-ignition**' instead of 'self-ignition' is generally used. Auto-ignitability is of high importance for the assignment of temperature classes in explosion protection (i. e. ATEX in Europe) of plants and equipment.

For solids and liquids adsorbed on a large surface, **self-heating** may occur by reaction with air with subsequent ignition. According to the CLP Regulation, Annex I, section 2.11, a self-heating substance or mixture is a liquid or solid substance or mixture, other than a pyrophoric liquid or solid, which, by reaction with air and without energy supply, is liable to self-heat; this substance or mixture differs from a pyrophoric liquid or solid in that it will ignite only when in large amounts (kilograms) and after long periods of time (hours or days).Therefore solids are considered under self heating substances in the chapter below.

Table R.7.1-9 Assignment of CLP hazard classes to the information requirement 'Self ignition temperature' according to REACH, Annex VII and the Test Method Regulation.

Information requirement according to Art. 10 (a) (vi) of the REACH Regulation (EC) No. 1907/2006 (the no. in brackets is the respective no. in the table in Annexes VII to IX to REACH)	**CLP Regulation (EC) No. 1272/2008** (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Chapter in revised R.7(a) guidance**	**Corresponding test method according to The Test Method Regulation 440/2008**	**Corresponding test method according to CLP Regulation**	**Chapter in the *Guidance on the Application of the CLP Criteria*** (ex RIP 3.6)
Self ignition temperature (7.12)	For gases and liquids*	7.1.12.1	A.15 Auto-ignition temperature (liquids and gases)	n.a.	n.a.
	For solids * Note: the UN Test N.4 is preferable to generate the information for this endpoint. Refer to R.7.1.10.7.	7.1.12.2, 7.1.10.7	A.16 Relative self-ignition temperature for solids	n.a.	Section 2.11

* Note that regardless of whether the hazard class or category is listed in Article 14 (4) (a) of REACH, the chemical safety assessment (when required) must be performed in accordance with Article 14 (3) of REACH. Furthermore, according to Article 10 (a) (iv) of REACH the technical dossier of a registration for a substance under the REACH Regulation must include information on classification and labelling of the substance as specified in section 4 of Annex VI to the REACH Regulation.

R.7.1.12.1 Auto-ignition

Type of property

For liquids and gases, the term '**auto-ignition**' instead of 'self-ignition' is generally used. Auto-ignitability is of high importance for the assignment of temperature classes in explosion protection (i. e. ATEX in Europe) of plants and equipment. In this chapter, only the **auto-ignition** phenomena will be discussed.

Definition

The degree of auto-ignitability is expressed in terms of the auto-ignition temperature. The auto-ignition temperature is the lowest temperature at which the test substance will ignite when mixed with air under the conditions defined in the test method.

Test method(s)

For testing Auto-ignition temperature, method A.15 of Regulation (EC) 440/2008 should be used, which references several national and international standards (e.g. EN 14522, etc.). The test procedure is applicable to gases, liquids and vapours which, in the presence of air, can be ignited by a hot surface.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for self-ignition temperature.

'The study does not need to be conducted:

- *if the substance is explosive or ignites spontaneously with air at room temperature; or*
- *for liquids non flammable in air, e.g. no flash point up to 200 °C; or*
- *for gases having no flammable range, or*
- *for solids,if the substance has a melting point ≤ 160 °C, or if preliminary results exclude self-heating of the substance up to 400 °C.'*

This means:

For gases:

Only gases classified as flammable according to the CLP Regulation have to be considered.

For liquids:

The auto-ignition temperature should be determined according to Directive EC 440/2008, method A.15. No data are required for liquids classified as:

- pyrophoric; or
- explosive, unstable or division 1.1 to 1.6; or
- organic peroxide; or

- self-reactive substance.

Further, the auto-ignition temperature does not have to be determined for liquids having no flash point up to 200 °C. In practice, liquids with a boiling point above 350 °C will not have a flash point below 200 °C. Therefore, determination of the auto-ignition temperature is not necessary in such cases if the flash point is not known.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

For the determination of the auto-ignition temperature, the weight of evidence approach is not possible. Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

For the determination of the auto-ignition temperature, (Q)SAR approaches are strongly discouraged for the purpose of classification/ risk assessment.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the auto-ignition temperature read-across is usually not possible. However interpolation may still be possible within homologous series.

However, it is not possible to read across from methyl compounds to ethyl and propyl compounds and vice versa.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. Substances which decompose below room temperature or which react vigorously with moisture may be difficult to test. In such cases, the test may be waived due to technical reasons.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

The auto-ignition temperature can be considerably reduced by the presence of catalytic impurities. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

Endpoint specific information in the registration dossier / in IUCLID

Material and methods

- description of the apparatus or reference to the standard or the test method applied;
- quantity of sample used.

Results and discussion

- the value or the range of the auto-ignition temperature;
- if testing is waived, the reasons for waiving must be documented in the dossier.

For liquids/gases: observations (e.g decomposition with air, reactions with moisture, etc.)

For solids see the below chapter R.7.1.12.2.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on auto flammability (self-ignition temperature) can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.12	VII 7.12	Auto flammability	E.4.13	3.11

R.7.1.12.2 Self-heating substances

For solids and liquids adsorbed on a large surface, **self-heating** may occur by reaction with air with subsequent ignition. According to the CLP Regulation Annex I, section 2.11 the following definition is provided:

'A self-heating substance or mixture is a liquid or solid substance or mixture, other than a pyrophoric liquid or solid, which, by reaction with air and without energy supply, is liable to self-heat; this substance or mixture differs from a pyrophoric liquid or solid in that it will ignite only when in large amounts (kilograms) and after long periods of time (hours or days).'

The ECHA *Guidance on the Application of the CLP Criteria* gives in Chapter 2.11 detailed information on the self-heating property, the CLP-classification, the relevant test method and the relation to the DSD and the transport of dangerous goods regulations.

See section R.7.1.10.7 of this guidance document for further details and information.

R.7.1.13 Oxidising properties

Some of the information requirements according to REACH Annex VII were phrased such that they correspond to 'indications of danger' as given in Annex II of DSD. For substances, classification and labelling according to the CLP Regulation has been mandatory since 1 December 2010 (and will become mandatory for mixtures (preparations) from 1 June 2015,

when the DSD and DPD will be repealed). Consequently, information requirements on oxidising properties are inherently covered by classification of the substance according to the CLP Regulation. However, the physical hazards according to CLP Regulation are structured completely differently from the physicochemical properties according to DSD (and therefore also REACH, Annex VII). This means that for some of the CLP hazard classes an unambiguous assignment to one of the headlines (information requirements) in Annex VII to REACH is not possible. The assignment of hazard classes to the headline 'oxidising properties' as shown in table below must therefore only be understood as a means to structure this document in accordance with Annex VII to REACH.

Table R.7.1-10 Assignment of CLP hazard classes to the information requirement 'Oxidising properties' according to REACH, Annex VII and correlation between the Test method Regulation and the test method according to CLP and supporting link with the Guidance on the application of the CLP Criteria.

Information requirement according to Art. 10 (a) (vi) of the REACH Regulation (EC) No. 1907/2006 (the no. in brackets is the respective no. in the table in Annexes VII to IX to REACH)	**CLP Regulation (EC) No. 1272/2008** (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Chapter in revised R.7(a) guidance**	**Corresponding test method according to The Test Method Regulation , Regulation (EC) 440/2008**	**Corresponding test method according to CLP Regulation**	**Chapter in the Guidance on the Application of the CLP Criteria** (ex RIP 3.6)
Oxidising properties (7.13)	Oxidising gases (2.4) *	7.1.13.1	n.a.	**ISO 10156**	**2.4**
	Oxidising liquids (2.13) *	7.1.13.2	A.21 Oxidising properties (liquids)	**UN Test O.2**	**2.13**
	Oxidising solids (2.14) *	7.1.13.3	A.17 Oxidising properties (solids)	**UN Test O.1**	**2.14**

* Note that regardless of whether the hazard class or category is listed in Article 14 (4)(a) of REACH the chemical safety assessment (when required) must be performed in accordance with Article 14 (3) REACH. Furthermore, according to Article 10(a)(iv) of REACH the technical dossier of a registration of a substance under the REACH Regulation must include information on classification and labelling of the substance as specified in section 4 of Annex VI to the REACH Regulation.

R.7.1.13.1 Oxidising gases

Definition

The following definition of oxidising gases is provided in CLP Annex I, section 2.4.1.:

'Oxidising gas means any gas or gas mixture which may, generally by providing oxygen, cause or contribute to the combustion of other material more than air does.'

The criteria *'more than air does'* is further defined in a Note under Table 2.4.1 in Section 2.4.1 as *'having an oxidising power greater than 23.5 % as determined by a method specified in ISO 10156 as amended'.*

Classification criteria and relevant information

All oxidising gases are classified as oxidising gas, Category 1 (Ox. Gas 1, H270). Detailed guidance on the classification criteria and the test method(s) can be found in the *Guidance on the application of the CLP Criteria*, section 2.4.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for oxidising properties.

'The study does not need to be conducted if:

- *the substance is explosive, or*
- *the substance is highly flammable, or*
- *the substance is an organic peroxide, or*
- *the substance is incapable of reacting exothermically with combustible materials, for example on the basis of the chemical structure (e.g. organic substances not containing oxygen or halogen atoms and these elements are not chemically bonded to nitrogen or oxygen, or inorganic substances not containing oxygen or halogen atoms).*

The full test does not need to be conducted for solids if the preliminary test clearly indicates that the test substance has oxidising properties.

Note that as there is no test method to determine the oxidising properties of gaseous mixtures, the evaluation of these properties must be realised by an estimation method based on the comparison of the oxidising potential of gases in a mixture with that of the oxidising potential of oxygen in air.'

According to above indents, the study therefore does not need to be conducted if the gas:

- is classified as highly flammable; or
- does not contain oxygen, fluorine and/or chlorine which are chemically bonded to elements other than carbon or hydrogen.

The other above cited indents are not relevant for this endpoint.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

There are not many gases that are oxidising. Most oxidising gases are identified as such in the UN-RTDG and in ISO 10156: 2010 *Gas cylinders - Gases and gas mixtures: - Determination of fire potential and oxidizing ability for the selection of cylinder valve outlets.*

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

There is no known scientific literature that refers to test results for gases that are not classified in ISO 10156 nor in the UN-RTDG.

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At present (Q)SAR is generally not applicable for the determination of oxidising limits of gases. Application of (Q)SAR is not possible. However, assessment of the chemical structure may be used to exclude oxidising behaviour of a substance. Possibly, this relation could be exploited in the development of future QSPR methods.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the oxidising gases read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

The normal level of impurities in the technical grade of oxidising gases does not impact the result of the test. Tests should be performed with the lowest concentration of impurities in the gas encountered in the normal manufacturing process and the moisture content should be less than or equal to 0.01 mol%. Therefore utmost care should be taken in the selection of the key study(ies), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

How to conclude on the DSD classification

All gases with a positive test result according to the test method described in ISO 10156 are classified ‘Oxidising O, R8’.

Endpoint specific information in the registration dosser / in IUCLID

Material and methods

- reference to the standard applied.

Results and discussion

- if the test is positive indicate that the gas is 'oxidising';
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.15	VII 7.13	Oxidising properties	E.4.16	3.14

Further information / references

Further information about classification and testing for oxidising gases can be found in the ECHA *Guidance on the application of CLP criteria*, section 2.4.

The test method is described in ISO 10156. The test is qualitative. If reaction is observed during the test, the gas to be evaluated is oxidizing.

For several gases, a 'coefficient of oxygen equivalency' (Ci) has been deduced from the explosion ranges observed during the tests. The Ci factors are listed in ISO 10156 along with the list of oxidising gases.

R.7.1.13.2 Oxidising liquids

Definition

The following definition of oxidising liquids is provided in CLP Annex I, section 2.13.1.:

'Oxidising liquid means a liquid substance or mixture which, while in itself not necessarily combustible, may, generally by yielding oxygen, cause, or contribute to, the combustion of other material.'

Classification criteria and relevant information

According to the CLP Regulation, a liquid is classified as an oxidising liquid if, in testing according to the UN Test O.2 of the UN-MTC (Part III, Section 34) it is at least as oxidising as a 65 % aqueous solution of nitric acid. The CLP Regulation has three categories for Oxidising Liquids. The category is also determined through the UN Test O.2, by comparison to various reference oxidisers.

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for oxidising properties.

'The study does not need to be conducted if:

- *the substance is explosive, or*
- *the substance is highly flammable, or*
- *the substance is an organic peroxide, or*
- *the substance is incapable of reacting exothermically with combustible materials, for example on the basis of the chemical structure (e.g. organic substances not containing oxygen or halogen atoms and these elements are not chemically bonded to nitrogen or oxygen, or inorganic substances not containing oxygen or halogen atoms).*

The full test does not need to be conducted for solids if the preliminary test clearly indicates that the test substance has oxidising properties.

Note that as there is no test method to determine the oxidising properties of gaseous mixtures, the evaluation of these properties must be realised by an estimation method based on the comparison of the oxidising potential of gases in a mixture with that of the oxidising potential of oxygen in air.'

The first indent states that explosive substances should not be tested for oxidising properties. For instance, organic substances with oxidising functional groups may be explosive and should first undergo the screening procedures for explosive properties in Annex 6 of the UN-MTC to rule out possible explosive behaviour. Such substances may also be thermally unstable and show self-reactive behaviour. Substances that have been classified as Explosives according to the CLP Regulation or have been assigned risk phrases R2 or R3 according the DSD, should normally not be tested for oxidising properties, since they are known to be explosive.

The second indent states that highly flammable substances do not have to be tested for oxidising properties. While it is not very clear what 'highly flammable' means in this case (whether it is or is not intended to mean 'extremely flammable' and 'flammable'), liquids that have a low flash point, or which are pyrophoric, are rarely oxidising. This implies that liquids classified as Flammable Liquids category 1 or 2, or as Pyrophoric Liquids, according to the CLP Regulation, normally do not need to be tested for oxidising properties. This corresponds to classification with risk phrases R12, R11 or R17 according to the DSD. If they contain oxidising functional groups, such substances may instead show self-reactive or explosive behaviour.

The third indent states that organic peroxides should not be tested for oxidising properties. Organic peroxides are distinguished by their chemical structure, and should be treated according to the procedures for the hazard class Organic Peroxides of the CLP Regulation, see Section R.7.1.10.9 of this document.

Waiving according to the fourth indent relies on the absence of particular molecular structural features. The wording is more precise in section 2.13.4 of Annex I to the CLP Regulation, which is in principle the same as the wording as in section 6 of Appendix 6 to the UN-MTC.

The last two paragraphs above quoted from Column 2 Specific rules for adaptation from Column 1 are not applicable for this endpoint.

According to 2.13.4.1 of Annex I to the CLP Regulation, an organic liquid does not have to be assessed for oxidising properties if:

a. *'the substance does not contain oxygen, fluorine or chlorine; or*
b. *the substance contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.'*

For inorganic liquids, assessment of oxidising properties does not have to be done if the substance does not contain any oxygen or halogen atoms, according to section 2.13.4.2 of Annex I to the CLP Regulation.

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

The UN Test O.2 of the UN-MCT is also used for classification according to the UN-RTDG,and consequently also in the various regulations on transport of dangerous goods e.g. ADR and RID. A liquid that has been classified as belonging to Division 5.1 according to the regulations on transport of dangerous goods on the basis of results from the UN Test O.2, is an Oxidising Liquid according to the criteria of the CLP Regulation.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

For the determination of whether a liquid is an oxidising liquid, weight of evidence is not possible. Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At the time of writing, no reliable (Q)SAR-methods exist for sufficiently accurate predictions of oxidising properties. As explained above, however, assessment of the chemical structure may be used to exclude oxidising behaviour of a substance. Possibly, this relation could be exploited in the development of future QSPR-methods. Such an assessment of chemical structure would formally form part of a Column 2 adaptation justification.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the whether a liquid is an oxidising liquid, read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies. Some oxidising substances may decompose when heated. Substances may occasionally react with cellulose in other ways than through oxidation of the cellulose (e.g. through breaking chemical bonds within the cellulose). See also section 2.13.4.4 of Annex I to the CLP Regulation.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

Minor impurities will usually not influence the test, unless they are very strong oxidisers. Expert judgement should be used to determine whether impurities may have an effect. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

A few substances may show other reactions than pure oxidation of the cellulose, or may decompose. If this is suspected, expert judgement should be sought. See also section 2.13.4.4 of Annex I to the CLP Regulation.

How to conclude on the DSD classification

Any substance classified as an oxidising liquid according to the CLP-criteria should normally be classified with risk phrase R8 or R9 according to the DSD. The DSD-criteria for classification with risk phrase R9 are not very precise, but if the CLP classification is Category 1, the substance should be classified with risk phrase R9 if the reaction with cellulose is violent, e.g. if spontaneous ignition occurs in the test.

In the DSD, the A.21 test method of Regulation (EC) 440/2008 is used for the assessment of oxidising properties of liquids. This method is in principle identical to the UN Test O.2 of the UN-MTC used in the CLP Regulation. However, the DSD does not make any division corresponding to the categories of the CLP, and therefore only one reference substance is used in the A.21 test method. Since the CLP Regulation method is used for classification of substances, it is strongly advisable to use the UN Test O.2 instead of the A.21 test method. This is because the O.2 test method will also give more detailed information on the oxidising behaviour of a substance (or mixture), since more reference mixtures are used.

Endpoint specific information in the registration dosser / in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied.

Results and discussion

- indicate the results of the spontaneous ignition test;
- indicate the mean pressure rise time for the test substance;
- indicate the mean pressure rise time for the reference substance(s);
- interpretation of results;
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.15	VII 7.13	Oxidising properties	E.4.16	3.14

Further information / references

The ECHA guidance document *Guidance on the Application of the CLP Criteria* gives in Chapter 2.13 detailed information on the oxidising property, the CLP-classification, the UN Test O.2 and the relation to the DSD and the transport of dangerous goods regulations.

R.7.1.13.3 Oxidising solids

Definition

The following definition of oxidising solids is provided in CLP Annex I, section 2.14.1:

'Oxidising solid means a solid substance or mixture which, while in itself is not necessarily combustible, may, generally by yielding oxygen, cause, or contribute to, the combustion of other material.'

Classification criteria and relevant information

According to the CLP Regulation, a solid is classified as an oxidising solid if in testing according to the UN Test O.1 of the UN-MTC (Part III, Section 34), it is at least as oxidising as potassium bromate in a 3:7 mixture with cellulose. The test is based on the burning behaviour of a mixture of cellulose and the tested solid. The CLP Regulation has three categories for oxidising solids. The category is also determined through the UN Test O.1 in the UN-MTC by comparison to reference mixtures of cellulose and potassium bromate[20].

Adaptation of the standard testing regime

ADAPTATION POSSIBILITIES ACCORDING TO COLUMN 2 OF ANNEX VII TO REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for oxidising properties.

'The study does not need to be conducted if:

- *the substance is explosive, or*
- *the substance is highly flammable, or*
- *the substance is an organic peroxide, or*
- *the substance is incapable of reacting exothermically with combustible materials, for example on the basis of the chemical structure (e.g. organic substances not containing oxygen or halogen atoms and these elements are not chemically bonded to nitrogen or oxygen, or inorganic substances not containing oxygen or halogen atoms).*

[20] At the time of writing, work is in progress at the UN-level to modify Test O.1: Test for oxidising solids. This includes changing the reference substance and introducing a gravimetric method for the measurement. For further information, see document UN/SCEGHS/23/INF.17 available at the following link: http://www.unece.org/fileadmin/DAM/trans/doc/2012/dgac10c4/UN-SCEGHS-23-INF17.doc-UN-SCETDG-41-INF.43e.pdf .

The full test does not need to be conducted for solids if the preliminary test clearly indicates that the test substance has oxidising properties.

Note that as there is no test method to determine the oxidising properties of gaseous mixtures, the evaluation of these properties must be realised by an estimation method based on the comparison of the oxidising potential of gases in a mixture with that of the oxidising potential of oxygen in air.'

The first indent states that explosive substances should not be tested for oxidising properties. For instance, organic substances with oxidising functional groups may be explosive and should first undergo the screening procedures for explosive properties in Annex 6 of the UN-MTC to rule out possible explosive behaviour. Such substances may also be thermally unstable and show self-reactive behaviour. Substances that have been classified as Explosives according to the CLP-regulation or have been assigned risk phrases R2 or R3 according the DSD, should normally not be tested for oxidising properties, since they are known to be explosive.

The second indent states that highly flammable substances do not have to be tested for oxidising properties. While it is not very clear what 'highly flammable' means in this case (whether it is or is not intended to mean 'extremly flammable' and 'flammable'), solids classified as Flammable Solids or as Pyrophoric Solids according to the CLP-regulation are rarely oxidising. This corresponds to classification with risk phrases R11 or R17 according to the DSD. If they contain oxidising functional groups, such substances may instead show self-reactive or explosive behaviour.

The third indent states that organic peroxides should not be tested for oxidising properties. Organic peroxides are distinguished by their chemical structure, and should be treated according to the procedures for the hazard class Organic Peroxides of the CLP-regulation, see Section R.7.1.10.9 of this document.

Waiving according to the fourth indent relies on the absence of particular molecular structural features. The wording is more precise in section 2.14.4 of Annex I to the CLP-regulation, which is in principle the same as the wording as in Section 6 of Appendix 6 to the UN-MTC.

The first note under last indent from REACH Annex VII, which allows waiving of further testing, namely *'[...] if the preliminary test clearly indicates that the test substance has oxidising properties'* is relevant only when using the A.17 test method of Regulation (EC) 440/2008, which is **not** the preferred test method since it belongs to the DSD classification system. The UN Test O.1 used for classification according to the CLP Regulation does not include any preliminary test.

The last note taken from Column 2 'Specific rules for adaptation from Column 1' is not applicable for this endpoint. For inorganic solids, assessment of oxidising properties does not have to be done if the substance does not contain any oxygen or halogen atoms, according to section 2.14.4.2 of Annex I to the CLP Regulation.

According to section 2.14.4.1 of Annex I to the CLP-regulation, an organic solid does not have to be assessed for oxidising properties if:

a. *'the substance does not contain oxygen, fluorine or chlorine; or*

b. *the substance contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.'*

ADAPTATION POSSIBILITIES ACCORDING TO ANNEX XI TO REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

The UN Test O.1 of the UN-MTC is also used for classification according to the UN-RTDG, and consequently also in the various regulations on transport of dangerous goods e.g. ADR and RID. A solid that has been classified as belonging to Division 5.1 according to the regulations on transport of dangerous goods on the basis of results from the UN Test O.1, is an oxidising solid according to the criteria of the CLP Regulation.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At the time of writing, no reliable (Q)SAR-methods exist for sufficiently accurate predictions of oxidising properties. As explained above, however, assessment of the chemical structure may be used to exclude oxidising behaviour of a substance. Possibly, this relation could be exploited in the development of future (Q)SPR-methods. Such an assessment of chemical structure would formally form part of a Column 2 adaptation argument.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the oxidising solids read-across is usually not possible. However interpolation may still be possible within homologous series.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. Some substances may decompose upon heating. Substances may occasionally react with cellulose in other ways than through oxidation of the cellulose.

FURTHER ADAPTATION POSSIBILITIES

Not foreseen.

Impurities; uncertainties

The UN Test O.1 is (currently) performed using the unaided eye as measuring instrument. Only by expert judgement and thorough experience can the result of the test be correctly judged, and even then uncertainties may arise.

Minor impurities will usually not influence the test, unless they are very strong oxidisers. Expert judgement should be used to determine whether impurities may have an effect.

A few substances may show other reactions than pure oxidation of the cellulose, or may decompose. If this is suspected, expert judgement should be sought. Particle size and size distribution can have an influence on the test results.

How to conclude on the DSD classification

Any substance classified as an oxidising solid according to the CLP Regulation criteria should normally be classified with risk phrase R8 or R9 according to the DSD. The DSD-criteria for classification with risk phrase R9 are not very precise, but if the CLP Regulation classification is Category 1, the substance should be classified with risk phrase R9 if the reaction with cellulose is violent.

In the DSD, the A.17 test method of Regulation (EC) 440/2008 is used for the assessment of oxidising properties of solids. Although the principle of this method is to a large extent the same as that of the UN Test O.1 of the UN-MTC, the experimental set-up, reference substance (barium nitrate) and measured quantity differ. Furthermore, the DSD does not make any division corresponding to the categories of the CLP. Since the CLP Regulation is used for classification of substances, it is not advisable to use the A.17 method (which belongs to the DSD classification system). Instead, the UN Test O.1 should be used, which will also give more detailed information on the oxidising behaviour of a substance (or mixture), since more reference mixtures are used.

Endpoints specific information in the registration dosser/in IUCLID

Material and methods

- description of the apparatus and dimensions or reference to the standard or the test method applied;
- particle size and distribution.

Results and discussion

- if testing is waived, the reasons for waiving must be documented in the dossier.

If the UN Test O.1 was used:

- indicate if a vigorous reaction was observed;
- indicate the maximum burning time for the test mixture;
- indicate the maximum burning time for the reference mixtures;
- interpretation of results, including any relevant special observations;
- estimated accuracy of the result (including bias and precision).

If A.17 test method was used:

- indicate if in the preliminary test, a vigorous reaction was observed;
- indicate the maximum burning rate for the test mixture;
- indicate the maximum burning rate for the reference mixture;
- interpretation of results, including any relevant special observations;
- estimated accuracy of the result (including bias and precision).

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on oxidising properties can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.15	VII 7.13	Oxidising properties	E.4.16	3.14

Further information / references

The ECHA *Guidance on the Application of the CLP Criteria* gives in Chapter 2.14 detailed information on the oxidising property, the CLP-classification, the UN Test O.1 and the relation to the DSD and the transport of dangerous goods regulations.

R.7.1.14 Granulometry

Advice to registrants with regard to nanomaterials characterisation of granulometry can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.3 Granulometry.

R.7.1.14.1 Type of property

Granulometry is not a specific physico-chemical property of a substance. The original particle size distribution is highly dependent on the industrial processing methods used and can also be affected by subsequent environmental or human transformations. Particle size is usually measured in micrometers (= 10^{-6} m; µm; 'microns').

Granulometry is of considerable importance for the toxic properties of a substance as it influences aspects such as:

- the route of exposure of humans and toxicity by inhalation;
- the choice of route of administration for animal testing;
- the efficiency of uptake in an organism;
- the distribution in the environment.

Granulometry is of importance for combustible dusts as it influences aspects such as the likelihood to form combustible/explosive dust - air mixtures.

In general all powder materials have a range of particle sizes (particle size distribution), a presentation of the particle size distribution (e.g. using a histogram of the particle size vs. mass, particle size vs. number of particles, etc.) is therefore necessary to interpret the data.

For inhalation exposure it is well know that the human toxicity will be related with the place of deposition into the respiratory tract. The location of deposition mainly depends on the properties of the particle (size, shape, density etc) that are commonly taken into account considering the aerodynamic diameter of the particle (see definition below). Thus, the general approach has been to use mass fractions (e.g. health related fractions as defined by EN 481 or the EPA PM Fractions). For instance, in Europe, from the publication of the EN 481 the OELs for powder materials have been defined for one or several fractions (inhalable, thoracic or respirable).

Photocentrifuge method - the method of determining the particle size distribution, which is described in ISO 13318-2:2007, is applicable to powders that can be dispersed in liquids, powders that are present in slurry form and some emulsions. Typical particle size range for analysis is from about 0.1 µm to 5 µm. The method is applicable to powders in which all particles have the same density and comparable shapes and do not undergo chemical or physical change in the suspension liquid. It is usually necessary that the particles have a density higher than that of the liquid.

Light extinction liquid-borne particle counter – in ISO 21501-3:2007 a calibration and verification method for a light extinction liquid-borne particle counter (LSLPC) is described, which is used to measure the size and particle number concentration of particles suspended in liquid. The light extinction method is based on single particle measurements and the typical size range of particles measured by this method is between 1 µm and 100 µm.

Light scattering liquid-borne particle counter - in ISO 21501-2:2007 a calibration and verification method for a light scattering liquid-borne particle counter (LSLPC) is described, which is used to measure the size and particle number concentration of particles suspended in liquid. The light scattering method is based on single particle measurements and the typical size range of particles measured by this method is between 0.1 µm and 10 µm.

Centrifugal X-ray method - the method of determining the particles size distribution described in ISO 13318-3:2004 is applicable to powders which can be dispersed in liquids or powders which are present in slurry form. The typical particle size range for analysis is from 0.1 µm to 5 µm. The method is applicable to powders in which all particles have the same effective density, chemical composition and comparable shapes.

The CEN document, EN 481 'Workplace Atmospheres – size fraction definitions for measurement of airborne particles' (CEN 1993) provides definitions of the inhalable, thoracic and respirable size fractions, and target specifications (conventions) for sampling instruments to measure these fractions. The current standard defines sampling conventions for particle size fractions which are to be used in assessing the possible health effects resulting from inhalation of airborne particles in the workplace. The different particle sizes defined in EN 481 are:

- inhalable fraction (the mass fraction of particles that can be inhaled by nose and mouth. Particles >100 µm are not included in the inhalable convention;
- thoracic fraction (the mass fraction of the inhaled particles that passes the larynx). The convention for thoracic fraction sets that 50% of the particles in air with an aerodynamic diameter of 10 µm belong to the thoracic fraction;
- respirable fraction (the mass fraction of the inhaled particles that reaches the alveoli) The convention for respirable fraction sets that 50% of particles with an aerodynamic diameter of 4 µm belong to the respirable fraction.

R.7.1.14.2 Definitions

Aerodynamic diameter: the diameter of a sphere of density 1 g cm^{-3} with the same terminal velocity (falling speed) due to gravitational force in calm air as the particle under the prevailing conditions of temperature, pressure and relative humidity (CEN, 1993). The aerodynamic diameter is used to compare partcles of different sizes, shapes and densities and it is a useful parameter to predict where in the respiratory tract such particles may be deposited. It is used in contrast to 'optical', 'measured' or 'geometric' diameters which are representations of actual diameters which in themselves cannot be related with the deposition within the respiratory tract.

Particle diffusion diameter: for particles of aerodynamic diameter less than 0.5 µm, the particle diffusion diameter should be used instead of the particle aerodynamic diameter. For diffusion, the appropriate *equivalent diameter* is the diffusion (mobility) diameter. This is defined as the diameter of a sphere with the same diffusion coefficient as the particle under the prevailing conditions of temperature, pressure and relative humidity.

The parameter of interest is the effective hydrodynamic radius, or effective Stoke's radius R_s. Particle size distribution (effective hydrodynamic radius) requires information on water insolubility. Fibre length and diameter distributions require information on the fibrous nature of the product and on stability of the fibrous shape under electron microscope conditions.

A fibre: is a water insoluble particle with an aspect ratio (length/diameter > 3) and diameter < 100 µm. Fibres of length < 5 µm need not be considered.

Particle: Minute piece of matter with defined physical boundaries. (ISO/TS 27687:2008)

Agglomerate: A collection of weakly bound particles of aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components (ISO/TS 27687:2008).

Aggreggate: Particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components (ISO/TS 27687:2008).

R.7.1.14.3 Test methods

Many methods are available for particle size measurements, but none of them is applicable to the entire size range (see Table R.7.1-11). Sieving, microscopic sedimentation and elutriation techniques are most commonly employed. Methods for determining particle size distribution are designed to provide information on the transportation and sedimentation of insoluble particles in water and air. An integrated testing strategy (ITS) detailing the appropriate methods for determination of particle size distribution of respirable and inhalable particles is shown in Figure R.7.1-6.

Details of methods for determining particle size distribution and for fibre length and diameter distributions are outlined in OECD TG 110 and in the 'Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances' (JRC, 2002).

The particle size distribution is carried out on the material under investigation and not as airborne dust.

The measurement principle of the method used will determine what kind of diameter of the particle can be determined: for instance, optical diameter when using light scattering or aerodynamic diameter when using impactors. Methods which determine the mass median aerodynamic diameter (MMAD) need the generation of representative test atmospheres using suitable generation equipment and correct sampling techniques. They can be used in case of airborne particles (dusts, smokes, fumes), nebulised particles (wet aerosol) or dispersed particles (dry aerosol).

Figure R.7.1-6 Integrated testing strategy for granulometry

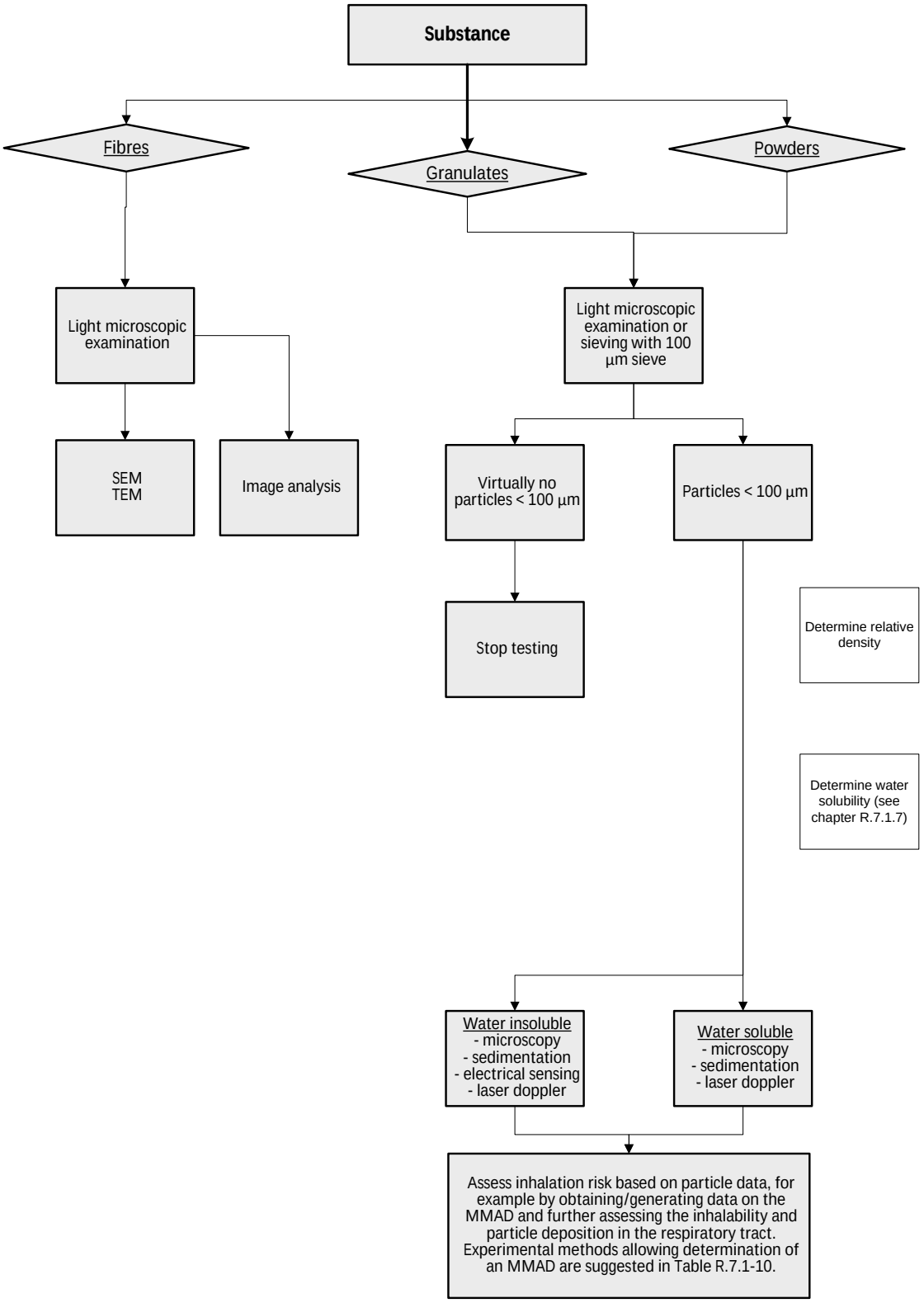

Table R.7.1-11 Methods to determine particle size distribution of a material

Method and details	Material and size range	MMAD
Microscopic examination It is preferable to prepare samples directly in order not to influence shape and size of the particles. This method determines size distribution of particles.	Particles of all kinds Size range: 0.5–5000 microns (light microscope) and <0.1–10 microns (SEM/TEM)	MMAD cannot be determined
Sieving Sieving using wire-mesh sieves and perforated sheet metal sieves is not suitable to determine the distribution of particles of respirable and inhalable size since their range is only 100-10,000 microns. Micro mesh sieves (range 5-100 micron) may give better results. However, since these sieves are generally operated in combination with mechanical or ultrasonic vibration, modification of median size and form may result. Sieving not suitable to determine distribution of particles of respirable size, but might be suitable to determine bigger particles.	Dry powders/granulates Size range: 100–10,000 microns (wire mesh/metal sieves) and 5-100 (micromesh)	MMAD cannot be determined
Sedimentation (gravitational settling) Method is based on gravitational settling of particles in liquid and the effective hydrodynamic radius is determined. Effective hydrodynamic radius distribution should be measured 3x with no two values differing by >20%. Requires sufficient numbers of radius intervals be used to resolve the radius distribution curve. Binary or ternary mixtures of latex spheres (2-100 microns) are recommended as calibration material. Method might be suitable to determine the distribution of particles of respirable and inhalable size.	Dry powders/granulates Size range: 2-200 microns	MMAD cannot be determined
Electrical Sensing Zone (e.g. Coulter) method Samples are suspended in an electrolytic solution. As the particle is drawn through an aperture, the change in conductance gives a measure of particle size. The important parameter is the settling velocity in the liquid phase, which depends on both density and diameter. Particles having a density of several g/cm^3 can be determined. Applicable to particles that are complete electrical isolators in the fluid. Difference in density between particles and fluid must not be too large. Method might be suitable to determine the distribution of particles of respirable and inhalable size.	Dry powders/granulates (non-conducting) Size range: 1-1000 microns	MMAD cannot be determined
Phase Doppler Anemometry Expensive technique. Particle size distribution can be measured either in air or in liquid. The method presupposes that the particles are spherical with	Dry powders/granulates Size range: 0.5-80 microns (in	MMAD cannot be determined

known refractive index. Method might be suitable to determine the distribution of particles of respirable and inhalable size.	air); 0.5-1000 microns (in liquid)	
Determination of fibre length and diameter distributions Light microscopy used to examine likelihood of fibres present by comparing similarities to known fibrous or fibre releasing substances or other data. Extreme care required during sample preparation to avoid fibre breaking and clumping. Care should also be taken to avoid contamination by airborne fibres. Samples might be prepared by (a) producing suspensions in water by gentle hand agitation or vortex mixing or (b) transfer of dry material onto copper tape either directly or by spraying of the dry fibres by use of atomiser or pipette. Length and diameter distributions should be measured independently at least twice and at least 70 fibres counted. No two values in a given histogram interval should differ by > 50% or 3 fibres, whichever is larger. The presence of long thin fibres would indicate a need for further, more precise measurements.	Fibrous products Size range: diameters as small as 0.1 micron and as large as 100 micron and lengths as small as 5 micron and as large as 300 micron	

It is advantageous to have accurate information about the propensity of materials to produce airborne dust (the *dustiness* of the material). No single method of dustiness testing is likely to represent and reproduce the various types of processing and handling used in industry. The measurement of dustiness depends on the test apparatus used, the properties of the dust and various environmental variables (i.e the dustiness is not a measurement of the 'dust as it is') . There are a number of methods for measuring the dustiness of bulk materials, based on the health related aerosol fractions defined in EN 481. Two methods (the rotating drum method and the continuous drop method) are detailed in EN 15051 'Workplace atmospheres – Measurement of the dustiness of bulk materials – Requirements and reference test methods' (CEN, 2006).

Dustiness is a relative term (derived from the amount of dust emitted during a standard test procedure). This is dependent on the method chosen, the condition and properties of the tested bulk material, and various environmental variables in which the tests are carried out. Thus, the two methods in EN 15051 may provide different results (the methods are intended to simulate handling processes) The standard is currently under revision (draft of European standard available) and the final publication is expected for 2013. The standard has been divided in 3 parts (a general part and one part for each of the methods). The methods (Table R.7.1-12) as described in the standard are used to determine dustiness in terms of the health related fractions defined by EN 481. Further analyse (e.g. analysing the contents on the dust collection stages) can be used to obtain the particle sizedistribution. These methods require the generation of representative test atmospheres using suitable generation equipment and correct sampling techniques.

Table R.7.1-12 Methods to generate/sample airborne dispersed or nebulised particles

Method and details	Material and size range	MMAD
Cascade impaction Cascade impactors can be used to obtain the size distribution of an aerosol (i.e in this context a dust cloud). Air samples are drawn through a device which consists of several stages on which particles are deposited on an impactation substrate. Particles will impact on a certain stage depending on their aerodynamic diameter . The cut- off size can be calculated from the jet velocities at each stage by weighing each stage before and after sampling and the MMAD derived from these calculations. This is a well established technique to measure the size distribution of particles (allowing calculating any mass fraction). Some models are specifically designed to give the 3 health related fractions defined by the EN 481. Please also check ISO/TR 27628:2007, which contains specific information on methods for bulk aerosol characterization and single particle analysis while using cascade impaction method.	Particles in an aerosol Size range: 0.1-20 and 0.5-80 microns	MMAD can be determined via an appropriate coupled analytical technique.
Laser scattering/diffraction In general, the scattering of the incident light gives distinct pattern which are measured by a detector. This technique is particle property dependent – i.e. material has unique scattering and diffraction properties which are also particle size dependent. It is important to calibrate the instrument with similar material (of the same size range as the material to be measured). Laser scattering techniques are suitable for geometric particles, viz spheres, cubes and monocrystals. Particle size will be established optically. The MMAD can be calculated by means of a calculation correction. Further information about corrections and limitations of the methods can be found in CEN/TR 16013-1 and CEN/TR 16013-2. Please also check ISO 13320:2009 Particle size analysis – Laser diffraction methods taking into account the possible limitations of the method, a the technique assumes a spherical particle shape in its optical model. The resulting particle size distribution is different from that obtained by methods based on other physical principles (e.g. sedimentation, sieving).	Particles of all kind Size range: 0.1 um to 3 mm (with special instrumentation and conditions, the size range can be extended above 3 mm and below 0.1 mm)	MMAD can be determined.
Rotating drum method (prEN 15051-2) This method is based on size selective sampling of an airborne dust cloud produced by the repeated lifting and dropping of a material in a rotating drum. Air drawn through the drum passes through a specially designed outlet and a 3-stage fractionating system consisting of two	Dry powders/granulates/friable products Size range: 0.5-10,000 microns	MMAD cannot be determined.

porous polyurethane foams and a membrane filter. The mass of dust collected on each collection stage is determined gravimetrically to give a direct measure of the biologically relevant size fractions. This method simulates a wide range of material handling processes in industry and determines the biologically relevant size functions of a material in the airborne state. This method is suitable to determine the respirable thoracic or inhalable fractions.		
Continuous drop method (prEN 15051-3) This method is based on the size selective sampling of an airborne dust cloud produced by the continuous single dropping of material in a slow vertical air current. The dust released by dropping material is conducted by the airflow to a sampling section where it is separated into the inhalable and respirable fractions. This method is suitable to determine the respirable and inhalable fractions.	Dry powders/granulates/friable products Size range: 0.5-10,000 microns	MMAD can be determined.

Table R.7.1-13 Methods that measure inhalable fractions only or that give no detailed distributions

Method and details	Material and size range	MMAD
Elutriation Particles are drawn out on a column at varying velocity. The velocity is used to calculate particle size and the weight of the remaining sample at a particular velocity is used to calculate the distribution. The method is limited to particles >15 microns. The method is not suitable to determine the distribution of particles of respirable size, but might be suitable to determine the distribution of particles of inhalable size	Dry powders/granulates Size range: 15-115 microns	MMAD cannot be determined.
Air jet sieve Air is aspirated through a weighted sample on a fine sieve and the weight loss measured. The method is capable of estimating the non-floatable fraction of the material under investigation. Aggregation of the particles will result in unreliable values. In addition, since the lower detection limit is only 10 micron, this method is not suitable to determine the distribution of particles of respirable size. The method is not suitable to determine the distribution of particles of the respirable fraction, but might be suitable to determine the distribution of particles between 10 and 10,000 microns..	Particles of all kind Size range: 10-10,000 microns	MMAD cannot be determined.
Cyclons The use of a cyclone is a simple approach to determining whether respirable and/or inhalable particles are present in the test atmospheres by constructing the cyclone cut off points at 4.25 and 100 microns. By measuring the weight of particles which pass through the cyclone it can be decided whether more sophisticated methods have to be applied to determine the size distribution of the particles smaller than 10 micron. This method is suitable to determine the respirable, thoracic or inhalable fraction.	Particles of all kind Size range: 0.1-200 microns	MMAD cannot be determined.

R.7.1.14.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VII to REACH

Column 2 of REACH Annex VII provides the following specific rules for adaptation of the standard information requirement for granulometry.

'*The study does not need to be conducted if the substance is marketed or used in a non solid or granular form.'*

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

As the granulometry of a substance is highly dependent on the industrial processing methods and possibly also on handling of the material, any published data on granulometry will be pertinent only to the particular sample or process.

There are a number of web sites and electronic databases that include compilations of and evaluations of data on particle properties. However, there appear to be a limited number of reference books that provide particle size data.

The equivalence of the various national and international standard methods for particle size distribution has not been tested and is not known.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

In some situations where data is available from multiple sources (e.g. information on particle size distribution of different batches, or information from different methods), a weight of evidence approach may be used. Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

There are no QSPR/(Q)SAR tools available for predicting particle size and the data will therefore need to be experimentally determined. Application of (Q)SAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies. Testing should always be possible for solids or granular substances.

Further adaptation possibilities

Not foreseen.

R.7.1.14.5 Impurities; uncertainties

There is a particular problem in relation to sedimentation and Coulter counter measurements. The effect of impurities on particle shape should be considered when measuring fibre length and diameter distributions.

The small quantities used as samples must be representative of product batches comprising many kilograms; therefore sampling and sample handling require great care.

Great care should also be taken due to the fact that non-conducting particles in a non-conducting liquid may be electrically charged resulting in non-representative settling of particles of a certain size. In addition, in the process of particle size distribution determination, it is very important to take the electrostatic charge of the particles into account. Electrostatically charged particles behave differently and may influence sampling.

It is useful to distinguish between aggregates and agglomerates. While an aggregate is held together by strong forces and may be considered to be permanent, agglomerates are held together with weak forces and may break up under certain circumstances. As small particles often form agglomerates, sample pre-treatment (e.g. the addition of dispersing agents, agitation or low-level ultrasonic treatment) may be required before the primary particle size can be determined. However, great care must be taken to avoid changing the particle size distribution.

R.7.1.14.6 Endpoint specific information in the registration dossier / in IUCLID

Material and methods

- sample preparation, such as any sonication, grinding, or addition of dispersion agents (if any);

- if a suspending medium is used (e.g. sedimentation test): indicate type of medium, temperature, pH, concentration and solubility of the substance in the suspending medium;

- the type of method used.

Results and discussion

- in the particle size field: mean and standard deviation;

- in the particle size distribution at different passages field: size and distribution;

- approximate information on particle shape (e.g. spherical, platelike, needle shaped) if available;

- for fibres: indicate both length and diameter of fibres.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on particle size distribution (Granulometry) can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.5	VII 7.14	Particle size distribution (Granulometry)	E.4.6	3.5

R.7.1.14.7 Further information / references

CEN 1993	EN 481: Workplace atmospheres. Size fraction definitions for measurement of airborne particles
CEN 2006	EN 15051: Workplace atmospheres. Measurement of the dustiness of bulk materials – Requirements and reference test methods
JRC (2002)	"Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances", ISBN 92-894-3704-9, EUR 20268 EN, http://publications.jrc.ec.europa.eu/repository/handle/111111111/5555
OECD TG 110	Test No. 110: Particle size distribution/fibre length and diameter distributions
prEN 15051-1 rev	Workplace exposure - Measurement of dustiness of bulk materials - Part 1: Requirements and choice of test methods
prEN 15051-2	Workplace exposure - Measurement of the dustiness of bulk materials - Part 2: Rotating drum method
prEN 15051-3	Workplace exposure - Measurement of the dustiness of bulk materials - Part 3: Continuous drop method
(ISO/TS 27687:2008)	Nanotechnologies-Terminology and definitions for nano-objects-Nanoparticle, nanofibre, and nanoplate
CEN/TR 16013-1:2010	Workplace exposure. Guide for the use of direct-reading instruments for aerosol monitoring. Choice of monitor for specific applications
CEN/TR 16013-2:2010	Workplace exposure. Guide for the use of direct-reading instruments for aerosol monitoring. Evaluation of airborne particle concentrations using optical particle counters

R.7.1.15 Adsorption / Desorption

Advice to registrants with regard to nanomaterials characterisation of adsorption/desorption can be found in *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.4 Adsorption/desorption.

R.7.1.15.1 Type of property

Adsorption/desorption is not a specific physicochemical property of a substance. This property indicates the binding capacity (or 'stickiness') of a substance to solid surfaces, and so is essential for understanding environmental partitioning behaviour.

Information on adsorption/desorption is an essential input to environmental exposure models, because:

- adsorption to suspended matter can be an important physical elimination process from water in sewage treatment plants (STPs). This in turn may mean that sewage sludge, if spread to land, is a major source of the substance in soil;
- adsorption to suspended matter in receiving waters affects both the concentration in surface water and the concentration in sediment;
- desorption of a substance from soil directly influences its mobility and potential to reach surface or groundwaters.

Consequently, information on adsorption/desorption is also an important factor in test strategies for assessing toxicity to sediment- or soil-dwelling organisms.

Substances that adsorb strongly to biological surfaces (e.g., gills, skin, etc.) may lead to toxic effects in higher organisms after biomagnification.

The information is also relevant for assessing environmental persistence. For example: degradation rates in sediment and soil are also assumed to be reduced by default if a substance is highly sorptive (since it is less bioavailable to microorganisms). This may lead to consideration of soil/sediment simulation testing in some cases.

Finally, there may be practical implications for test performance: Substances that adsorb strongly to surfaces can be difficult to test in aquatic systems.

R.7.1.15.2 Definition

Adsorption is caused by temporary (reversible) or permanent bonding between the substance and a surface (e.g. due to van der Waals interactions, hydrogen bonding to hydroxyl groups, ionic interactions, covalent bonding, etc.). The OECD guidances offer further information (OECD 2000a, OECD 2000b, OECD 2001, OECD 2002).

The organic carbon normalized adsorption coefficient (K_{oc}) is the ratio of a substance concentration sorbed in the organic matter component of soil or sediment to that in the aqueous phase at equilibrium. In other words, $K_{oc} = K_d/f_{oc}$, where K_d is the distribution coefficient for adsorption, and f_{oc} the organic carbon content – the fraction organic carbon present in the soil or sediment. In turn, K_d is the experimental ratio of a substance's concentration in the soil (C_s) to that in the aqueous phase (C_{aq}) at equilibrium; namely $K_d = C_s/C_{aq}$. The organic matter normalized distribution coefficient (K_{om}) is similarly defined, but refers to the organic matter content of soil rather than the organic carbon content (OECD, 2000a).

R.7.1.15.3 Test method(s)

The adsorption of a substance to sewage sludge, sediment and/or soil can be measured or estimated using a variety of methods, which are tabulated in

Table R.**7.1-14** in order of increasing complexity. The dissociation constant (if appropriate) should be known before testing. Information on vapour pressure, solubility in water and organic solvents, octanol-water partition coefficient and stability/degradability is also useful.

Table R.7.1-14 Methods for the measurement of adsorption

Method and Description	Applicability / Notes
Adsorption control within an inherent biodegradability test (OECD TG 302B) Estimate of the extent of adsorption to STP sludge made from the elimination level in a Zahn-Wellens inherent biodegradation test. (e.g. OECD TG 302B). 3-hour value recommended. Values beyond 24 hours not normally used. Where data are not available for adsorption up to 24 hours, data from time scales beyond this can only be used if adsorption is the only removal mechanism, with an upper limit of 7 days.	Highly adsorptive substances that are water soluble
HPLC method: OECD TG 121; EU C.19: Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (Original Guideline, adopted 22 January 2001) Calibration with reference substances (preferably structurally related to the test substance) of known K_{oc} allows the K_{oc} of the test substance to be estimated. Test substance K_{oc} value should lie within the calibration range of the reference substances.	Measurement of log K_{oc} in the range 1.5 to 5.0. Validated for several chemical types, see test guideline for details. Poorly soluble and volatile substances as well as mixtures. Ionisable substances: test both ionised and unionised forms in appropriate buffer solutions where at least 10 % of the test compound will be dissociated within pH range 5.5 to 7.5. May not be suitable for: substances that react with the column, solvent or other test system components; surface active substances; substances that interact in a specific way with inorganic soil components such as clay minerals; inorganic compounds; moderate to strong acids and bases.
Batch test of adsorption of substances on activated sludge (ISO 18749) Screening method to determine the degree of adsorption of substances on activated or primary sludge in sewage treatment plants (ISO, 2004). The method does not differentiate between adsorption and other elimination methods (such as complex formation, flocculation, precipitation, sedimentation or biodegradation).	Suitable for substances that: are water soluble, or allow for stable suspensions/dispersions/emulsions, are not significantly removed by abiotic processes (e.g. stripping/foaming), do not de-flocculate activated sludge, are not readily biodegradable, and have a sufficiently sensitive analytical method.

Sediment and soil adsorption/desorption isotherm (OPPTS 835.1220) Screening method according to US-EPA guideline (OPPTS, 1996) using three soil types.	
Batch equilibrium method (OECD TG 106; EU C.18: Absorption – Desorption Using a Batch Equilibrium Method (Updated Guideline, adopted 21 January 2000) Test uses a range of actual soils and so represents a more realistic scenario than the HPLC (OECD 121) method.	Used for substances with K_{oc} values that cannot be reliably determined using other techniques (e.g. surfactants). Requires a quantitative analytical method for the substance, reliable over the range of test concentrations. For ionisable substances, soil types should cover a wide range of pH. Adjustments for poorly soluble substances given in the test guideline.
OECD TG 312: Leaching in Soil Columns (Original Guideline, adopted 13 April 2004) K_d values can be derived from column leaching studies.	Appropriate study design to estimate K_d values particularly for unstable test substances that degrade significantly during the equilibrium time of 'shake flask' sorption studies
Simulation tests and direct field measurement: including OECD guidance document no. 22 (OECD, 2000b). Monolith lysimeters can be used to study the fate and behaviour of substances in an undisturbed soil profile under outdoor conditions. They allow for monitoring of the volume of leaching/drainage water as well as the concentrations of a substance and its transformation products. They are mainly used in pesticide studies. Field leaching studies can also be carried out where hydrodynamically isolated soil layers are analysed *in situ*. Although such studies are the most realistic, their reproducibility and representativity may be limited (e.g. due to the effects of large-scale soil structure, weather events, the soil conditions at the time of application, etc.). Since data from these methods are unlikely to be encountered for the vast majority of industrial substances, they are not considered further here. Further information can be found in guidance for pesticide registration.	

R.7.1.15.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex VIII and IX to REACH

Screening information on adsorption (and desorption) is required for substances manufactured or imported in quantities of 10 t/y or more. Depending on the results, further information (for example, a test) may be required for substances manufactured or imported in quantities of 100 t/y or more.

Column 2 of REACH Annexes VIII and IX provides two exemptions.

'The study does not need to be conducted if:

- *based on the physicochemical properties the substance can be expected to have a low potential for adsorption (e.g. the substance has a low octanol water partition coefficient), or*
- *the substance and its relevant degradation products decompose rapidly.'*

Or in other words, the substance and its relevant degradation products decompose rapidly. Therefore, if a substance hydrolyses, it might be more appropriate to also determine the degree of adsorption of the hydrolysis products.

In practice, a cutoff value of log K_{ow} = 3 can be applied for adsorption potential. However, caution should be exercised in using this criterion, as substances that are water soluble and have a low octanol-water partition coefficient do not necessarily always have a low adsorption potential. A *measured* adsorption coefficient is usually needed for ionising substances, since it is important to have information on pH-dependence (cationic substances in particular generally adsorb strongly). Similarly, measured values will normally be needed for surface active substances (e.g. surfactants), because K_{ow} values (predicted or measured) are likely to be poor predictors of adsorption for these types of substance. For ionisable substances, partition coefficients should also be corrected according to the pH of the environment being assessed (see Annex 2). For complex mixtures (e.g. UVCBs), a single value of K_{oc} will not be definitive. In such cases a range of values or a representative value can be given, depending on the substance.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

For all organic substances manufactured or supplied in quantities of 10 tonnes per year or more, the K_{oc} should be estimated using read-across or QSPR methods as a first step. If the property is likely to be a significant determinant in the calculation of risk (e.g. following a sensitivity analysis), then a test should be conducted to provide a more reliable value for substances manufactured or supplied in quantities of 100 t/y or more. In general, confirmatory testing would not be expected for non-ionising substances with a log K_{ow} value below 3, or for substances that degrade rapidly (in which case the degradation products may be more relevant). The HPLC method may be used as a first step in testing, with the batch equilibrium method being considered only if more definitive data become necessary for the Chemical Safety Assessment. Column leaching studies might be an option under some circumstances (e.g. for unstable test substances that degrade significantly during the equilibrium time of shake flask sorption studies).

If estimation methods are not appropriate (e.g. because the substance is a surfactant or ionisable at environmentally-relevant pH), then a batch equilibrium test may need to be considered at the 10 tonnes per year band, and would be essential at the 100 tonnes per year band.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

Soil sorption (K_{oc}) of organic non-ionic substances can often be estimated from their octanol-water partition coefficient (K_{ow}), as well as from other properties such as aqueous solubility. Such methods, including QSPR, are useful in the first instance to indicate the qualitative/quantitative adsorption coefficient of a substance. In some instances an estimated value may be sufficient for this endpoint. In all such cases the estimated method must be proven to be valid for the type of substance considered (see the general guidance for use and applicability of QSPR), and if possible a sensitivity analysis should be conducted with values generated from different models. Using a range of values in the CSA will help to highlight if the adsorption coefficient is an important factor for environmental behaviour of the substance. In general an estimated value will be sufficient if it is indicated that the adsorption coefficient will not affect the CSA, i.e. no risk is identified for the sediment/soil compartments. Estimated values are essential for substances for which experimental measurement is not feasible i.e. for *difficult substances*. Estimated values are also useful for comparing screening tests [e.g. HPLC method (OECD 121; EC C19)]. A number of reviews of K_{oc} prediction have been published recently (Lyman 1990, Reinhard & Drefahl 1999, Doucette 2000, Delle Site 2001, Doucette 2003, Dearden 2004). That of Doucette (2000) contains a number of worked examples of the estimation of log K_{oc} values. Additional information on the K_{oc} can be found in Gerstl (1990), Briggs (1981) and Nendza (1998).

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

Read-across and/or QSPR prediction for K_{oc} are important predictive tools and should be the first method used to predict Koc if reliable measured data do not exist and the model is valid for the substance. However if these options do not give meaningful and valid information or if K_{oc} is an important factor in the CSA (i.e. risks are indicated for sediment/soil compartments based on a predicted value and log K_{ow} > 3), then an experimental value should be measured.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies. In general, partition coefficients that are measured with a suitable standard method are preferred (and they are usually essential for surfactants and ionic substances that dissociate at environmentally relevant pH).

Further adaptation possibilities

Not foreseen. The K_{oc} is not directly relevant for environmental classification or the PBT assessment. However, it is a key property for exposure assessment so the information requirement should not be waived.

R.7.1.15.5 Impurities;uncertainties

Impurities can have an impact on the measurement of adsorption/desorption. Expert judgement should be used when considering whether impurities may affect the determination of the adsorption/desorption. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.15.6 Endpoint specific information in the registration dossier / in IUCLID

HPLC method (OECD TG 121, EU C.19)

Materials and methods

- description of the HPLC equipment and operating conditions (column, mobile phase, means of detection, temperature);
- dead time and method used for its determination;
- reference substances (identity, purity, Koc, retention times) with results of at least 6 measurements with at least one of them above and one below the expected value for the test substance;
- quantities of test and reference substances introduced in the column.

Results and discussion

- average retention data and estimated d log Koc value for test compound;
- all values of log Koc derived from individual measurements.

Batch equilibrium method (OECD TG 106, EU C.18)

Materials and methods

- details on soil types (nature and sampling site(s), organic C, clay content and soil texture, and pH, if relevant Cation Exchange Capacity);
- information on the test substance (nominal and analytical test concentrations, stability and adsorption on the surface of the test vessel, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant);
- details on test conditions (e.g. soil/solution ratio, number of replicates and controls, sterility, test temperature, and pH of the aqueous phase before and after contact with the soil);
- details on sampling (e.g. frequency, method);
- details on the analytical methods used for determination of the substance (detection limit, recovery %).

Results and discussion

- soil dry mass, total volume of aqueous phase, concentration of test substance in solution and/or soil after agitation and centrifugation, equilibration time, Koc, if appropriate mass balance;
- explanations of corrections made in the calculations, if relevant (e.g. blank run).

Leaching in soil columns (OECD TG 312)

Materials and methods

- details on soil types (nature and sampling site(s), organic C, clay content and soil texture, Cation Exchange Capacity, bulk density (for disturbed soil), water holding capacity and pH;
- information on the test substance (amount of test substance and, if appropriate, reference substance applied, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant);
- details on test conditions (number of replicates and controls, test temperature, amount, frequency and duration of application of artificial rain);
- details on the analytical methods used for determination of the substance (detection limit, recovery %);
- reference substance used.

Results and discussion

- Koc, tables of results expressed as concentrations and as % of applied dose for soil segments and leachates;

- mass balance, if appropriate;
- leachate volumes;
- leaching distances and, where appropriate, relative mobility factors.

Adsorption control within an inherent biodegradability test (OECD TG 302B)

Materials and methods

- details on inoculum;
- information on the test substance (toxicity to bacteria, test concentration);
- details on test conditions (blank controls used, inoculum and test compound ratio (as DOC));
- details on sampling (frequency);
- details on the analytical methods used for determination of the DOC or COD;
- reference substance.

Results and discussion

- estimate of the extent of adsorption to STP sludge made from the elimination level in this Zahn-Wellens inherent biodegradation test, based on the 3-hour value if possible;
- values beyond 24 hours should not normally be used but where data is not available for adsorption up to 24 hours, data from time scales beyond this can only be used if adsorption is the only removal mechanism, with an upper limit of 7 days;
- if relevant results of testing of inhibition of biodegradation.

Simulation test/field measurement (OECD TG 22)

Materials and methods

- details on soil types (nature and sampling site(s); if relevant: organic C, clay content and soil texture, Cation Exchange Capacity and pH;
- details on lysimeter;
- information on the test substance (nominal and analytical test concentrations, solubilising agent if relevant (and justification for its use), radiochemical purity if relevant);
- details on test climate conditions (e.g. air temperature, solar radiation, humidity, potential evaporation or rate of artificial rainfall), soil temperature and soil moisture and duration of the study;
- details on sampling (frequency, method);
- details on the analytical methods used for determination of the test substance (detection limit, recovery %).

Results and discussion

- concentration of test substance in soil layers; Koc, if appropriate mass balance and concentrations and as % of applied dose for soil segments and leachates;
- explanations of corrections made in the calculations, if relevant (e.g. blank run).

Distribution modelling

Materials and methods

- model name and version;
- date of the model development;
- model type description e.g. steady-state, dynamic, fugacity, Gaussian, Level I-IV, etc.;
- environmental compartments which the model covers;
- information on model segmentation and environmental properties;

- input parameters (minimum information required for assessing the partitioning and degradation behaviour):
    - vapour pressure;
    - water solubility;
    - molecular weight;
    - octanol-water partition coefficient;
    - information on ready biodegradability;
    - for inorganic substances: it is recommended to have information on the partition coefficients and possible abiotic transformation products;
- temperature effect.

Results and discussion

- key exposure routes and distribution of the substance among them.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on adsorption/desorption can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
5.4.1	VIII 9.3.1	Adsorption / desorption	E.5.5.2	4.1.4
5.4.2	/	Henry's Law constant	E.5.5.3	4.1.4
5.4.3	X 9.3.4	Distribution modelling	E.5.5.4	4.1.4
5.4.4	X 9.3.4	Other distribution data	E.5.5.5	4.1.4

R.7.1.15.7 Further information / references

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Poole S.K. and Poole C.F. (1999) Chromatographic models for the sorption of neutral organic compounds by soil from air and water. *J. Chromatogr. A* 845, 381-400.

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R.7.1.16 Stability in organic solvents and identity of relevant degradation products

R.7.1.16.1 Type of property

The stability in organic solvents is required for substances manufactured or imported in quantities of ≥ 100 t/a only if their stability in organic solvent is considered critical (REACH Annex IX, section 7.15).

There are rare occasions when it is important to have information on the stability of a compound in an organic solvent, to ensure confidence in the test results. However, for many substances, the stability in organic solvents will not be critical and testing need not be conducted.

Examples of when stability in organic solvents could be important are:

- for certain solubility measurements (e.g. octanol–water partition coefficient);
- to check on the stability of reagent solutions, fortification standards or calibration standards;
- when a test substance is dosed as a solution in an organic solvent (e.g. ecotoxicity studies);
- when a test substance is extracted from an environmental sample, plant or animal tissue or diet matrix (arising from a variety of physicochemical property, ecotoxicity and animal toxicity studies) into an organic solvent and stored pending analytical measurement.

R.7.1.16.2 Definition

A study of the stability of a test compound in an organic solvent is normally undertaken for a specific time period to confirm whether the test compound is stable under these conditions for the duration of the storage of the organic solvent or extract containing the test substance. Often several time periods are selected to check whether there is any particular downward trend in stability over time.

The stability of the test substance at a particular time period during the study is normally expressed as a percentage of the concentration of the test substance in the solvent extract, at that time period compared with the initial starting concentration of the test substance at t = 0, namely:

$$\frac{C_t}{C_0} \times 100\ \%$$

where C_t is the concentration of test substance in solvent extract at $t = t_1, t_2, t_3 \ldots, t_n$; and C_0 is the concentration of test substance in solvent extract at t = 0.

R.7.1.16.3 Test method(s)

A number of physical, chemical and biological processes can result in a decline in the actual concentration of a test substance in an organic solvent over time. Information on the stability of a test substance in a solvent is desirable, particularly when samples are to be stored. However, there does not appear to be any generally accepted methodology for performing such stability studies. Factors affecting the rate of degradation include rates of hydrolysis, of photolysis and of oxidation.

Typically, one or more concentrations of the test substance in the solvent are made up and analysed immediately after preparation (i.e. t = 0). They are then stored in appropriate vessels under the required test conditions (e.g. temperature, absence of light) and analysed, along with a freshly prepared solution of the test substance at the original test concentration(s), at regular intervals during the period of interest.

At each time of analysis, a sample is withdrawn from storage and mixed thoroughly before taking any aliquot for analysis. The analysis is carried out using the recommended method to determine whether any significant loss of the test substance has occurred during storage. It is important to analyse freshly made standards of the test substance in the organic solvent at the same time as analysing stored samples, so that any losses that may occur of the test substance during sampling, sample treatment and analysis are taken into consideration.

It is important to be able to have a check on the temperature to ensure that the temperature regime has been maintained throughout the period of the stability study.

Unlabelled reference material of suitable known purity may be used where a reliable method of analysis is available. Where an analytical method is still under development or is unlikely to be sufficiently sensitive, radio-labelled compounds should be used if available. Use of radio-labelled compounds can shorten the analysis time and help facilitate identification of any degradation products, should the test substance not be stable in the organic solvent.

Recovery or spiking experiments should normally be run. The number of spiking levels or the range of concentrations tested within a project should be left to the judgement of the analyst.

Further information should be obtained by checks on the stability of standards of the test substance in organic solvents as part of routine analytical protocols, to confirm whether the test substance is unstable under normal storage conditions.

Further tests may be necessary to identify storage conditions which minimise any degradation of the test substance not only in organic solvents, but also during the conducting of other tests, such as water solubility, surface tension and in the preparation of test media for ecotoxicity studies (OECD, 2000). Identification of the degradation product(s) will allow an assessment of whether they are likely to be more toxic than the parent material in subsequent ecotoxicity studies.

R.7.1.16.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex IX to REACH

Column 2 of REACH Annex IX provides the following specific rules for adaptation of the standard information requirement for stability in organic solvents and identity of relevant degradation products:

'The study does not need to be conducted if the substance is inorganic.'

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Stability data of substances in organic solvents are not normally reported in standard published sources of physicochemical data. Relevant sources of basic information regarding stability and storage conditions of substances are the Hazardous Substances Data Base (HSDB) and Sax's 'Dangerous Properties of Industrial Materials'.

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

At present (Q)SAR is generally not applicable for determination of stability in organic solvent and degradation products. Application of (Q)SAR is not possible.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

At present grouping and read across are not applicable.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered, if none of the waiving possibilities applies.

Further adaptation possibilities

Not foreseen.

R.7.1.16.5 Impurities; uncertainties

Impurities can have an impact on the measurement of stability in organic solvent and degradation products. Expert judgment should be used when considering whether impurities may affect the determination of the stability in organic solvent and degradation products. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.16.6 Endpoint specific information in the registration dossier / in IUCLID

This endpoint needs to be fulfilled on a case by case basis. As several different methods can be used to document this intrinsic property, we recommend the same strategy for drafting robust study summaries as described for the other endpoints. The general aspects described in

section 2 of the Practical guide 3: How to report robust study summaries should also be applied for this endpoint. All endpoint specific characteristics should be described in such a way that the robust study summary allows an independent assessment of the endpoints reliability and completeness. The objectives, methods, results and conclusions of the full study report should be reported in a transparent manner as described for all other endpoints in this guidance.

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

REFERENCE TO OTHER ECHA GUIDANCE DOCUMENTS

Further detailed guidance on stability in organic solvents can be found in:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.17	IX 7.15	Stability in organic solvents and identity of relevant degradation products	E.4.18	3.15

R.7.1.16.7 Further information / references

OECD Series on Testing and Assessment Number 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO(2000)6 (http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2000)6&doclanguage=en).

R.7.1.17 Dissociation constant

R.7.1.17.1 Type of property

Information on the dissociation constant is **supplementary data** for hazard assessment (OECD TG 112, 1981). The dissociation of a substance in water is of importance in assessing its impact upon the environment and may also influence the ADME of a substance and consequently its effects on human health. It governs the form of the substance which in turn determines its behaviour and transport. It may affect the adsorption of the substance on soils and sediments and absorption into biological cells.

The dissociation constant may also be an important factor in deciding which method or conditions should be used to determine the octanol-water partition coefficient (K_{ow}) and soil adsorption partition coefficient (K_{oc}). Slight changes in pH can considerably affect the form in which the substance is present in solution, especially if the pK_a value is within the environmentally-relevant pH range[21]. The dissociated and non-dissociated species may have significantly different water solubilities and partition coefficients. Therefore, significantly different bioavailability and toxicity may result. It is important to note that the dissolution of salts from their crystal lattice into individual ions is not intended to be covered by the endpoint dissociation constant. Therefore this section refers only to acid dissociation (pK_a).

[21] Fresh surface waters have pH values in the range 4-9, whereas marine environments have a stable pH of about 8. pH normally varies between 5.5 and 7.5 for agricultural soils and sewage treatment plant tanks.

R.7.1.17.2 Definition

Dissociation is the reversible splitting of a substance into two or more chemical species, which may be ionic (OECD TG 112, 1981). The process can be represented as:

$$RX \leftrightarrows R^+ + X^-$$

The dissociation constant (K) for this process is expressed as the ratio of concentrations of the species on either side of the equation in water at equilibrium:

$$K = \frac{[R^+][X^-]}{[RX]}$$

Where the cation R+ is hydrogen, the substance can be considered an acid, and so this constant becomes an acid dissociation constant (K_a).

$$K_a = \frac{[H^+][X^-]}{[HX]}$$

A substance can have more than one acidic (or basic[22]) group, and the dissociation constant can be derived for each dissociation step in a similar way.

The K_a is related to pH as follows (where p is –log10):

$$pK_a = pH - \log_{10}\left(\frac{[X^-]}{[HX]}\right)$$

In practice for a simple substance having one dissociating group, the pK_a is equivalent to the pH at which the ionised and non-ionised forms are present in equal concentration (i.e. the substance has undergone 50% dissociation).

It is important to differentiate between dissociation and hydrolysis as hydrolysis is a separate standard information requirement according to Annex VIII of the REACH regulation. Hydrolysis is defined as reaction of a substance RX with water, with the net exchange of the group X with OH at the reaction centre (OECD TG 111, 2004).

$$RX + H_2O \rightarrow ROH + HX$$

R.7.1.17.3 Test method(s)

OECD test guideline 112 (Dissociation constants in water, adopted May 1981) describes three laboratory methods to determine the pK_a of a substance. The three methods are appropriate for particular types of substances as described in the test guideline[23].

[22] Base strength is expressed as the acidity of the conjugate acid. The term pK_b was once used to express basicity so that the same scale could be used alongside acidity – care should be taken when citing older sources to check which term has been used. For consistency, dissociation of bases should preferably be expressed using the pK_a of the conjugate acid.

[23] The test method is available at the following link: http://www.oecd-ilibrary.org/environment/test-no-112-dissociation-constants-in-water_9789264069725-en

R.7.1.17.4 Adaptation of the standard testing regime

Adaptation possibilities according to column 2 of Annex IX to REACH

Column 2 of REACH Annex IX provides the following specific rules for adaptation of the standard information requirement for dissociation constant:

'A study does not need to be conducted if:

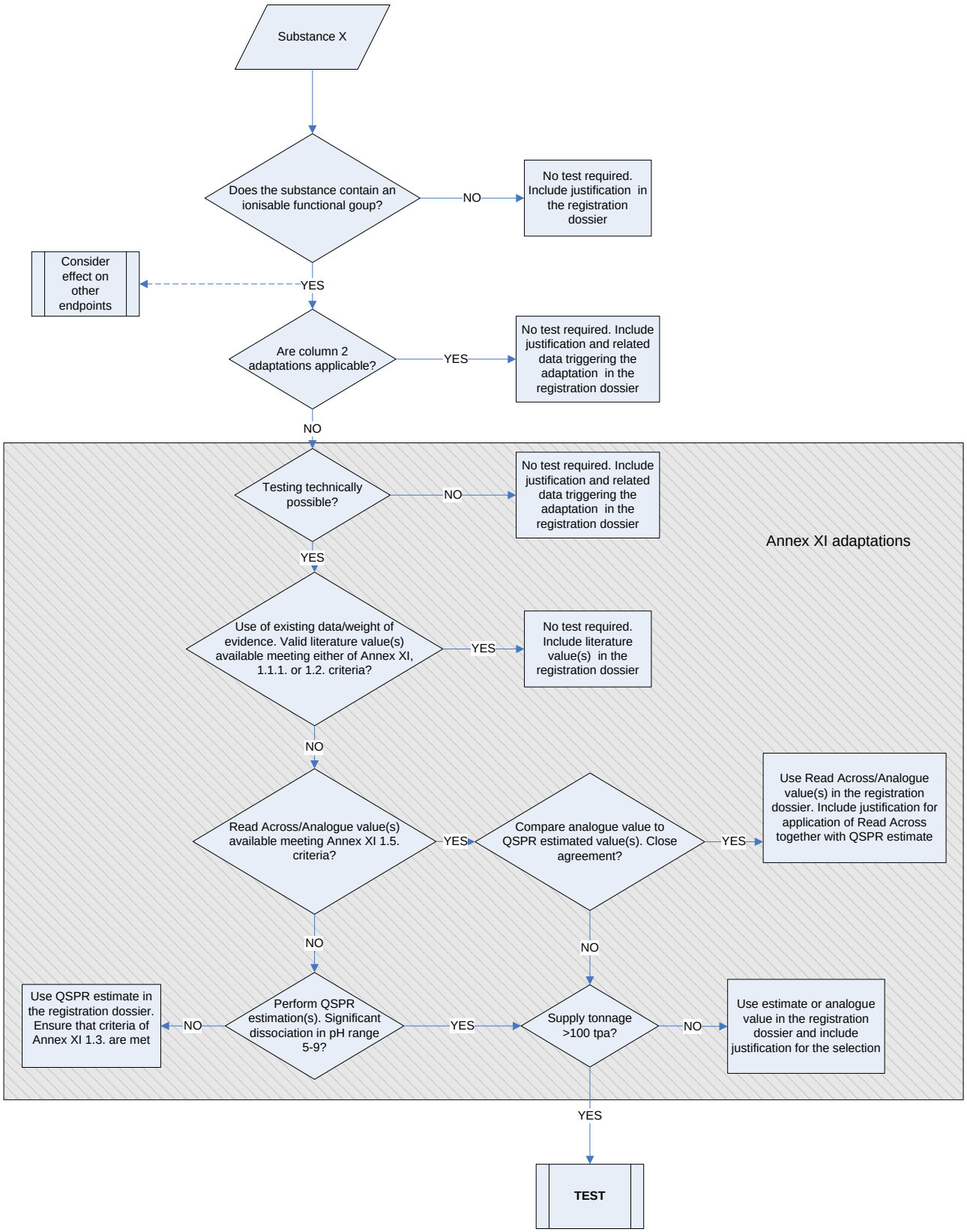
- *the substance is hydrolytically unstable (half-life less than 12 hours) or is readily oxidisable in water; or*

- *it is scientifically not possible to perform the test (e.g. because the analytical method is not sensitive enough).'*

In all cases where the above specific rules for adaptation are used to waive testing, evidence demonstrating the existence of that property of the substance which triggers the adaptation rule should be provided in the IUCLID dossier, e.g. if the test is not performed because the substance is hydrolytically unstable (half life < 12 hours) then the dossier must contain valid data on the hydrolysis clearly indicating a half life < 12 hours.

It is important to note that OECD TG 112 allows the use of a small amount of a water-miscible solvent to aid dissolution of sparingly soluble substances. Therefore low solubility will only prevent performance of the test in the context of the column 2 rules above for substances which remain highly insoluble and undetectable by analytical techniques in the presence of water miscible solvents.

Figure R.7.1-7 Integrated testing strategy for dissociation constant



Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

Many literature sources for dissociation constant exist; some reference textbooks and on-line sources are listed in section R.7.1.1.2. These should be searched for published, valid data. As mentioned in section 1.1.1 of Annex XI to REACH a number of conditions need to be met before any such data can be used. Namely:

- *'adequacy for the purpose of classification and/or risk assessment;*
- *sufficient documentation is provided to assess the adequacy of the study; and*
- *the data are valid for the endpoint being investigated and the study is performed using an acceptable level of quality assurance.'*

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used provided that data from a number of distinct sources indicate a similar value for the dissociation constant which is supported by one or more relevant QSPR predictions.The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

Estimated pK_a data can be generated by valid QSPR methods. In general, pK_a values that are measured with a suitable method are preferred to QSPR predictions. If an estimated pK_a value suggests that the substance will dissociate significantly at environmentally relevant pH, a test may be required to confirm the result.

There have been a few attempts to model pK_a values of diverse sets of substances. Klopman and Fercu (1994) used their MCASE methodology to model the pK_a values of a set of 2464 organic acids, and obtained good predictions; a test set of about 600 organic acids yielded a standard error of 0.5 pK_a unit. Klamt *et al.* (2003) employed their COSMO-RS methodology to predict pK_a values of 64 organic and inorganic acids, with a standard error of 0.49 pK_a unit. A comparison of commercially available software for the prediction of pK_a was done by Dearden *et al.* (2007).

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For most ionisable substances supplied at greater than 100 t/y that are predicted to dissociate at environmentally relevant pHs, a test will typically be required for dissociation constant. Similar substances (analogues) for which measured pK_a data according to a reliable method are available may be considered for read-across. Such values should be reinforced by estimated methods for pK_a (e.g. the result of a QSPR prediction; see section above). In some instances it may be acceptable to read-across dissociation constant from an analogue.

However if there is significant variation between the analogue read-across and the predicted pK_a then a test should be conducted.

TESTING IS TECHNICALLY NOT POSSIBLE

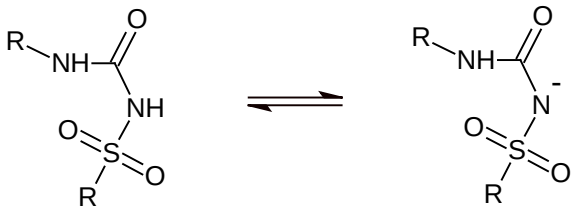
Testing should always be considered if none of the waiving possibilities applies. Instances where testing is technically not possible as a consequence of the properties of the substance are expected to be limited to highly reactive or unstable substances, and substances which in contact with water emit flammable gases.

Further adaptation possibilities

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

No dissociating groups

If the substance cannot dissociate due to a lack of relevant functional groups, the dissociation constant is irrelevant and testing information does not need to be provided. However, ionisable groups might not always be obvious (e.g. in sulphonyl urea herbicides, which contain the function $-S(=O)_2NH.C(=O)NH-$, the acid group is $S(=O)_2NH$).



If a substance is much more soluble in water than expected, this may be an indication that dissociation has occurred.

UVCBs

For complex mixtures (e.g. UVCBs) containing ionisable components the assessment of pK_a is clearly complicated. Estimation of the representative constituent's pK_a values, if appropriate, should be considered.

R.7.1.17.5 Impurities; uncertainties

Impurities can have an impact on the measurement of dissociation constant. Expert judgement should be used when considering whether impurities may affect the determination of the dissociation constant. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

The presence of multiple dissociation/equilibrium reactions can complicate determination of the dissociation constant(s). In cases where multiple dissociation reactions can take place due to the presence of numerous dissociating groups and/or the presence of tautomerisation and/or zwitterionic forms, care should be taken in the interpretation of experimental results. QSPR predictions for such substances should also be carefully analysed as the models may not account for concurrent equilibria/dissociations. Additionally QSPR predictions may not account for intramolecular hydrogen bonding effects which can have a pronounced effect on the

observed dissociation constant. In some cases, formation of intramolecular hydrogen bonding depends on the cis/trans isomerism of the substance, as is the case for the isomers fumaric and maleic acid. Care should be taken when using QSPR predictions for such molecules, as cis/trans isomerism is typically not taken into account.

The extent of ionisation may vary according to pH, ionic strength and/or the level of common ions in the test medium (common ion effect), and relatively small changes may significantly alter the equilibrium between dissociated and non-dissociated species.

R.7.1.17.6 Endpoint specific information in the registration dossier / in IUCLID

Knowledge of an ionisable substance's pK_a is important for all such substances. For substances supplied at levels below 100 tonnes per annum dissociation constant is not a testing requirement. Ideally however, a literature value, analogue value and/or QSPR prediction can be obtained and provided for such substances, especially if dissociation is relevant for interpreting the results of other physicochemical or fate and (eco)toxicological tests and for chemical safety assessment. For ionisable substances supplied at tonnages greater than 100 t/y, dissociation constant is a standard information requirement.

For substances which contain multiple ionisable functionalities, all measured macro pK_a values should be reported and preferably assigned to specific micro-reactions.

With regard to study summaries of experimental data, the IUCLID dossier should contain all relevant information regarding the endpoint and as a minimum the items listed below:

Materials and methods

- type of method;
- test guideline followed.

Test Materials

- test material identity.

Results and discussion

- concentration of the substance;
- test results as pK_a-value(s);
- temperature of the test medium (ºC);
- if testing is waived, the reasons for waiving must be documented in the dossier.

Any deviation from the guideline method used (and reasons for it) or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on dissociation constant can be found in:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.21	IX 7.16	Dissociation constant	E.4.22	3.16

R.7.1.17.7 References on dissociation constant

Balogh G.T., Gyarmati B., Nagy B., Molnar L. and Keseru G.M. Comparative evaluation of in silico pK_a prediction tools on the Gold Standard dataset. *QSAR Comb Sci* (2009) 28:1148-1155.

Dearden J.C., Cronin M.T.D., and Lappin D.C. A comparison of commercially available software for the prediction of pK_a. *J. Pharm. Pharmacol.* (2007) 59, Suppl. 1, A-7.

Klamt A., Eckert F., Diedenhofen M. and Beck M.E. (2003) First principles calculations of aqueous pK(a) values for organic and inorganic acids using COSMO-RS reveal an inconsistency in the slope of the pK(a) scale. *J. Phys. Chem. A* 107, 9380-9386.

Klopman G. and Fercu D. (1994) Application of the multiple computer automated structure evaluation methodology to a quantitative structure-activity relationship study of acidity. *J. Comput. Chem.* (1994) 15, 1041-1050.

Liao C. and Nicklaus M.C. Comparison of nine programs predicting pK_a values of pharmaceutical substances. *J. Chem. Inf. Model.* (2009) 49, 2801-2812.

Manchester J, Walkup G, Rivin O. and You Z.P. Evaluation of pK_a estimation methods on 211 druglike compounds. *J Chem Inf Model* (2010) 50, 565-571.

Meloun M. and Bordovská S. Benchmarking and validating algorithms that estimate pK_a values of drugs based on their molecular structure. *Anal. Bioanal. Chem.* (2007) 389, 1267-1281.

OECD (1981). Dissociation constants in water (titration method – spectrophotometric method – conductometric method). Organisation for Economic Co-operation and Development (OECD) Guideline for the testing of chemicals no 112.

R.7.1.18 Viscosity

R.7.1.18.1 Type of property

Viscosity is a property:

- needed for substance characterization;
- needed for the classification of aspiration hazard of liquids;
- which gives an indication of the penetration of the substance within soil.

R.7.1.18.2 Definition

Viscosity: viscosity is the (inner) resistance of a substance (gas, liquid) to a shift caused by laminar flow.

Dynamic viscosity (= dynamic viscosity coefficient) *h:*

Quantifies the property 'viscosity' by the quotient shear stress t / shear rate $\dot{\gamma}$ *(h= t/$\dot{\gamma}$)*

Kinematic viscosity (= kinematic viscosity coefficient) *n:*

is given by the quotient dynamic viscosity to density *(n= h/r).*

R.7.1.18.3 Test method(s)

Five different types of test methods are standardized for liquid substances:

- capillary viscometer;
- flow cup;
- rotational viscometer;
- rolling ball viscometer;
- drawn-shear viscometer.

There exist a lot of standardized determination methods with sometimes very specialised application ranges with respect to products, especially mixtures. For substances (within the scope of the REACH Regulation) the following standardised determination methods are recommended:

- Capillary viscometer:
  - EN ISO 3104, EN ISO 3105, DIN 51562, BS 188, NF 60-100, ASTM D445, ASTM D4486;
- Flowcup:
  - EN ISO 2431;
- Rotational viscometer:
  - EN ISO 3219, DIN 53019;
- Rolling ball viscometer:
  - DIN 53015.

For newtonian liquids (liquids for which the viscosity is independent of the shear stress and shear rate) any determination method may be used within the scope and applicability specifications. For non-newtonian liquids (liquids for which the viscosity depends on the shear rate) only the use of rotational viscometers is possible. Because the viscosity is remarkably temperature dependent each determination must be accompanied by the temperature at which the measurement was made. It is recommended to use the mean of two test runs. It is also recommended to determine the viscosity at at least two different temperatures. The classification criteria for aspiration hazard refer to kinematic viscosity at 40 °C.

If explosives, pyrophorics or self-reactives are to be characterized, determination of the viscosity may not be practicable. For pyrophorics and self-reactives testing under inert gas should be considered. In any case the determination method has to be chosen carefully.

The use of the most recent update of the standard is advised; they are accessible via numerous websites, see R.7.1.1.3.

R.7.1.18.4 Adaptation of the standart testing regime

Within the REACH Regulation requirements testing of viscosity is only of interest for liquid substances.

Adaptation possibilities according to column 2 of Annex IX to REACH

Column 2 of REACH Annex IX does not provide any specific rules for adaptation from column 1.

Adaptation possibilities according to Annex XI to REACH

USE OF EXISTING DATA: DATA ON PHYSICAL-CHEMICAL PROPERTIES FROM EXPERIMENTS NOT CARRIED OUT ACCORDING TO GLP OR THE TEST METHODS REFERRED TO IN ARTICLE 13 (3) OF REACH

If experimental data are available (study reports or literature data) meeting the criteria in section 1.1.1 of Annex XI to REACH, these could be used to meet the endpoint data requirements. If an estimation method is used as a source of information according to Column 2 of Annex VII, the QSAR model must meet the criteria set out in section 1.3 of Annex XI to REACH.

WEIGHT OF EVIDENCE

Where no single source of existing data (study reports, QSAR, literature data) is considered sufficiently reliable, thus not fully meeting the criteria in section 1.1.1 of Annex XI to REACH, or where several sources of similar reliability with deviating results exist, a weight of evidence approach may be used. The criteria in section 1.2 of Annex XI to REACH must then be met.

(Q)SAR

For the determination of the viscosity, (Q)SAR approaches are discouraged for the purpose of classification / risk assessment, except when the mean absolute error of the (Q)SAR is less than 5 %.

GROUPING OF SUBSTANCES AND READ-ACROSS APPROACH

For the determination of the viscosity read across is not possible.

TESTING IS TECHNICALLY NOT POSSIBLE

Testing should always be considered if none of the waiving possibilities applies. But the testing is technically not possible:

- if the substance is a solid;
- if liquid explosives, pyrophorics or self-reactives are to be characterized, determination of the viscosity may not be practicable (see above section Test method(s)).

Further adaptation possibilities

- the viscosity does not have to be determined experimentally if conclusive and consistent literature data are available;
- data for viscosity generated with the same tests and classification principles as specified in the CLP Regulation generated in conjunction with transport classification can satisfy the REACH requirements on a case-by-case basis.

As stated in Annex IX of REACH, when for certain endpoints, it is proposed to not provide information for other reasons than those mentioned in column 2 of that Annex or in Annex XI of REACH, this fact and the reasons must also be clearly stated. Such an approach may then be used.

R.7.1.18.5 Impurities; uncertainties

The influence of impurities is negligible if their concentration is below 1 %. The influence of higher concentrations may be significant. There exists no generalised tendency of the influence on the viscosity. Therefore utmost care should be taken in the selection of the key study(s), or weight-of-evidence approaches, that the data selected is representative of the substance being registered by the respective companies.

R.7.1.18.6 Endpoint specific information in the registration dossier / in IUCLID

MATERIALS AND METHODS

- type of method;
- test guideline followed.

Results and discussion

- viscosity value and unit according to the used test method;
- preferred units are m Pa·s (for dynamic viscosity) and mm^2/s (for static viscosity) but other units are also accepted;
- each measured value should be accompanied with temperature (in °C). Usually two values are needed. Preferably one value is measured at approximately 20 °C and another at an approximately 20 °C higher temperature. Two determinations of viscosity should be measured for each temperature;
- for non-Newtonian liquids, the results obtained are preferably in the form of flow curves, which should be interpreted;

- individual and mean values should be provided at each temperature (from OECD Guideline 114 'Viscosity of liquids').

Any deviation from the guideline method used or any other special consideration should be reported. In cases where there is more than one source of data, the endpoint summary under results and discussion should provide a justification for the selection of the key study chapter.

Reference to other ECHA Guidance Documents

Further detailed guidance on viscosity can be found in the following chapters:

IUCLID Section	REACH Annex	Endpoint title	IUCLID 5 End User Manual Chapter	ECHA Practical Guide 3
4.22	IX 7.17	Viscosity	E.4.23	3.17

R.7.1.19 Shape

Please *check Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.3.3 Recommendations for shape.

R.7.1.20 Surface area

Please check *Appendix R7-1 Recommendations for nanomaterials applicable to: Chapter R7a Endpoint specific guidance*, section 2.2.3.4 Recommendations for surface area.

R.7.1.21 Further information to be submitted for classification and labelling in hazard classes of the substance in accordance with article 10 (a) (iv) REACH

The criteria listed in the table below should be provided for general registration purposes according to Article 10 (a) (iv) and section 4 of Annex VI to REACH. The assignment of hazard classes to relevant subchapters in R.7.1.21.1 to R.R.7.21.3 should therefore only be understood as a means to structure this document in accordance with Annexes VII to XI to the REACH Regulation.

Table R.7.1-15 Information to be submitted for general registration purposes according to Article 10 (a) (iv) REACH, CLP hazards classes and corresponding tests methods according to the Test Method Regulation and CLP[24]

CLP Regulation (EC) No. 1272/2008 (the no. in brackets is the respective chapter no. in Annex I to CLP)	**Corresponding test method according to the Test Method Regulation, Regulation (EC) No. 440/2008**	**Chapter in revised R.7(a) guidance**	**Information requirement according to REACH Regulation (EC) No. 1907/2006**	**Corresponding test method according to CLP Regulation**
Flammable aerosols (2.3)[25]	n.a.	7.1.21.1	See Article 10 (a) (iv) REACH requirements	Test methods according to 75/324/EC amended by 2008/47/EC (harmonised with **UN-MTC Section 31)**
Gases under pressure (2.5)	n.a.	7.1.21.2	See Article 10 (a) (iv) REACH requirements	n.a.
Corrosive to metals (2.16)	n.a.	7.1.21.3	See Article 10 (a) (iv) REACH requirements	**UN Test C.1 (UN-MTC Section 37.4)**

R.7.1.21.1 Flammable aerosols

For further guidance on these please check the *Guidance on the application of the CLP Criteria*, chapter 2.3[26].

R.7.1.21.2 Gases under pressure

For further guidance please check the *Guidance on the Application of the CLP Criteria* chapter 2.5.

R.7.1.21.3 Corrosive to metals

For further guidance please check the *Guidance on the Application of the CLP Criteria* chapter 2.16.

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[24] Please note that REACH information requirements regarding classification and labelling in accordance with Article 10(a)(iv) of the REACH Regulation are not limited to the items listed in this table. This table stresses that, while the REACH Regulation does not require the generation of information regarding the following hazard classes (Article 10(a)(vi) of the REACH Regulation, see Table R.7.1-1), any information available on these hazard classes must be included in a REACH registration dossier for a substance pursuant to Article 10(a)(iv) of the REACH Regulation.

[25] The 4th ATP to the CLP Regulation amends the criteria in the CLP Annex I, Section 2.3 Flammable aerosols by changing the scope and title to Section 2.3 Aerosols.

[26] The 4th ATP to the CLP Regulation amends the criteria in the CLP Annex I, Section 2.3 Flammable aerosols by changing the scope and title to Section 2.3 Aerosols. Consequently the *Guidance on the Application of the CLP Criteria*, Part 2: Physical hazards has been restructured to take account of the 4th ATP, which applies to substances from 1 December 2014 and to mixtures from 1 June 2015. Once the 4th ATP is applied a Guidance corrigendum will be made to delete the outdated sub-chapter 2.3.1 Flammable aerosols in the *Guidance on the Application of the CLP criteria*.

Appendix 1 to Section R.7.1

Appendix R.7.1-1 Henry's law constant and evaporation rate

The Henry's law constant (HLC) is one of the most important factors in determining the environmental fate of chemicals. Henry's law states that the mass of gas dissolved by a given volume of solvent is proportional to the pressure of the gas with which it is in equilibrium. HLC is the ratio of the equilibrium concentration of the chemical in the gas phase (C_G) and that in the liquid phase (C_L):

$$\mathrm{HLC} = \frac{C_G}{C_L}$$

Therefore, HLC quantifies the partitioning of substances between the aqueous phase and the gas phase such as rivers, lakes and seas with respect to the atmosphere (gas phase). Indeed, this constant is a fundamental input for fugacity models that estimate the multimedia partitioning of chemicals (Mackay, 1991). As HLC is a ratio of two concentrations, it is without unit if both concentrations are expressed in the same unit. Some prefer to express the gas concentration in pascals and the liquid concentration in mol/m^3, thus giving the unit Pa·m^3/mol for the HLC.

For many chemicals, volatilisation can be an extremely important removal process, with half lives as low as several hours. HLCs can give qualitative indications of the importance of volatilisation. For substances with HLC values less than 0.01 Pa·m^3/mol, the substance is less volatile than water and as water evaporates the concentration of the substance in the aqueous phase will increase; for substances with HLC values around 100 Pa·m^3/mol, volatilisation will be rapid.

However, the degree of volatilisation of substances from the aquatic environment is highly dependent on the environmental parameters for the specific water bodies in question, such as the depth and the gas exchange coefficient (influenced e.g. by wind speed and water flow rate). The HLC cannot be used for evaluation of the removal of a substance from the water phase without considering these factors. As the n-octanol/water partition coefficient (K_{ow}) is used to predict bioaccumulation potential in air-breathing organisms, this aspect is especially important in a PBT context.

For example, where a substance has both a low vapour pressure and low water solubility, HLC can be relatively large if calculated using the ratio of vapour pressure and water solubility, which might imply that volatilisation is an important fate process. In practice, adsorption to dissolved organic carbon is likely to be much more relevant, and volatilisation will be lower than the HLC value suggests.

EXPERIMENTAL DETERMINATION OF HENRY'S LAW CONSTANT

The experimental approaches can be classified into two major groups: dynamic equilibration approach (often referred to as the *gas purge* approach) and the static equilibration approach. The following table briefly summarises the reviewing work done by Staudinger and Roberts (1996).

Table R.7.1-16 Experimental approaches for the determination of HLC

Approach	Average Relative Standard Deviations (RSDs) / Notes
Dynamic approach	
Batch air stripping (bubble column) Henry's law constant (HLC) values are determined by measuring the rate of loss of the substance of interest from water by isothermally stripping with a gas (typically air) in a suitable bubble column apparatus.	Average RSDs determined from different literature sources ranged from 2.8 to 21
Concurrent flow (wetted wall column) Values are determined based on the use of a wetted wall (desorption) column. The wetted wall column equilibrates an organic solute between a thin film of water and a concurrent flow of gas. Substance-laden water is introduced into the wetted wall column where it comes in contact with a substance-free gas stream flowing concurrently. HLC: The knowledge of flow rates and compound masses present in the separated phase streams enables the direct calculation of HLC.	Average RSDs determined from different literature sources ranged from 19 to 52 Preliminary work must be performed to ensure that phase equilibrium is reached.
Static approach	
Single equilibration A known mass of a substance is introduced into an air-tight vessel with a known volume of water and air. When the equilibrium is attained the substance concentration is determined in one or both phases.	Average RSDs determined from different literature sources ranged from 2.8 to 30
Multiple Equilibration A liquid sample containing a known quantity of solute is allowed to equilibrate with a known volume of solute-free air. The air is the expelled and a new equilibration with the same amount of solute-free air is started. This process can be repeated until the number of equilibrations exhausts the mass of solute remaining in the system.	RSDs ranged from 0.7 to 3.5 This method is applicable for substances with 0.1 £ HLC £ 2 The experimental error is reduced with a larger number of equilibrations.
EPICS Technique HLC is determined by measuring the gas headspace concentration ratios from pairs of sealed bottles. Relative rather than absolute air-phase concentrations are required.	Average RSDs determined from different literature sources ranged from 2.9 to 19
Variable Headspace The method is based upon the measurement of the relative equilibrium air-phase concentration (gas chromatography peak areas) from aliquots of the same solution in multiple containers having different headspace-to-liquid volume ratios.	Average RSDs determined from different literature sources ranged from 0.5 to 7.9

A data-analysis of reviewed experimental studies for HLC can be found in Staudinger and Roberts (1996). HLC values can also be found in one or more of the following references: Sander (1999), CRC Handbook of Chemistry and Physics (2000), the NIST Chemistry WebBook

(1998), and 'The Handbook of Environmental Data on Organic Chemicals' (Verschueren K, 2001).

MAIN FACTORS AFFECTING HENRY'S LAW CONSTANT VALUES

Staudinger and Roberts (1996) thoroughly explain all the factors affecting HLC values and report equations that quantify the effect of temperature and pH. According to their work, in a majority of cases temperature is the main parameter affecting HLC values for natural waters with moderate contamination (1 mg/ml or less). Other conditions that have influence on HLC values are listed in Table R.7.1-17 (Staudinger and Roberts, 1996):

Table R.7.1-17 Conditions that have influence on HLC values

pH	Important for compound (substance) classes that dissociate to a significant extent in water because only nondissociated species undergo air-water exchange. For most natural waters (6 < pH < 8) the apparent HLC will be significantly less than the intrinsic HLC.
Compound Hydration	Important for aldehydes, which hydrate nearly completely in water, resulting in HLC apparent being several orders of magnitude lower than the intrinsic constant.
Compound concentration/ Complex mixtures effects	If a solution cannot be regarded as diluted (e.g. concentration approaching 10.0 mg/ml) HLC apparent will be lower than HLC values determined at lower concentrations.
Dissolved salts	If the ionic strength of a solution is high (e.g. seawater) the apparent HLC will be higher than the HLC determined in pure water.
Suspended solids /Dissolved Organic Matter (DOM)	If a compound is easily adsorbed (e.g. pesticides) the apparent HLC will be higher than the HLC determined in pure water.
Surfactants	Compounds with high Kow are expected to have an effect on HLC by lowering its value. Recorded effects increase in direct proportion with Kow.

It is worth noting that because of the complex nature of the water matrix the net effect of a possible combination of the parameters listed above may be more than the simple sum of individual effects (Staudinger and Roberts, 1996).

QSPR PREDICTION OF HENRY'S LAW CONSTANT

The prediction of HLC has been reviewed by Schwarzenbach *et al.* (1993), Reinhard and Drefahl (1999), Mackay *et al.* (2000) and Dearden and Schüürmann (2003). The most important approaches are:

- Ratio of water solubility (c_w) to vapour pressure (vp);
- Estimation using connectivity indices;
- Estimation using group and bond contribution methods.

The first method for estimating HLC is not strictly a QSAR method as it uses the water solubility (c_w) and vapour pressure (vp). It is not a highly accurate method, but neither is the measurement of HLC, especially for substances with very high or very low HLC values. vp/c_w can be converted to the dimensionless form of HLC (ratio of concentrations in air and water, c_a/c_w) or K_{aw} by the following equation, which is valid at 25 °C:

$$c_a/c_w = 40.874\ vp/c_w$$

Since both water solubility and vapour pressure can be calculated by QSAR methods, then this approach might in some circumstances be a QSAR based method. The method is limited to substances of low water solubility (< 1.0 mol/L). If QSAR calculated values are used for vp and/or c_w, then the respective uncertainties must be considered. For miscible compounds or compounds with water solubility > 1 mol/L the vp/c_w method is not valid.

The second method is based on a combination of connectivity indices and calculated polarisability (Nirmalakhandan and Speece, 1988). A relatively narrow range of chemical types was used to develop the model, so it is not widely applicable. Moreover, Schüürmann and Rothenbacher [1992] found it to have poor predictive power.

Most prediction methods for HLC use a group or bond contribution approach, although some have used physicochemical properties [Dearden *et al.* 2000]. The group and bond contribution methods were first used by Hine and Mookerjee [1974], who obtained, for a set of 263 diverse simple organic chemicals, a standard deviation of 0.41 log unit for the group contribution method and one of 0.42 for the bond contribution method. Cabani *et al.* [1981] claimed an improvement in the group contribution method over that of Hine and Mookerjee, whilst Meylan and Howard [1991] extended the bond contribution method and obtained, for a set of 345 diverse chemicals, a standard error of 0.34 log unit.

EVAPORATION RATE

Evaporation rates generally have an inverse relationship to boiling points, i.e. the higher the boiling point, the lower the rate of evaporation. Knowledge of the evaporation rate of spills of volatile liquids can be useful in several respects. If it is known that a spill of a high vapour pressure liquid will evaporate completely in a short period of time, it may be preferable to isolate the area and avoid any intervention or clean-up. The evaporation rate also controls the atmospheric concentration of the vapour and hence the threat of explosion or fire. Data on the volatility properties of the liquid, its temperature, the wind speed, and the spill dimensions are used to calculate the evaporation rate and hence the fraction evaporated at any time.

The substance's tendency to partition into the atmosphere is controlled by the vapour pressure, which is essentially the maximum vapour pressure that a pure substance can exert in the atmosphere. This can be viewed as a kind of *solubility* of the chemical in the atmosphere. Using the ideal gas law ($PV=nRT$), the vapour pressure P in the pressure unit pascal (Pa) can be converted into a solubility (mol/m^3), where the gas constant R is 8.314 $Pa.m^3/mol{\cdot}K$ and T is absolute temperature (K).

Conversion from vapour pressure into concentration in air under ambient temperature:

$$\%\ volume = vapour\ pressure\ (Pa)/101\ 325 \times 100$$

$$or\ ppm = vapour\ pressure\ (Pa)/101\ 325 \times 1\ 000\ 000$$

Since the molar volume is the same for all ideal gases (equal volumes of all gases under the same conditions of temperature and pressure contain the same number of molecule) ppm ≡ volume (i.e. ml/m^3). To convert to weight per unit volume:

$$X\ ppm = X \times MW/24.041\ mg/m^3,\ 1\ mg/m^3 = 24.041/MW\ ppm$$

In the formulation of paints and related products, solvents are chosen based on their evaporation characteristics appropriate to the application technique and the curing temperature. To a large extent the evaporation rate of a solvent determines where and how it can be used. In determining the evaporation rate of solvents, n-butyl acetate is used as the

standard and is assigned an evaporation rate value of 1. Other solvents are assigned evaporation rate values that indicate how fast they evaporate in relation to n-butyl acetate. For instance, a solvent that evaporates three times as fast as n-butyl acetate would be assigned a value of 3, whereas a solvent that evaporates half as fast as n-butyl acetate would be assigned a value of 0.5.

The rate of evaporation is determined using ASTM D3539-87. A known volume of liquid is spread on a known area of filter paper that is suspended from a sensitive balance in a cabinet. Dry air or nitrogen at 25 ºC is passed through the cabinet at a known rate. The loss of weight is determined and plotted against time.

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

R.7.2.1 Introduction

Irrespective of whether a substance can become systemically available, changes at the site of first contact (skin, eye, mucous membrane/ gastro-intestinal tract, or mucous membrane/ respiratory tract) can be caused. These changes are considered local effects. A distinction in local effects can be made between those observed after single and those after repeated exposure. In this guidance document, the focus will be on local effects after single ocular or dermal exposure. However, wherever possible, use should also be made of existing repeated dose data as far as they may contain valuable information for the purpose of assessing and classifying effects after single ocular or dermal exposure.

Substances causing local effects after single exposure can be further distinguished as irritant or corrosive substances, depending on the severity, reversibility or irreversibility of the effects observed. *Corrosive substances* are those which may destroy living tissues with which they come into contact. *Irritant substances* are non-corrosive substances which, through immediate contact with the tissue under consideration may cause inflammation. These tissues are in the present context skin, eye (cornea and conjunctiva) and mucous epithelia such as the respiratory tract. Criteria for classification of irritant and corrosive substances are given in Annex VI to Directive 67/548/EEC[27].

Substances may also cause irritant effects only after repeated exposure, for example organic solvents. This type of chemicals may have defatting properties (Ad-hoc Working group on Defatting substances, 1997). Chemicals that have a similar mechanism need to be considered for labelling with the risk phrase 'repeated exposure may cause skin dryness or cracking'.

Information on the mechanism underlying corrosion and irritation from skin, eye and respiratory tract are given in Appendices 1-3 to Section R.7.2 : Appendix R.7.2-2 Mechanisms of local toxicities: skin corrosion/irritation, eye and respiratory irritation.

R.7.2.1.1 Definitions of skin- and eye irritation / corrosion / respiratory irritation

Dermal irritation: Defined in OECD TG 404/EU B.4 as *"…the production of reversible damage of the skin following the application of a test substance for up to 4 hours"*.

Dermal irritation after repeated exposure: Substances which may cause skin dryness, flaking or cracking upon repeated exposure but which can not be considered a skin irritant.

Dermal corrosion: Defined in OECD TG 404/EU B.4 as *"…the production of irreversible damage to skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars…."*.

Eye irritation: Defined in OECD TG 405/EU B.5 as *"…the production of changes in the eye following application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application"*.

[27] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

Eye corrosion: Defined in OECD TG 405/EU B.5 as *"…the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application".*

Respiratory tract irritation: There is no EU or OECD TG for respiratory irritation and testing for respiratory irritation is not required under REACH. Respiratory irritation is often used to describe either or both of two different toxicological effects, *sensory irritation* and *local cytotoxic effects*.

Risk phrases and hazard codes to be considered within the EU classification and labelling information system (EC, 2001):

a) Corrosion

- Corrosive: Risk phrase "R34", "Causes burns". Hazard code: "C"
  Full thickness destruction of the skin occurs as a result of up to 4 hours exposure.

- Corrosive: Risk phrase R35, "Causes severe burns". Hazard code: "C"
  Full thickness destruction of the skin occurs as a result of up to 3 minutes exposure.

b) Irritation

- Irritant: Risk phrase "R38", "Irritating to skin". Hazard code: "Xi",

- Irritant: Risk phrase "R66", "Repeated exposure may cause skin dryness or cracking". Hazard code: "Xi" [a]

- Irritant: Risk phrase "R36", "Irritating to eyes". Hazard code: "Xi"

- Irritant: Risk phrase: "R41", "Risk of serious damage to eyes". Hazard code: "Xi"

- Irritant: Risk phrase: "R37", "Irritating to respiratory system". Hazard code: "Xi"

Note that cytotoxic irritation of the respiratory tract, if observed in repeated dose studies at critical concentrations and if composed of a clearly nectrotic character, has been classified according to the criteria for R48.

Information on the Globally Harmonised System (GHS) for the classification and labelling of chemicals can be found at (UN/ECE, 2003):
http://www.unece.org/trans/danger/publi/ghs/ghs_rev01/01files_e.html

Note that dermal/respiratory irritation following repeated exposure are not discussed in the present context, since this report focuses on acute effects after single exposure. However, data from repeated exposure studies may be useful in certain cases (e.g. if the substance was identified as a corrosive or strong irritant after the first application or for deriving quantitative information). Nevertheless, for the sake of completeness, both the definition of dermal irritation after repeated exposure as well as the related risk phrase (R66) are given here. More guidance on local effects after repeated exposure can be found in Section R.7.5 on repeated dose toxicity.

R.7.2.1.2 Objective of the guidance on skin- and eye irritation / corrosion / respiratory irritation

The general objectives are:

a. to establish whether information from physical/chemical data, from non-testing methods (grouping, QSARs and expert systems), from *in vitro* studies, from animal studies or human experience provide evidence that the substance is, or is likely to be, corrosive.

b. to establish whether information from physical/chemical data, from non-testing methods (grouping, QSARs and expert systems), from *in vitro* studies, from animal studies or human experience provide evidence of significant skin, eye or respiratory irritation.

c. to establish the time of onset and the extent and severity of the responses and information on reversibility.

d. to gather, in the process of hazard identification, any quantitative data on dose-response relationships that might allow the derivation of DNELs essential for a complete risk assessment.

If a risk assessment is necessary, both the severity of the identified hazard (in so far at it can be judged from the test data) and the probability of the occurrence of an acute corrosive or irritant response in humans must be assessed based on the likelihood of any exposure to the substance and in relation to the route, pattern and extent of the expected exposure.

Please note that there are currently no standard tests and no OECD TG available for respiratory irritation and there is no testing requirement for respiratory irritation under REACH. Consequently respiratory irritation is not included in the testing strategies suggested in this report.

Nevertheless, account should be taken of any existing and available data that provide evidence of the respiratory irritation potential of a substance. Moreover, the data on local dermal or ocular corrosion/irritation might contain information that is relevant for the respiratory endpoint and this should be considered accordingly. It is for instance a reasonable precaution to assume that corrosive (and severely irritating) substances would also cause respiratory irritation when vaporised or in form of aerosol, though formal classification with R37 is not justified in this case. Furthermore, information from cases where symptoms have been described associated with occupational exposures can be used on a case-by-case basis to characterise the respiratory irritation potency of a substance. Information from acute and repeated dose inhalation toxicity studies may also be considered sufficient to show that the substance causes respiratory irritation at a specific concentration level or range. The data need to be carefully evaluated with regard to the exposure conditions (sufficient documentation required). Possible confounding factors should be taken into account.

R.7.2.2 Information requirements on skin / eye irritation / corrosion

The information requirement for irritation and corrosion that shall be submitted for registration and evaluation purposes is specified in REACH Annexes VI to XI. According to Annex VI, the registrant should gather and evaluate all available information before considering further testing. These include physico-chemical properties, (Q)SAR, grouping, *in vitro* data, animal studies, and human data. Furthermore, information on exposure, use and risk management measures should also be collected and evaluated.

If these data are inadequate for hazard and risk assessment, further testing should be carried out in accordance with the requirement in REACH Annexes VII (^{3}1tpa) and VIII (^{3}10tpa).

INFORMATION REQUIREMENTS FOR QUANTITIES OF ≥1 TPA (ANNEX VII)

If new testing data are necessary, these must be derived from *in vitro* methods only. Annex VII does not foresee *in vivo* testing for irritancy or corrosivity.

The standard information required (column 1) at this tonnage level for skin corrosion/irritation can be satisfied by following four steps: (1) assessment of the available human and animal data, (2) assessment of the acid or alkaline reserve, (3) *in vitro* skin corrosivity study, (4) an *in vitro* skin irritation study.

Column 2 lists specific adaptations that specify when step 3 or 4 do not have to be conducted. These are:

1. when the available information already indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes.

2. the substance is flammable in air at room temperature (Please note that this rule should actually read: “the substance is spontaneously flammable in air at room temperature”).

3. the substance is classified as very toxic in contract with skin.

4. an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2000 mg / kg body weight).

The standard information required (column 1) at this tonnage level for eye irritation can be satisfied by following four steps: (1) assessment of the available human and animal data, (2) assessment of the acid or alkaline reserve, (3) *in vitro* eye irritation study.

Column 2 lists specific adaptation that specify when step 3 *in vitro* eye irritation testing is not necessary. These are:

1. when the available information already indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes.
2. the substance is flammable in air at room temperature (Please note that this rule should actually read: “the substance is spontaneously flammable in air at room temperature”).

Information requirement for quantities of ≥10 tpa (Annex VIII)

For substances manufactured or imported in quantities of ≥10 tpa *in vivo* testing is required to meet the standard information requirements of Annex VIII column 1. Column 2 lists specific rules that allow deviating from the standard testing regime. More importantly, the standard testing regime of Annex VII and VIII can be adapted by the rules laid down in Annex XI, e.g. allowing to avoid unnecessary animal testing as required in Annex VIII (see Section R.7.2.4.1 for possible alternatives). For detailed information, see the REACH legislative text.

In summary these rules for adapting the standard testing are for:

a. skin irritation:

   · the substance is classified as corrosive to the skin or as a skin irritant or

   · the substance is a strong acid (pH < 2) or base (pH > 11.5) or

- the substance is flammable in air at room temperature (Please note that this rule should actually read: “the substance is spontaneously flammable in air at room temperature”) or
- the substance is classified as very toxic in contact with skin or
- or an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2000 mg/kg body weight).

b. eye irritation:

- the substance is classified as irritating to eyes with risk of serious damage to eye or
- the substance is classified as corrosive to the skin and provided that the registrant classified the substance as eye irritant or
- the substance is a strong acid (pH<2,0) or base (pH > 11,5) or
- the substance is flammable in air at room temperature (Please note that this rule should actually read: “the substance is spontaneously flammable in air at room temperature”).

Guidance on the application of these rules is given in the integrated testing strategies described in Section R.7.2.6.

R.7.2.3 Information and its sources on irritation / corrosion

R.7.2.3.1 Non-human data on irritation / corrosion

Non-testing data on irritation / corrosion

Physico-chemical properties

Information of relevance to irritation/corrosion can be inferred from basic physico-chemical characteristics of a substance (extreme pH). Substances with *extreme* pH values will be inevitably skin corrosives or severe eye irritants:

IF pH≤2 or pH≥11.5, THEN predict to be corrosive to skin and severely irritating to eyes. See also Section R.7.2.4.1)

Grouping, (Q)SARs and expert systems

Non-testing methods can be divided into three categories: 1) grouping approaches (read-across, SARs and categories), 2) QSARs, and 3) expert systems, generally incorporating multiple (Q)SARs, expert rules and data. These methods can be used for the assessment of skin and eye irritation and corrosion, if they provide relevant and reliable data for the chemical of interest. Generally this means that the use of non-testing methods should be justified by means of detailed descriptions. In the case of QSARs and expert systems, the justification is provided by means of a QSAR Model Reporting Format (QMRF). In this guidance document, it is not possible to provide QMRFs for all existing models. However, QMRFs for potentially useful models are available from the JRC QSAR Model Database, which will be accessible via the website (http://qsardb.jrc.it). More detailed guidance on QSAR models, their use and reporting formats, including the QMRF, is provided in Section R.6.1.

In the case of skin irritation and corrosion, many of the models have a mechanistic basis, which provides additional information on the relevance of the model.

SAR and read-across on skin irritation and corrosion:

SARs and read-across are treated together because the existence of a SAR (structural alert or set of fragments) provides one means of justifying read-across.
The occurrence of structural analogues that exhibit corrosion (or irritation) potential can be used to predict the effect in the substance of interest and derogate from further assessment, as indicated in the OECD testing strategy for skin irritation/corrosion (OECD, 2001). Negative data from structural analogues may also be used to make predictions in certain cases, provided that there are no other substructures in the substance that are thought likely to cause the effect. Structural alerts are generally considered to reflect some kind of chemical or biochemical reactivity that underlies the toxicological effect.

The non-reactive chemicals, which lack alerts for reactivity, will normally not exhibit irritant or corrosive effects. However, irritant effects such as irritant contact dermatitis can occur in the case of exposure to organic solvents, which have defatting properties. Chemicals that have a similar mechanism need to be considered for 'Repeated exposure may cause skin dryness or cracking' (R66) (Ad-hoc Working group on Defatting Substances, 1997).

An example of a simple SAR is the use of the hydroperoxide group as an alert for corrosivity, which is mechanistically based on the fact that hydroperoxides are both acidic and oxidisers. Another SAR is the peroxide group (R1-C-O-O- R2), based on the fact that peroxides are oxidising agents. These SARs are mentioned in the Classification and Labelling guide (EC, 2004). The validity of these models, however, is not given there. Rorije et al. (2007) showed that 75 and 60% of the hydroperoxides and peroxides are classified for corrosivity and irritancy, respectively.

A variety of SARs for predicting the presence of irritation or corrosion have been described by Hulzebos et al. (2001, 2003, 2005), and others have been incorporated into the BfR rulebase and the SICRET tool (Walker et al., 2005, see Appendix R.7.2-2 QSARs and expert systems for skin irritation and corrosion).

Read-across has been used to a limited extent in the New Chemicals notification procedure for the classification of skin irritants (Hoffmann et al., 2005). As of May 2006, one substance has been classified as R38 by read-across from an analogue, and seven substances have been unclassified for R38 on the basis of read-across from analogues that were not found to meet the classification criteria for skin irritation (Thomas Cole, ECB, personal communication).

QSARs and expert systems on skin irritation and corrosion:

QSARs and expert systems for skin irritation and corrosion have been described in several reviews (Hulzebos et al., 2001, 2003, 2005; Patlewicz et al., 2003; Gallegos Saliner et al., 2006). A few examples are presented in Appendix R.7.2-2 QSARs and expert systems for skin irritation and corrosion, including literature-based QSAR models, commercial models, and expert systems.

Most of the QSARs reported in the literature have been developed from small data sets of specific groups of compounds, although in some cases more diverse and larger datasets were also examined. In general, it has been suggested that basic physico-chemical parameters such as acidity, basicity, hydrophobicity, and molecular size as well as electrophilic reactivity, are useful to predict the toxic potential of homologous chemicals. In contrast, models intended to predict the toxic potential of heterogeneous groups of chemicals emphasise the commonality of structural features.

Commercial models are coded in the form of expert systems, which are computer programs that guide hazard assessment by predicting toxicity endpoints of certain chemical structures based on the available information. Expert systems can be based on an automated rule-

induction system (e.g. TOPKAT, HazardExpert and MultiCASE), or on a knowledge-based system (e.g. DEREK for Windows, the BfR-DSS, and SICRET). More details on commercial expert systems are reported in Appendix R.7.2-2.

Not all of the models were developed with EU regulatory purposes in mind, so it is important to assess in each case whether the endpoint or effect being predicted corresponds with the regulatory endpoint of interest. In principle, such models could be redeveloped (re-parameterised) by using updated or alternative datasets, and used instead of the published models. The BfR model (also reported in Appendix R.7.2-2) has been developed to predict EU regulatory endpoints, and it has been recently validated (Rorije & Hulzebos, 2005 and Gallegos Saliner et al., 2007).

Use of (Q)SAR models for skin corrosion:

In the case of classification models for skin corrosion, where it is not indicated in the supporting documentation whether the predicted classification should be R34 or R35, it is recommended to treat the prediction as equivalent to R35 (severe corrosive). Very few models are available (see Gallegos Saliner et al., 2006 for review). Available models tend to focus on defined chemical classes (e.g. acids, bases, phenols) and might be useful as an alternative to *in vitro* testing for such chemicals.

SARs and read-across for eye irritation and corrosion:

The occurrence of structural analogues that exhibit corrosion (or irritation) potential can also be used to predict the effect in the substance of interest and derogate from further assessment. Negative data from structural analogues may also be used to make predictions in certain cases, provided that there are no other substructures present that are thought likely to cause the effect.

Read-across has been used in the New Chemicals notification procedure for the classification of eye irritants. An example is provided by the classification as R36 of Neodol HS, a branched alcohol ethoxy sulphate, by read-across from structurally related anionic surfactants. The adequacy of the read-across was justified in multiple ways:

i. by comparing the *in vitro* results of Neodol HS with that of SLS in the Cytosensor Microphysiometer Test. Since SLS is classified as R36 and used as the positive control in this assay, and since the test result showed that Neodol has a lower eye irritancy than SLS, it was argued that Neodol HS should also be (conservatively) classified as R36;

ii. by referring to the Critical Micelle Concentration (CMC). Below this concentration, the surfactant is in the monomer form, which has irritant properties, whereas above the CMC, the surfactant form micelles, which are less irritant. Thus, the higher the CMC, the greater the proportion of monomers present, and the more likely the surfactant will be an irritant. Neodol HC was shown to have a lower CMC than similar chemicals classified as R36;

iii. by referring to the fact that alkyl ethoxy sulphates, such as Neodol HC, tend to be weaker eye irritants than alkyl sulphates and sulphonates, and that alkyl sulphates and sulphonates with similar chain lengths to Neodol HC are classified as R36.

This illustrates the use of *in vitro* data to support read-across by comparing the *in vitro* effect of the chemical of interest with that of a suitable benchmark chemical.

QSARs and expert systems for eye irritation and corrosion:

An extensive review of the current state-of-the-art has been published by the ECB (Gallegos Saliner et al. 2006). In Appendix R.7.2-3 some examples are given to illustrate currently available models and the techniques that have been used to develop them. These models include literature-based QSAR models, commercial models, and expert systems.

From the scientific literature, it appears that more emphasis has been placed on the QSAR modelling of ocular irritation compared with dermal irritation. Examples of models based on classical regression and classification techniques, together with more innovative approaches, are collected in Appendix R.7.2-3.

The most widely used commercial expert systems for assessing eye irritation are the same as those used for assessing skin irritation and corrosion. Details on automated rule-induction systems (e.g. TOPKAT and MultiCASE), and on knowledge-based systems (e.g. DEREK for Windows, and the BfR-DSS) are reported in Appendix R.7.2-3.

Not all of the models were developed with EU regulatory purposes in mind, so it is important to assess in each case whether the endpoint or effect being predicted corresponds with the regulatory endpoint of interest. In the case of the more transparent, literature-based models, the examples could be more useful in terms of illustrating the feasibility of developing a model by using defined descriptors and by applying a defined statistical approach to a suitable dataset. If alternative or extended datasets are available, such models could be redeveloped (re-parameterised) and used instead of the published models. The BfR model for the prediction of eye irritation has been developed to predict EU regulatory endpoints, and it has been recently validated (Tsakovska et al., 2005 and Tsakovska et al., 2007).

Use of (Q)SAR models for eye irritation / corrosion:

In the case of classification models for eye irritation, the classification criteria used in the model develop should be compared with the EU classification criteria, to assess the relevance of the model. Where it is not indicated in the supporting literature whether the predicted classification should be R36 or R41, the risk phrase chosen should be supported with expert judgment.

Table R.7.2-1 Overview of available (Q)SARs for skin and eye irritation / corrosion and the availability of QSAR model reporting formats (QMRFs), in which the application of the OECD principles for QSARs is illustrated

Category of model or source	Reference or name of the model	Type of model	Applicability domain	Draft QMRF* developed
Literature models	Barratt, 1995	Statistical model	Acids, Bases , Phenols and pKa,	no
	Berner et al.,1988, 1990a, 1990b	Mathematical model	pKa related acids	no
	Nangia et al., 1996	Mathematical model	pKa related for bases	no
	Barratt, 1996b	Statistical model	Electrophiles	no
	Smith et al. 2000 a,b	Statistical model	Esters	no
	Barratt, 1996b	Statistical model	Neutral organics	no
	Gerner et al., 2004, 2005; Walker et al.,	Rule-based model	New Chemicals Database, organic	yes

	2004		chemicals	
Computerised models	TOPKAT commercial	Mathematical model using connectivity descriptors	Organic chemicals	yes
	DerekfW, commercial	Expert system using structural alerts	Organic chemicals and some metals	yes
	MultiCASE, commercial	Mathematical model using fragments	Organic chemicals	no
	Hazard expert, commercial	Organic chemicals using structural alerts	Organic chemicals	no
	BfR rulebase, free, available in-house at BfR	Rule-based model	New Chemicals Database, organic chemicals	yes
Review papers	Hulzebos et al., 2001, 2003, 2005	N.A.	N.A.	N.A.
	Patlewicz et al., 2003	N.A..	N.A.	N.A.
	Gallegos Saliner et al (2006)	N.A..	N.A.	N.A.

*) QMRF: (Q)SAR model reporting format see Section R.6.1 (available at http://qsardb.jrc.it).

**) see Annex II and III for more information on these models

Testing data for irritation / corrosion

The internationally accepted testing methods for skin irritation and eye irritation are described in OECD TGs. Those regarding skin effects can be found in TGs 404, 430, 431 and 435 (EU B.4, B.40, B.40bis), those for the endpoint eye in TG 405 (EU 5). The testing strategies developed (see Section R.7.2.6) emphasise the need to evaluate all available information (including physico-chemical properties) before attempting any *in vivo* testing. They both employ screening elements designed to avoid, as far as possible, *in vivo* testing of corrosive substances and to limit *in vivo* testing of severely irritating substances. In particular, it is recommended to test *in vitro* for skin corrosion (method B.40) before any attempts to assess skin or eye irritation/corrosion by animal testing and when no other information is available. There is no method for respiratory irritation in Annex V of Directive 67/548/EC.[28]

[28] All the test methods previously included in Annex V to Directive 67/548/EEC will be incorporated in a new Test Methods (TM) Regulation that is currently (February 2008) under adoption. The TM Regulation will be adapted to technical progress whenever a new test method has been developed, scientifically validated and accepted for regulatory use by the National Coordinators of the Member states.

In vitro data

In vitro tests for skin corrosivity:

Accepted *in vitro* tests for skin corrosivity are listed in Annex V of Directive 67/548/EC[28] and as OECD TG (EU, 2000; OECD 2004ab; OECD 2006).
These are the following (see also Table R.7.2-2):

i. The Transcutaneous Electrical Resistance (TER using rat skin) test (OECD TG 430/EU B.40)

ii. Human Skin Model tests (OECD TG 431/EU B.40 bis)

iii. The in vitro Membrane Barrier test method (not yet included as EU B.40 method; OECD TG 435)

For acceptable use in OECD TG 431/EU B.40 bis , human skin models need to satisfy the conditions for general and functional models given in the guideline. Models currently accepted as valid are EPISKIN™ and EpiDerm™ (Fentem *et al*., 1998; Liebsch *et al*., 2000; ECVAM, 1998; ECVAM, 2000); SkinEthic™ has undergone testing for this purpose (Kandárová *et al*., 2006) and been endorsed by ESAC as a method able to distinguish between corrosive and non-corrosive chemicals within the context of OECD TG 431/EU B.40 bis.

The TER assay and the human skin model assays do not allow the sub-categorisation of corrosive substances as permitted in the GHS.

The *in vitro* Membrane Barrier Test Method for Skin Corrosion (commercially available as Corrositex®) is recognised to enable identification of corrosive substances and mixtures and allowing the sub-categorisation of corrosive substances as required under the GHS. However, a limitation of the test is that many non-corrosive substances and preparations and some corrosive substances and preparations do not qualify for testing (i.e., test substances and preparations not causing a colour change in the Chemical Detection System; aqueous substances with a pH in the range of 4.5 to 8.5 often do not qualify for testing). Both ECVAM and ICCVAM have therefore concluded that this test may only be used for determining the corrosivity/ non-corrosivity of a specific categories of substances, *e.g.,* organic and inorganic acids, acid derivatives, and bases (ECVAM, 2001; ICCVAM, 2002). The test is accepted for testing purposes related to the transportation of chemicals of these specific classes by the US Department of Transport (US DOT, 2002).

In vitro tests for skin irritation:

After prevalidation (Fentem et al., 2001) and extensive optimisation (Zuang *et al*., 2002; Cotović *et al*., 2005; Kandárová *et al*., 2005), two human skin assays EPISKIN™ (EPISKIN SNC, France) and EpiDerm™ (MatTek Inc., USA), have undergone a formal ECVAM validation (2003-2006) and are currenly undergoing ESAC peer review.
Irritant substances are identified in the human skin assays by:

i. their ability to induce a decrease in cell viability (measured by the MTT test) below defined threshold levels.

ii. their ability to release inflammatory mediators (Interleukin 1-α) when the cell viabiliy is above the defined threshold levels.

If the ESAC peer-review concludes that the test(s) are scientifically valid they will be forwarded to the EU and OECD for regulatory acceptance. At the time of writing this report it is expected that the EPISKIN text will be validated and endorsed as a full replacement of the *in vivo* test.

In this case the test should be used for Annex VII and for Annex VIII under provisions laid down in Annex XI 1.4 to avoid *in vivo* testing for skin irritation under the standard testing regime in compliance with Article 25 of the REACH legislation.

The Skin integrity function test (SIFT; Heylings *et al*., 2003), which uses the electrical resistance of mouse skin and transepidermal water loss across mouse skin as endpoints, was discontinued after phase 1 of the validation study.

The validation trial was designed to test these assays against current EU irritant/non irritant classifications. A post-hoc assessment using GHS classifications was subsequently undertaken (see Section R.7.2.4).

***In vitro* tests for eye irritation:**

At present there are no validated or OECD adopted *in vitro* tests for eye irritation. Within the EU, the 64th Competent Authority (CA) meeting November 2002, agreed that where there are positive results in the non-validated *in vitro* tests below, a substance can be considered a severe eye irritant (R41) and can be labelled accordingly (negative results require further testing *in vivo;* EC, 2006a):

i. isolated rabbit eye (IRE) test

ii. isolated chicken eye (ICE) test

iii. bovine corneal opacity & permeability (BCOP) test

iv. hen's egg test – chorio-allantoic membrane (HET-CAM) test.The above tests are currently undergoing evaluation by ICCVAM (with ECVAM collaboration) as to their validation status for the identification of severe eye irritants (ICCVAM, 2006).

There are two human corneal epithelium models available commercially, EpiOcular™ (MatTek Inc.) and SkinEthic™ HCE (SkinEthic, France) which have undergone assessment in industry-organised trials from which pre-validation and validation data have been submitted to ECVAM for evaluation. ECVAM is also taking the lead in the evaluation of promising cell cytotoxicity/cell function-based *in vitro* methods (e.g., red blood cell haemolysis, neutral red release, fluorescein leakage and silicon microphysiometer). These assays have previously undergone validation studies which were not successful, but they may be currently used as screening tests within companies and may be considered suitable for particular chemical domains following evaluation of supporting data.

The above tests are mainly concerned with modelling the immediate effects of chemicals on the cornea**.** *In vivo* eye irritation endpoints which are not covered by the above-mentioned optimised protocols are the following:

i. persistence/reversibility of effects

ii. effects on conjunctivae or other eye tissue

iii. mechanical irritation produced by solid materials

Integrated Testing Strategies combining the different tests according to their applicability domain and capacity to classify in the different ranges of irritation will be developed, once the individual tests will be completely evaluated (Scott et al., in preparation).

Table R.7.2-2 Validation status, regulatory acceptance, relevant guidelines

Area of concern	Test	validation status, reg. acceptance, use, limitations	OECD guideline	Dir 67/548/EEC	ECVAM-Invittox Nr.
skin corrosion					
	TER (1)	Validated	TG 430	Part of annex V	115
	EpiDerm	Validated	TG 431	Part of annex V	119
	EPISKIN	Validated	TG 431	Part of annex V	118
	SkinEthic	Validated	N.A.	N.A.	No protocol
	Corrositex	Validated	TG 435	Not yet	116
skin irritation					
	EpiDerm	Validated	not yet	Not yet	No protocol
	EPISKIN	Validated	not yet	Not yet	No protocol
	SIFT (2)	Only prevalidation so far. Applicability domain limited.	N.A.	N.A.	No protocol
eye irritation					
	IRE (3)	Pending, but regulatory acceptance for severe irritants *	N.A.	N.A.	85
	ICE (4)	Pending, but regulatory acceptance for severe irritants*	N.A.	N.A.	80
	BCOP (5)	Pending, but regulatory acceptance for severe irritants*	N.A.	N.A.	98, 124
	HET-CAM (6)	Pending, but regulatory acceptance for severe irritants*	N.A.	N.A.	47, 96
	RBC (7)	Pending. Used by industry for screening purposes.	N.A.	N.A.	37, 99
	FL (8)	Pending. Used by industry for screening purposes.	N.A.	N.A.	71, 82, 120
	NRR	Pending. Used by industry for	N.A.	N.A.	54

	(9)	screening purposes.			
	CMP / SMP (10)	Pending. Used by industry for screening purposes.	N.A.	N.A.	97, 102
	EpiOcular TM	Pending. Used by industry for screening purposes.	N.A.	N.A.	No protocol
	SkinEthic TM	Pending. Used by industry for screening purposes.	N.A.	N.A.	No protocol
*) see: EC 2004.					
1) TER = Transcutaneous Electrical Resistance. 2) SIFT = Skin Integrity Function Test in Mouse. 3) IRE = Isolated Rabbit Eye. 4) ICE = Isolated Chicken Eye. 5) BCOP = Bovine Corneal					
Opacity and Permeability. 6) HET-CAM = Hen's Egg Test on Chorioallantoic Membrane. 7) RBC = Red Blood Cell Haemolysis Test. 8) FL = Fluorescein Leakage. 9) NRR = Neutral Red					
Release. 10) CMP / SMP = Cytosensor Microphysiometer / Silicon Microphysiometer					

status within Dir 67/548/EEC and availability of invittox protocols of relevant tests in the field of skin corrosion, skin irritation and eye irritation.

Animal data

Skin and eye irritation:

Annex VI of the Dangerous Substances Directive (Directive 67/548/EEC) defines both skin and eye irritation as a local toxic effect, and, as such, an assessment of irritation is normally part of the acute testing phase of a toxicity programme and it is an early requirement of all regulatory programmes. As a consequence, testing for irritation has, historically, used animal models and a variety of test methodologies depending upon, for example, the laboratory undertaking the test, the era and intended application.
Current approaches for irritation testing are covered by:

i. OECD TG 404, Acute Dermal Irritation/Corrosion (adopted 12 May 1981; most recently updated 24 April 2002);

ii. Commission Directive 2004/73/EC, Method B4, Acute Toxicity: Dermal Irritation/Corrosion.

iii. OECD TG 405, Acute Eye Irritation/Corrosion (adopted 12 May 1981; most recently updated 24 April 2002)

iv. Commission Directive 2004/73/EC, EU B.5, Acute Toxicity: Eye Irritation/Corrosion.

The guidelines for skin and eye irritation testing require a tiered approach, using one animal (the rabbit is the preferred species) initially, which in the absence of severe effects is followed by a further two animals (a total of three animals).

Both OECD and EU methods use the scoring system developed by Draize (1944). The EU criteria for classification are based on the mean tissue scores obtained over the first 24-72 hour period after exposure and on the reversibility or irreversibility of the effects observed. Currently for both eye and skin, *irritants* (labels R36 and R38, respectively) cause significant inflammation of the eye (conjunctiva redness/oedema, cornea and/or iris) and/or skin (erythema and/oedema) but these effects are transient i.e. the affected sites are repaired within the observation period of the test. A *severe eye irritant* causes considerable damage to the cornea and/or iris and is labelled with R41. The criteria for R41 include persistence of effects (any score), irreversible staining of the eye and/or criteria for the degree of severity. Guidance on how industry interprets eye irritation data in the light of EU classification and labelling is summarised in a publication by ECETOC (1997).

A corrosive substance causes full thickness destruction of the skin tissue and is classified as *corrosive* and assigned a label (R34 or R35) depending upon the exposure time (3 min and 4 hours, respectively).

For existing substances, the use of methods other than those specified in Annex V of Directive 67/548/EC [29], or corresponding OECD methods, such as LVET (Griffith et al., 1980) may be accepted on a case-by-case basis.

In addition to the OECD guidelines and Commission Directives mentioned above, further animal data may be available from:

[29] All the test methods previously included in Annex V to Directive 67/548/EEC will be incorporated in a new Test Methods (TM) Regulation that is currently (February 2008) under adoption. The TM Regulation will be adapted to technical progress whenever a new test method has been developed, scientifically validated and accepted for regulatory use by the National Coordinators of the Member states.

i. Acute dermal toxicity test (OECD TG 402/EU B.3)

ii. Skin sensitization (OECD TGs 406 and 429/EU B.6 and B.42)

See Section R.7.2.6 for comments on how to use information from these tests in an Integrated Testing Strategy for skin and eye irritation/corrosion.
Data on chemosensory effects obtained in the Alarie test for respiratory irritation (Alarie, 1973, Arts et *al.,* 2006) may be useful as supportive evidence for human eye irritation after exposure to airborne chemicals (e.g. vapours).

Respiratory irritation:

There are currently no OECD adopted test guidelines that deal specifically with respiratory tract irritation. The type of information from animal studies that could inform on the respiratory irritation potential of the chemical concerned are the Alarie assay (information on sensory irritation, Alarie, 2000; ASTM, 2004) and single or repeated inhalation exposure studies (information on (histo)pathological changes).

In rodents, sensory irritation leads to a concentration-dependent reduction in the respiratory rate (breath-holding) mediated via the trigeminal nerve reflex; this reflex effect on respiration can be measured experimentally as the RD_{50} value in the Alarie assay.

Single inhalation exposure studies may provide information on nasal irritation such as rhinitis, whereas histopathological examination of respiratory tract tissues of animals repeatedly exposed by inhalation (28-day and 90-day inhalation studies) may provide information on inflammatory/cytotoxic effects such as hyperemia, edema, inflammation or mucosal thickening.

Data from bronchoalveolar lavage may give additional information on the inflammatory response.

R.7.2.3.2 Human data for irritation / corrosion

Existing human data include historical data that should be taken into account when evaluating intrinsic hazards of chemicals. *New* testing in humans for hazard identification purposes is not acceptable for ethical reasons.

Existing data can be obtained from case reports, poison information centres, medical clinics, and occupational experience or from epidemiological studies. Their quality and relevance for hazard assessment should be critically reviewed. However, in general human data can be used to determine a corrosive or irritating potential of a substance. Good quality and relevant human data have precedence over other data. However, lack of positive findings in humans does not necessarily overrule good quality animal data that are positive.

Specifically with regard to respiratory irritation, there is a view in the occupational health literature that sensory irritation may be a more sensitive effect than overt tissue-damaging irritation, given that its biological function is to serve as an immediate warning against substances inhaled during a short period of time which could damage the airways, and that it triggers physiological reflexes that limit inhalation volumes and protect the airways. However, there is a lack of documented evidence to indicate that this is a generic position that would necessarily apply to all inhaled irritants.

R.7.2.4 Evaluation of available information on irritation/corrosion

R.7.2.4.1 Non-human data on irritation/corrosion

Non-testing data on irritation/corrosion (skin and eye)

Physico-chemical properties

According to the current EU and OECD guidelines, substances should not be tested in animals for irritation/corrosion if they can be predicted to be corrosive from their physico-chemical properties. In particular, substances exhibiting strong acidity (pH ≤2) or alkalinity (pH ≥11.5) in solution are predicted to be corrosive, and should not be tested. However, no conclusion can be made regarding corrosivity when the pH has an intermediate value (when 2< pH <11.5).

Physico-chemical properties for skin corrosion/irritation:

Chemicals that have other pH values will need to be considered further for their potential for skin and eye irritation/corrosion.

The following decision rule can be used in a tiered testing strategy:

IF pH ≤ 2 or pH ≥ 11.5 THEN assume the chemical to be corrosive (R34 or R35).

This model is included in OECD testing strategy for skin irritation and corrosion (OECD, 2001). Several studies have investigated and confirmed the usefulness of pH as a predictor of corrosion (Worth & Cronin, 2001) and as an element in tiered testing strategies (Worth, 2004).

However, where extreme pH is the only basis of classification as corrosive, it may also be important to take into consideration the acid/alkaline reserve, a measure of the buffering capacity of a chemical substance (Young *et al*., 1988; Botham *et al*., 1998; Young & How, 1994), as mentioned in the OECD test guideline 404. However, the buffering capacity should not be used alone to exonerate from classification as corrosive. Indeed, when the Acid/Alkaline reserve suggests that the substance might be non-corrosive, further in vitro testing should be considered.

Physico-chemical properties for eye irritation:

A chemical known or predicted to be corrosive to the skin is automatically considered to be severely irritating to the eye (R41). However, no conclusion can be made regarding eye irritation potential when the pH has an intermediate value (when 2< pH <11.5). Thus, the following decision rule may be used in a tiered testing strategy:

IF pH ≤2 or pH ≥11.5 THEN consider the chemical for classification as a severe eye irritant.

To predict the eye irritation potential of non-corrosive chemicals, the distribution of pH values for irritants and non-irritants in a data set of 165 chemicals has been analysed (Worth, 2000). The irritants spanned a wide range of pH values from 0 to about 12, whereas the non-irritants spanned a much narrower range from about 3 to 9. Using the cut off values generated by classification tree analysis, the following model was formulated:

IF pH <3.2 or if pH >8.6, then consider the chemical for eye irritation classification; otherwise make no prediction.

According to the way the model was developed, *irritant* can either be R41 or R36. Further information and/or reasoning is needed to conclude on the risk phrases. The more severe classification (R41) should be assumed if no further information is available.

This model had a sensitivity of 53% (and therefore a false negative rate of 47%), a specificity of 97% (and therefore a false positive rate of 3%), and a concordance of 76%. A QSAR Model Reporting Format (QMRF) has been developed (see Section R.6.1 and JRC QSAR Model Database: http://qsardb.jrc.it).

Based on these statistics, this model is not recommended for the stand-alone discrimination between eye irritants and non-irritants. However, could be used in the context of a tiered testing strategy to identify eye irritants (due to its very low false positive rate) but not non-irritants (due to its relatively high false negative rate).

Grouping, (Q)SARs and expert systems

Guidance has been developed by the ECB (Worth et al., 2005) on how to apply (Q)SARs for regulatory use. Guidance on how to assess the validity and suitability of (Q)SAR models and adequacy of their predictions is given in Section R.6.1, and guidance on the use of read-across/category approaches is given in Section R.6.2.

First the model should be descripted in accordance with OECD principles on (Q)SARs (OECD, 2004c), and documented by means of a QMRF. Interpretation of the model is additionally needed. For example a model based on the logarithm of the octanol/water partition coefficient (K_{ow}) might indicate how the log K_{ow} should be derived, measured, calculated, with which program, whether ionised chemicals can be used as well. For more complicated parameters e.g. the quantum descriptors HOMO (Highest Occupied Molecular Orbital energy) and LUMO (Lowest Unoccupied Molecular Orbital energy) this is even more crucial as the calculation outcome depend on the configuration state of the molecule. The performance parameters for the model (i.e. correlation coefficient, sensitivity/specificity, etc.) have to be reported. When the predictivity of a model is assessed, it should be assessed whether the test set is within the applicability domain of the model. The guidance given by the authors/builders of the model should be a starting point.

The second step is to evaluate the prediction of a specific chemical. The OECD principles on (Q)SARs can be used again. One of the most important principle is the chemical's fit in the applicability domain (i.e. is the submitted chemical similar to the training set and does information exist on the predictivity) The outcome of the prediction should be assessed and documented in the form of a QPRF.

The third and last step of the evaluation explicitly needs to meet regulatory requirements. In this last evaluation the (Q)SAR prediction is weighed against the possible mechanism of skin irritation and corrosion. It has to be compared with the effects that can be observed in the *in vivo* test, to see whether all skin irritation/corrosion pathways are covered. In this last step, the hazard of defatting properties has to be assessed as well. (Q)SAR models have to be evaluated in considering the possible mechanism and how this would relate to EU hazard classification.[30]

The mechanism of irritation and corrosion has toxicodynamic and toxicokinetic parameters. Models that solely predict irritation and corrosionon toxicodynamics properties such as acidity or basicity, electrophilicity, other reactivity, surfactant activity, solving membranes, have to be additionally evaluated for their toxicokinetic parameters. These parameters can be physical chemical parameters or others and indicate the potential to cross the skin (stratum corneum) and be active in the living tissue underneath the stratum corneum. Also models that solely predict (the absence of) activity, irritation and corrosion, e.g. by physical chemical properties that illustrate the toxicokinetic behaviour of chemicals, have to be evaluated for their activity (toxicodynamics).

[30] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

For example, the BfR physico-chemical rulebase predicts the absence of skin and eye irritation. Evaluations of the BfR rulebases for the prediction of no skin irritation/corrosion (Rorije and Hulzebos, 2005; Gallegos Saliner *et al*, 2007) and for the prediction of no eye irritation (Tsakovska *et al*, 2005) have been carried out independently. However, when the absence of irritation cannot be excluded, further information on the structure of the chemical is needed to predict presence of irritation/corrosion.

The absence of skin and eye irritation and corrosion is well predicted with the BfR rulebase and therefore these rules can be applied.

There is no other model yet available which sufficiently describes the absence of effects. Neutral organics are expected not to be irritants, however their defatting potential should be discussed. The definition of a neutral organic is a chemical which do not have potential reaction centres, even after skin metabolism. The absence of reactivity needs to be described in sufficient detail or be substantiated with other information.

The presence of effects has been well established using the pH cut off values for high acidity and basicity and can be applied. Structural alerts for the presence of effects can be used, however further characterisation of the effect needs to be described in sufficient detail or be substantiated with other information. For instance, the BfR structural rulebases for the prediction of skin irritation/corrosion (Rorije *et al*., 2007 and Gallegos Saliner *et al*. 2007) and for the prediction of eye irritation (Tsakovska *et al*., 2007) have been recently validated.

Testing data on irritation / corrosion (skin and eye)

In vitro data

There are OECD adopted guidelines for tests (see Section R.7.2.3) under which substances can be classified as corrosive. A negative result in these tests should be supported by a *Weight of Evidence* determination using other existing information, e.g. pH, (Q)SAR, human and/or animal data. These tests do not provide information on skin irritation and, therefore, further information is required to evaluate the skin irritation potential of non-corrosives. If a substance is shown to be non-corrosive in an *in vitro* test, unless this is confirmed by other data, an *in vivo* test needs to be conducted at the appropriate tonnage level.

Annex VII of REACH requires information from *in vitro* tests for skin and eye irritation, not from animal tests.

In order to accept an *in vitro* skin or eye irritation test under Annex VII or VIII, it is of great importance that a proper quality assessment of any such reports should be done.

As a consequence of the general rules in Annex XI, data from the following types of tests may be accepted as described below.

Skin irritation

Validated Tests:

The human skin model tests, EpiDerm™ and EPISKIN™ have undergone formal validation by ECVAM. The predictive capacity (expressed as sensitivity and specificity by comparison of in vitro data with animal data from the Draize skin irritation test carried out according to OECD TG 404) of the EPISKIN skin irritation test (SIT) using two endpoints (cytotoxicity (MTT test) and Interleukin 1-alpha release) was 90.7% (sensitivity) and 78.8% (specificity). Thus the test is considered scientifically valid for the prediction of irritant and non-irritant chemicals for Annex VII, and also Annex VIII according to the rules laid down in Annex XI.

The sensitivity and specificity of the EpiDerm SIT using one endpoint (cytotoxicity by MTT reduction measurement) was 60,1% and 88,8%, respectively. In its current form, the test is suitable for the identification of irritant chemicals as it has a low false positive rate, but not for the identification of non-irritant chemicals because of a high false negative rate. A positive result from the assay could thus be used for classification as irritant at Annex VII and VIII levels, but since negative data would however need to be supported by additional data, the EPISKIN™ test (SIT; see above) is the preferred method.

The methods were primarily validated against the EU classification scheme (irritants vs non-irritants; R38 vs no-label). A post-hoc evaluation of the EPISKIN assay performance against the GHS classification showed that the assay is not able to discriminate the GHS mild irritants from the GHS non-irritants and GHS irritants.

According to the Proposal for a EU Regulation on Classification and Labelling of Substances and Mixtures based on the GHS, GHS non-irritants and GHS mild irritants will become EU non-irritants (EC, 2006b). Considering this proposal for the new EU classification system and based on the results of the skin irritation validation study, the assay discriminated the irritants (GHS irritants) from the non-irritants (GHS mild and GHS non-irritants) with a sensitivity of 100% and a specificity of 64.4%. Note, that the final published EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS) should be taken into account (see Section R.7 Introduction).

Non-validated tests:

Positive data from the following tests may be accepted and used under Annexes VII and VIII (exploiting the possibilities provided by Annex XI Section 1.4). Negative results would however need to be supported by other data (see skin ITS box 9c).

The Skin integrity function test (SIFT) has completed a pre-validation followed by an optimisation phase, but more work is required for full validation. The test in its current state has a specific applicability domain (surfactants). In contrast, the pig's ear test and PREDISKIN™ assay only underwent a prevalidation study.

Eye irritation:

Positive outcome from four *in vitro* assays, the BCOP, ICE, IRE and HET-CAM are accepted by the EU to classify severe eye irritants under Annex VII and Annex VIII using the adaptations of the standard testing regime specified in Annex XI. They have undergone a formal retrospective evaluation, and their scientific validity has been the subject of a statement by ESAC (2007).

For the lower ranges of irritancy no assay is currently accepted by regulators but the following assays exist: Two reconstituted human tissue models, the EpiOcular™ and SkinEthic™ HCE models, have undergone corporate validation (EpiOcular: Blazka et al, 1999, 2000, 2003) and prevalidation trials (SkinEthic: van Goethem et al., 2006) respectively. The results are undergoing a formal evaluation. Positive data from these may be accepted under Annex VII and VIII (see adaptation rules in Annex XI) if there is sufficient background information on the performance of the assay.

Four cytotoxicity and cell/tissue function based assays such as the Red Blood Cell haemolysis test, the Neutral Red Release assay, the Fluorescein Leakage test and the Silicon/Cytosensor Microphysiometer assay are currently undergoing a retrospective evaluation. Companies may use several of these for internal purposes and resultant positive data may be appropriate for Annex VII and VIII (see adaptation rules in Annex XI).

Quality Aspects:

In such a quality assessment that will lay the basis for later possible *Weight of Evidence* considerations, see Sections R.4.4 and R.5.2.1.2 for aspects that need to be taken into account in such a WoE.

Animal data

Well-reported studies particularly if conducted in accordance with principles of GLP, can be used to identify substances which would be considered to be, or not to be, corrosive or irritant to the skin or eye. There may be a number of skin or eye irritation studies already available for an existing substance, none of which are fully equivalent to a EU test method such as those in the Annex V to Directive 67/548/EEC. If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the skin and/or eye irritation potential of the substance.

If the results from a variety of studies are unclear, based on the criteria given below for evaluation of the data, the registrant will need to decide which of the studies are most reliable, relevant for the endpoint in question and will be adequate for classification purposes.

Particular attention should be given to the persistence of irritating effects, even those which do not lead to classification. Effects such as erythema, oedema, fissuring, scaling, desquamation, hyperplasia and opacity which do not reverse within the test period may indicate that a substance will cause persistent damage to the human skin and eye.

Data from studies other than skin or eye irritation studies (e.g. other toxicological studies on the substance in which local responses of skin, eye mucous membranes and/or respiratory system have been reported) may provide useful information though they may not be well reported in relation to, for example, the basic requirements for information on skin and eye irritation. However, information from studies in animals on mucous membrane and/or respiratory system irritation can be very useful for risk assessment provided the irritation is clearly substance-induced, and particularly if it can be related to exposure levels.

Quality Aspects

Data from *existing* irritation studies in animals must be taken into account before further testing is considered. A quality assessment of any such reports should be done using, for example, the system developed by Klimisch et al. (1997), as described in Section R.4.2, and a judgement will need to be made as to whether any further testing is required. Some examples to note are:

i. Was the animal species the rabbit or was it another such as rat or mouse? Rat and mouse, as species, are not as sensitive as the rabbit for irritation testing.

ii. How many animals were used? Current methodology requires 3 but 6 was frequently used in the past.

iii. How many dose levels were used? If dilutions were included, what solvent was used (as this may have influenced absorption)? Which dose volume was used?

iv. For skin, which exposure period was used? Single or repeated exposure?

v. The method used to apply the chemical substance to the skin should be noted i.e. whether occluded or semi-occluded, whether the application site was washed after treatment.

vi. Check the observation period used post exposure. Shorter periods than in the current guideline may be adequate for non-irritants but may require a more severe

classification for irritants when the observation period is too short to measure full recovery.

vii. For eye irritation, was initial pain noted after instillation of the test substance, was the substance washed out of the eye, was fluorescent staining used?

viii. For eye irritation, how was the test material applied into the eye?

Irritation scores from old reports, reports produced for regulatory submission in the USA or in publications may be expressed as a Primary Irritation Score. Without the original data it is not always possible to convert these scores accurately into the scoring system used in the EU. For extremes i.e. where there is either no irritation or severe irritation, it may not be necessary to look further, but average irritation scores pose a problem and judgement may be required to avoid repeat testing.

Observations such as the above can all be used to assess whether the existing animal test report available can be used reliably to predict the irritation potential of a substance, thus avoiding further testing.

Specific considerations for eye irritation

A refinement of the classical Draize test is the rabbit low volume eye test (LVET). The test protocol deviates from OECD TG 405 in that in the LVET, 10uL is directly applied onto the cornea. The grading scale and the data interpretation in the LVET is exactly the same as those used in OECD TG 405. The validity of the LVET is currently under review of ECVAM for the detergent and cleaning preparations applicability domain. Anatomical and physiological considerations for rabbit and human eyes indicate that a dose volume of 10uL is appropriate (A.I.S.E. 2006): the tear volume in both rabbit and man is approximately the same (~ 7-8uL), and after blinking, the volume capacity in the human eye is ~10uL. These considerations suggest that the LVET is also potentially a suitable test to demonstrate toxicological effects on man of potential eye hazards of substances. The LVET has been used in industry safety evaluation of single chemicals (Griffith et al, 1980) and detergent and cleaning preparations (Freeberg *et al*, 1984; Freeberg *et al.* 1986a,b; Cormier *et al.,* 1995; Roggeband et al, 2000), and has shown to be a very good predictor of the effects on man. It still overpredicts, but much less so than the classical Draize test of OECD TG 405.

In summary, available data from the LVET on substances and preparations should be considered and must be carefully evaluated. For the classification of substances however it must be taken into account that the test up to now has a limited applicability domain (detergent and cleaning products). Consequently, positive LVET data (be it R36 or R41) are a trigger for the appropriate classification for eye irritancy, but negative data from LVET as a *stand alone method* (in the absence of any other information) are not conclusive for *no classification*.

Specific considerations for respiratory irritation

All data available should be evaluated to estimate a substance potential to induce respiratory tract irritation. Sources of information could be:

Human data:

- Experience from occupational exposure
- Published data on volunteers (objective measurements, psychophysical methods, and subjective reporting)
- Other data (e.g. from nasal lavage)

Animal data:

- Alarie assay
- Data from other inhalation studies (acute, repeated exposure):
    1. Clinical symptoms of dyspnoea or breathing difficulties,
    2. Histomorphology of the respiratory tract,
    3. Lavage examination (nasal, bronchoalveolar)

Data indicating the cytotoxic type of respiratory irritation, which were mainly gained from histopathological examinations of tissues, are considered in the DNEL derivation for the acute toxic effects or for the repeated dose toxic effects (Section R.8.2.1 and Appendix R.8-8).

With respect to the sensory irritation response, the evidence from all sources has to be considered for the quantitative risk assessment procedure.

Although the Alarie test for various reasons has never become an OECD TG, results of the Alarie assay can be used for hazard identification of sensory irritation as the Alarie test detects the potential of a substance to stimulate the trigeminal nerve. Like in acute inhalation toxicity testing, results from Alarie tests may show high inter-laboratory variability. Therefore, the use of Alarie data for deriving quantitative information for instance to establish short-term DNELs for irritation should be done with caution (i.e. taking into account the actual breathing pattern, whether a response plateau is being reached; see the review by Bos *et al*, 1992). In that review it was shown that data of the Alarie test could not be used to establish TLV values for lifetime exposure. It can be expected that a substance that is capable to stimulate the trigeminal nerve in mice will also have this potential in humans. However, because the human response at an exposure concentration equal to the RD_{50} cannot quantitatively be determined and because responses in the Alarie-test of less than 10-12% are considered to be within the expected normal variation (Boylstein, 1996; Doty *et al*, 2004; ASTM, 2004), use of the Alarie-bioassay in a quantitative risk assessment, if any, is suggested to start from an RD10 rather than from an RD_{50}.

Although anatomical differences in rodents and humans do exist (f.i. rodents are obligate nose breathers and humans not), sensory irritation will be present in both but the location and the type of effect may differ, i.e. in rodents a decrease in breathing frequency may be observed whereas in humans this may result in coughing.

Sensory irritation does not necessarily lead to tissue damage. Effects characterising overt tissue damage are covered by inhalation studies for acute or repeated exposure toxicity. In this sense the Alarie assay is not designed to predict such pathological changes (Bos *et al*, 2002). If available from other studies with the inhalation route (acute and repeated exposure) the characterisation of histomorphological lesions at the respiratory tract could be used as supplemental information.

Although both the Alarie test and for instance human nasal pungency threshold determinations are aimed to test for sensory irritation, correlation of the results of the Alarie test with such human data is difficult as the first is looking at rather strong effects upon exposure for at least 20 min (a 50% decrease in breathing frequency may be experienced by humans as unbearable) whereas human data are based on, for instance, very short exposure durations (sniffing for a few seconds). The results of a study by Cometto-Muniz et al. (1994) indicated that RD_{50} values in animals are not easily comparable with ‘nasal pungency thresholds’ in humans (see also Bos *et al*, 2002).

R.7.2.4.2 Human data for irritation/corrosion

Human data for skin corrosion, skin irritation and eye irritation

Well-documented *existing* human data of different sources can often provide very useful information on skin and/or respiratory irritation, sometimes for a range of exposure levels. Often the only useful information on respiratory irritation is obtained from human experience (occupational settings). The usefulness of all human data on irritation will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. Experience has shown that it is difficult to obtain useful data on substance-induced eye irritation, but data may be available on human ocular responses to certain types of preparations (e.g. Freeberg *et al*, 1986a).

The quality and relevance of existing human data for hazard assessment should be critically reviewed. For example, in occupational studies with mixed exposure it is important that the substance causing the irritation or corrosion has been accurately identified. There may also be a significant level of uncertainty in human data due to poor reporting and lack of specific information on exposure.

Examples of how existing human data can be used in hazard classification for irritancy are provided in a recent ECETOC monograph (ECETOC, 2002).

Human data on local skin effects may be obtained from existing data on single or repeated exposure. The exposure could be of accidental nature or prolonged, for example in occupational settings. The exposure is usually difficult to quantify. When looking at the effects, corrosivity is characterised by destruction of skin tissue, namely visible necrosis through the epidermis and into the dermis. Corrosive reactions are typified by ulcers, bleeding and bloody scabs. After recovery the skin will be discoloured due to blanching of the skin, complete areas of alopecia and scars (see Chapter 3.2 of GHS), i.e. corrosivity is an irreversible damage. With this characterisation it should be possible to discern corrosive properties in humans. However, to distinguish between “Causes severe burns”, R35, and “Causes burns”, R34, (3 minutes’ and 4 hours’ exposure in rabbits, respectively) may not be so obvious in practice. A clear case for R35 classification would be an accidental splash which gave rise to necrosis of the skin. In cases where it is obvious that a prolonged exposure is needed (not to be mixed with delayed effects) before necrosis occurs, R34 seems more reasonable. If the distinction between R35 and R34 is not clearly apparent then the more stringent classification should be chosen. Discrimination between corrosives and skin irritants in rabbits is made on the effects caused after 4 hours’ exposure. Irritants to the skin cause a significant inflammation which is reversible.

Severe eye irritants (R41) give more severe corneal opacity and iritis than eye irritants (R36). R41 compounds induce considerable tissue damage which can result in serious physical decay of vision. The effects normally do not reverse within 21 days (relates to animals); see Chapter 3.3 of the GHS. In contrast, the effects of R36 compounds are reversible within 21 days. In humans, a sight control by a physician would reveal a decay of vision. If it is not transient but persistent it implies classification with R41. If the discrimination between R41 and R36 is not obvious, then R41 should be chosen.

Human data for respiratory irritation

Consideration should be given to real-life human observational experience, if this is properly collected and documented (Arts *et al*, 2006), e.g. data from well-designed workplace surveys, worker health monitoring programmes. For substances with an array of industrial uses and with abundant human evidence, the symptoms of respiratory irritation can sometimes be associated with certain concentrations of the irritants in the workplace air and might thus allow derivation of DNELs. However, the exposure details need to be well documented and due consideration should be given to possible confounding factors.

Data on sensory irritation of the airways may be available from volunteer studies including objective measurements of respiratory tract irritation such as electrophysiological responses, data from lateralization threshold testing, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids. Including anosmics as subjects could exclude odor as a bias.

R.7.2.4.3 Exposure considerations for irritation / corrosion

Exposure-based waiving from testing is not applicable to the endpoints of skin corrosion, skin and eye irritation. Exposure-based waiving from testing as specified in Annex XI (3) applies to Sections 8.6 and 8.7 of Annex VIII, Annex IX and Annex X according to the REACH text.

R.7.2.4.4 Remaining uncertainty on irritation / corrosion

Usually it is possible unequivocally to identify (or accept) a substance as being corrosive, whatever type of study provides the information.

There may be a significant level of uncertainty in human data on irritant effects (because of poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, small numbers of subjects, etc.).

Data from studies in animals according to internationally accepted test methods will usually give very good information on the skin or eye irritancy of a substance in the test species, and, in general, it is assumed that substances which are irritant in Annex V studies in animals will be skin and/or eye irritants in humans, and those which are not irritant in Annex V studies will not be irritant in humans. Good data, often clearly related to exposure levels, can be obtained on respiratory and mucous membrane irritation, from well-designed and well-reported inhalation studies in animals. However, inconsistent results from a number of similar studies increases the uncertainty in deriving data from animal studies.

The data obtained from *in vitro* studies may include many dose levels and replicates: when such a study has a well-defined mechanistic basis and indicates that a substance is expected to be irritating, this may suffice for defined hazard identification purposes.

R.7.2.5 Conclusions for irritation / corrosion

R.7.2.5.1 Concluding on suitability for Classification and Labelling

In order to conclude on C&L, all the available information needs to be taken into account, and consideration should be given to both Annex VI of the Directive 67/548/EEC[31] and the various remarks (as they relate to classification and labelling) made throughout this guidance document.

R.7.2.5.2 Concluding on suitability for Chemical Safety Assessment

A dose-response assessment is difficult to make for irritation and corrosion simply because up to the present time most data have been produced with undiluted chemicals in accordance with test guidelines and traditional practice (which continues today). From a risk characterisation perspective it is therefore advisable to use the outcome of the classification procedure, i.e. a substance that is classified is assumed to be sufficiently characterised. However, a complete risk assessment requires both hazard, as well as dose-response data. Consequently, if the latter are available, they must be taken into account (see flowchart 1).

[31] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS)

For instance,dose-response information might be available from sub-acute dermal, repeated dose dermal and inhalation toxicity studies as well as from human experience.

Guidance on the possibilities for derivation of DNELs for skin and eye irritation/corrosion and respiratory irritation is given in Appendix R.8-9.

However, with specific regard to respiratory irritation, special attention needs to be given to as to whether extrapolation of the dose-response assessments from animal tests to the human situation is possible (see Section R.7.2.4.2).

R.7.2.5.3 Information not adequate

A *Weight of Evidence* approach comparing available adequate information with the tonnage-triggered information requirements by REACH may result in the conclusion that the requirements are not fulfilled. In order to proceed in further information gathering the following testing strategies can be adopted (see Section R.7.2.6).

R.7.2.6 Integrated Testing Strategy (ITS) for irritation / corrosion

R.7.2.6.1 Objective / General principles

For substances with no or very few data, the following sequential test strategy is recommended for developing adequate and scientifically sound data for assessment/evaluation and classification of the corrosive and irritating properties of substances. For existing substances with insufficient data, this strategy can also be used to decide which additional data, beside those available, are needed.

The objective of the testing strategies is to give guidance on a stepwise approach to hazard identification with regard to skin and eye irritation/corrosion. A principle of the strategy is that the results of one study are evaluated before another study is initiated. The strategy seeks to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

Some guidance for testing is provided by the specific rules for adaptation from standard information requirements, as described in column 2 of Annexes VII-X, together with some general rules for adaptation from standard information requirements in Annex XI.

R.7.2.6.2 Testing strategy for irritation / corrosion

Risk assessment of the irritating potential of a substance is normally made in a qualitative way provided the substance has been classified as being irritant or corrosive to skin. Existing test guidelines do not contain dose-response assessment, so that a quantitative analysis will often not be possible. Therefore, hazard identification and appropriate classification is the key determinant in the information strategy below. As a consequence, the use of *Assessment Factors* is of limited use in order to take into account uncertainty of data. However, the registrant is encouraged to keep and use all quantitative data that might be encountered in the process of retrieving hazard information in the context of the present ITS and to perform a complete risk assessment, comprising hazard as well as quantitative information.

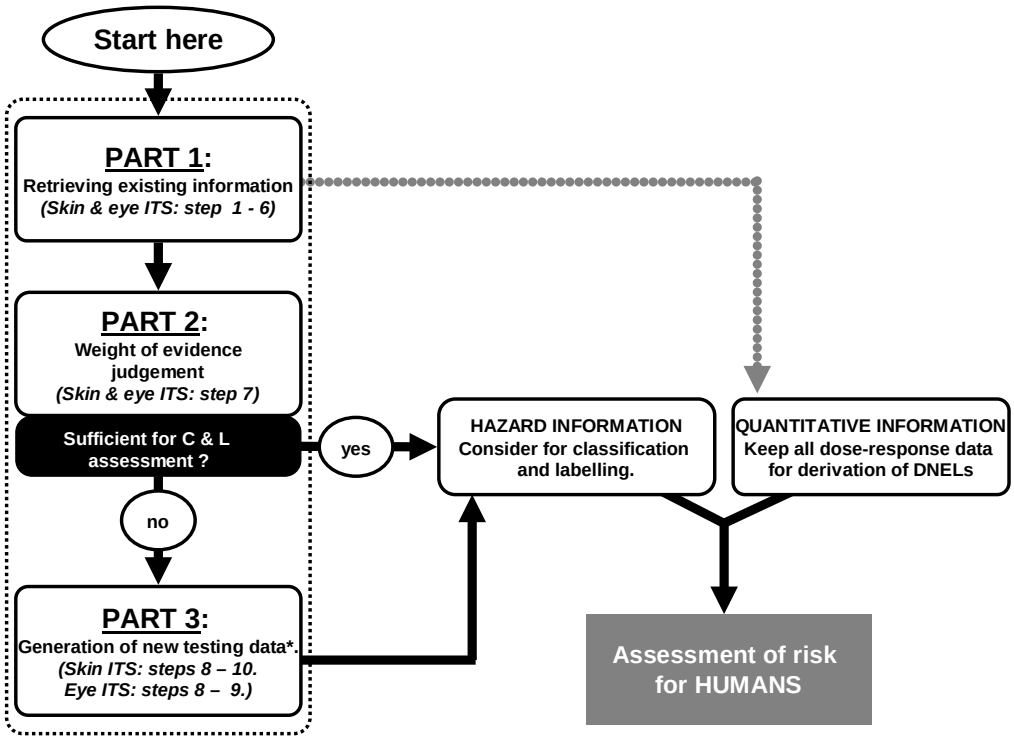
It is recommended that the information strategy is followed to step 6 (Figure R.7.2-1 & Figure R.7.2-2) in all cases and thereafter the weight of the evidence (WoE) analysis is performed. Clearly, not all steps will necessarily be accompanied by data, but it is important, that all potential data sources are explored prior to starting the WoE analysis. Note that before the WoE analysis in step 7, no new *in vitro* or *in vivo* tests should be conducted: Instead the assessment should be solely based on existing data. Furthermore, prior to perform any new *in vivo* test, the use of *in vitro* methods should be fully exploited (see Article 25 of REACH) by

using the general rules of Annex XI which allow to adapt the standard testing regime set out in Annexes VIII to X.

If the substance is not classified for skin irritation/corrosion, no risk assessment for this endpoint is performed, regardless of the exposure. Please note that there is no option for exposure-based waiving for this endpoint in the REACH regulation.

The following flow chart (Figure R.7.2-1)gives an overview of the overall strategy for defining a testing strategy for irritation and corrosion.

Figure R.7.2-1 Overview of the Integrated Testing Strategy for irritation/corrosion



*Generation of new testing data according to Annex VII to VIII and with due observation of the rules for adaptation of the standard testing regime laid down in Annex XI.

The ITS presented here comprises three sequential parts (see flow chart below): Part 1 (in light grey) is about retrieving existing information (step 1 to 6), part 2 (in dark grey) represents a *Weight of Evidence* (*WoE*) analysis and judgement (step 7) and part 3 (white background) is about the generation of new information by testing (step 8 to 10).
In the information retrieval part, existing and available information from the literature and databases is gathered and considered in a stepwise process. At the end of this part all information collected is analysed using a WoE approach (step 7). It is therefore necessary to run through all steps before arriving at step 7. This means that in cases of "yes, consider to classifiy…", the registrant should nevertheless proceed to the next step. However, the ITS may be exited in the sole exception if the substance is spontaneously flammable at room temperature in contact with air or water (box 1a). In this case, testing is not required.

In the information generation part, new information on the irritation potential of substances is created by means of *in vitro* or, as a last resort (see Article 25 of the REACh legislation), *in vivo* testing. Therefore, before concluding the WoE analysis in step 7, new *in vivo* tests should not be conducted.

Figure R.7.2-2 Integrated testing strategy (ITS) for assessing the skin corrosion and skin irritation potential of substances

Step	Information	Conclusion
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable) in contact with air(pyrophoric) or water at room temperature? → ↓ NO ↓	YES: No testing required. No need to proceed.
1b	Is the substance an organic hydro peroxide or an organic peroxide? → ↓ NO ↓	YES: Consider to classify as ■ corrosive (R34; "causes burns") if the substance is a hydro peroxide or ■ irritating as R38 ("Irritating to skin") if the substance is a peroxide. OR Provide evidence for the contrary Proceed to next step
1c	Is the pH of the substance lower than 2 or higher than 11.5? [a] → ↓ NO ↓	YES: Consider to classify as corrosive. Where classification is based upon consideration of pH alone (see step 7!), R35 should be applied. Proceed to next step
1d	Are there other physical or chemical properties that indicate that the substance is irritating/corrosive? → ↓ NO ↓	YES: Use this information for WoE analysis (step 7). Proceed to next step
Existing human data		
2	Are there adequate existing human data[b] which provide evidence that the substance is an irritant or corrosive → ↓ NO ↓	YES: Consider to classify accordingly. Proceed to next step
Existing animal data from irritation/corrosivity studies		
3	Are there data from existing studies *on irritation and corrosion* in laboratory animals, which provide sound conclusive evidence that the substance is a corrosive, irritant or non-irritant? → ↓	YES: Consider to classify accordingly (either R35 or R34 or R38 or no classification).

	NO ↓	Proceed to next step
Existing data from general toxicity studies via the dermal route and from sensitization studies		
4a	Is the substance acutely toxic (LD_{50}£400 mg/kg bw) or very toxic (LD_{50}£50 mg/kg bw) via the dermal route? [c] → ↓ NO ↓	YES: The substance will be classified for its acute dermal toxicity. Proceed to next step
4b	Has the substance proven to be a corrosive, irritant or non-irritant in a suitable acute dermal toxicity test? [d] → ↓ NO ↓	YES: If test conditions are consistent with OECD 404, consider to classify accordingly (R35 or R34 or R38 or no classification). Proceed to next step
4c	Has the substance proven to be a corrosive or an irritant in sensitisation studies or after repeated exposure? [e] → ↓ NO ↓	YES: This information cannot be used for considering a concrete classification conclusion but must be used exclusively within the integrated WoE judgement. Proceed to next step
Existing (Q)SAR data and read-across		
5a	Are there structurally related substances (suitable "read-across" or grouping), which are classified as corrosive (R34, R35) on the skin, or do suitable QSAR methods indicate corrosion potential of the substance? [f] → ↓ NO ↓	YES: Consider to classify as R35 Proceed to next step
5b	Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritant on the skin (R38), or do suitable (Q)SAR methods indicate irritating potential of the substance? [f] → ↓ NO ↓	YES: Consider to classify as R38. Proceed to next step
Existing in vitro data		
6a	Has the substance demonstrated corrosive properties in an OECD adopted *in vitro* test? → ↓ NO [g]	YES: Consider to classify as corrosive. If discrimination between R34 and R35 is not possible, R35 must be chosen.

	↓	Proceed to next step
6b	Are there acceptable data from a validated *in vitro* test (adopted by OECD or not), which provide evidence that the substance is an irritant or non-irritant? → ↓ NO ↓	YES: Consider to classify accordingly (R38 or no classification). Proceed to next step
6c	Are there data from a non-validated *in vitro* test, which provide sound conclusive evidence that the substance is an irritant[h]? → ↓ NO ↓	Yes: Consider to classify as R38, Proceed to next step
Weight of evidence analysis		
7	Taking all existing and relevant data (steps 1-6) into account, is there sufficient information to make a decision of whether classification/labelling is necessary, and – if so – how to classify and label? → ↓ NO ↓	YES: Classify accordingly (R35 or R34 or R38 or no classification)
New in vitro/ex vivo tests for corrosivity (Annex VII)		
8	Does the substance demonstrate corrosive properties in an OECD adopted *in vitro* or *ex vivo* tests for skin corrosion? → ↓ NO [g] ↓	YES: Classify R34 or R35. If discrimination between R34 and R35 is not possible, R35 must be chosen.
New in vitro/ex vivo tests for irritation (Annex VII)		
9a	Does the substance demonstrate irritating or non-irritating properties in validated *in vitro* tests (adopted by OECD or not) for skin irritation? → ↓ NO ↓	YES: Classify accordingly
9b	Does the substance demonstrate irritating properties in a non-validated *in vitro* test for skin irritation[h]? → ↓ NO ↓	YES: Classify accordingly.
New in vivo test for irritation (Annex VIII) [i]		

10	Does the substance demonstrate irritancy in an OECD adopted *in vivo* test? → ↓ NO ↓ No classification	YES: Classify accordingly.

Notes to the information scheme skin irritation/corrosion

[a)] Note that if the buffering capacity suggests that the substance may not be corrosive, further data are needed to confirm this.

[b)] data from case reports, occupational experience, poison information centres or from clinical studies.

[c)] if the substance is acutely toxic (LD_{50}£400 mg/kg bw) or very toxic (LD_{50}£50 mg/kg bw) via the dermal route further testing for irritation/corrosion would result in severe suffering or death of the animal. Thus, further testing is not required and sufficient labelling (warning) is provided by the risk phrases: “R24: toxic in contact with the skin” or “R27: very toxic in contact with the skin” and the symbol with T or T^+, shown below. Please note, that although the derogation regarding acute toxicity (LD_{50}£400 mg/kg bw) is not a specific rule for adaptation from column 1 in REACH, it is considered here to be scientific common sense.

[d)] Has the substance proven to be either an irritant or a corrosive in an acute dermal toxicity test carried out with rabbits with the undiluted test substance (liquids) or with a suitable suspension (solids)? In case of signs of skin corrosion: classify as R35. In all other cases: calculate or estimate the amount of test substance per cm^2 and compare this to the test substance concentration of 80 µl or 80 mg/cm^2 employed in the OECD TG 404 for dermal irritation/corrosion test with rabbits. If in the same range and adequate scoring of skin effects is provided: classify or not as R38. In case conclusive negative data was obtained in rabbits, stop. If not in the same range and inadequate scoring of skin effects: use for WoE analysis and proceed.

In case the test was performed in other species, which may be less sensitive, evaluation must be made with caution. Usually, the rat is the preferred species for toxicity studies within the EU. The limit dose level of 2000 mg/kg bw of a solid is normally applied as a 50% suspension in a dose volume of 4 ml/kg bw onto a skin surface area of ca 5x5 cm. Assuming a mean body weight of 250 grams, a dose of 1 ml of the suspension will be applied to an area of 25 cm^2, i.e 20 mg test substance per cm^2. In case of an undiluted liquid, 0,5 ml is applied to 25 cm^2, i.e. 20 µl/cm^2. Considering the fact that the rat skin is less sensitive compared to rabbit skin, much lower exposures are employed and, in general, the scoring of dermal effects is performed less accurate, the results of dermal toxicity testing in rats will not be adequate for classification with respect to skin irritation. Only in case of evidence of skin corrosivity in the rat dermal toxicity test, the test substance can be classified as R35. All other data should be used for WoE.

[e)] Regarding data from skin sensitiation studies, the skin of guinea pigs is less sensitive than the skin of rats which is less sensitive than the skin of rabbits. Only in case of evidence of skin corrosivity in the sensitization test (Maximization or Buhler) with the neat material or dilutions of solids in water, physiological saline or vegetable oil, the test substance should be classified as R35. However, care should be exercised when interpreting findings from guinea pig studies, particularly from maximisation protocols, as intradermal injection with adjuvant readily causes necrosis. All other data should be used for WoE only. Information on irritating properties from skin sensitisation tests cannot be used to conclude a specific classification regarding acute skin irritation but may be used in a WoE analysis. In general, irritation data from the Local Lymph Node Assay are not usable. The test substance is applied to the dorsum of the ear by open topical application, and specific vehicles for enhancement of skin penetration are used.

[f)] Conclusion on no classification can be made if the in silico model has been shown to predict adequately the absence of the classified effect and also fulfils the requirements of Annex XI.

[g)] No classification for corrosivity if a negative result can be supported by a WoE determination using other existing information, e.g. pH, SAR, human and/or animal data (according to OECD TG 430 and 431/EU B.40 and B.40 bis). If not corrosive, the irritating potential needs to be determined, proceed.

[h)] Conclusion on no classification can only be made if it has been concluded in the evaluation process that the test allows the identification of non-irritants and the data are used in a WoE approach following Annex XI 1.2.

[i)] In the light of a recently finished ECVAM validation trial, the *in vivo* test might be avoided in the near future by using the EPISKIN *in vitro* model. At the time of writing this report, the model has not finally be endorsed by ESAC, but it is likely that it will be recommended as a stand-alone replacement method for the animal test. *In vivo* testing as specified in Annex VIII for the appropriate tonnages might therefore be avoided using the tool of Annex XI 1.4 *in vitro* methods, that allows adaptation of the standard testing regime using suitable and, for the case of negative identification, validated *in vitro* tests.

The ITS for eye irritation is completely analogous in structure to that of skin corrosion, irritation. The ITS consists of an information retrieval part (steps 0a to 6 in light grey) and a part on the generation of new information by testing (step 8 to 9, no background colour). These two parts are separated by a WoE analysis and judgement (step 7 in dark grey).
In the information retrieval part, existing and available information from the literature and databases is gathered and considered in a stepwise process. At the end of this part all information collected is analysed using a WoE approach (step 7). It is therefore necessary to run through all steps before arriving at step 7. This means that in cases of “yes, consider to classify…”, one should nevertheless proceed to the next step (“Proceed to next step”). An exception is a “yes” in one or all of the following boxex: 0a, 1a or 1c: if the substance is classified as a skin corrosive or its pH is < 2 and > 11.5 (taking the buffer capacity into due consideration), the process of information retrieval can stop at this point, since the substance’s eye irritation potential is implicit in this classification. If the substance is spontaneously flammable at room temperature in contact with air (pyrophoric) or water, testing is not required.

In the information generation part (steps 8 to 9), new information on the irritation potential of substances is created by means of *in vitro* or, as a last resort (see article 25 of the REACH legislation), *in vivo* testing. Therefore, before concluding the WoE analysis in step 7, new *in vivo* tests should not be conducted.

Figure R.7.2-3 Integrated testing strategy (ITS) for assessing the eye irritation potential of substances.

Step	Information	Conclusion
Conclusion of the information strategy on skin irritation / corrosion		
0a	Is the substance classified as a skin corrosive? → ↓ NO ↓	YES: when assigned R34 or R35, the risk of severe damage to eyes is considered implicit. No need to proceed.
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable in contact with air (pyrophoric) or water at room temperature? → ↓ NO ↓	YES: no testing required No need to proceed
1b	Is the substance an organic hydro peroxide or an organic peroxide? → ↓ NO ↓	YES: Consider to classify for ■ corrosivity (hydro-peroxide) using R34 ("causes burns'), thus implicitly also for severe ocular irritancy (R41 “risk of serious damage to eyes”) or ■ for irritation (peroxide) using R36 (“irritating to eyes”). Proceed to next step
1c	Is the pH of the substance lower than 2 or higher than 11.5? [a] → ↓ NO ↓	YES: when assigned R35, the risk of severe damage to eyes is considered implicit. No need to proceed
1d	Are there other physical or chemical properties that indicate that the substance is irritating to the eye [b]? → ↓ NO ↓	YES: Use this information for WoE analysis (step 7). Proceed to next step
Existing human data		
2	Are there adequate existing human data [c] which provide evidence that the substance is irritating to the eye? → ↓ NO ↓	YES: Consider to classify (R41 or R36), or use for WoE analysis (step 7). Proceed to next step

Existing animal data from eye irritation studies		
3	Are there data from existing studies *on eye irritation* in laboratory animals, which provide sound conclusive evidence that the substance is an eye irritant or non-irritant? → ↓ NO ↓	YES: Consider to classify accordingly (R41 or R36 or no classification). Proceed to next step
Existing data on acute dermal toxicity		
4	Is the substance acutely toxic (LD_{50}£400 mg/kg bw) or very toxic (LD_{50}£50 mg/kg bw) via the dermal route? [d] → ↓ NO ↓	YES: The substance will be classified for its acute dermal toxicity. Proceed to next step
Existing (Q)SAR data and read-across		
5	Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritating to the eye, or do valid QSAR methods indicate eye irritation of the substance? [e]→ ↓ NO ↓	YES: Consider to classify accordingly (R41 or R36). If discrimination between R41 and R36 is not possible, R41 must be chosen. Proceed to next step
Existing in vitro data		
6a	Are there data from a validated *in vitro* test (adopted by OECD or not), which provide evidence that the substance is an eye irritant or non-irritant? → ↓ NO ↓	YES: Consider to classify accordingly (R36, R41 or no classification). If discrimination between R41 and R36 is not possible, R41 must be chosen. Proceed to next step
6b	Are there acceptable data from a non-validated *in vitro* test, which provide evidence that the substance is an irritant to the eye [f]? → ↓ NO ↓	YES: Consider to classify R41, Proceed to next step
Weight of evidence analysis		
7	Taking all existing and relevant data (steps 1 – 6) into account, is there sufficient information to make a decision of whether classification / labelling is necessary, and – if so – how to classify and label? →	YES: Classify for accordingly (R36, R41 or no classification).

	↓ NO ↓	
New in vitro/ex vivo tests for eye irritation (Annex VII)		
8a	Does the substance demonstrate irritating or non-irritating properties in validated *in vitro* or *ex vivo* tests (adopted by OECD or not) for eye irritation? → ↓ NO ↓	YES: Classify accordingly (R36, R41 or no classification). If discrimination between R41 and R36 is not possible, R41 must be chosen.
8b	Does the substance demonstrate severe irritating properties in acceptable non-validated *in vitro* or *ex vivo* tests for eye irritation (at present only IRE, ICE, BCOP and HET-CAM) [f]? → ↓ NO ↓	YES: Classify R41
New in vivo test for eye irritation (Annex VIII)		
9	Does the substance demonstrate irritancy in an OECD adopted *in vivo* test? → ↓ NO ↓ No classification	YES: Classify accordingly.

Notes to the information scheme eye irritation

[a] Note that if the buffering capacity suggests the substance be non-corrosive, further data are needed to confirm this.

[b] If pH < 3.2 or pH > 8.6, the substance is very likely to be an eye irritant.

[c] Data from case reports, occupational experience, poison information centres or from clinical studies.

[d] If the substance is acutely toxic (LD_{50}£400 mg/kg bw) or very toxic (LD_{50}£50 mg/kg bw) via the dermal route further testing for eye irritation would result in severe suffering or death of the animal. Thus, further testing is not required and sufficient labelling (warning) is provided by the risk phrases: “R24: toxic in contact with the skin” or “R27: very toxic in contact with the skin” and the symbol with T or T^+, shown below.

[e] Conclusion on no classification can be made if the model has been shown to adequately predict the absence of the classified effect and if it fulfils the requirements of Annex XI.

[f] Conclusion on no classification can only be made if it has been concluded in the evaluation process that the test allows the identification of non-irritants and the data are used in a WoE approach following Annex XI 1.2.

Appendices 1-3 to Section R.7.2

Appendix R.7.2-1 Mechanisms of local toxicities: skin corrosion / irritation, eye and respiratory irritation

Content of Appendix 7.2-1

Mechanisms of skin corrosion and irritation

Mechanisms of eye irritation

Mechanisms of respiratory irritation

MECHANISMS OF SKIN CORROSION AND IRRITATION

Clinically, different types of irritant contact dermatitis (ICD) exist, and have been classified on the basis of differences in morphology and mode of onset, as: acute irritant dermatitis (primary irritation); irritant reaction; delayed, acute irritant contact dermatitis; cumulative irritant dermatitis; traumatic irritant dermatitis, pustular and acneiform irritant dermatitis; non-erythematuous irritant dermatitis; and subjective irritation (Lammintausta & Maibach, 1990).

Two different pathogenetic pathways may be involved in ICD. Acute ICD is characterised by an inflammatory reaction which mimics allergic contact dermatitis, with the release of inflammatory mediators and cytokines. Chronic ICD, on the other hand, is characterised by disturbed barrier function, associated with an increased epidermal turnover which leads clinically to lichenification (Berardesca and Distante, 1994).

The clinically relevant elements of skin irritation are a disturbance of the desquamation process, resulting in scaling or hyperkeratosis (chronic effects), i.e. epidermal events, and an inflammatory response with vasodilation and redness in combination with extravasation of water, which may be observed as papules, vesicles and/or bullae and oedema (acute effects), i.e. events essentially taking place in the dermis (Serup, 1995). The onset of irritation takes place at the stratum corneum level and later in the dermis, whereas early events in sensitisation occur in the dermis. Variations in the skin reactions are dependent on the degree of injury induced, as well as on the effects of an irritant substance on different cell populations. For example, pigmentary alterations are due to effects on melanocytes, whereas ulcerations are due to extensive keratinocyte necrosis (skin corrosion). The release of cytokines and mediators can be initiated by a number of cells, including living keratinocytes and those of the stratum corneum, which thus modulate inflammation and repair (Sondergard *et al*., 1974; Hawk *et al*., 1983; Barker *et al*., 1991; Baadsgaard and Wang, 1991; Hunziker *et al*., 1992; Berardesca *&* Distante, 1994).

The physico-chemical properties, concentration, volume and contact time of the irritant give rise to variations in the skin response. Furthermore, inter-individual differences exist, based on age, gender, race, skin colour and history of any previous skin disease. In the same individual, reactivity differs according to differences in skin thickness and skin sensitivity to irritation of the different body regions. Finally, a greater sensitivity to some irritants (DMSO, propylene glycol, SLS and soap) has been reported during winter, because of the reduced hydration state of the skin (Frosch and Pilz, 1995). Although clinically different types of irritant reactions can be observed, they are all based on cellular and biochemical mechanisms which induce the irritant response. It is not yet possible to conclude whether the observed clinical differences are actually due to differences in biochemical mechanisms, and further investigations are needed.

According to Barratt (1995) and further elaborated by Walker et al. (2004), for organic chemicals, the mechanisms leading to skin irritation are normally described by a two-stage

process where a chemical first has to penetrate the *stratum corneum* and then trigger a biological response in deeper epidermal or dermal layers.

For strong inorganic acids and bases, no *stratum corneum* penetration is needed because they erode the *stratum corneum*. According to the Technical Guidance Document (TGD) supporting Commission Directive 93/67/EEC on risk assessment for new notified and existing substances (EC, 2003), the percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts is slow, since these chemicals are binding to macromolecules in skin. As a result of binding, corrosion can occur as the *stratum corneum* is eroded. Reactivity can be caused by electrophiles and/or pro-electrophiles. Electrophiles contain atoms, such as N, O or halogens attached to a C-atom, which makes that specific C-atom positively charged and therefore reactive with electron-rich regions of peptides and proteins. This causes irritation via covalent binding to the skin.

At this time, the following mechanisms are proposed for inducing skin irritation or skin corrosion by affecting the structure and function of the *stratum corneum* :

1. Mechanisms of skin irritation:

   - Reaction with skin proteins and interference with lipids in the *stratum corneum* by surface-active agents (denaturation of proteins, disruption of plasma membrane lipids)

   - Dissolving of plasma membrane lipids and thus defatting and disintegration of skin by low molecular weight organic chemicals.

2. Mechanisms of skin corrosion:

   - Erosion of the *stratum corneum* by most inorganic acids and bases and by strong
     organic acids with pH <2.0 and bases with pH >11.5 and

   - Binding to skin components in the *stratum corneum* by cationic surfactants and percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts.

3. Mechanisms that may lead to both skin irritation and corrosion:

   - Penetration of the *stratum corneum* by anionic or non-surfactant organic chemicals with sufficient hydrophobic and hydrophilic properties and

   - Elicitation of a inflammatory and/or cytotoxic response in the epidermis or dermis.
     The severity of these responses may determine whether irritation or corrosion occurs.

MECHANISMS OF EYE IRRITATION

Eye injury can be caused by many insults. These can be physical such puncture by sharp objects. Eye injury can be caused by chemicals such as systemic drugs that can enter into the eye through the blood stream (examples are Cyclosporine, Vaccines, Intravenous immunoglobulines, Intravenous streptokinase). Various degrees of eye injury can also be caused by direct (topical) contact with chemicals or chemical mixtures such as acids, alkalis, solvents or surfactants. These materials may contact the eye intentionally e.g. through the use of eye drops, medications, products intended for use around the eyes but also unintentionally

e.g. accidental spills and splashes of consumer products or accidental exposures in the workplace.

In general, chemicals or chemical mixtures which contact the eye directly may cause local effects on the frontal tissues and substructures of the eye e.g. cornea, conjunctiva, iris, lachrymal system and eye lids. There are several modes of action by which topical chemicals and chemical mixtures cause eye injury (see Table R.7.2-3).

Table R.7.2-3 Categories of irritant chemicals and their typical mode of action in eye irritation.

Chemical/chemical mixtures	Mode of Action
Inert chemicals	May cause effect due to large size. Protrusions may cause direct puncture of the eye
Acids	May react directly with eye proteins and cause coagulation or precipitation resulting in relatively localised injury
Bases (Alkalis)	May actively dissolve cell membranes. May penetrate to the deeper layers of the eye tissue
Solvents	May dissolve lipids in plasma membranes of epithelial and underlying cells resulting in loss of the cells affected and, as a result, tissue degradation, that might be – depending on the repair mechanisms (cell proliferation, tissue restoration) transient.
Lachrymators	May stimulate the sensory nerve endings in the corneal epithelium causing an increase in tearing.

The degree of eye injury is usually dependent on the characteristics (chemical category/class) and concentration of the chemical or chemical mixture. Acids and alkalis usually cause immediate irritation to the eyes. Other substances may cause eye injuries that start as mild but progress to be more severe at a later period.

Upon exposure of the ocular surface to eye irritants, inflammation of the conjunctiva can be induced. This includes dilation of the blood vessels causing redness, increased effusion of water causing swelling (oedema/chemosis) and an increase in the secretion of mucous leading to an increase in discharge. Visual acuity can be impaired. Irritants may also produce an increase in tear production and changes to the tear film integrity such as increased wetness. Iritis may result from direct irritation or become a secondary reaction to the corneal injury. Once the iris is inflamed, infiltration of fluids can follow which affects the ability to adjust the size of the pupil and decreases the reaction to light leading to decreased visual acuity. Due to the richness of nerves in the iris, irritation also causes subjective symptoms such as itching, burning and stinging.

Eye injury can be reversible or irreversible depending on the degree of damage and degree of repair. Damage to the corneal epithelium alone can repair quickly, often with no permanent eye damage. The cornea may still repair fairly well if the damage goes beyond the basement membrane into the superficial part of the stroma but the repair process may take days or even weeks to occur. Once the damage extends significantly into the stroma, corneal ulceration can occur due to the subsequent series of inflammatory processes. If damage extends to and beyond the endothelium, corneal perforation may occur which is irreversible and may cause permanent loss of vision. Eye injury can cause different degrees of functional loss e.g. increase of tear production, opacification of the cornea, oedema and so decrease visual acuity.

The body has its own defence mechanisms e.g. sensing the pain, stinging and burning and the eyelids will blink to avoid full exposure to the chemical. Increased tear production and blinking of the eyes with the help of the drainage apparatus help to dilute or clear the causative agent. Such defence mechanisms are highly developed in man with rapid blinking and profuse tear production resulting from exposure of the eye to a foreign material that is irritating. It is well reported in the literature that species differences occur in the rate of blinking and tear production mechanism that can influence how effectively foreign materials are removed from the eye.

MECHANISMS OF RESPIRATORY IRRITATION

The term "respiratory tract irritation" is often used to indicate either or both of two different toxicological effects. These are i) cytotoxic effects in the affected tissue, and ii) sensory irritation. The first type of irritation is comparable to deremal and eye irritation.

Cytotoxic irritant effects are characterised by inflammation (increased blood flow (hyperemia), local infiltration with white blood cells, swelling, oedema) and there may also be haemorrhage, and eventual necrosis and other pathological changes. The effects are in principle reversible.

Chronic irritation can lead to repeated episodes of cell proliferation in the affected tissues, and this may increase the risk of tumor development. The nature of effects depends on the chemical compound and its primarily targeted region, the severity of effects depends on the concentration and duration of exposure. In general, repeated exposure studies in animals tend to focus on observing (histo)pathological evidence for tissue damage rather than for sensory irritant effects. In case overt tissue damage (mucosal erosion and ulceration) occurs, a non-specific cytotoxic action at the site of contact along the respiration route can be assumed. Depending on the concentration and duration of exposure a severity gradient of lesions from anterior to posterior regions can be observed (in contrast to effects in certain mucosa types depending on the metabolic activation of the test substance) and, depending on the severity and the extent of the lesions, adjacent submucosal tissues can also be affected (e.g., by cartilage destruction). Such lesions are not fully reversible due to scar formation or replacement of the original mucosa, or may induce other serious health effects as marked bleeding or persistent airway obstruction.

"Sensory irritation" refers to the local and central reflex interaction of a substance with the autonomic nerve receptors, which are widely distributed in the mucosal tissues of the eyes and upper respiratory tract. Compound or compound-group specific target sites of sensory irritation generating different responses can be identified: a) nasal (and eye) irritation, i.e. interaction with the trigeminal nerve, b) pharyngeal irritation, i.e. interaction with the glossopharyngeal nerve, and c) larynx and lower respiratory tract, i.e. interaction with the vagus nerve.

Sensory irritation leads to unpleasant sensations such as pain, burning, pungency, and tingling. The severity depends on the airborne concentration of the irritant rather than on the duration of exposure. Sensory irritation is a receptor-mediated effect, and usually occurs almost immediately upon exposure to the inhaled irritant. It leads to reflex involuntary responses such as sneezing, lacrimation, rhinorrhea, coughing, vasodilatation of blood vessels in the nasal passages, and changes in the rate and depth of respiration. In humans, protective behavioural responses such as covering the nose and mouth can also occur. Sensory irritation is distinct from odor sensation, which is mediated via different nerve pathways (olfactory). However, there is evidence that odor perception and other cognitive influences can affect the perception of sensory irritation in humans.

In rodents, sensory irritation leads to a reflex reduction in the respiratory rate (breath-holding); this reflex effect on respiration can be measured experimentally (determination of the RD_{50} value in the Alarie assay) although results may vary considerably depending on the species and strain of rodents, on the exposure duration (time should be long enough to induce

changes), and results also show inter-laboratory variability. Investigations into the correlation of the results of the Alarie test with human data are difficult since the parameters examined in humans and mice are different and adequate human data to determine a human equivalent to the RD_{50} is not available at the moment. The results of a study by Cometto-Muniz et al. (1994) indicate that RD_{50} values in animals are not easily comparable with 'nasal pungency thresholds' in humans.

As indicated, human data are mostly based on subjective experiences and need to be carefully controlled in order to prevent confounding by odour perception (Dalton, 2003; Doty *et al*., 2004). Validated questionnaires have been developed for the investigation of sensory irritation responses in human volunteers. During recent years, emphasis was given to develop a spectrum of objective measurements (see review by Arts *et al*., 2006).

There is a view in the occupational health literature that sensory irritation may be a more sensitive effect than overt tissue-damaging irritation (which is a non-receptor mediated unspecific mode inducing cell death at the site of contact). Sensory irritation-related effects are fully reversible given that its biological function is to serve as a warning against inhaled substances that could damage the airways, and that it triggers physiological reflexes that limit inhalation volumes and protect the airways. However, there is a lack of documented evidence to indicate that this is a generic position that would necessarily apply to all inhaled irritants. It should be noted that no clear relationship between the RD_{50} value and the onset of histologically observable lesions in animals has been observed.Appendix R.7.2-2 - QSARs and expert systems for skin irritation and corrosion.

Appendix R.7.2-2 QSARs and expert systems for skin irritation and corrosion

Content of Appendix 7.2-2

LITERATURE-BASED QSAR MODELS

In the open scientific literature, (Q)SARs have been based on continuous (e.g. Primary Irritation Indices) or categorical (e.g. EU classifications) measures of skin irritation.

For defined classes of chemicals, categorical QSARs have been reported for discriminating between corrosives and non-corrosives (Barratt 1996a, 1996b), and between skin irritants and non-irritants (Smith *et al.*, 2000a; Smith *et al.*, 2000b). These studies did not actually provide a transparent algorithm for classifying chemicals, so they are of limited value for regulatory use. However, they illustrate the feasibility of developing such models, so it should be possible for a QSAR specialist to redevelop the models in such a way that an algorithm is clearly defined.

A linear discriminant model for distinguishing between irritant and non-irritant liquid esters in human volunteers was reported by Smith *et al*. (2000a). As mentioned above the exact algorithm is not clear. In addition the primary irritation index for human irritation may need translation when these scores are considered for classification. However, the results could be informative for future model development for esters, since they indicate that irritant esters can be distinguished from non-irritants on the basis of a limited number of physico-chemical parameters.

For defined classes of chemicals, continuous QSARs for predicting the Primary Irritation Index (PII) have also been published (Barratt 1996b; Hayashi *et al.*, 1999; Kodithala *et al.*, 2002). For example, the application of stepwise regression analysis to a set of 52 neutral and electrophilic organic chemicals produced the following model:

$$PII = 1.047 \log P - 0.244\ MV + 0.888\ DM + 0.353$$

$$N=52,\ r^2 = 0.422,\ r_{cv}^2 = 0.201,\ s=1.376,\ F=11.70$$

This equation indicates that the PII has a positive dependence on log P (logarithm of the octanol-water partition coefficient) and DM (dipole moment), and a negative dependence on MV (molecular volume). This model has a low goodness-of-fit (r^2) and a poor predictivity (as reflected by r_{cv}^2), so is not recommended for regulatory use. Nevertheless, the model does reveal three potentially useful descriptors for the development of new models for PII prediction. More research is needed into the development of models for predicting PII and it should be considered whether the information generated could be used in the setting of DNELs.

Some limited evidence indicates that the reactive effects of acids and bases can be predicted by using the acid/base dissociation constant (pKa), which can itself be predicted by using commercially available software products, such as the SPARC program. Evidence for the usefulness of pKa as a predictor of skin irritation for acids has been provided by Berner *et al.* (1988, 1990a, 1990b), whereas evidence for the usefulness of pKa as a predictor of skin

irritation for bases has been provided by Nangia *et al*. (1996). Barratt also used pKa for predicting the effects of acids and bases (Barratt, 1995). These studies did not address the question of how to use pKa where there are multiple functional groups in the chemical of interest, and therefore multiple ionization constants. Based on current knowledge, no clear recommendations can be made about how to use pKa information.

COMMERCIAL MODELS

TOPKAT, which is commercialised by Accelrys (http://www.accelrys.com/products/topkat), incorporates models to discriminate severe irritants from non-severe irritants, as well as mild/moderate irritants from non-irritants. These models are based on work by Enslein *et al*. (1987), but due to a lack of documentation, it is not clear whether the current version of the software encodes the models that were originally published. A QMRF for the TOPKAT skin irritation model is provided as an appendix. The algorithm of the TOPKAT is not transparent. The model predicts a probability of a weak/mild/moderate and severe irritation. It states that probabilities <0.3 and >0.7 give sufficient certainty of the prediction. The model gives the sensitivity and specificity values of the specific classes such as acyclic etc, which are mostly around or above 90%. It also shows similar structures from the TOPKAT perspective including the experimental result. The TOPKAT predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. The models indicate whether the prediction is in the applicability domain of the model. Due to the limitations of the model (lack of transparency for the algorithm, no external validation, no mechanistic reasoning), it cannot be used as stand alone method. The TOPKAT prediction should be supported with mechanistic reasoning, using other models or expert judgment.

There is a rulebase for irritation in **Derek for Windows** (Sanderson & Earnshaw, 1991; Combes & Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.chem.leeds.ac.uk). To predict toxicity, the program checks whether any alerts within the query structure match previously characterised toxicophores (substructure with potential toxic effect) in the knowledge base. The reasoning engine then assesses the likelihood of a structure being toxic, and a message indicating the nature of the toxicological hazard is provided together with relevant literature references. There are nine levels of confidence: certain, probable, plausible, equivocal, doubted, improbable, impossible, open, contradicted. The DerekfW8.0 rulebase has 25 structural alerts for the prediction of skin irritancy/corrosion; four alerts are specific to eye irritancy, and some combined for the respiratory irritation and gastrointestinal tract, but none is specific to skin irritancy or corrosivity. If DerekfW does not make a prediction of irritancy or corrosivity, it cannot be concluded that there is no effect – it could mean that none of known alerts was found to be present in the chemical of interest or it was outside the applicability domain of that specific alert. The DerekfW model is transparent in its algorithm, when the model is fired showing the structural alert and its limitations. The alert is supported with literature references and sometimes with example chemicals, although this is not sufficient to consider them validated. The example chemicals support the mechanistic reasoning. The DerekfW model can be used for positive identification of skin irritation. The confidence levels have to be translated to consider them for classification. Due to the limitations (lack of validation) it cannot be used as stand alone method, though the mechanistic reasoning provides supporting information. The DerekfW model cannot be used to predict non-irritation/corrosion as the model only contains alerts that detect the presence of irritation/corrosion.

HazardExpert is a rule-based software tool developed and commericalised by CompuDrug Chemistry Ltd. (http://www.compudrug.com) for predicting the toxicity of organic compounds in humans and in animals (Smithing & Darvas 1992). HazardExpert uses a fragment-based approach to predict toxicokinetic effects and various human health effects, including membrane irritation. Since this endpoint is not clearly defined in HazardExpert, it is recommended not to use it directly for the assessment of skin or eye irritation. However, it could be used as supplementary information in a *Weight of Evidence* approach for positive prediction.

The Multiple Computer Automated Structure Evaluation (**MultiCASE**) program, developed by MultiCASE Inc. (http://www.multicase.com), is an automated rule induction tool that automatically identifies molecular fragments likely to be relevant to the activity of molecules (Klopman, 1992; Klopman *et al.*, 1993). It also provides an indication of the importance of these fragments in relation to the potency of the molecules containing them. MultiCASE can be used to predict various human health endpoints, including eye irritation (Klopman *et al.*, 1993; Rosenkranz *et al.*, 1998). However, it is not clear how to relate the MultiCASE scoring system to Draize scores or regulatory classifications. In principle, the MultiCASE model can be used for positive and negative indications of skin irritation. The structural alert is provided as well as information on its internal validation. The MultiCASE model also indicates whether it is in the applicability domain of the model. The MultiCASE predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. Due to limitations (lack of external validation and mechanistic reasoning) the model cannot be used as a stand alone method. The prediction should be supported with mechanistic reasoning using other models or expert judgment.

The Danish EPA has developed an in-house MultiCASE model for predicting severe versus mild skin irritation based on 800 test results taken from RTECS (Registry of Toxic Effects of Chemical Substances), the HSDB (Hazardous Substances Data Bank) and the official list of EU-classified substances (Annex I of Directive 67/548/EEC). It is not clear how the RTECS and HSDB classification criteria for irritation comply with the EU criteria. Due to limitations in the information for assessing the reliability of the prediction, these predictions are difficult to use in the regulatory context.

BFR DECISION SUPPORT SYSTEM

A decision support system (DSS) developed by the German Federal Institute for Risk Assessment (BfR) uses physico-chemical exclusion rules to predict the absence of skin irritation/corrosion potential in combination with structural inclusion rules (SARs) to predict the presence of such potential (Gerner *et al.*, 2004; Walker *et al.*, 2004). The exclusion rules are based on physico-chemical properties such as molecular weight, aqueous solubility, and log K_{ow}, whereas the inclusion rules are based on substructural molecular features. The physico-chemical rules implicitly take into account bioavailability (skin penetration) whereas the structural rules take reactivity into account. The physico-chemical and structural rulebases are designed to predict the EU risk phrases for skin irritation (R38) and skin corrosion (R34 and R35). Further details are given in QSAR Reporting Format for the BfR skin and eye irritation rulebases (http://qsardb.jrc.it).

The exclusion rules have the following general form:

IF (physico-chemical property) A THEN predict the absence of toxic effect B

Example: IF Log K_{ow} < -3.1 THEN the chemical does not need to be considered for classification

The structural inclusion rules take the following general form:

IF (substructure) A THEN predict the occurrence of toxic effect B

Example: IF *Chlorosilane* THEN the chemical needs to be considered for "corrosive" classification

The performance of the BfR physico-chemical rulebase for predicting the absence of skin effects has been validated by the RIVM (Rorije & Hulzebos, 2005), whereas the structural rulebase for predicting the occurrence of skin effects has been validated by the ECB (Gallegos Saliner *et al.*, 2007). The endpoint is EU classification, the algorithms and domain of

applicability are transparent, the rules and alerts are independently validated by ECB and RIVM (Gallegos Saliner *et al.*, 2007, Rorije & Hulzebos, 2005). Though the rules are empirically derived, a mechanism of action can be deduced. For chemicals in the applicability domain of the rulebase, the rules may be used on their own to predict the presence or absence of hazard. Thus, the resulting predictions can be used as the basis for classification. It should be determined, on a case-by-case basis, whether the predictions for a given chemical provide a sufficient basis for classification, or whether additional information is needed in a weight-of-evidence approach.

SICRET

The so-called “Skin Irritation Corrosion Rules Estimation Tool” (SICRET), has been developed by Walker *et al.* (2005) to estimate whether chemicals are likely to cause skin irritation or skin corrosion SICRET is not actually a computer-based tool but a tiered approach based on the use of physico-chemical property limits, structural alerts and *in vitro* tests to classify chemicals that cause skin irritation or skin corrosion. The physico-chemical rules and alerts include those in the BfR rulebases as well as some additional rules and alerts published by Hulzebos *et al.* (2001, 2003, 2005).

Appendix R.7.2-3 QSARs and expert systems for eye irritation and corrosion

Content of Appendix 7.2-3

Literature-based QSAR models

Commercial models

BfR decision support system

LITERATURE-BASED QSAR MODELS

In the open scientific literature, (Q)SARs have been based on continuous (e.g. molar eye scores) or categorical (e.g. EU classifications) measures of eye irritation. Examples of mathematical (continuous) models have been published models by Sugai *et al.* (1991) & Cronin *et al.* (1994), whereas examples of categorical models have been published by Sugai *et al.* (1990) and by Barratt (1997).

Regression models based on solvatochromic parameters can be used for predicting the degree of eye irritation, as illustrated by Abraham and coworkers (Abraham, 1994; Abraham *et al.*, 1998). The mecahnistic basis of these models is that a substance is transferred from a pure organic liquid to an organic solvent phase consisting of the tear film and cell membranes on the surface of the eye. The more soluble the organic liquid in the initial phase, the greater the degree of irritation is. These models are worthy of further characterisation. However, for routine regulatory use, information on a number of so-called Abraham descriptors would also need to be made available.

Neural network approaches can also be used to model eye irritation (e.g. Patlewicz *et al.*, 2000). At present, however, many of these models lack the transparency, especially in the algorithm. However if the training sets are provided as well as validation information they could possibly be used in a *Weight of Evidence* approach. Mechanistic reasoning should also be provided.

An approach called Membrane-Interaction QSAR analysis, developed by Kulkarni *et al.*(2001), provides a means of incorporating molecular dynamic simulations to generate membrane–solute interaction properties. The development and application of models based on molecular simulations requires the use of specialised expertise and software. They could be used to increase understanding of the mechanisms of eye irritation.

A classification approach called Embedded Cluster Modelling (ECM) provides a means of generating *elliptic models* in two or more dimensions (Worth & Cronin, 2000), so that irritants can be transparently identified as those chemicals located within the boundaries of the ellipse. The statistical significance of these “embedded clusters’ can be verified by cluster significance analysis (CSA), as illustrated for an eye irritation dataset by (Cronin, 1996).

Applying the methods of ECM and CSA, the following model, applicable to undiluted organic liquids, was developed by Worth & Cronin (2000):

Classify an undiluted, organic liquid as an eye irritant if:

$$(\log P\text{-}1.07)^2 / 2.06^2 + (dV1 + 0.98)^2 / 0.99^2 £ 1$$

This model was based on 73 diverse organic chemicals, using two descriptors: LogP (which accounts for diffusion) and a size-independent molecular connectivity index (dV1, which accounts for the degree of branching and cyclicity). The sensitivity, specificity and concordance of the model were 73%, 78% and 75%, respectively, whereas the positive and negative

predictivities were 77% and 74% respectively. The model is an explicit algorithm with a defined applicability domain and predicts EU classifications directly.

The different methods were applied to a dataset of 119 organic liquids classified as I or NI according to EU classification criteria. The classification models (CMs) were developed by applying linear discriminant analysis (LDA), binary logistic regression (BLR), and classification tree (CT) analyses, using a single predictor variable (molecular weight), and assigning equal probabilities for the two classes (I/NI). The cut off values below which a chemical should be predicted to be irritating to the eye were 121, 77, and 137 g/mol, in the LDA, BLR, and CT classification models, respectively (Table R.7.2-4) (Worth & Cronin, 2003).

Table R.7.2-4 Classification results of the different models of eye irritancy

CM (p<0.01)	Cut off value	Sensitivity	Specificity	Accuracy
Linear Discriminant Analysis (LDA)	if MW ≤ 121 g/mol, then predict I; otherwise, predict NI	73	62	65
Binary Logistic Regression (BLR)	if MW ≤ 77 g/mol, then predict I; otherwise, predict NI	27	93	76
Classification Tree (CT)	if MW ≤ 137 g/mol, then predict I; otherwise, predict NI	97	49	61

All of these models are simple to apply and are associated with a transparent algorithm. The statistics illustrate the inevitable trade-offs that result from the selection of different cut off values. Thus, the BLR model does not identify many irritants (only 27%), but is does so with a high degree of confidence (i.e. low false positive rate of 7%). Conversely, the CT does not identify many of the non-irritants (49%), but it has a low false negative rate of 3%). Thus, the combined use of the BLR and CT models could be useful for distinguishing between eye irritants and non-irritants.

COMMERCIAL MODELS

The **TOPKAT** software includes models for eye irritation based on structural fragments. These models were originally developed by Enslein *et al.* (1988), but the algorithms are not well defined in the TOPKAT documentation The TOPKAT algorithm is not transparent. The model predicts a probability of a weak/mild/moderate and severe irritation. It states that probabilities <0.3 and >0.7 give sufficient certainty of the prediction. The model gives the sensitivity and specificity values of the specific classes such as acyclic, which are mostly around or above 90%. It also shows similar structures from the TOPKAT perspective including the experimental result. The TOPKAT predictions weak/mild/moderate and severe irritation need to be translated to consider them for classification. The models indicate whether the prediction is in the applicability domain of the model. Due to the limitations of the model (lack of transparency for the algorithm, no external validation, no mechanistic reasoning), it cannot be used as stand alone method. The TOPKAT prediction should be underlined with a mechanistic reasoning, using other models or expert judgment.

There is a rulebase for irritation in **Derek for Windows** (Sanderson & Earnshaw, 1991; Combes & Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.chem.leeds.ac.uk). See for a general outline the skin irritation section on (Q)SARs. The DerekfW8.0 rulebase has four four alerts are specific to eye irritancy. If DerekfW does not make a prediction of irritancy or corrosivity, it cannot be concluded that there is no effect – it could mean that none of known alerts was found to be present in the chemical of interest or it was outside the applicability domain of that specific alert. The DerekfW model is transparent in its algorithm, when the model is fired showing the structural alert and its limitations. The alert is underlined with literature references and sometimes with example chemicals, which is not sufficient to consider them internally validated. The example chemicals

underline the mechanistic reasoning. The DerekfW model can be used for positive identification of skin irritation. The confidence levels have to be translated to consider them for classification. Due to the limitations (lack of internal and external validation) it cannot be used as stand alone method, though the mechanistic reasoning possibly provides sufficient information. The DerekfW model cannot be used to predict for non-irritation/corrosion as the model only contains alerts that detect the presence of irritation/corrosion.

The fragment-based **MultiCASE** approach has been used to model eye irritation (Klopman *et al.*, 1993; Enslein *et al.*, 1988; Rosenkranz *et al.*, 1998; Klopman (1998). The publications on these models do not define the algorithms. In principle, the MultiCASE model can be used for positive and negative indication for eye irritation. The structural alert is provided as well as the internal validation. The MultiCASE model also indicates whether it is in the applicability domain of the model. The MultiCASE predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. Due to limitations (lack of external validation and mechanistic reasoning) the model cannot be used as a stand alone method. The prediction should be underlined with mechanistic reasoning using other models or expert judgment.

BFR DECISION SUPPORT SYSTEM

The decision support system (DSS) developed by the German Federal Institute for Risk Assessment (BfR) uses physico-chemical exclusion rules to predict the absence of eye irritation/corrosion potential in combination with structural inclusion rules (SARs) to predict the presence of such potential (Gerner *et al.*, 2005). These rules are used analogously to those described in the skin irritation and corrosion section above. The physico-chemical and structural rulebases are designed to predict the EU risk phrases for eye irritation (R36) and severe eye irritation/corrosion (R41). Independent validation exercises by the ECB support the performance of the physico-chemical rulebase for predicting the absence of eye effects (Tsakovska *et al.*, 2005), as well as the performance of the structural rulebase for predicting the occurrence of eye effects (Tsakovska *et al.*, 2007).

R.7.2.7 Useful links

JRC QSAR Model Database: http://qsardb.jrc.it

ECVAM page: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam

ECVAM database service on alternative methods to animal experimentation (DB-ALM): http://ecvam-dbalm.jrc.ec.europa.eu/

R.7.2.8 References on skin and eye irritation/corrosion and respiratory irritation

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R.7.3 Skin and respiratory sensitisation

R.7.3.1 Introduction

A number of diseases are recognised as being, or presumed to be, allergic in nature. These include asthma, rhinitis, conjunctivitis, allergic contact dermatitis, urticaria and food allergies (the latter is not discussed in this document). In this Section, the endpoints discussed are those traditionally associated with occupational and consumer exposure to chemicals (proteins are not discussed in this document). Photosensitisation is potentially important but its mechanism of action is poorly understood, and it is not discussed in this document.

R.7.3.1.1 Definition of skin and respiratory sensitisation

A sensitizer is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin the characteristic adverse health effects of allergic contact dermatitis or atopic dermatitis may be provoked. After inhalation exposure, adverse health effects include asthma (and related respiratory symptoms such as rhinitis) or extrinsic allergic alveolitis.

Respiratory hypersensitivity is a term that is used to describe asthma and other related respiratory conditions, irrespective of the mechanism (immunological or non-immunological) by which they are caused. In contrast, dermal allergy is based on an immunological mechanism.

It is perhaps helpful to attempt to define the term chemical respiratory hypersensitivity. One approach taken by the UK Health and Safety Executive was to describe the induction phase as the process of rendering the airways unusually sensitive (hypersensitive) such that following subsequent inhalation exposure an asthmatic reaction might be elicited associated with classical symptoms of airway narrowing, chest-tightening and bronchial restriction (HSE, 1997). Other approaches to definition of relevant terms are available elsewhere. For instance, various definitions are provided for specific sensitising agents in the workplace – all of which imply a mechanism whereby hypersensitivity of the respiratory tract is induced as the result of workplace exposure – and that this may result later in the development of occupational asthma (Bernstein et al., 1993). Lists of chemicals cited here, by the HSE, and elsewhere, as causes of respiratory sensitisation and occupational asthma are very similar, and in some instances identical (Chan-Yeung et al., 1993). Among the chemicals populating these lists are: diisocyanates, acid anhydrides, certain platinum salts, some reactive dyes, cyanuric chloride, and plicatic acid (from Western Red Cedar).

When directly considering human data in this document, the clinical diagnostic terms asthma, rhinitis and extrinsic allergic alveolitis have been retained.

These definitions are reflected in the criteria for the classification of skin and respiratory sensitizers, which provide a useful tool against which the hazardous properties of a substance can be judged. These criteria are given in the 22nd Adaptation to Technical Progress to Directive 67/548/EEC [Directive 96/54/EC, Official Journal L248; pp 227-229]; Annex VI has been recast in the 28th Adaptation to Technical Progress (ATP) (Directive 2001/59, Official Journal L225; pp 1- 333).

R.7.3.1.2 Objective of the guidance on skin and respiratory sensitisation

The general objectives are to determine:

- whether there are (Q)SAR data, existing *in vitro* or *in vivo* data, or human evidence indicating that the agent has skin or respiratory sensitisation potential

- whether the agent has skin sensitisation potential based on new tests according to the strategy as presented in this document.

Therefore, in the sections on skin sensitisation and respiratory sensitisation firstly an overview of types of data is given that may provide information on sensitisation, followed by guidance on the process of judging the available data in terms of adequacy, completeness and remaining uncertainty. In Section R.7.3.7 guidance is given on application of the data to reach a conclusion on suitability for classification and labelling and possibly potency. Finally in Section R.7.3.8 an integrated testing strategy (ITS) for skin sensitisation and an integrated evaluation strategy (IES) for respiratory sensitisation is presented.

R.7.3.1.3 Mechanisms of immunologically-mediated hypersensitivity

Among the key steps required for a chemical to induce sensitisation via skin contact are gaining access to the viable epidermis, protein binding, metabolic activation (if required), internalization and processing by Langerhans cells (LC), transport of antigen by LC to draining lymph nodes, and presentation to and recognition by T lymphocytes. For chemicals that sensitise via the respiratory tract, the relevant mechanisms are believed to be essentially similar, although gaining access to the respiratory epithelium may be somewhat easier than at skin surfaces due to the lack of a stratum corneum. Moreover, because the lining of the respiratory tract, the professional antigen presenting cells, and regulatory mechanisms in the respiratory tract differ from those in the skin, they all may have an impact on the type of immune response evoked. Although the site of induction of an adaptive immune response to a chemical allergen may be influenced by local conditions and local immunoregulatory mechanisms, the fact remains that the inherent properties of the chemical itself play a major role in determining whether an immune responses is induced and the qualitative characteristics of that response.

Although it is sometimes assumed that immune responses induced following encounter with antigen in or on the skin are often of selective Th_1-type, this is not necessarily the case. It is clear that cutaneous immune responses can be of either Th_1- or Th_2-type according to the nature of the antigen.

In the respiratory tract, chemical respiratory allergens appear to preferentially elicit Th_2-immune responses (Maestrelli et al., 1997); observations that are consistent with experimental experience in mice (Dearman et al., 2002; Herrick et al., 2003; Farraj et al., 2004), and possibly also rats (Arts et al., 1998). Th2 type immune responses are characterised by the production of cytokines such as IL4 and IL5 and by the production of IgE antibodies. However, the mechanisms through which chemicals are able to induce sensitisation of the respiratory tract are not fully understood and there remains controversy about the roles played by IgE antibody-mediated mechanisms, and whether IgE represents a mandatory universal requirement for the induction by chemicals of allergic sensitisation of the respiratory tract. The area is complicated because although for all chemical respiratory allergens there are patients who display serum IgE antibodies of the appropriate specificity, in other instances (and particularly with respect to the diisocyanates) there are symptomatic subjects in whom it is not possible to detect IgE antibody. There are two, non-mutually exclusive, possibilities. The first is that IgE does play a central role but that for one or more of various reasons it is not being detected accurately in the serum of patients with occupational asthma. The second is that allergic sensitisation of the respiratory tract by chemicals can be effected through IgE antibody-independent immunological mechanisms (Kimber et al., 2002 and 2005). These may also include Th_1-type immune responses. In this context it has been reported, for instance, that inhalation challenge of sensitised rodents with contact allergens may elicit respiratory allergic reactions (Garssen et al., 1991; Garcia et al., 1992; Buckley et al., 1994; Zwart et al., 1994; Satoh et al., 1995; Arts et al., 1998). This comes as no surprise because it is clear that contact sensitisation is systemic in nature and that there is no reason to suppose that encounter of sensitised animals with the relevant contact allergen at respiratory epithelial

surfaces will not cause an adverse immunologic reaction. However, it is important to note that in reality only a very few precedents for the elicitation of pulmonary reactions by skin sensitising chemicals in humans have been observed, and in practice it may not represent a significant health issue.

In addition, there is a growing body of evidence that effective sensitisation of the respiratory tract by chemicals defined as respiratory allergens (such as for instance the acid anhydrides, diisocyanates and others) can and does occur in response to dermal contact (reviewed by Kimber et al., 2002). There are also experimental animal data and human evidence for sensitisation by inhalation and skin effects following dermal challenge (Kimber et al., 2002, Baur et al., 1984, Ebino et al., 2001, Stadler et al., 1984). Therefore, it is not necessarily the case that chemicals that cause allergic dermal reactions require sensitisation via the skin, or that chemicals that cause allergic airway reactions require sensitisation via the respiratory tract.

R.7.3.2 Information requirements for skin and respiratory sensitisation

The information requirements for sensitisation are described in REACH Annexes VI to XI, where the information that shall be submitted for registration purposes is specified.

Column 1 of Annex VII clearly informs on the standard information requirement for skin sensitisation data for substances produced or imported in quantities of ≥1 t/y.

The assessment of skin sensitisation shall comprise the following consecutive steps:

1. *an assessment of the available human, animal and alternative data,*

2. *In vivo testing*

Column 2 of Annex VII lists specific rules according to which the required standard information may be omitted, replaced by other information, or adapted in another way. If the conditions are met under which column 2 of this Annex allows adaptations, the fact and the reasons for each adaptation should be clearly indicated in the registration. For skin sensitisation column 2 reads:

Step 2 does not need to be conducted if:

- *the available information indicates that the substance should be classified for skin sensitisation or corrosivity; or*

- *the substance is a strong acid (pH<2.0) or base (pH>11.5); or*

- *the substance is flammable in air at room temperature.*

The Murine Local Lymph Node Assay (LLNA) is the first-choice method for in vivo testing. Only in exceptional circumstances should another test be used. Justification for the use of another test shall be provided. This means that in certain cases other in vivo methods may be conducted. In such cases convincing scientific justification for the use of another test shall be provided.

No information requirements are present for respiratory sensitisation. Respiratory sensitizers are indicated for harmonised classification and labelling in REACH Article 115, and respiratory sensitisation is mentioned in Annex I and XV which deal with respectively chemical safety report and preparation of these dossiers.

In addition to these specific rules, the required standard information set may be adapted according to the general rules contained in Annex XI. In this case as well, the fact and the reasons for each adaptation should be clearly indicated in the registration.

General requirements for generation of information on intrinsic properties of substances are given in REACH Article 13 which states that this information may be generated by means other than tests, provided the conditions specified in Annex XI are met.

R.7.3.3 Information for skin sensitisation and its sources

R.7.3.3.1 Non-human data for skin sensitisation

Non-testing data for skin sensitisation

Non-testing methods for skin sensitisation cover a breadth of different approaches namely read-across/chemical categories, chemistry considerations and (Q)SARs. Read-across/chemical categories are described in Sections R.6.1 and R.6.2.

A compendium of available (Q)SARs is not in existence at the present time, work is being carried out by ECB to develop an inventory of evaluated (Q)SARs which will populate the (Q)SAR Application Toolbox, a larger project currently led by the OECD. The JRC QSAR Model Database is being designed to help a user determine the validity and applicability of a model for a specific chemical and purpose. This is relevant to the assessment of adequacy. The OECD principles (described on Website http://www.oecd.org/document/23) will help to characterise the validity of a given model. Preliminary practical guidance on their interpretation has been developed (Worth et al., 2005). Evaluated (Q)SARs will be documented in (Q)SAR Reporting Formats (see Section R.6.1.9). More generic information on evaluating QSARs, their predictions and reporting formats is provided in Section R.6.1.6.

Exploring the reaction chemistry of compounds forms the basis of most read-across justifications and many of the available skin sensitisation (Q)SARs. The skin sensitisation potential of a chemical is related to its ability to react with skin proteins to form covalently linked conjugates and recognition of these by the immune system. In the vast majority of cases, this is dependent on electrophilic reactivity of the skin sensitizer or a derivative produced (usually by oxidation) *in vivo* or abiotically (Barratt et al., 1997). There are various types of electrophile-nucleophile reactions in skin sensitisation, perhaps the most frequently encountered are: Michael-type reactions; S_N2 reactions; S_NAr reactions; acylation reactions and Schiff-base formation. These chemical reaction mechanisms can serve as a means of describing the domain of applicability (the scope) of a (Q)SAR or form the basis for grouping chemicals into chemical categories. Recent work in this area has been described in (Aptula et al., 2005, Aptula and Roberts 2006, Roberts et al., 2007).

There are relatively few (Q)SARs for skin sensitisation reported in the peer reviewed literature. Available models include local and global (Q)SARs as well as expert systems.

Local (Q)SAR models

The majority of local models available have been developed for direct-acting electrophiles using the relative alkylation index (RAI) approach. This is a mathematical model derived by Roberts and Williams (1982). It is based on the concept that the degree of sensitisation produced at induction, and the magnitude of the sensitisation response at challenge, depends on the degree of covalent binding (haptenation; alkylation) to carrier protein occurring at induction and challenge. The RAI is an index of the relative degree of carrier protein haptenation and was derived from differential equations modelling competition between the carrier haptenation reaction in a hydrophobic environment and removal of the sensitizer

through partitioning into polar lymphatic fluid. In its most general form the RAI is expressed as:

$$RAI = \log D + a \log k + b \log P \quad (1)$$

Thus the degree of haptenation increases with increasing dose D of sensitizer, with increasing reactivity (as quantified by the rate constant or relative rate constant *k* for the reaction of the sensitizer with a model nucleophile) and with increasing hydrophobicity (as quantified by log P, P being the octanol/water partition coefficient). This RAI model has been used to evaluate a wide range of different datasets of skin sensitising chemicals. Examples include sulfonate esters (Roberst and Basketter 2000), sulfones (Roberts and Williams 1982), primary alkyl bromides (Basketter et al., 1992), acrylates (Roberts 1987), aldehydes and diketones (Patlewicz et al., 2001, Patlewicz et al., 2002, Patlewicz et al., 2004, Roberts et al., 1999, Roberts and Patlewicz 2002, Patlewicz et al., 2003).

This approach has been shown to be mechanistically robust but the breadth of available models so far is still somewhat limited. These types of models assume a reasonable appreciation of chemistry.

The covalent hypothesis has served and continues to be the most promising way of developing mechanistically based robust QSARs. These are local in that their scope is characterised by a mechanistic reactivity domain as outlined in Aptula et al., 2005, Aptula and Roberts 2006, Roberts et al., 2007. An example of this type of mechanistic model has been recently published (Roberts et al., 2006). In the RAI model, log*k*, has been typically modelled by experimental rate constants, substituents' constants or molecular orbital parameters. More effort is needed to encode reactivity into descriptors, this could be achieved through the systematic generation of *in vitro* reactivity data as outlined in (Aptula and Roberts 2006, Aptula et al., 2006b, Schultz et al., 2006, Gerberick et al., 2004) and in the next section.

Global statistical models

Global Statistical models usually involve the development of empirical QSARs by application of statistical methods to sets of biological data and structural descriptors.

These are perceived to have the advantage of being able to make predictions for a wider range of chemicals. In some cases, the scope/domain of these models are well described, in most other cases a degree of judgement is required in determining whether the training set of the model is relevant for the chemical of interest. Criticism often levied at these types of models is that they lack mechanistic interpretability. The descriptors might appear to lack physical meaning or are difficult to interpret from a chemistry perspective. The sorts of descriptors used may encode chemical reactivity/electrophilicity e.g. LUMO (the energy of the lowest molecular orbital) and partitioning effects e.g. Log P, but more commonplace is that a large number of descriptors are calculated that encode structural, topological and/or geometrical information. A number have been reported in the recent literature, examples include those developed using LLNA data (Devillers 2000, Estrada et al., 2003, Fedorowicz et al., 2005, Fedorowicz et al., 2005, Li et al., 2005, Miller et al., 2005, Ren et al., 2006, Li et al., 2007).

Expert systems

There are several commercial (Q)SAR models for skin sensitisation available. Examples include TOPKAT, CASE, Derek for Windows and TIMES.

Statistical Models

TOPKAT (current version 6.2) marketed by Accelrys Inc (San Diego, USA) comprises two suites of models; one for aromatics (excluding chemicals with 1 benzene ring) and the other for aliphatics and chemicals with 1 benzene ring. The first set of models discriminate between

non-sensitizers and sensitizers, a probability is calculated for the submitted chemical structure. If the probability is greater than or equal to 0.7, the chemical is predicted to be a sensitizer, a non-sensitizer would have a probability of less or equal to 0.30. The second set of models resolve the potency: weak/moderate vs. strong where a probability of 0.7 or more indicates a strong sensitizer and a probability below 0.30 indicates a weak or moderate sensitizer. Probability values between 0.30 and 0.70 are referred to as indeterminate. An optimum prediction space algorithm ensures that predictions are only made for chemicals within the model applicability domain (Enslein et al., 1997, http://www.accelrys.com/products/topkat/).

CASE methodology and all its variants were developed by Klopman and Rosenkranz. There are a multitude of models for a variety of endpoints and hardware platforms. The CASE approach uses a probability assessment to determine whether a structural fragment is associated with toxicity (Cronin et al., 2003). The MCASE models that have been developed for skin sensitisation are described further in primary articles (Gealy et al., 1996, Graham et al., 1996, Johnson et al., 1997). There are two sensitisation modules available for purchase from MultiCase Inc (Ohio, USA) (http://www.multicase.com/products/prod0911.htm). In addition the (Q)SAR estimates for one MCASE skin sensitisation model are included in the Danish Environmental Protection Agency (EPA) (Q)SAR database which is currently hosted on the European Chemicals Bureau (ECB) website http://ecb.jrc.it/QSAR/ .

Knowledge based systems

Derek for Windows (DfW) is a knowledge-based expert system created with knowledge of structure-toxicity relationships and an emphasis on the need to understand mechanisms of action and metabolism. It is marketed and developed by LHASA Ltd (Leeds, UK) a not-for-profit company and educational charity (http://www.lhasalimited.org/index.php).

Within DfW (version 9), there are 361 alerts covering a wide range of toxicological endpoints. An alert consists of a toxicophore, a substructure known or thought to be responsible for the toxicity alongside associated literature references, comments and examples. The skin sensitisation knowledge base in DfW was initially developed in collaboration with Unilever in 1993 using its historical database of guinea pig maximisation test (GPMT) data for 294 chemicals and contained approximately forty alerts (Barratt et al., 1994). Since that time, the knowledge base has undergone extensive improvements as more data have become available (Payne and Walsh 1994). The current version (version 9) contains seventy alerts for skin sensitisation and the closely-related endpoint of photoallergenicity (Barratt et al., 2000, Langton et al., 2006).

Hybrids

TIssue MEtabolism Simulator (TIMES) software has been developed to integrate a skin metabolism simulator with 3D-QSARs for evaluating reactivity of chemicals in order to predict their skin sensitisation potency (Dimitrov et al., 2005, Dimitrov et al., 2005). The simulator contains 236 hierarchically ordered spontaneous and enzyme controlled reactions. Covalent interactions of chemicals/metabolites with skin proteins are described by 47 alerting groups. 3D-QSARs (COREPA) are applied for some of these alerting groups.

Clearly there are a breadth of different (Q)SARs and expert systems available for the estimation of skin sensitisation hazard. The approaches are quite varied and each has been developed on different sets of *in vivo* data (principally GPMT and LLNA). Whilst efforts have been made to characterise a number of the literature based models in terms of the OECD principles for QSAR validation (see Roberts et al., 2007 as an example), further work is still required for some of the commercial systems (ECETOC 2003). In addition, in many cases these models have been demonstrated to be reasonable for predicting skin sensitizers correctly but are limited in predicting non-sensitizers correctly (Roberts et al., 2007, ECETOC 2003). For this reason, careful interpretation of model predictions needs to be considered in light of other

information e.g. analogue read-across (other similar chemicals with respect to their mechanistic domain).

Further work should explore encoding more knowledge/rules for non-reactive chemicals as well as those chemicals likely to undergo chemical or metabolic transformation.

Consideration of which model(s) to apply will be dependent on the specific chemical of interest, the underlying training set data and the applicability domain. These issues are described more fully in Section R.6.1. An example is illustrated here; if the chemical falls into a chemistry reactivity domain that is well characterised, then a local (Q)SAR model developed for this domain (such as those previously described) will give rise to the most robust prediction of skin sensitisation. Where the mechanism is not understood or not known *a priori* one or more of the expert systems such as TOPKAT, Derek for Windows or the others already described will be best placed to provide an estimate. These systems whilst not wholly transparent do provide a reasonable amount of supporting information to enable the robustness of a prediction to be evaluated. This is discussed in more detail in Section R.7.3.4.1.

Testing data for skin sensitisation

In vitro data

At present, no officially adopted EU-OECD *in vitro* tests for skin sensitisation exist. However, several systems are in the course of development (Eskes et al., 2005), based on an improved understanding of the biochemical and immunological mechanisms underlying the process (Worth et al., 2002). Currently, *in vitro* assays to detect the sensitising properties of a chemical are under development for the following areas:

- **Epidermal bioavailability**: skin penetration is a prerequisite for skin sensitisation. Information about the skin penetration properties can help to evaluate the potential of a chemical to be identified as a skin sensitizer (ECVAM, 2007).

- **Chemical reactivity**: since the majority of chemical allergens is electrophilic and reacts with nucleophilic amino acids, peptide reactivity assays can give an indication of skin sensitisation potency or potential to form a complete antigen (Gerberick et al., 2004, Aptula et al., 2006b).

- **Cell-based assays**: the knowledge that changes occur in epidermal Langerhans cells as a result of exposure to chemical allergens (e.g. the expression of surface markers and/or cytokines release) and that Langerhans cells can be replaced by blood derived dendritic-like cells or cell lines have been applied to design in vitro alternative tests (Kimber et al., 2001, Tuschl et al., 2000, Casati et al., 2005, Ryan et al., 2005, Sakaguchi et al., 2006, Aeby et al., 2004, Azam et al., 2006, Python et al., 2007). These systems have been shown to selectively express various mediators and/or markers of activation following exposure to chemical sensitizers and attempts to develop robust assays have started. Beside Langerhans cells, keratinocytes play a prominent role in the sensitisation process (Corsini et al., 1998, van Och et al., 2005, Vandebriel et al., 2005). In addition to chemical processing, LC activation requires the binding of cytokines produced by keratinocytes as a result of initial chemical exposure. Moreover the assessment of keratinocytes cytokine expression as a function of the ability of chemicals to induce cutaneous sensitisation is also the object of several investigations (Aiba et al., 2000, Herouet et al., 2000). Keratinocytes have been tested both in primary cultures, in co-culture with dendritic cells and as reconstituted epidermis (Casati et al., 2005, Kubilus et al., 1986, Coquette et al., 2003). The use of reconstituted skin models for the assessment of contact allergens is under investigation.

Owing to the complexity of the mechanisms of skin sensitisation, a single test will probably not be able to replace the currently required animal procedures. Efforts are still needed to identify the most relevant endpoints in the optimisation of existing tests. However, a combination of several *in vitro* tests, covering the relevant mechanistic steps of skin sensitisation, into a test battery could possibly lead to replacement of *in vivo* tests (Eskes et al., 2005). How the outputs from these tests could be combined is not as yet determined, although a general strategy has been presented (Jowsey et al., 2006). Until that date, *in vitro* tests may be used as supportive evidence in combination with other types of data for the identification of allergens (see Section R.7.3.8.3 for an ITS based on a WoE approach).

Animal data

Guideline-compliant tests

For new *in vivo* testing of skin sensitisation potential, the murine local lymph node assay (LLNA) is the REACH Annex VII-endorsed method. This assay has been validated internationally and has been shown to have clear animal welfare benefits and scientific advantages compared with the guinea pig tests described below. The LLNA is designed to detect the potential of chemicals to induce sensitisation as a function of lymphocyte proliferative responses induced in regional lymph nodes. This method is described in OECD TG 429/EU B.42.

Two further animal test methods for skin sensitisation are described in OECD TG 406/EU B.6: the guinea pig maximisation test (GPMT) and the Buehler test. The GPMT is an adjuvant-type test in which the acquisition of sensitisation is potentiated by the use of Freund's Complete Adjuvant (FCA) and in which both intradermal and topical exposure are used during the induction phase. The Buehler test is a non-adjuvant method involving for the induction phase topical application only.

Both the GPMT and the Buehler test are able to detect chemicals with moderate to strong sensitisation potential, as well as those with relatively weak sensitisation potential. In such methods activity is measured as a function of challenge-induced dermal hypersensitivity reactions elicited in test animals compared with controls. Since the LLNA is the preferred method for new *in vivo* testing, the use of the standard guinea pig tests to obtain new data on skin sensitisation potential will be acceptable only in exceptional circumstances and will require scientific justification. However, existing data of good quality deriving from such tests will be acceptable and will, if providing clear results, preclude the need for further *in vivo* testing.

ECETOC Monograph 29 (2000) contains a useful discussion of these tests.

Non-guideline compliant tests and refinements to the standard assays

Existing data may be available from tests that do not have an OECD guideline, for example:

i. other guinea pig skin sensitisation test methods (such as the Draize test, optimisation test, split adjuvant test, open epicutaneous test);

ii. additional tests (such as the mouse ear swelling test);

Information may also be available from other endpoints, for example, repeated dose dermal studies that show effects indicative of an allergic response, such as persistent erythema and/or oedema.

For new testing, refinements to the existing guideline methods may also be possible. In such cases, care should be taken to ensure that any modifications or deviations from standard methodologies are scientifically justified. For example, it might be feasible to conduct a

reduced version of the LLNA (rLLNA) in which assessments are made on the basis of results from a vehicle control and a single (highest) concentration of the test substance (Eskes et al., 2005). In such cases, it is recommended that expert advice be sought before commencing the tests.

R.7.3.3.2 Human data on skin sensitisation

Human data on cutaneous (allergic contact dermatitis and urticarial) reactions may come from a variety of sources:

- consumer experience and comments, preferably followed up by professionals (e.g. diagnostic patch tests)
- diagnostic clinical studies (e.g. patch tests, repeated open application tests)
- records of workers' experience, accidents, and exposure studies including medical surveillance
- case reports in the general scientific and medical literature
- consumer tests (monitoring by questionnaire and/or medical surveillance)
- epidemiological studies
- human experimental studies such as the human repeat insult patch test (Stotts, 1980) and the human maximisation test (Kligman, 1966), although it should be noted that *new* experimental testing for hazard identification in humans, including HRIPT and HMT, is not acceptable for ethical reasons.

R.7.3.4 Evaluation of available information on skin sensitisation

For both steps of the effects assessment, i.e. hazard identification and dose (concentration)-response (effect) assessment, it is very important to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of the data. The completeness of the data refers to the conclusion on the comparison between the available adequate information and the information that is required under the REACH proposal for the applicable tonnage level of the substance. Such a conclusion relies on WoE approaches, mentioned in REACH Annex XI Section 1.2, which categorise available information based on the methods used: *guideline tests, non-guideline tests*, and other types of information which may justify adaptation of the standard testing regime. Such a WoE approach also includes an evaluation of the available data as a whole, i.e. both over or across endpoints: i.e. for a sensitive evaluation of sensitisation effects, it is necessary to efficiently integrate the information gathered for sensitisation with that obtained from the study of skin and eye irritation (and acute dermal toxicity).

This approach provides a basis to decide whether further information is needed on endpoints for which specific data appear inadequate or not available, or whether the requirements are fulfilled.

For this specific endpoint some additional remarks are made on the adequacy of the various types of data that may be available.

R.7.3.4.1 Non-human data on skin sensitisation

Non-testing data on skin sensitisation

The evaluation and assessment of a chemical using (Q)SARs is dependent on both the chemical of interest and the (Q)SAR model(s) used to make a prediction. Here we attempt to provide some specific advice for skin sensitisation. More general advice on (Q)SARs including evaluation of OECD principles is described in Section R.6.1.3).

One of the first steps to consider is what information already exists on chemicals *similar* to the one of interest. Chemical similarity is a widely used concept in toxicology, and is based on the hypothesis that similar compounds have similar biological activities. This forms the underlying basis for developing (Q)SARs. In the case of skin sensitisation, the most robust means of comparing two or more chemicals is through an evaluation of their likely chemical reactivity. Recent work in this area has been investigating means of encoding reactivity for the different mechanistic domains in form of rules (Aptula and Roberts 2006, Aptula et al., 2006). (Note: This approach might involve the systematic generation of *in vitro* reactivity data for these different mechanistic domains. (see Aptula et al., 2006 as an example) .If the chemical reactivity is not known, or can not be determined through experimentation then a pragmatic means of identifying similar chemicals can be done through a substructural/analogue search.

There are a number of available computational tools and databases that facilitate the search and retrieval of similar analogues. Some like Leadscope (http://www.leadscope.com) are commercial, others like Chemfinder (www.chemfinder.com), ChemID (http://chem.sis.nlm.nih.gov/chemidplus/) or DssTox (http://www.epa.gov/nheerl/dsstox/) are freely available to use on the internet.

Some of the available search engines are linked to databases (through hyperlinks and indexes) whereas other facilities such as DssTox provide a repository of available QSAR datasets which can be downloaded for subsequent use in appropriate QSAR /database software tools.

Many of currently available tools containing public data have focussed on endpoints such as carcinogenicity, mutagenicity or acute toxicity. This means that an additional search is needed to identify skin sensitisation data. Much of the available skin sensitisation experimental data resides in peer reviewed publications. Cronin and Basketter (1994) published the results of over 270 *in vivo* skin sensitisation tests (mainly from the guinea pig maximisation test). All data were obtained in the same laboratory and represent one of the few occasions when large amounts of information from corporate databases was released into the open literature. A larger database of animal and human studies for 1034 compounds is described by Graham et al. (1996), the MCASE database. A comparatively large number of data have been published for the local lymph node assay, examples include publications by Ashby et al. (1995) and Gerberick et al (2005).

These publications are invaluable to identify analogues with associated skin sensitisation test data.

The second step involves an assessment of the similarity of the analogues identified. Considerations will include whether:

- the same endpoint is considered
- there are any additional functional groups or additional substituents that might influence the reactivity and sensitising behaviour (applicability domain considerations)
- the physico-chemical parameters similar (e.g. LogP, applicability domain considerations)

- there are impurities that influence the sensitisation profile
- the likely chemical mechanism is the same

These considerations may help identify an available local (Q)SAR for that chemical class/mechanistic group.

If an appropriate local model can not be identified then a third step of evaluating a chemical using one of the available global models/expert systems is merited.

Here a prediction needs to be evaluated in the context of the likely chemistry and the available *like* chemicals available within the training set. i.e. is the compound of interest within the scope of the model and are similar chemicals in the training set of the model well predicted. This type of information provides additional weight to whether the estimate derived is meaningful and relevant. For global models available in the literature, the training sets and the algorithm(s) are usually available to allow such comparisons to be made.

For expert systems such as Derek for Windows, TOPKAT etc, the training sets and to an extent the algorithms or descriptors used are often kept latent within the software. Some supporting information is provided on the robustness and relevance for a given prediction. For example, within DfW it is possible to see representative example chemicals and explanations of the mechanistic basis for the SAR developed. Within TOPKAT, it is possible to obtain an assessment of whether the chemicals falls within the applicability domain of the model (both with respect to the fragment and descriptor space), whether it is an example chemical in the database as well as perform a similarity assessment to identify analogues. Similar functionalities and features are present in many of the other commercial expert systems available.

Although the main factors driving skin sensitisation (and therefore the (Q)SARs) is the underlying premise of the electrophilicity of a chemical, other factors such as hydrophobicity encoded in the octanol/water partition coefficient (log P) may also be considered as playing a role in the modifying the sensitisation response observed. Within DfW, an assessment of the likely skin penetration ability is made using the algorithm by Potts and Guy. This relates the Kp value to log P and MW (Potts and Guy 1992). It is then possible to rationalise the output in terms of bands of penetration potential. Some have been described in (Howes et al., 1996).

Specific model and prediction information can be described in more detail in reporting formats ((Q)SAR Reporting Format). This summarises the pertinent information to consider for given model when evaluating an estimate as well as the estimate itself. More details are provided in Section R.6.1.

Other information such as results in other assays such as the Ames test (a common feature of genotoxic substances is that they can bind covalently to DNA and cause direct DNA damage) or aquatic toxicity tests may provide supporting information about the electrophilicity of the chemical of interest and hence its likely sensitisation ability. Some of this work is still at an early stage but correlations have been explored between mutagens and sensitizers (Wolfreys and Basketter 2004) and between aquatic toxicants and sensitizers (Aptula et al., 2006).

Testing data on skin sensitisation

In vitro data

Even though a number of *in vitro* methods are currently under development, none of these methods has yet undergone a formal validation process. According to Annex XI, *in vitro* data obtained with non-validated methods can only be used in a WoE approach. If such data are considered for the evaluation, expert judgement is needed to assess their reliability. In particular, attention should be paid to the level of optimisation of the method that should meet

at least the ECVAM criteria for entering pre-validation (Curren et al., 1995), including evidence of the reproducibility of the method, its mechanistic relevance and predictive capacity (Balls et al., 1995, Hartung et al., 2004, Worth et al., 2001).

In vitro assays only cover a (specific) part of the process of sensitisation that occurs *in vivo*, therefore it is unlikely that a single method will be able to substitute for the animal test.

Animal data

Well reported studies using internationally acceptable protocols, particularly if conducted in accordance with the principles of GLP, can be used for hazard identification. Other studies (see Section R.7.3.3.1 and below), not fully equivalent to OECD test protocols, can, in some circumstances, provide useful information. Particular attention should be paid to the quality of these tests and the use of appropriate positive and negative controls. The specificity and sensitivity of all animal tests should be monitored through the inclusion of appropriate positive and negative controls. In this context, positive controls are the 6-monthly sensitivity checks with an appropriate positive control substance, and negative controls are the vehicle-treated control animals included as part of each test.

Guideline-compliant tests

For the conduct and interpretation of the LLNA the following points should be considered:

i. the vehicle in which the test material and controls have been applied;

ii. the concentrations of test material that have been used;

iii. any evidence for local or systemic toxicity, or skin inflammation resulting from application of the test material;

iv. whether the data are consistent with a biological dose response;

v. the submitting laboratory should be able to demonstrate its competency to conduct the LLNA.

OECD TG 429/EU B.42 provides guidance on the recommended vehicles, number of animals per group, concentrations of test chemical to be applied and substances to be used as a positive control. A preliminary study or evaluation of existing acute toxicity/dermal irritation data is normally conducted to determine the highest concentration of test substance that is soluble in the vehicle but does not cause unacceptable local or systemic toxicity. The submission of historical control data will demonstrate the ability of the test laboratory to produce consistent responses. Based on the use of radioactive labelling, chemicals that result in a stimulation index (SI) of ≥3 at one or more test concentrations are considered to be positive for skin sensitisation. Both positive and negative responses in the LLNA conducted as described in OECD TG 429/EU B.42 meet the data requirements for classification of a substance as a skin sensitizer: no further testing is required.

Alternative vehicles to those listed in OECD TG 429/EU B.42 may be used in the LLNA if sufficient scientific justification is provided. OECD TG 429/EU B.42 also states that endpoints other than radioactive labelling may be used to assess proliferation, on condition that justification and scientific support, which will include full citations and a description of the methodology, are provided.

The guinea pig test methods described in OECD TG 406/EU B.6, the GPMT (Magnusson et al., 1969, Schlede et al., 1995) and the Buehler, can also be used for hazard identification. Recommendations on conducting and analysing these methods are provided by Steiling et al.,

2001. Particular attention should be paid to the quality of these tests with consideration given to the following points:

i. numbers of test and control guinea pigs;

ii. number or percentage of test and control animals displaying skin reactions;

iii. whether skin irritation was observed at the induction phase;

iv. whether the maximal non-irritating concentration was used at the challenge phase;

v. the choice of an appropriate vehicle (ideally, one that solubilises or gives a stable suspension or emulsion of the test material, is free of allergenic potential, is non-irritating, enhances delivery across the stratum corneum, and is relevant to the usage conditions of the test material, although it is recognised that it will not always be possible to meet all these conditions);

vi. whether there are signs of systemic toxicity (a sighting study should be performed to determine an appropriate induction dose that causes irritation but not systemic toxicity);

vii. staining of the skin by the test material that may obscure any skin reactions (other procedures, such as chemical depilation of the reaction site, histopathological examination or the measurement of skin fold thickness may be carried out in such cases);

viii. results of rechallenge treatments if performed;

ix. checking of strain sensitivity at regular intervals by using an appropriate control substance (as specified in OECD guidelines and EU Test Methods). Currently (2007), the recommended interval is 6 months.

The investigation of doubtful reactions in guinea pig tests, particularly those associated with evidence of skin irritation following first challenge, may benefit from rechallenge of the test animals. In cases where reactions may have been masked by staining of the skin, other reliable procedures may be used to assist with interpretation; where such methods are used, the submitting laboratory should provide evidence of their value.

Non-guideline compliant tests and refinements to the standard assays

The submitted dossier should include scientific justification for conducting any new test that is a modification or deviation from guideline methods. In such cases, it would be advisable to seek appropriate expert advice on the suitability of the assay before testing is begun.

For hazard identification, it may be possible to use a reduced LLNA (rLLNA) (Kimber et al., 2006) which reduces the use of animals by requiring only a single (high) dose group (≥10%) and a concurrent negative control group. A preliminary study or evaluation of existing acute toxicity/dermal irritation data is normally conducted to determine the highest concentration of test substance that is soluble in the vehicle, but that does not cause unacceptable local or systemic toxicity. As with the full LLNA, although a concurrent positive control group is not required, registrants would be required to submit historical positive control data supportive of their competence. The rLLNA should be used only in appropriate circumstances:

i. where hazard identification is the primary objective and

ii. where potency data are not required

As in the standard (OECD guideline-compliant) LLNA, group sizes should comprise four or five animals. A positive result in a rLLNA will suffice in circumstances where risk assessment and/or risk management is NOT required. Registrants should be aware that the rLLNA is less scientifically rigorous than the standard LLNA, with an associated increased level of uncertainty.

Historically, guinea pig studies that are not fully equivalent to OECD test protocols have been conducted and can provide useful hazard information. These studies include, but are not limited to, the following: Draize test, optimisation test, split adjuvant test, open epicutaneous test and the cumulative contact enhancement test. In the case of positive results the substance may be considered as a potential skin sensitizer. If, taking into account the above quality criteria, especially the positive and negative control data, there is a clear negative result, i.e. no animals displaying any signs of sensitisation reactions, then no further animal testing is required. Where there is a low level of response, the quality of the study is questionable, or where unacceptably low concentrations of the test material have been used for induction and/or challenge, further testing may be required.

R.7.3.4.2 Human data on skin sensitisation

When reliable and relevant human data are available, they can be useful for hazard identification and even preferable over animal data. However, lack of positive findings in humans does not necessarily overrule positive and good quality animal data.

Well conducted human studies can provide very valuable information on skin sensitisation. However, in some instances (due to lack of information on exposure, a small number of subjects, concomitant exposure to other substances, local or regional differences in patient referral etc) there may be a significant level of uncertainty associated with human data. Moreover, diagnostic tests are carried out to see if an individual is sensitised to a specific agent, and not to determine whether the agent can cause sensitisation.

For evaluation purposes, existing human experience data for skin sensitisation should contain sufficient information about:

- the test protocol used (study design, controls)
- the substance or preparation studied (should be the main, and ideally, the only substance or preparation present which may possess the hazard under investigation)
- the extent of exposure (magnitude, frequency and duration)
- the frequency of effects (versus number of persons exposed)
- the persistence or absence of health effects (objective description and evaluation)
- the presence of confounding factors (e.g. pre-existing dermal health effects, medication; presence of other skin sensitizers)
- the relevance with respect to the group size, statistics, documentation
- the *healthy worker* effect

Evidence of skin sensitising activity derived from diagnostic testing may reflect the induction of skin sensitisation to that substance or cross-reaction with a chemically very similar substance. In both situations, the normal conclusion would be that this provides positive evidence of the skin sensitising activity of the chemical used in the diagnostic test.

Human experimental studies on skin sensitisation are not normally conducted and are generally discouraged. Where human data are available, then quality criteria and ethical considerations are presented in ECETOC monograph no 32.

Ultimately, where a very large number of individuals (e.g.10^5) have frequent (daily) skin exposure for at least two years and there is an active system in place to pick up complaints and adverse reaction reports (including via dermatology clinics), and where no or only a very few isolated cases of allergic contact dermatitis are observed then the substance is unlikely to be a significant skin sensitizer. However, information from other sources should also be considered in making a judgement on the substance's ability to induce skin sensitisation.

It is emphasised that testing with human volunteers is strongly discouraged, but when there are good quality data already available they should be used as appropriate in well justified cases.

R.7.3.5 Information and its sources on respiratory sensitisation

R.7.3.5.1 Non-human data on respiratory sensitisation

Non-testing data on respiratory sensitisation

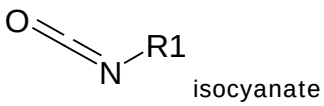
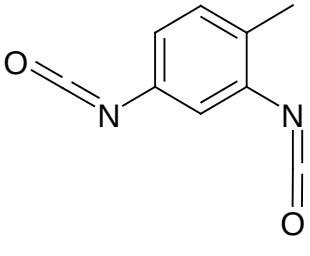
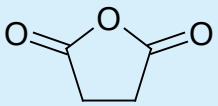
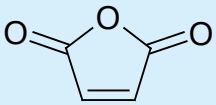
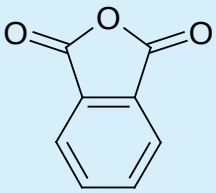
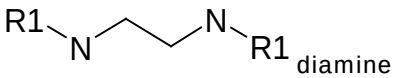
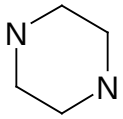
Attempts to model respiratory sensitisation have been hampered by a lack of a predictive test protocol for assessing chemical respiratory sensitisation. (Q)SAR models are available but these have largely been based on data for chemicals reported to cause respiratory hypersensitivity in humans. Examples of some structural alerts are shown in Table R.7.3-1.

Agius et al (1991) made qualitative observations concerning the chemical structure of chemicals causing occupational asthma. This work drew attention to the large proportion of chemical asthmagens with at least two reactive groups, e.g., ethylene diamine and toluene diisocyanate. The earlier work was followed up by a simple statistical analysis of the occurrence of structural fragments associated with activity, with similar conclusions (Agius et al, 1994 and 2000).

The MCASE group has developed three models for respiratory hypersensitivity (Karol et al., 1996, Graham et al., 1997, Cunningham et al., 2005). The Danish (Q)SAR Database has an in-house model for respiratory hypersensitivity for which estimates can be extracted from the on-line database (available at http://ecb.jrc.it/QSAR). Derek for Windows contains several alerts derived from a set of respiratory sensitisers/asthmogens (Payne et al., 1995).

Whilst the available structural alerts (SAR) are transparent and easily to apply (Aigus et al., 1991, 1994 and 2000, Payne et al., 1995), it should be stressed that these are derived on the basis of chemical asthmagens not specifically chemical respiratory allergens. A need therefore remains to develop new (Q)SARs as and when a robust predictive test method becomes available.

Table R.7.3-1 Examples of structural alerts for respiratory sensitisation

Structural Alert Description	Examples of structures
 isocyanate	 Toluene-2,4-diisocyanate
 cyclic anhydride	  maleic anhydride trimellitic anhydride
 diamine	 piperazine

Testing data for respiratory sensitisation

In vitro data

No *in vitro* tests specific for respiratory sensitisation are available yet, owing to the complexity of the mechanisms of the sensitisation process.

Efforts are still needed to identify the most relevant endpoints in the optimisation of existing tests. However, a combination of several *in vitro* tests, covering the relevant mechanistic steps of respiratory sensitisation, into a test battery could eventually lead to replacement of the *in vivo* tests.

Animal data

At present, although a number of test protocols has been published to detect respiratory allergenicity of low molecular weight compounds, none of these are validated nor are these widely accepted. One approach that might be of some value in characterising the likely respiratory sensitising activity of chemicals is application of the LLNA, or of other tests for measuring skin sensitisation potential. Although the LLNA was developed and validated for the identification of contact allergens, there is evidence that chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995). That is, chemicals known to cause respiratory allergy and occupational asthma have been shown to test positive in the LLNA. Among such chemicals are acid anhydrides (such as trimellitic anhydride and phthalic anhydride), diisocyanates (including diphenylmethane diisocyanate and hexamethylene diisocyanate) and certain reactive dyes. In fact, the view currently is that most, if not all, chemical respiratory allergens are able to elicit positive responses in the LLNA, or in other tests for skin sensitisation, such as the M&K (guinea pig maximisation) test. This is true even of those chemical respiratory allergens, such as phthalic anhydride, for instance, that are

implicated virtually exclusively with the induction of chemical respiratory allergy and have rarely, if ever, been shown to cause allergic contact dermatitis. Against this background and in combination with other data it might be possible to conclude in a WoE assessment that chemicals that (at an appropriate test concentration and test conditions, i.e. skin penetration should have occurred) are negative in the LLNA, as well as being considered as not being skin sensitizers, can also be regarded as lacking the potential to cause allergic sensitisation of the respiratory tract.

One approach that has been proposed for the identification of chemicals that have the potential to cause allergic sensitisation of the respiratory tract is one in which activity is measured as a function of the profiles of cytokines produced by draining lymph node cells in mice exposed more chronically (over a 2 week period) to the test chemical (Dearman et al., 2002). This method is predicated on an understanding that allergic sensitisation of the respiratory tract is favoured by selective Th_2-type immune responses and that in many instances chemical respiratory allergy and occupational asthma are associated with IgE antibody. Using this approach chemical respiratory allergens are identified as a function of their ability to stimulate in mice the selective development of preferential Th2-type immune responses associated with a predominance of type 2 cytokine secretion by draining lymph node cells (Dearman et al., 2002 and 2003). Specifically, chemical contact allergens promote Th1 responses characterised by an enhanced production of IFN-gamma, whereas chemical respiratory allergens promote Th_2 responses characterised by enhanced production of IL-4, IL-5 and IL-13. Many variables other than the compound itself, such as concentration used to induce sensitisation, duration of the sensitisation period, and presence or absence of mitogens to reveal differences in cytokine expression have all been noted to have impact on the outcome (Van Och et al., 2002). There are general guidelines now available for the conduct of the method (Dearman et al., 2003), however, this method has not yet been formally validated nor is it widely accepted.

Another, relatively simple approach may serve the purpose to specifically predict sensitisation of the respiratory tract: i.e. increases in total serum IgE antibodies after induction. This method is based on statistically significant increases in total serum IgE (see review by Arts and Kuper, 2007).

Methods that use both an induction and an inhalation elicitation or challenge phase and which include different parameters such as total and/or specific IgE antibody determinations, lung function testing, tests for a specific hyperreactivity (e.g. methacholine challenges), bronchoalveolar lavage measurements, and histopathological examination of the entire respiratory tract, may provide (additional) information on the potential of chemicals to cause respiratory sensitisation. These methods usually use high IgE-responding animal strains; to test for Th1-mediated responses low IgE-responding strains should typically be used. Several of these models have been reviewed recently (Arts and Kuper, 2007).

There are currently no predictive methods to identify chemicals that induce asthma through non-immunological mechanisms, however, when performing challenge tests including non-sensitised but challenged controls information can be obtained on non-immunological effects of these chemicals.

R.7.3.5.2 Human data on respiratory sensitisation

Human data on respiratory reactions (asthma, rhinitis, alveolitis) may come from a variety of sources:

- consumer experience and comments, preferably followed up by professionals (e.g. bronchial provocation tests, skin prick tests and measurements of specific IgE serum levels)

- records of workers' experience, accidents, and exposure studies including medical surveillance
- case reports in the general scientific and medical literature
- consumer tests (monitoring by questionnaire and/or medical surveillance)
- epidemiological studies

R.7.3.6 Evaluation of available information for respiratory sensitisation

R.7.3.6.1 Non-human data for respiratory sensitisation

Non-testing data for respiratory sensitisation

Given the lack of available (Q)SARs for respiratory sensitisation, it is not possible to provide any additional guidance.

Testing data for respiratory sensitisation

In vitro data

Presently (March 2007) there are no *in vitro* tests available to assess respiratory sensitisation. If such a method were to become available then it would need to be assessed for its relevance and reliability (Hartung et al., 2004).

Animal data

Although the LLNA does not represent a method for the specific identification of chemical respiratory allergens, there is evidence that chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995). The interpretation is therefore that a chemical which fails to induce a positive response in the LLNA (at an appropriate test concentration) most probably lacks the potential for respiratory allergy. Conversely, it cannot be wholly excluded that a chemical that induces a positive response in the LLNA, might sensitise the respiratory tract upon inhalation or via dermal exposure. Any potential hazard for respiratory sensitisation could only be positively identified by further testing, although such testing is neither validated nor widely accepted.

One further approach to the identification of chemicals that have the potential to induce allergic sensitisation of the respiratory tract is *cytokine fingerprinting* (Dearman et al., 2002; see Section R.7.3.5.1). This method is predicated on an understanding that allergic sensitisation of the respiratory tract is favoured by selective Th_2-type immune responses and that in many instances chemical respiratory allergy and occupational asthma are associated with IgE antibody.

In addition, there are other approaches that have been proposed and these have been reviewed recently (Arts and Kuper, 2007) - although again it is important to emphasise that there are currently available no fully evaluated or validated animal models for the predictive identification of chemical respiratory allergens.

As indicated previously, some chemicals may have the potential to induce pulmonary reactions via Th1-type immune responses. Studies with typical skin allergens such as DNCB, DNFB and picryl chloride (trinitrochlorobenzene) in BALB/c mice, guinea pigs or Wistar rats have shown the potential of these chemicals to induce allergic reactions in the lungs that are independent

of IgE (Garssen et al., 1991; Garcia et al., 1992; Buckley et al., 1994; Zwart et al., 1994; Satoh et al., 1995; and see for a review Arts and Kuper, 2007). Sensitisation and challenge with DNCB resulted in laryngitis in low IgE-responding Wistar rats (Arts et al., 1998). [In addition, cellular immune responses to these sensitizers were shown to be associated with hyperreactivity of the airways to non-specific stimuli (Garssen et al., 1991).] For these reasons, it might be the case that people who are sensitised via the skin might suffer adverse pulmonary reactions if they were to inhale sufficient amounts of the contact allergen to which they were sensitised. As indicated previously, very few precedents for the elicitation of pulmonary reactions by skin sensitising chemicals in humans have been observed. In practice it appears not to represent a health issue.

R.7.3.6.2 Human data for respiratory sensitisation

Although human studies may provide some information on respiratory hypersensitivity, the data are frequently limited and subject to the same constraints as human skin sensitisation data.

For evaluation purposes, existing human experience data for respiratory sensitisation should contain sufficient information about:

- the test protocol used (study design, controls)
- the substance or preparation studied (should be the main, and ideally, the only substance or preparation present which may possess the hazard under investigation)
- the extent of exposure (magnitude, frequency and duration)
- the frequency of effects (versus number of persons exposed)
- the persistence or absence of health effects (objective description and evaluation)
- the presence of confounding factors (e.g. pre-existing respiratory health effects, medication; presence of other respiratory sensitizers)
- the relevance with respect to the group size, statistics, documentation
- the healthy worker effect

Evidence of respiratory sensitising activity derived from diagnostic testing may reflect the induction of respiratory sensitisation to that substance or cross-reaction with a chemically very similar substance. In both situations, the normal conclusion would be that this provides positive evidence for the respiratory sensitising activity of the chemical used in the diagnostic test.

For respiratory sensitisation, no clinical test protocols for experimental studies exist but tests may have been conducted for diagnostic purposes, e.g. bronchial provocation test. The test should meet the above general criteria, e.g. be conducted according to a relevant design including appropriate controls, address confounding factors such as medication, smoking or exposure to other substances, etc. Furthermore, the differentiation between the symptoms of respiratory irritancy and allergy can be very difficult. Thus, expert judgment is required to determine the usefulness of such data for the evaluation on a case-by-case basis.

Although predictive models are under validation, there is as yet no internationally recognized animal method for identification of respiratory sensitisation. Thus human data are usually evidence for hazard identification.

Where there is evidence that significant occupational inhalation exposure to a chemical has not resulted in the development of respiratory allergy, or related symptoms, then it may be possible to draw the conclusion that the chemical lacks the potential for sensitisation of the respiratory tract. Thus, for instance, where there is evidence that a large cohort of subjects have had opportunity for regular inhalation exposure to a chemical for a sustained period of time in the absence of respiratory symptoms, or related health complaints, then this will provide reassurance regarding the absence of a respiratory sensitisation hazard.

R.7.3.7 Conclusions on skin and respiratory sensitisation

The preceding paragraphs on skin and respiratory sensitisation are summarised in the separately provided summary tables. However, it is emphasised that the complete guidance text should be read in order to gain a correct and complete view of the described area.

R.7.3.7.1 Remaining uncertainty on sensitisation

Reliable data can be generated on skin sensitisation from well designed and well conducted studies in animals. The use of adjuvant in the GPMT may lower the threshold for irritation and so lead to false positive reactions, which can therefore complicate interpretation (running a pre-test with FCA treated animals can provide helpful information). In international trials, the LLNA has been shown to be reliable, but like the guinea pig tests is dependent on the vehicle used, and it can occasionally give false positive results with irritants. Careful consideration should be given to circumstances where exposure may be sub-optimal due to difficulties in achieving a good solution and/or a solution of sufficient concentration. In some circumstances inconsistent results from guinea pig studies, or between guinea pig and LLNA studies, might increase the uncertainty of making a correct interpretation. Finally, for existing human data consideration must be given to whether inter-individual variability is such that it is not scientifically sound to generalize from a limited test panel.

When considering whether or not a substance is a respiratory sensitizer, observations of idiosyncratic reactions in only a few individuals with hyper-reactive airways are not sufficient to indicate the need for classification.

Major uncertainties remain in our understanding of the factors that determine whether or not a substance is an allergen, and if so, what makes it a skin or a respiratory sensitizer.

R.7.3.7.2 Concluding on suitability for Classification and Labelling

REACH demands that all available information for a chemical is gathered and any lack of information is reported.

Skin sensitizers

Standard information required for skin sensitisation is described in Annex VII of REACH, i.e. for any substance manufactured or imported in quantity of 1 ton or more.

A substance can be classified as *skin sensitizer* following the flow chart for integrated testing strategy (ITS) reported in Table R.7.3-1 in Section R.7.3.8.3.

According to Directive 67/548/EEC[32], labelling for skin sensitisation is with symbol Xi, the indication of danger *irritant* and the risk phrase R43 (R43: May cause sensitisation by skin contact).

Respiratory sensitizers

In REACH, respiratory sensitizers are indicated for harmonised classification and labelling and regulated in Annex I of Directive 67/548/EEC. Annex XV in REACH lays down general principles for preparing dossiers to propose and justify harmonised classification and labelling of CMRs (carcinogenic, mutagenic, toxic for reproduction) and respiratory sensitizers.

Potential hazard for respiratory sensitisation cannot be easily addressed, as validated testing methods are currently not available. A probable hazard for respiratory sensitisation should be mentioned in the Safety Data Sheet.

Although no testing strategy is available, a substance could be classified as *respiratory sensitizer* by following the flow chart for integrated evaluation strategy (IES) reported in Section R.7.3.8.3 which is based on existing evidence.

According to Directive 67/548/EEC, labelling for *respiratory sensitizers* is with symbol Xn, the indication of danger *harmful* and the risk phrase R42 (R42: May cause sensitisation by inhalation). Concluding on suitability for chemical safety assessment: dose response assessment and potency

There is evidence that for both skin sensitisation and respiratory hypersensitivity dose-response relationships exist (although these are frequently less well defined in the case of respiratory hypersensitivity). The dose of agent required to induce sensitisation in a previously naïve subject or animal is usually greater than that required to elicit a reaction in a previously sensitised subject or animal; therefore the dose-response relationship for the two phases will differ. Little or nothing is known about dose-response relationships in the development of respiratory hypersensitivity by non-immunological mechanisms.

It is frequently difficult to obtain dose-response information from either existing human or guinea pig data where only a single concentration of the test material has been examined. With human data, exposure measurements may not have been taken at the same time as the disease was evaluated, adding to the difficulty of determining a dose response.

Dose-response data however, can be generated from local lymph node assays or, in exceptional cases, using specially designed guinea pig test methods. Such types of data can give data on induction and elicitation thresholds in these models, but it must be remembered these cannot be translated directly to human thresholds.

Measurement of potency

Appropriate dose-response data can provide important information on the potency of the material being tested. This can facilitate the development of more accurate risk assessments. This section refers to potency in the induction phase of sensitisation.

Neither the standard LLNA nor the GPMT/Buehler is specifically designed to evaluate the skin sensitising potency of test compounds, instead they are used to identify sensitisation potential for classification purposes. However, all could be used for some estimate of potency. The relative potency of compounds may be indicated by the percentage of positive animals in the

[32] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS). See section R.7 Introduction

guinea pig studies in relation to the concentrations tested. Likewise, in the LLNA, the EC3 value (the dose estimated to cause a 3-fold increase in local lymph node proliferative activity) can be used as a measure of relative potency (ECETOC, 2000). Often linear interpolation of a critical effect dose from the EC3 is proposed (ECETOC), but more advanced statistical approaches basing conclusions on the characteristic of the dose response curve and variability of the results is also used (Basketter et al., 1999, van Och et al., 2000). The dose-response data generated by the LLNA makes this test more informative than guinea pig assays for the assessment of skin sensitising potency. EC3 data correlate well with human skin sensitisation induction thresholds derived from historical predictive testing (Schneider et al., 2004; Griem, 2003; Basketter et al., 2005b). Accordingly, there are proposals for how this information may be used in a regulatory sense (Basketter et al., 2005b) and for risk assessment.

Derivation of a DNEL

Potency information, such as the LLNA EC3 value, can be utilised for the derivation of no-effect levels, that is – in this instance - the threshold required for the induction of skin sensitisation. It should be noted that thresholds for skin sensitisation should be expressed in terms of dose per unit area. As mentioned above, the EC3 value correlates well with thresholds observed in previously published human predictive test data and with clinical experience (reviewed in Basketter et al., 2007a). The EC3 value can then be extrapolated by the application of assessment factors (reflecting e.g. intra and inter-individual variability and vehicle matrix effects) to derive no-effect levels (expressed in µg/cm^{2}of skin) for use of specific skin sensitizers in defined exposure situations (Gerberick et al., 2001; Felter et al., 2002 and 2003; Basketter et al., 2006). The approach is commonly referred to as quantitative risk assessment (QRA) and has been deployed, with considerable effect, to identify safe exposure levels for a range of skin sensitising chemicals (Zachariae et al., 2003; Basketter et al., 2003). Most recently, this has been reported extensively for fragrance and preservative sensitizers (Api et al., 2007; Basketter et al., 2007b).

Guidance on how to use the potency information for qualitative assessment (see also Section E.3.4.2) and how to derive a DNEL as a second step in the safety assessment of sensitizers is given in Appendix R.8-10.

R.7.3.7.3 Additional considerations

Chemical allergy is commonly designated as being associated with skin sensitisation (allergic contact dermatitis), or with sensitisation of the respiratory tract (asthma and rhinitis). In view of this it is sometimes assumed that allergic sensitisation of the respiratory tract will result only from inhalation exposure to the causative chemical, and that skin sensitisation necessarily results only from dermal exposure. This is misleading, and it is important for the purposes of risk management to acknowledge that sensitisation may be acquired by other routes of exposure. Since adaptive immune responses are essentially systemic in nature, sensitisation of skin surfaces may theoretically develop from encounter with contact allergens via routes of exposure other than dermal contact (although in practice this appears to be uncommon). Similarly, there is evidence from both experimental and human studies which indicate that effective sensitisation of the respiratory tract can result from dermal contact with a chemical respiratory allergen. Thus, in this case, it appears that the quality of immune response necessary for acquisition of sensitisation of the respiratory tract can be skin contact with chemical respiratory allergens (Kimber et al., 2002). Such considerations have important implications for risk management. Thus, for instance, there is a growing view that effective prevention of respiratory sensitisation requires protection of both skin and respiratory tracts. This includes the cautious use of known contact allergens in products to which consumers are (or may be) exposed via inhalation, such as sprays. The generic advice is that appropriate strategies to minimise the risk of sensitisation to chemical allergens will require consideration of providing protection of all relevant routes of exposure.

R.7.3.7.4 Information not adequate

A WoE approach, comparing available adequate information with the tonnage-triggered information requirements by REACH, may result in the conclusion that the requirements are not fulfilled. In order to proceed in further information gathering the testing strategy given in the next Section R.7.3.8 can be adopted.

R.7.3.8 Integrated testing strategy (ITS) for sensitisation

R.7.3.8.1 Objective / General principles

Ensure that the objective of this testing strategy is to give guidance on a stepwise approach to hazard identification with regard to the endpoint; a key principle of the strategy is that the results of one study are evaluated before another is initiated. The strategy should seek to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

R.7.3.8.2 Preliminary considerations

The guidance given in Sections R.7.3.2 to R.7.3.4 above will have enabled the identification of the data gaps that need to be filled in to meet the requirements of REACH as defined in Annexes VI to XI. Careful consideration of existing toxicological data, exposure characteristics and current risk management procedures is recommended to ascertain whether the fundamental objectives of the ITS (see above) have already been met. Give guidance on other factors that might mitigate data requirements for the endpoint of interest e.g. possession of other toxic properties, characteristics that make testing technically not possible.

R.7.3.8.3 Testing strategies for sensitisation

Develop a testing strategy for the endpoint that takes account of existing data on toxicity, exposure characteristics as well as the specific rules for adaptation from standard information requirements, as described in column 2 of Annexes VII-X, together with some general rules for adaptation from standard information requirements in Annex XI.

Figure R.7.3-1 Integrated testing strategy for skin sensitisation

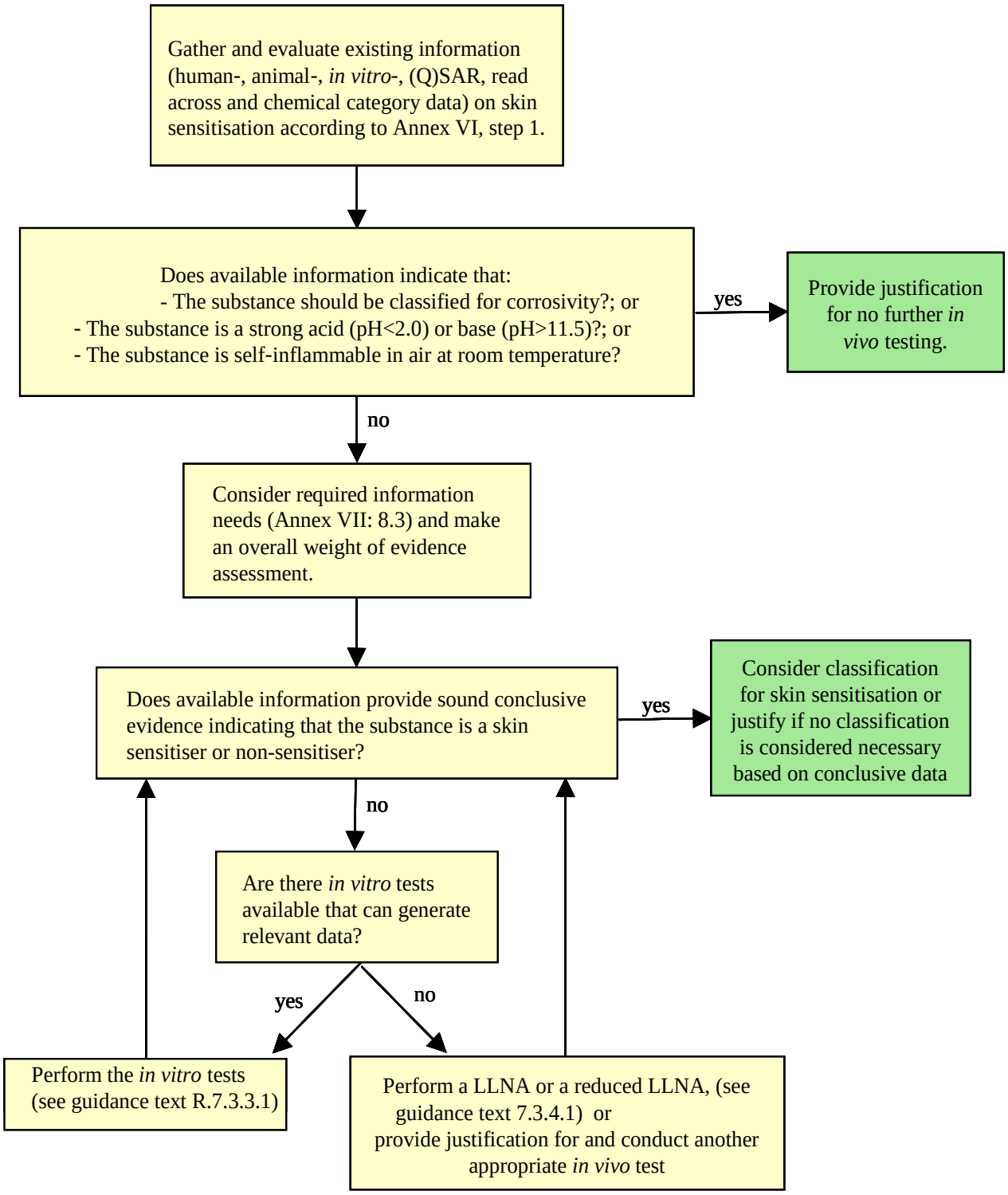
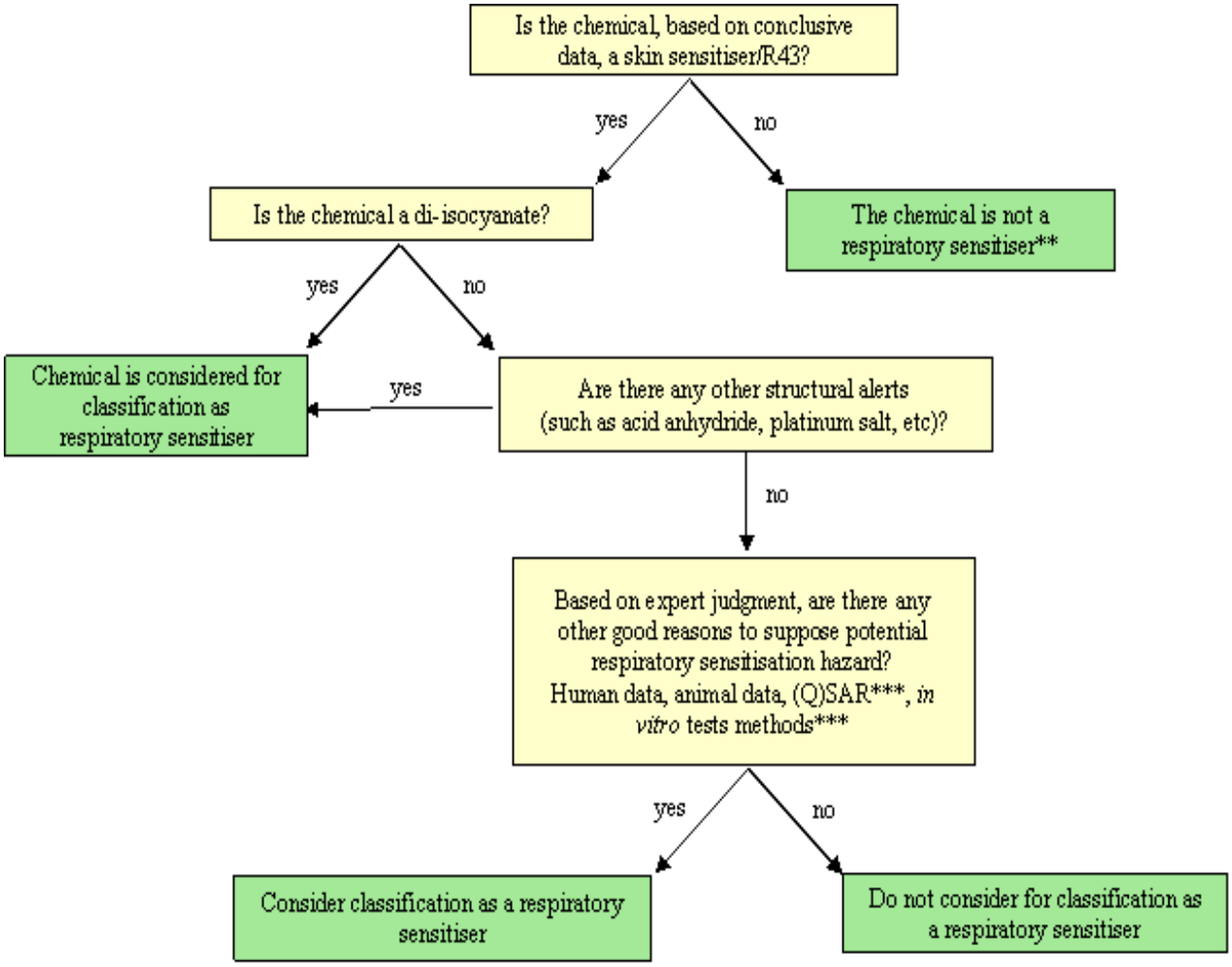


Figure R.7.3-2 Integrated evaluating strategy for respiratory sensitisation data*



* In contrast to tests for skin sensitisation, the performance of tests for respiratory sensitisation is currently not required under REACH. Therefore the present IES scheme depicts a strategy for evaluating existing data.
** This does not discount the possibility that the chemical may induce respiratory hypersensitivity through non-immunological mechanisms. Chemicals that act through such mechanisms are usually identified on the basis of evidence from human exposure.
*** not yet available

R.7.3.9 References on skin and respiratory sensitisation

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R.7.4 Acute toxicity

R.7.4.1 Introduction

Assessment of the acute toxic potential of a chemical is necessary to determine the adverse health effects that might occur following accidental or deliberate short-term exposure. The nature and severity of the acute toxic effects are dependent upon various factors, such as the mechanism of toxicity and bioavailability of the chemical, the route and duration of exposure and the total amount of chemical to which the person or animal is exposed.

R.7.4.1.1 Definition of acute toxicity

The term *acute toxicity* is used to describe the adverse effects, which may result from a single exposure (i.e. a single exposure or multiple exposures within 24 hours) to a substance. In the context of this guidance, exposure relates to the oral, dermal or inhalation routes. The adverse effects can be seen as clinical signs of toxicity (for animals, refer to OECD Guideline Document 19, 2000), abnormal body weight changes, and/or pathological changes in organs and tissues, which in some cases may result in death. In addition to acute systemic effects, some substances may have the potential to cause local irritation or corrosion of the gastro-intestinal tract, skin or respiratory tract following a single exposure. Acute irritant or corrosive effects due to the direct action of the chemical on the exposed tissue are not specifically covered by this document, although their occurrence may contribute to the acute toxicity of the chemical and must be reported. The endpoints of skin and eye irritation/corrosion and respiratory irritation are addressed in Section R.7.2.

At the cellular level acute toxicity can be related to three main types of toxic effect, (i) general basal cytotoxicity (ii) selective cytotoxicity and (iii) cell-specific function toxicity. Acute toxicity may also result from chemicals interfering with extracellular processes (ECVAM workshop report 16, 1996). Toxicity to the whole organism also depends on the degree of dependence of the whole organism on the specific function affected.

R.7.4.1.2 Objective of the guidance on acute toxicity

A chemical substance may induce systemic and/or local effects. This document is concerned with assessment of systemic effects following acute exposure.

Generally the objectives are to establish:

- whether a single exposure (or multiple exposures within 24 hours) to the substance of interest could be associated with adverse effects on human health; and/or
- what types of toxic effects are induced, their time of onset, duration and severity (all to be related to dose); and/or
- the dose-response relationships to determine the LD_{50}, the LC_{50}, the discriminating dose, or the acute toxic class; and/or
- when possible, the slope of the dose-response curve; and/or
- when possible, whether there are marked sex differences in response to the substance; and
- what information enables the classification and labelling of the substance for acute toxicity

The indices of LD_{50} and LC_{50} are statistically-derived values relating to the dose that is expected to cause death in 50% of treated animals in a given period; these values do not provide information on all aspects of acute toxicity. Indeed, information on lethality is not an essential requirement for the classification decision or risk assessment. Other parameters and observations and their type of dose response may yield valuable information. The potential to avoid acute toxicity testing should be carefully exploited by application of read-across or other non-testing means. Furthermore, there is an overriding obligation to minimize the use of animals in any assessment of acute toxicity.

For risk assessment, further considerations on the nature and reversibility of the toxic effects are necessary.

R.7.4.2 Information requirements for acute toxicity

The standard information requirements for acute toxicity under the REACH Regulations are as follows:

Annex VII (≥1 t/y): acute toxicity via the oral route of exposure is required;

Column 2 of Annex VII details specific rules for adaptation of these information requirements, notably allowing for the waiving of acute oral toxicity testing if the substance is corrosive to the skin or if a study on acute toxicity by the inhalation route is available.

Annex VIII -X (≥ 10 t/y): acute toxicity via the oral and dermal or inhalation route of exposure.

Column 2 of Annex VIII details specific rules for adaptation, notably requiring information on at least one other route of exposure depending on the nature of the substance and the likely route of human exposure (for details see Annex VIII Section 8.5); as for Annex VII, allowance is made for the waiving of acute oral toxicity testing if the substance is corrosive to the skin.

If there is any reason (alert from existing data) for a concern of acute toxicity at non-corrosive levels, one could point out needs to address this.

R.7.4.3 Information and its sources on acute toxicity

Information on acute toxicity, as detailed below, can be obtained from a variety of sources including unpublished studies, data bases and publications such as books, scientific journals, criteria documents, monographs and other publications (see Chapter R.3 for further general guidance).

R.7.4.3.1 Non-human data on acute toxicity

Non-testing data on acute toxicity

Non-testing data can be provided by the following approaches: a) structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), collectively called (Q)SARs; b) expert systems incorporating (Q)SARs and/or expert rules; and c) grouping methods (read-across and categories. These approaches can be used to assess acute toxicity if they provide relevant and reliable (adequate) data for the chemical of interest. Guidance on how to assess the relevance and reliability of non-testing data is provided in the general guidance on (Q)SARs in Section R.6.1 and on grouping approaches in Section R.6.2. Non-testing methods should be documented according to the appropriate reporting formats (see Sections R.6.1.9 and R.6.2.6). In the case of (Q)SARs and expert systems, a detailed description of available models is provided in the JRC QSAR Model Database (http://qsardb.jrc.it/).

Compared with some endpoints, there are relatively few (Q)SAR models and expert systems capable of predicting acute toxicity. Available approaches have been reviewed in the literature (Cronin *et al*., 1995,2003; Lessigiarska *et al*., 2005; Tsakovska et al., 2006). On the basis of these reviews, the following conclusions can be made: a) the relatively small number of models for *in vivo* toxicity is related to the nature of the endpoint – acute toxicity measurements are usually related to whole body phenomena and are therefore very complex. The complexity of the mechanisms involved leads to difficulties in the QSAR modelling process; b) most QSAR models identify hydrophobicity as a parameter of high importance for the modelled toxicity. In addition, many models indicate the role of the electronic and steric effects; c) most literature-based models are restricted to single classes of chemicals, such as phenols, alcohols, anilines. Models based on more heterogeneous data sets are those incorporated in the expert systems.

In the sections below some examples are given in order to illustrate the potential possibility for applying the (Q)SAR approaches for the acute toxicity endpoint for predictive purposes or to investigate the mechanisms of toxicity.

(Q)SAR models

QSARs on inhalation toxicity

Some simple regression models have been developed for predicting the inhalational toxicity of volatile substances, and these can be used reliably within their domains of applicability. Typically, parameters such as vapour pressure (VP) and boiling point (BP) have been found to be useful predictors of the acute toxic effect (e.g. LC_{50} value). These models are based on the assumption that toxicity occurs by the non-specific mechanism of narcosis, and that the LC_{50} data are based on tests in which a steady-state concentration has been reached in the blood. These models are suitable only for systemic acting volatile compounds.

For example, acute (non-lethal) neurotoxicity data for the neurotropic effects of some common solvents on both rats (whole-body exposures for 4h) and mice (whole-body exposures for 2h), taken from Frantik et al (1996), were subjected to QSAR analysis by Cronin (1996). Stepwise regression analysis of the 4-hr toxicity data causing the 30% depression in response ($log1/ECR_{30}$) in rats gave the following equation:

$$log1/ECR_{30} = 0.361\ ClogP - 0.117\ {}^{0}c - 1.76$$

$$n = 37\ R^2 = 0.817 \quad s = 0.280 \quad F = 35.2$$

This relationship demonstrates a partial dependence of neurotoxicity with the octanol-water partition coefficient, logP. The negative correlation with the zero-order molecular connectivity ${}^{0}c$ is thought to be an indication that the membrane permeability of blood-brain barrier is reduced for large molecules.

Stepwise regression for mouse neurotoxicity gave the following equation:

$$log1/ECM_{30} = 0.212\ ClogP + 0.00767\ BP - 0.176\ {}^{0}c - 2.03$$

$$n = 39\ R^2 = 0.811 \quad s = 0.271 \quad F = 22.4$$

in which BP is the boiling point of the substance (BP is inversely related to vapour pressure).

The application of principal components analysis (PCA), to separate compounds of high neurotoxicity from those of low neurotoxicity, suggested that in addition to partitioning through a membrane (determined by logP and molecular size), aqueous solubility and volatility

are also important factors governing neurotoxicity (Cronin, 1996). Metabolism to more toxic compounds is suggested as a possible cause of compounds appearing as outliers in the QSARs.

QSARs for predicting LD_{50}

There are references in the literature to a few models for predicting LD_{50}, generally for small sets of compounds. For example, Hansch & Kurup (2003) developed the following QSAR to predict the toxicity of barbiturates (LD_{50}) in for female white mice, using toxicity data from Cope and Hancock (1939):

$$\log 1/LD_{50} = -1.44 \log P + 0.16 NVE - 8.70$$

$$n = 11 \quad R^2 = 0.924 \quad s = 0.077 \quad R^2_{cv} = 0.879$$

where NVE is the number of valence electrons (used as a measure of polarisability).

QSARs for predicting human toxicity

The same descriptors were used to predict the LD_{100} of miscellaneous drugs to humans, using toxicity data from King (1985):

$$\log 1/C = 0.61 \log P + 0.017 NVE + 1.44$$

$$n = 36 \quad R^2 = 0.850 \quad s = 0.438 \quad R^2cv = 0.817$$

QSARs for predicting *in vitro* effects

A number of QSAR models for predicting *in vitro* effects are cited in the literature (reviewed in Tsakovskaet al., 2006), but these are not directly relevant to the assessment of acute toxicity for regulatory purposes. In general, these models have been developed to investigate the mechanisms of cytotoxic action, and they outline the role of hydrophobicity as well electronic descriptors, including electrotopological state descriptors (Lessigiarska *et al*., 2006), bond dissociation energies (Selassie *et al*., 1999), and dissociation constants (Moridani *et al*., 2003). While these models are not directly relevant to the assessment of acute toxicity, the fact that reliable QSARs can be developed for the *in vitro* cytotoxicity of defined groups of chemicals indicates that the approach of modelling *in vitro* data should be further explored with a view to integrating such QSARs into the ITS for acute toxicity. For example, a battery of QSARs could be developed for predicting the *in vitro* data of a validated *in vitro* test, and then used to supplement or replace *in vivo* testing.

Expert systems

For heterogeneous groups of compounds, expert systems are available in which rule bases express generalised relationships between chemical structure and toxicity. In knowledge-based experts systems (see also Section R.6.1), such as HazardExpert, such rules are derived from human expert opinion. In statistically based expert systems, such as TOPKAT and MultiCASE, statistical methods were used to derive (Q)SAR models (see also Section R.6.1).

HazardExpert

HazardExpert is a module of Pallas software developed by CompuDrug Limited (http://www.compudrug.com). The program works by searching the query structure for known toxicophores, which are stored in the “Toxic Fragments Knowledge Base” and which include substructures exerting both positive and negative modulator effects. Once a toxicophore has been identified, this triggers estimates for a number of toxicity endpoints, including neurotoxicity. The default knowledge base of the system is based on a US-EPA report (Brink and Walker, 1987) and scientific information collected by CompuDrug Limited. This program

can be linked to MetabolExpert, another module of the Pallas software, to predict the toxicity of the parent compound and its metabolites. Information on the validity of the model is not available. Investigations on the validity and applicability of HazardExpert are needed before recommendations can be made about its regulatory use.

TOPKAT

The TOPKAT software package employs cross-validated quantitative structure-toxicity relationship (QSTR) models for assessing various measures of toxicity (http://accelrys.com/products/discovery-studio/toxicology/). The Rat Oral LD_{50} module of TOPKAT includes 19 QSAR regression models for different chemical classes. The models are based on a number of structural, topological and electrophysiological indices, and they make predictions of the oral acute median lethal dose in the rat (LD_{50}).

The TOPKAT rat oral LD_{50} models are based on experimental data from the RTECS. Since RTECS lists the most toxic value when multiple values exist, the TOPKAT model tends to overestimate the toxicity of query structures.

The Rat Inhalation LC_{50} module of TOPKAT contains five submodels related to different chemical classes.

TOPKAT models, including the models for acute oral toxicity, have been used by Danish EPA to evaluate the dangerous properties of around 47 000 organic substances on the EINECS list [17]. An external evaluation of this model using 1840 chemicals not contained in the TOPKAT database gave poor results (R^2 = 0.31). However, 86% of estimations fall within a factor of 10 from test results (DK EPA study).

The Danish EPA concluded that the TOPKAT model is sufficient to give an indication of the least strict classification for acute toxicity, Xn; R22. An Internet version of the Danish QSAR database is accessible from the ECB website (http://qsardb.jrc.it).

MultiCASE

The MultiCASE software (http://www.multicase.com) contains an acute toxicity module, which consists of a rat LD_{50} model based on 7920 compounds from compilations by FDA, NTP and WHO data. Information on the validity of the model is not available. Investigations on the validity and applicability of MultiCASE are needed before recommendations can be made about its regulatory use.

Testing data on acute toxicity

In vitro data

There are currently no *in vitro* tests that have been officially adopted by the EU or OECD for assessment of acute toxicity.

However, a number of *in vitro* tests for acute toxicity are undergoing a validation process:

Two In vitro basal cytotoxicity assays for predicting starting doses for in vivo oral toxicity tests and lethal concentrations in man have undergone peer review by ICCVAM, namely the BALB/c 3T3 NRU & normal human keratinocyte (NHK) NRU assays (http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_brd.htm).

Two in vitro tests pre-validated: TER and PCP in 2 renal cell lines (test battery). The loss of monolayer integrity is often an early indicator of nephrotoxicity in intact renal epithelia in vitro and reflects loss of renal function in vivo. Trans-epithelial resistance (TER), coupled with

enhanced paracellular permeability (PCP), is a good measure of this integrity. (Duff et al., 2002). These tests should be used in a WoE approach as alerts or correctors in respect to the basal cytotoxicity assays. Their contribution is under evaluation in A-Cute-Tox (see below).

A ECVAM validated test, the CFU-GM, to predict anticancer agents induced myelotoxicity in humans, is now under evaluation to widen its applicability domain to chemicals' induced toxicity (http://ecvam-dbalm.jrc.ec.europa.eu/). If sufficiently validated and suited to the purpose of assessment of acute toxicity, this could be included in a WoE.

The integrated project A-Cute-Tox (A 5-year 6th FP project initiated in 2005) is addressing the possible replacement of the acute oral systemic toxicity tests (http://www.acutetox.org/). Particular attention should be given in the future to results of the project.

Animal data

Data may be available, particularly for phase-in substances, from a wide variety of animal studies, which give different amounts of direct or indirect information on the acute toxicity of a substance; e.g.:

- OECD TG 420 (EU B.1 bis) Acute oral toxicity – Fixed dose procedure
- OECD TG 423 (EU B.1tris) Acute oral toxicity – Acute toxic class method
- OECD TG 425 Acute oral toxicity – Up-and-down procedure
- OECD 401 (EU B.1) Acute Oral Toxicity (method deleted from the OECD Guidelines for testing of chemicals and from Annex V to Directive 67/548/EEC; see below)
- OECD TG 402 (EU B.3) Acute dermal toxicity
- OECD TG 403 (EU B.2) Acute inhalation toxicity
- Draft OECD TG 433 "Acute Inhalation Toxicity, Fixed Dose Procedure";
- Draft OECD TG 436 "Acute Inhalation Toxicity, Acute Toxic Class Method";
- Draft OECD TG 434 "Acute Dermal Toxicity, Fixed Dose Procedure";
- ICH compliant studies;
- Mechanistic and toxicokinetic studies;
- Studies in non-rodent species.

Traditionally, acute toxicity tests on animals have used mortality as the main observational endpoint, usually in order to determine LD_{50} or LC_{50} values. These values were regarded as key information for hazard assessment and supportive information for risk assessment. However, derivation of a precise LD_{50} or LC_{50} value is no longer considered essential. Indeed, some of the current standard acute toxicity test guidelines, such as the fixed dose procedures (OECD 420, EU B.1 bis and draft OECD 433), use signs of non-lethal toxicity and have animal welfare advantages over the other guidelines.

Existing OECD TG 401 (EU B.1) data would normally be acceptable but testing using this deleted method must no longer be performed.

In addition to current regulatory methods, acute toxicity data on animals may be obtained by conducting a literature search and reviewing all available published and unpublished toxicological or general data, and the official/existing acute toxicological reference values. For more extensive general guidance see Section R.3.1.

Utilising all the available information from sources such as those above, a *Weight of Evidence* approach should be taken to maximise use of existing data and minimise the commissioning of new testing.

When several data are available, a hierarchal strategy should be used to focus on the most relevant.

R.7.4.3.2 Human data on acute toxicity

Acute toxicity data on humans may be available from:

- Epidemiological data identifying hazardous properties and dose-response relationships;
- Routine data collection, poisons data, adverse event notification schemes, coroner's report;
- Biological monitoring/personal sampling;
- Human kinetic studies – observational clinical studies;
- Published and unpublished industry studies;
- National poisoning centres.

The main obstacles to the use of human data are their limited availability and often limited information on levels of exposure (ECETOC, 2004).

R.7.4.3.3 Exposure considerations for acute toxicity

With regard to acute toxicity, exposure considerations are detailed in column 2 in Annex VIII, but not in Annex XI. If there is only one demonstrated route of exposure, this route must be addressed. Where the potential for human exposure exists, the most likely route, or routes, of exposure should be determined so that the potential for acute toxicity by these routes can be assessed. Determination of the most likely route of exposure will have to take into account not only how the substance is manufactured and handled, including engineering controls that are in place to limit exposure, but also the physico-chemical properties of the substance, for instance, whether the substance is a solid or liquid, the particle size and proportion of respirable and inhalable particles, vapour pressure and log P.

R.7.4.4 Evaluation of available information on acute toxicity

The detailed generic guidance provided in Chapter R.4 on the process of judging and ranking the available data for its adequacy (reliability and relevance), completeness and remaining uncertainty is relevant to information on acute toxicity.

R.7.4.4.1 Non-human data on acute toxicity

Non-testing data on acute toxicity

Physico-chemical properties[33]

It may be possible to infer from the physico-chemical characteristics of a substance whether it is likely to be corrosive or absorbed following exposure by a particular route and, produce acute toxic effects. Physico-chemical properties may be important in the case of the inhalation route (vapour pressure, MMAD, log K_{ow}), determining the technical feasibility of the testing and acting upon the distribution in the airways in particular for *local-acting substances*. Indeed, some physico-chemical properties of the substance or mixture could be the basis for waiving testing. In particular, it should be considered for low volatility substances, which are defined as having vapour pressures **<** 1×10^{-5} kPa (7.5×10^{-5} mmHg) for indoor uses, and **<** 1×10^{-4} kPa (7.5×10^{-4} mmHg) for outdoor uses. Furthermore, inhalable particles are capable of entering the respiratory tract via the nose and/or mouth, and are generally smaller than 100 µm in diameter. Particles larger than 100 µm are less likely to be inhalable. In that way, particular attention should be driven on results of aerosol particle size determination.

[33] Refer also to Tables R.12-1 to R.12-6 in Section R.7.12

In particular, for substances in powder form, particle size of the material decisively influences the deposition behaviour in the respiratory tract and potential toxic effects. Particle size considerations (determined by e.g. granulometry testing, OECD 110) can be useful for:

- selecting a representative sample for acute inhalation toxicity testing;
- assessing the respirable and inhalable fractions, preferably based on aerodynamic particle size;
- justifying derogations from testing, for instance, when read-cross (or chemical grouping approach) data can be associated with results from particle size distribution analyses (see Section R.6.2).

Physico-chemical properties are also important for determination of the potential of exposure through the skin, for example, log K_{ow}, molecular weight and volume, molar refraction, degree of hydrogen bonding, melting point (Hostýnek, 1998).

Read-across to structurally or mechanistically similar substances (SAR)

Generic guidance on the application of grouping approaches is provided in Section R.6.2.

(Q)SAR

Several (Q)SAR systems are available that can be used to make predictions about, for example, dermal penetration or metabolic pathways (see cross-cutting QSAR guidance for list of models). However, these systems have not been extensively validated against appropriate experimental data and it has not been yet verified if the results genuinely reflect the situation *in vivo*. That is why the modelled data can be used for hazard identification and risk assessment purposes only as part of a WoE approach.

The complexity of the acute toxicity endpoint (possibility of multiple mechanisms) is one of the reasons for limited availability and predictivity of QSAR models. In the absence of complete validation information, available models could be used as a part of the WoE approach for hazard identification and risk assessment purposes after precise evaluation of the information derived from the model.

Evaluation of the validity of the method

An evaluation of model validity according to OECD principles should be available, as described in Section R.6.1.

Evaluation of the reliability of the individual prediction

The reliability of individual (Q)SAR predictions should be evaluated, as described in Section R.6.1.
The evaluations of model validity and estimate reliability should be documented according to the appropriate reporting formats, as described in Section R.6.1.
In the case of grouping approaches, adequacy should be assessed and documented according to guidance described in Section R.6.2.

Testing data on acute toxicity

In vitro data

The *in vitro* tests that are currently available provide supplementary information, which may be used to determine starting doses for *in vivo* studies, assist evaluation of data from animal studies, especially in identification of species differences, or to increase understanding of the toxicological mechanism of action of the substance. They cannot be used to replace testing in animals completely, although this may be possible in the future.

The outcome of the EU-US (ECVAM-ICCVAM) validation study on the Use of In Vitro Basal Cytotoxicity Test Methods For Estimating Starting Doses For Acute Oral Systemic Toxicity (http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_brd.htm) was that the Peer Review Panel agreed that the applicable validation criteria have been adequately addressed for using these *in vitro* test methods in a WoE approach to determine the starting dose for acute oral *in vivo* toxicity protocols. Moreover, on the basis of a preliminary analysis of data, there is the indication that the cytotoxicity tests might be useful in predicting low toxicity substances (LD_{50}[3] 2g/kg body weight) and that they might therefore be used to filter these out in the future. This application needs to be validated with a wider range of compounds.

In vitro data may be useful for predicting acute toxicity in humans providing that the domain of applicability for the test method is appropriate for the class of chemical under evaluation and a range of test concentrations have been investigated that permit calculation of an IC_{50} (inhibitory concentration 50%) value. Indeed, on the basis of a preliminary comparison of data, there is the indication that the results of *in vitro* cytotoxicity tests may be more predictive of acute oral toxicity in humans than rat or mouse data. This aspect needs to be further investigated.

Generic guidance is given in Chapter R.4 for judging the applicability and validity of the outcome of various study methods, assessing the quality of the conduct of a study (including how to establish whether the substance falls within the applicability domain of the method and the validation status for the given domain) and aspects such as vehicle, number of duplicates, exposure/ incubation time, GLP-compliance or comparable quality description.

Animal data

Acute toxicity tests on animals have primarily used mortality as the main observational endpoint, usually in order to determine LD_{50} or LC_{50} values, although some of the current standard protocols, such as the fixed dose procedure (OECD TG 420, EU B.1 bis), use evident signs of toxicity in place of mortality. In many cases, there will be little information on the cause of death or mechanism underlying the toxicity, and only limited information on pathological changes in specific tissues or clinical signs, such as behavioural or activity changes.

Many acute toxicity studies on chemicals of low toxicity are performed as limit tests. For more harmful chemicals choice of optimum starting dose will minimize use of animals. When multiple dose levels are assessed, characterisation of the dose-response relationship may be possible and signs of toxicity identified at lower dose levels may be useful in estimating LOAELs or NOAELs for acute toxicity. For local acting substances, mortality after inhalation may occur due to tissue damage in the respiratory tract. In these cases, the severity of local effects may be related to the dose or concentration level and therefore, it might be possible to identify a LOAEL or NOAEL. For systemic toxicity, there could be some evidence of target organ toxicity (pathological findings have to be documented) or signs of toxicity based on clinical observations.

Whichever approach is used in determining acute toxicity critical information needs to be derived from the data to be used in risk assessment. It is important to identify those dose levels which produce signs of toxicity, the relationship of the severity of these with dose and the level at which toxicity is not observed (i.e. the acute NOAEL).

In addition to current available OECD or EU test methods (see Section R.7.4.3), alternative *in vivo* test methods for assessment of acute dermal and inhalation toxicity are in the process for adoption and use for regulatory purposes. Whichever test is used to evaluate acute toxicity on animals, the evaluation of studies takes into account the reliability based on the approach of Klimisch *et al*. (1997) (standardised methods, GLP, detailed description of the publication), the relevance, and the adequacy of the data for the purposes of evaluating the given hazard from

acute exposure (for more guidance see Section R.4.2). The best studies are those that give a precise description of the nature and reversibility of the toxic effect, the number of subjects, gender, the number of animals affected by the observed effects and the exposure conditions (atmosphere generation for inhalation, duration and concentration or dose). The relevance of the data should be determined in describing the lethal or non-lethal endpoint being measured or estimated.

In addition, when several studies results are available for one substance, the most relevant one should be selected; data from others studies that have been evaluated should be considered as supportive data for the full evaluation of the substance.

The classification criteria for acute inhalation toxicity relate to a 4-hour experimental exposure period. If data for a 4-hour period are not available then extrapolation of the results to 4 hours are often achieved using Haber's Law (C.t = k). However, there are limits to the validity of such extrapolations, and it is recommended that the Haber's Law approach should not be applied to experimental exposure durations of less than 30 minutes or greater than 8 hours in order to determine the 4-hour LC_{50} for C&L purposes.

Nowadays a modification of Haber's Law is used ($C^n.t = k$) as for many substances it has been shown that n is not equal to 1 (Haber's Law). In case extrapolation of exposure duration is required, the *n* value should be considered. If this *n* value is not available from literature, a default value may be used. It is recommended to set n = 3 for extrapolation to shorter duration than the duration for which the LC_{50} or EC_{50} was observed and to set n = 1 for extrapolation to longer duration (ACUTEX TGD, 2006), also taking the range of approximately 30 minutes to 8 hours into account.

Experimentally, when concentration-response data are needed for specific purposes, OECD TG 403 (EU B.2) or the CxT approach could be taken into consideration. The OECD TG 403/(EU B.2 will result in a concentration-response curve at a single exposure duration, the CxT approach will result in a concentration-time-response curve, taking different exposure durations into account. The CxT approach (under consideration for the revision of OECD TG 403) uses two animals per CxT combination and exposure durations may vary from about 15 minutes up to approximately 6 hours. This approach may provide detailed information on the concentration-time-response relationship in particular useful for risk assessment and determination of NOAEL/LOAEL.

R.7.4.4.2 Human data on acute toxicity

When available, epidemiological studies, case reports, information from medical surveillance or volunteer studies may be crucial for acute toxicity and can provide evidence of effects that are undetectable in animal studies (e.g. symptoms like nausea or headache). Nevertheless, the conduct of human studies is not recommended.

Such data could also be useful to identify particular sensitive sub-populations like new born, children, patients with diseases (in particular with chronic respiratory, e. g. asthma, BPOC).

Additional guidance should be provided on the reliability and the relevance of human studies because there are no standardised guidelines for such studies (except for odour threshold determination) and these are not usually conducted according to GLP. Such guidance is provided in Section R.4.3.3.

R.7.4.4.3 Exposure considerations on acute toxicity

Particular attention should be addressed to the potential routes of exposure in humans to select the appropriate testing strategy.

Generic aspects of data waivers based on exposure considerations are presented in Section R.5.1. Information on the role of exposure information in the testing strategies for acute toxicity is presented in Section R.7.4.6.

R.7.4.4.4 Remaining uncertainty on acute toxicity

In most cases, remaining uncertainties will exist due to the absence of valid human acute toxicity data, and so appropriate assessment factors should be applied. Toxicokinetic data could help in deriving chemical-specific interspecies assessment factors. As acute toxicity testing does not usually include clinical chemistry, haematology and detailed histopathology and functional observations, an additional assessment factor may need to be applied when a NOAEL or LOAEL from these studies is used to derive DNELs (for more guidance on the setting of DNELs for acute toxicity, see Chapter R.8, Appendix R.8-8).

R.7.4.5 Conclusions on acute toxicity

R.7.4.5.1 Concluding on suitability for Classification and Labelling

In order to achieve classification and labelling, Annex VI of the Dangerous Substances Directive 67/548/EEC[34] must be applied. The criteria for classification are based on specific 'cut offs' based on the LD_{50} or LC_{50}, although determination of a precise LD_{50} or LC_{50} value is not essential for classification purposes. This is because the LD_{50}/LC_{50} is not an absolute value (Schütz, 1969) since many factors influence its reproducibility (Zbinden and Flury-Roversi, 1981).

Ideally, classification and labelling should be achieved using data generated from studies conducted in accordance with officially adopted OECD test guidelines, or test methods incorporated for the time being into Annex V of Directive 67/548/EEC[35]. Such studies will permit identification of the LD_{50}, LC_{50}, the discriminating dose (fixed dose procedures), or a range of exposure where lethality and/or severe toxicity is expected (acute toxic class methods). For materials of low toxicity (no mortalities expected at the upper dose limit) testing is restricted to this dose level (the limit test) and if absence of mortalities is confirmed, classification of the substance with respect to acute toxicity is not required.

In the Up-and-Down Procedure (OECD TG 425), where individual animals are dosed sequentially, estimation of the LD_{50} with a confidence interval is possible and this can be used for classification purposes. Data generated in the fixed dose/concentration procedures (OECD TG 420, draft 433 and 434 and EU B.1 bis) and the acute toxic class methods (OECD TG 423, draft TG 436 and EU B.1 tris) are equally sufficient for classification purposes. In the fixed dose/concentration procedures, the discriminating dose is identified as the dose causing evident toxicity but not mortality, and must be one of the four dose levels specified in the test method. Evident toxicity is a general term describing clear signs of toxicity such that at the next highest dose level, either severe pain and enduring signs of severe distress, moribund status or probable mortality can be expected in most animals. In the acute toxic class methods, the range of exposure where death is expected is determined by testing at one or more of the four fixed doses. The OECD and EU guidelines for fixed dose procedure and acute toxic class methods include flow charts that allow conclusions to be drawn with respect to GHS

[34] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

[35] The new Test Methods Regulation is currently (February 2008) under adoption and contains all the test methods previously included in Annex V to Directive 67/548/EEC.

classification. In addition the flow charts in the acute toxic class methods allow identification of LD_{50} or LC_{50} cut offs. In the absence of GLP compliant data generated in accordance with OECD or EU methods, all other available information should be considered. Each individual set of data (e.g. a non-GLP study) must be assessed for reliability and relevance as stated in Section R.7.4.4 and any unsuitable data (i.e. that considered unreliable or not relevant) should be disregarded. When experimental data for acute toxicity are available in several animal species, scientific judgement should be used in selecting the most relevant data from among the valid, well-performed tests. When equally reliable data from several species are available, priority should be given to the data relating to the most sensitive species, unless there are reasons to believe that this species is not an appropriate model for humans. If definitive classification and labelling cannot be achieved from any individual source, but multiple sets of data all lead to the same conclusion, then, the WoE approach might be sufficient to classify and a robust proposal detailing this should be put forward.

Where evidence is available from both humans and animals and there is a conflict between the findings, the quality and reliability of the evidence from both sources shall be evaluated in order to resolve the question of classification. Generally, data of good quality and reliability in humans shall have precedence over other data. However, well designed and conducted epidemiological studies may lack the sufficient number of subjects to detect relatively rare, but nevertheless important, effects. Also, the interpretation of many studies is hampered by difficulties in identifying and taking account of confounding factors. Positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness and quality of both the human and animal data.

If the existing data are contradictory, not concordant or insufficient to reliably determine the appropriate classification and labelling of the substance, additional *in vitro* studies, QSARs, read-across should be considered before conducting any OECD or EU compliant *in vivo* study. In that way *in vitro* data could have a supporting role in a read-across or chemical grouping approach. Study data, which permit an assessment of dose response relationship, should be considered for risk assessment and classification and labelling.

Of particular importance in classifying for inhalation toxicity is the use of well-articulated values in the high toxicity categories for dusts and mists. Inhaled particles between 1 and 4 microns mean mass aerodynamic diameter (MMAD) will deposit in all regions of the rat respiratory tract. This particle size range corresponds to a maximum dose of about 2 mg/L (draft OECD GD 39). In order to achieve applicability of animal experiments to human exposure, dusts and mists would ideally be tested in this range in rats. The cut off values in the table for dusts and mists allow clear distinctions to be made for materials with a wide range of toxicities measured under varying test conditions.

Currently, non-animal test data (e.g. *in vitro*, QSARs and read-across data) cannot be used as stand-alone for classification and labelling purposes, but can be used for classification to support a read-across argument. In future they might be used in different purposes when such methods have been formally validated and incorporated into official test guidelines, and when classification systems have been adapted to take account of such data.

R.7.4.5.2 Concluding on suitability for Chemical Safety Assessment

For chemical safety assessment, both standard OECD/EU test guideline data and all applicable data considered both reliable and relevant should be used. A quantitative rather than qualitative assessment is preferred to conclude on the risk posed by a substance with regards to acute toxicity dependent on the data available and the potential exposure to the substance during the use pattern/lifecycle of the substance. If quantitative data are not available, the nature and the severity of the specific acute toxic effects can be used to make specific recommendations with respect to handling and use of the substance.

Information on acute toxicity is not normally limited to availability of a LD_{50} or LC_{50} value. Additional information which is important for the chemical safety assessment will be both qualitative and quantitative and will include parameters such as the nature and severity of the clinical signs of toxicity, local irritant effects, the time of onset and reversibility of the toxic effects, the occurrence of delayed signs of toxicity, body weight effects dose response relationships (the slope of the dose response curve), sex-related effects, specific organs and tissues affected, the highest non-toxic and lowest lethal dose (adapted from ECETOC Monograph No. 6, 1985).

If a NOAEL can be identified this can be used in determination of a DNEL. However, depending upon the nature of the acute toxicity information available, this may not always be possible. For instance, data from an OECD/EU test method may permit calculation of an LD_{50}/LC_{50} value, or identification of the range of exposure where lethality is expected, or the dose at which evident toxicity is observed, but may not provide information on the dose level at which no adverse effects on health are observed. If the data permits construction of a dose-response curve, then derivation of the NOAEL may be possible. When a limit test has been conducted, and no adverse effects on health have been observed, then the limit dose can be regarded as the NOAEL. If adverse effects on health are seen at the limit dose then it is unlikely that lower dose levels will have been investigated and in this case identification of the NOAEL will not be possible. If data is available for several species, then the most sensitive species should be chosen for the purposes of the Chemical Safety Assessment, provided it is the most relevant to humans.

If human data on acute toxicity is available, it is unlikely that this will be derived from carefully controlled studies or from a significant number of individuals. In this situation, it may not be appropriate to determine a DNEL from this data alone, but the information should certainly be considered in the WoE and may be used to confirm the validity of animal data. In addition, human data should be used in the risk assessment process to be able to determine DNEL for particular sensitive sub-populations like new-born, children or those in poor health (patients).

More extensive guidance on the setting of DNELs for acute toxicity, see Chapter R.8, Appendix R.8-8.

The anticipated effects from physico-chemical properties and bioavailability data on the acute toxicity profile of the substance must also be considered in the Chemical Safety Assessment.

R.7.4.5.3 Information not adequate

A WoE approach, comparing available adequate information with the tonnage-triggered information requirements by REACH, may result in the conclusion that the requirements are not fulfilled.

In absence of data from test guidelines or equivalent methods, data from other endpoints could be helpful for the determination of acute toxicity potential. For example, data could be provided by subchronic toxicity or neurotoxicity studies, as in general the design of these studies includes a pilot study to determine dose of departure for the main test. In order to proceed with further information gathering the following testing strategy can be adopted.

R.7.4.6 Integrated Testing Strategy (ITS) for acute toxicity

R.7.4.6.1 Objective / General principles

The main objective of this Integrated Testing Strategy (ITS) is to provide advice on how the REACH Annex VII and VIII information requirements for acute toxicity can be met using the most humane methods. If the ITS is followed, the information generated will be sufficient to make a classification decision with respect to acute toxicity hazard and may provide data for

the risk assessment and DNEL derivation. In addition, assessment of acute toxicity may provide information that is valuable for the conduct of repeated dose toxicity studies, such as identification of target organ toxicity and dose selection.

By adhering to the criteria outlined in the previous chapters, informed decisions may be made on whether sufficient data already exist to cover the objectives, or whether further testing is required.

If further testing is deemed necessary, the use of the most appropriate study in accordance with the REACH proposal is considered rather than a *one study fits all* approach. An overarching principle is that all data requirements are met in the most efficient and humane manner so that animal usage and costs are minimized.

R.7.4.6.2 Preliminary considerations

The standard information requirements for acute toxicity under the REACH regulations are given in Section R.7.4.2.

According to REACH, acute toxicity studies should not be conducted if a substance is known to be corrosive. However, if there are health concerns regarding exposure to non-corrosive concentrations, then acute toxicity assessment may be considered appropriate. In such cases, a specific protocol should be developed as standard LC_{50} or any other *in vivo* acute toxicity testing cannot be performed. For example, *in vitro* data on basal cytotoxicity could be used to establish the most appropriate range of concentrations to be tested.

Regardless of tonnage level, before any testing is triggered, careful consideration of existing toxicological data, exposure characteristics and current risk management procedures is recommended to ascertain whether the fundamental objectives of the ITS have already been met. This consideration should take account of discussions that have taken place under other regulatory schemes, such as ESR, DPD, BPD and the EU hazard classification scheme. If it is concluded that further testing is required, then a series of decision points are defined to help shape the scope of an appropriate testing program.

The following four-stage process has been developed for clear decision-making:

Stage 1. gather existing information according to Annex VI

Stage 2. consider information needs according to the relevant Annex VII to X

Stage 3. identify data gaps (and adequacy of all available data for classification and labelling and/or risk assessment, or to fulfil the criteria for waiving)

Stage 4. generate new data / propose testing strategy

R.7.4.6.3 Testing strategy for acute toxicity (see Figure R.7.4-1)

Stage 1. Gathering of existing information

The starting point of the ITS is the review of existing data (e.g. human or animal data, physico-chemical properties, (Q)SARs, *in vitro* test data). For non-corrosive substances, the results of skin and eye irritation and skin sensitisation studies (Annex VII) may provide useful information on the potential for systemic toxicity.

In the ITS, all existing human and test data (e.g. from clinical reports, poisoning cases, animal studies, corrosivity, physico-chemical properties) should be considered. Some information from the existing data e.g. *in vitro* studies (*de novo in vitro* basal cytotoxicity and dermal penetration studies), systemic effects observed in other studies, route of human exposure,

physico-chemical properties, dermal or respiratory toxicity of structurally-related substances, might primarily be used for the selection of either an acute *in vivo* inhalation test or an acute *in vivo* dermal test. No specific reference is made to valid (Q)SAR models/approaches or to valid *in vitro* methods, but such data should be assessed when available or generated.

Section R.7.4.3 presents a detailed discussion of the sources that may provide relevant information for the assessment of acute toxicity.

Stage 2. Considerations on information needs

A detailed evaluation of the existing information collated in Stage 1 is conducted to allow an informed decision on the testing needs to fulfil the REACH requirements. It is important to ensure that the available data are relevant and reliable to fulfil these requirements.

It should be noted that if a substance is predicted to be corrosive then further consideration should be given as to whether or not an acute oral test can be justified (in particular in relation with animal welfare considerations). Justifications for conducting a study must be provided in order to minimise the animal use. If the substance is considered likely to be corrosive, no acute toxicity testing should normally be conducted (see above). Where information on corrosivity is not available then *in vitro* corrosivity tests should be conducted.

The standard information requirements for acute toxicity under the REACH regulations are given in Section R.7.4.2.

When acute toxicity via a second route is required, the choice of the second route (dermal or inhalation) depends on the nature of the substance and the likely route of human exposure. However, information on only one route of exposure may be sufficient and justified (based on physico-chemical, toxicokinetic or human data and review of all possible exposure scenarios; for example with gases only inhalation route could be evaluated as no relevant human exposure may occur by oral or dermal route; for liquid with high viscosity, no testing by inhalation route should be conducted).

If human exposure is possible via inhalation, or if physico-chemical properties indicate that such exposure may occur, then testing via this route for acute toxicity should be conducted. Data from skin/eye irritation, skin sensitisation and acute oral toxicity should be used as indicators to help testing via inhalation (for example, substance with only potential local toxicity; choice of exposure concentrations). If no systemic effects are shown during acute oral testing, then the requirement to conduct inhalation testing should be considered on a case-by-case basis.

Consideration of the need for assessment of acute dermal toxicity should be given if the inhalation route is not considered appropriate. In some cases, it may be possible to draw conclusions about the potential for acute dermal toxicity without further testing, on the basis of the data available from acute oral toxicity and/or dermal absorption studies. Evidence for the potential of high dermal absorption should be considered on a case-by-case basis taking into account physico-chemical properties e.g. Log Kow, water solubility, molecular weight and melting point of the substance. Testing for acute dermal toxicity is indicated if:

Systemic toxicity is observed in skin/eye irritation and/or skin sensitisation studies;

Death is observed in an acute oral toxicity test and there is potential for dermal absorption;

Systemic toxicity is observed in an acute oral toxicity test and there is potential for high dermal absorption (determined following e.g. OECD TG 428, EU B.45)

There is the potential for high dermal exposure (case-by-case basis)

Stage 3. Identification of data gaps / adequacy of data

The purpose of this step is to identify what additional information is required in order to classify the substance and to perform a risk assessment.

The available information may include data generated using study protocols that differ from the standard regulatory tests. The evaluation should include whether the available information meets or exceeds the data requirements from standard regulatory study protocols. Therefore it may be possible that the tonnage-driven minimum needs can be met through combined data obtained from several sources.

At this stage, it is also necessary to verify if the available information is adequate for hazard characterisation. For this process, all relevant information should be taken account of in a weight of evidence assessment. Quantitative data on the dose response relationship for the critical toxicological effects and/or estimations of the either the LC_{50}/LD_{50} values or the Discriminating Dose will be important for assessing the hazard classification and can be used in the risk assessment. Information from testing for other toxicological endpoints (e.g. repeated dose toxicity) may also be useful for the risk assessment (see also Chapter R.8, Appendix R.8-8). Mathematical modelling should be considered for estimating a threshold exposure level (e.g. benchmark dose), as an alternative to generating additional *in vivo* data.

For the inhalation route, standard protocols involve a 4-hour exposure. If data for other time periods are available (e.g. for 0.5 to 8 hours), extrapolation to a 4-hour exposure period can be achieved using a modification of Haber's Law ($C^n.t = k$). If this «n» value is not available from the literature, a default value may be used; it is recommended to set n = 3 for extrapolation to shorter duration than the duration for which the LC_{50} or EC_{50} was observed and to set n = 1 for extrapolation to longer duration (ACUTEX TGD, 2006). Experimentally, the value of n can be determined using the CxT approach (draft revision OECD TG 403).

If the data and subsequent decisions are deemed consistent with an adequate hazard characterisation and are sufficient to classify the substance or to conduct a risk assessment, then no further testing for acute toxicity is recommended.

In some cases, the substance may be excluded from acute toxicity testing if it does not appear as scientifically necessary (Annex XI). This might be the case for example if

A WoE analysis demonstrates that the available information is sufficient for an adequate hazard characterisation and the exposure to the substance is adequately controlled;

The substance is not bio-available via a specific route and possible local effects are adequately characterised (example, no dermal absorption for dermal route)

For inhalation route, no testing is required if it is not technically possible to generate a testing atmosphere, the vapour pressure is very low (<0.1 Pa at 20°C) or the particle size is > 100 mm

Finally, the conclusion that no further testing is required may be reached when the data meet the requirements for classification for toxic effects or if the substance has already been classified for acute toxic effects.

Where evidence is available from both humans and animals and there is a conflict between the findings, the evidence should be evaluated towards understanding the toxicological basis for these divergent findings. Issues relating to the quality and reliability of the data should also be taken into account. Generally, data of good quality and reliability in humans shall take precedence over other data. However, well-designed and conducted epidemiological studies may lack a sufficient number of subjects to detect relatively rare but still significant effects, to assess potentially confounding factors. Positive results from well-conducted animal studies are

not necessarily negated by the lack of positive human experience but require an assessment of the robustness and quality of both the human and animal data.

If the remaining data are contradictory, not concordant or insufficient to determine reliably the appropriate classification and labelling of the substance, additional *in vitro* studies, QSARs, read-across should be considered before conducting any OECD compliant *in vivo* study. Study data, which permit an assessment of dose response relationship, should be considered particularly valuable for risk assessment purposes.

Stage 4. Generation of new data / proposal for testing strategy

If sufficient data for risk assessment and classification purposes are already available, no further testing will be required. If data gaps need to be filled, new data shall be generated (Annexes VII & VIII). Due to animal welfare considerations, new tests on animals should only be performed as a last resort when all other sources of information have been exhausted.

The standard OECD guidelines should normally be used as these provide the necessary information on acute toxicity hazard in a way that balances the need to protect human health with animal welfare concerns (see Section R.7.4.3 and the above guidance for Stage 3).

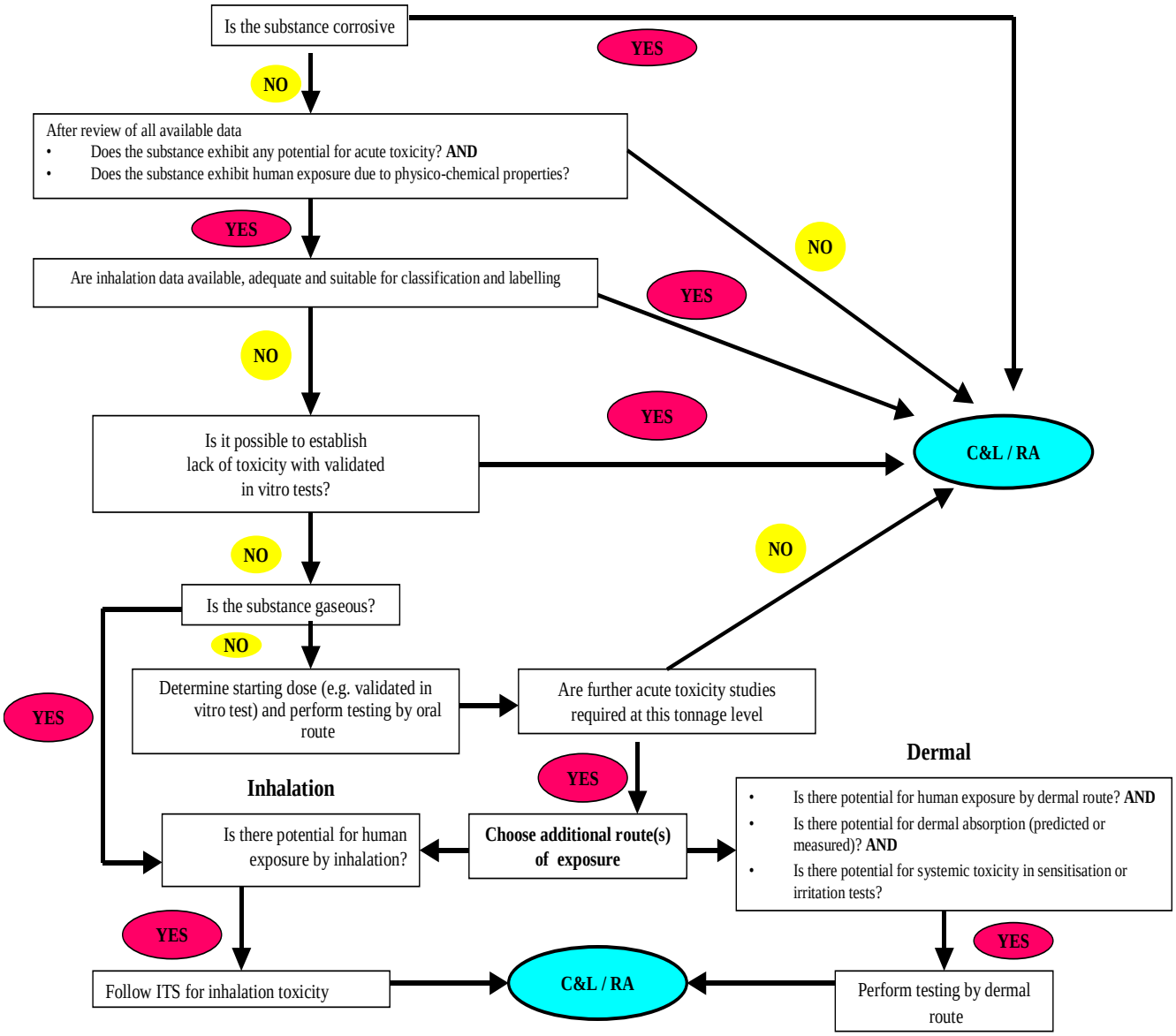
The route of exposure to be used for acute toxicity evaluation depends on the nature of the substance (e.g. gas or not, molecular weight, log K_{ow}) and should reflect the most likely route of human exposure. If any specific human exposure may be identified, further testing for risk assessment should be considered as proposed in Annex VIII. If any human exposure by inhalation is identified, then the testing strategy by inhalation should be proposed (Figure R.7.4-2).

First considerations should be based on defining the potential of the substance for acute toxicity. For such a question, information may be provided by existing data from SARs, QSARs, chemical categories approaches and available *in vitro* and *in vivo* data. If no potential for toxicity is shown, then no further testing is required and a decision on classification can be taken. Such information may also provide relevant information in risk assessment considerations.

Following the general testing strategy, dose selection appears to be an important aspect in order to select the most appropriate starting point. When validated *in vitro* tests are available, as shown by the joint ECVAM-ICCVAM study, these may provide relevant results, and help the dose selection for oral route testing (see Section R.7.4.4.1).

For substances in the ≥10 t/y tonnage band, testing by the dermal route should be considered if a human exposure is identified, or if results from physico-chemical properties and in particular skin irritation/sensitisation tests show any dermal absorption or any systemic toxicity. Depending on such information, dermal testing should be conducted or not following standard protocols (see Section R.7.4.3).

Figure R.7.4-1 ITS for acute toxicity endpoint

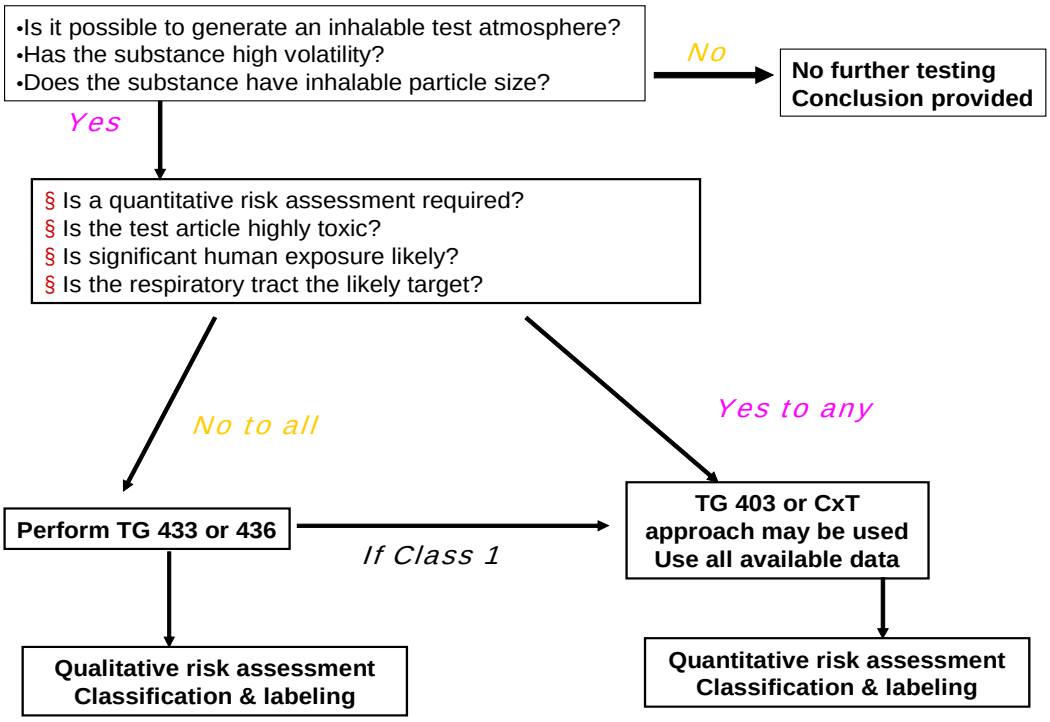


(*) if the substance is corrosive but there are health concerns regarding exposure to non-corrosive concentrations, then acute toxicity assessment may be considered appropriate
(**) Testing by inhalation may be required if the substance is a gas, a liquid or a solid with a high vapour pressure, or a solid with inhalable particle size (particular substances in powder form nanoparticles, fibres…)

A specific testing strategy (Figure R.7.4-2) is proposed for the inhalation route. Primary considerations should be based on the in(ability) to generate a suitable atmosphere depending on the physico-chemical properties (for example, low volatility, solid, particle size >100 mm (see also Section R.7.4.4.1). In this situation, no human exposure may be identified and no further testing is required.

Wherever possible, assessment of acute inhalation toxicity should be conducted in accordance with OECD TG's 433 and 436 (official adoption in process) since they have been designed to use less animals than OECD TG 403 and EU B.2. In addition, OECD TG 433 does not require mortality as endpoint. However, in some circumstances, i.e. if a dose response curve is needed for risk assessment purposes, testing according to OECD TG 403, EU B.2 or the CxT approach may be considered appropriate (see also draft OECD Guidance Document 39).

Figure R.7.4-2 ITS for acute inhalation toxicity endpoint (see also draft OECD GD 39)



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R.7.5 Repeated dose toxicity

R.7.5.1 Introduction

Repeated dose toxicity studies provide information on possible adverse general toxicological effects likely to arise from repeated exposure to a substance. Furthermore, these studies may provide information on e.g. reproductive toxicity and carcinogenicity, even though they are not specifically designed to investigate these endpoints.

Organs and tissues investigated in repeated dose toxicity studies include vital organs such as heart, brain, liver, kidneys, pancreas, spleen, immune system, lungs etc. Effects examined may include changes in morphology, physiology, growth or life span, behaviour which result in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in the susceptibility to the harmful effects of other environmental influences. Therefore, it is important that the possible adverse general toxicological effects are assessed for chemical substances that may be present in the environment.

R.7.5.1.1 Definition of repeated dose toxicity

The term *repeated dose toxicity* comprises the general toxicological effects occurring as a result of repeated daily dosing with, or exposure to, a substance for a part of the expected lifespan (sub-acute or sub-chronic exposure) or for the major part of the lifespan, in case of chronic exposure.

The term *general toxicological effects* (in this report often referred to as *general toxicity*) includes effects on, e.g. body weight and/or body weight gain, absolute and/or relative organ and tissue weights, alterations in clinical chemistry, urinalysis and/or haematological parameters, functional disturbances in the nervous system as well as in organs and tissues in general, and pathological alterations in organs and tissues as examined macroscopically and microscopically. Repeated dose toxicity studies may also examine parameters, which have the potential to identify specific manifestations of toxicity such as e.g., neurotoxicity, immunotoxicity, endocrine-mediated effects, reproductive toxicity and carcinogenicity.

An *adverse effect* is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub) population that results in an impairment of functional capacity, or an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (OECD, 2003).

A chemical substance may induce systemic and/or local effects.

- A *local effect* is an effect that is observed at the site of first contact, caused irrespective of whether a substance is systemically available.

- A *systemic effect* is defined as an effect that is normally observed distant from the site of first contact, i.e., after having passed through a physiological barrier (mucous membrane of the gastro-intestinal tract or of the respiratory tract, or the skin) and becomes systemically available.

- It should be noted, however, that toxic effects on surface epithelia may reflect indirect effects as a consequence of systemic toxicity or secondary to systemic distribution of the substance or its active metabolite(s).

R.7.5.1.2 Objective of the guidance on repeated dose toxicity

The objectives of assessing repeated dose toxicity are to evaluate:

- whether exposure of humans to a substance has been associated with adverse toxicological effects occurring as a result of repeated daily exposure for a part of the expected lifetime or for the major part of the lifetime; these human studies potentially may also identify populations that have higher susceptibility;
- whether administration of a substance to experimental animals causes adverse toxicological effects as a result of repeated daily exposure for a part of the expected lifespan or for the major part of the lifespan; effects that are predictive of possible adverse human health effects;
- the target organs, potential cumulative effects and the reversibility of the adverse toxicological effects;
- the dose-response relationship and threshold for any of the adverse toxicological effects observed in the repeated dose toxicity studies;
- the basis for risk characterisation and classification and labelling of substances for repeated dose toxicity.

R.7.5.2 Information requirements for repeated dose toxicity

Section R.2.1 provides general guidance on the information requirements of REACH. For repeated dose toxicity, all available information relevant for the endpoint needs to be evaluated and classification considered at each tonnage level. The following standard information requirements on repeated dose toxicity are specified in REACH Annexes VII-X:

In **Annex VII** (≥ 1 t/y), no test requirements on repeated dose toxicity are specified additional to the available information relevant for repeated dose toxicity.

In **Annex VIII** (≥ 10 t/y), a short-term repeated dose toxicity study (28 days) is usually required, in one species, male and female, using the most appropriate route of administration, having regard to the likely route of human exposure.

In **Annex IX** (≥ 100 t/y), a sub-chronic repeated dose toxicity study (90-days) is usually required, in one species (90-day study: rodent), male and female, and a short-term repeated dose toxicity study (28 days) is the minimum requirement, using the most appropriate route of administration, having regard to the likely route of human exposure. It should be noted that the 28-day test is not required at this tonnage level if already provided as part of Annex VIII requirements or if the 90-day study is proposed at this tonnage level.

In **Annex X** (≥ 1000 t/y), no specific test requirements additional to those required in Annexes VIII-IX for repeated dose toxicity is required at this tonnage level.

Column 1 of the REACH Annexes VII to X establishes the standard information required for all chemical substances and Column 2 lists specific rules according to which the required standard information requirements for individual endpoints may be modified (adapted) by waiving requirement for certain information, or in certain cases, defining the need for additional or different information. (see Section R.2.1 for further details).

In addition to the specific rules for adaptation listed in column 2 of the Annexes VII to X, the required standard information may also be adapted according to Annex XI, which specifies general rules for adaptation of the standard testing requirements set out in Annexes VII-X in

cases where 1) testing does not appear scientifically necessary, 2) testing is technically not possible, and 3) testing may be omitted based on the exposure scenarios developed in the CSA (substance-tailored exposure-driven testing) (see Section R.5.1 (Exposure based waiving)).

It should also be noted that the introductory sections to Annexes VII-X point at a specific adaptation to the standard information requirements as *in vivo* testing shall be avoided with corrosive substances at concentration/dose levels causing corrosivity.

Factors that can influence the standard information requirements include the results of other toxicity studies, immediate disintegration of the substance, accumulation of the substance or its metabolites in certain tissues and organs, failure to identify a NOAEL in the required test at a given tonnage level, toxicity of particular concern, exposure route, structural relationships with a known toxic substance, physico-chemical properties of the substance, and use and human exposure patterns. These adaptations are detailed in the stepwise ITS presented in Section R.7.5.6.

R.7.5.3 Information and its sources on repeated dose toxicity

Toxicological information, including repeated dose toxicity, can be obtained from unpublished studies, data bases and publications such as books, scientific journals, criteria documents, monographs and other publications (see Chapter R.3 for further general guidance). Information relevant for repeated dose toxicity can also be obtained from data on other endpoints, structural analogues and physico-chemical properties.

Before new tests are carried out to determine the hazardous properties of a chemical substance, all available information, shall be assessed, according to REACH Annex VI, step 1. (See Chapter R.4 for general guidance on evaluation of information).

R.7.5.3.1 Non-human data on repeated dose toxicity

Non-testing data on repeated dose toxicity

Physico-chemical data

The physico-chemical properties of a chemical substance are essential elements in deciding on the appropriate administration route to be applied in experimental *in vivo* repeated dose toxicity studies as well as to decide on exemption from testing in cases where testing is technically not possible.

(Q)SAR models

The OECD has recently prepared a report on the use of (Q)SAR in the various member countries (OECD, 2006), which provides clear insight in how these tools are being used in the various OECD member countries. A review conducted by ECETOC on the use of (Q)SARs within current regulatory decision-making frameworks in EU, North America, and Japan, and within industry concluded that applicability of currently available (Q)SARs for chronic mammalian toxicity, certainly as a stand-alone approach, was very limited at that time (ECETOC 2003).

The ECB has started building a freely accessible inventory of evaluated (Q)SAR models which help to identify valid (Q)SARs for regulatory purposes (see also cross cutting guidance on (Q)SARs). If there are any models relevant for the underlying endpoint these will be included in the ECB inventory.

More extensive guidance on the availability and application of (Q)SARs is available in Section R.6.1.

Structurally or mechanistically related substance(s) (read-across/chemical category)

The concept of grouping, including both read-across and the related chemical category concept has been developed under the OECD HPV program (OECD 2007a. This is an approach which might be used to fill data gaps without the need for conducting tests when specific conditions, as specified in REACH Annex XI Section 1.3, are met.

Extensive guidance on the application of chemical categories/read across is available in Section R.6.2.

Testing data on repeated dose toxicity

In vitro data

Currently, no available alternatives to animal testing are accepted for regulatory purposes for detecting toxicity after repeated exposure. Numerous *in vitro* systems have been developed over the last decades and have been discussed and summarized in recent ECVAM reports on repeated dose toxicity testing (Worth & Balls 2002, Prieto et al., 2005, and Prieto et al., 2006). At present, the *in vitro* models listed in these reports are at research and development level and cannot be used for repeated dose toxicity predictive purposes, although they are very useful to study individual types of organ toxicity or in assessing mechanistic aspects of target organ toxicity, on the tissue, cellular and molecular level. Some of the drawbacks are for instance the limited possibilities of current cell culture systems to account for kinetics and biotransformation, and the difficulty to derive from *in vitro* systems values such as NOAELs. Further development and optimisation of current *in vitro* systems as well as the selection of endpoints relevant to general as well as cell-type-specific mechanisms of toxicity or expression of toxic effects *in vivo* is ongoing. New technologies such as genomics, transcriptomics, proteomics and metabolomics could help in the identification of specific markers of toxicity that occur early in the process of long-term toxic responses and that are mechanistically linked to the underlying pathology. A recent ECVAM workshop report (Prieto et al., 2006) includes a proposed approach to assess repeated dose toxicity *in vitro* by integrating physiologically-based kinetic (PBK) modelling, the use of biomarkers, and omics technologies. However, this integrated approach is still under development and evaluation and is not ready for regulatory purposes.

The latest information on the status of alternative methods that are under development can be obtained from the ECVAM website (current address: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam) and other international centres for validation of alternative methods.

Human *in vitro* data, particularly on kinetics and metabolism, may assist in study interpretation thereby avoiding the need for unnecessary animal experimentation.

At present, available *in vitro* test data from well-characterised target organ and target system models on, e.g. mode of action(s) / mechanism(s) of toxicity may be useful in the interpretation of observed repeated dose toxicity.

Animal data

The most appropriate data on repeated dose toxicity for use in hazard characterisation and risk assessment are primarily obtained from studies in experimental animals conforming to internationally agreed test guidelines. In some circumstances repeated dose toxicity studies not conforming to conventional test guidelines may also provide relevant information for this endpoint.

The information that can be obtained from the available EU/OECD test guideline studies for repeated dose toxicity is briefly summarised below. Table R.7.5-2 summarises the parameters examined in these OECD test guideline studies in more detail to facilitate overview of the similarities and differences between the various studies. It should be noted that the test guidelines given in Annex V to Directive 67/548/EEC[36] (http://ecb.jrc.it/testing-methods/) are generally comparable to the OECD test guidelines (http://www.oecd.org/env/testguidelines). Further details of the study protocols are described in the respective test guidelines.

Repeated dose 28-day toxicity studies:

Separate guidelines are available for studies using oral administration (EU B.7 / OECD TG 407), dermal application (EU B.9 / OECD TG 410), or inhalation (EU B.8 / OECD TG 412). The principle of these study protocols is identical although the OECD TG 407 protocol includes additional parameters compared to those for dermal and inhalation administration, enabling the identification of a neurotoxic potential, immunological effects or reproductive organ toxicity.

The 28-day studies provide information on the toxicological effects arising from exposure to the substance during a relatively limited period of the animal's life span.

Repeated dose 90-day toxicity studies:

Separate guidelines are available for studies using oral administration (OECD TG 408/409 / EU B.26/B.27 in rodent/non-rodent species, respectively), dermal application (OECD TG 411/EU B.28), or inhalation (OECD TG 413/EU B.29). The principle of these study protocols is identical although the revised OECD TG 408 protocol includes additional parameters compared to those for dermal and inhalation administration, enabling the identification of a neurotoxic potential, immunological effects or reproductive organ toxicity.

The 90-day studies provide information on the general toxicological effects arising from sub-chronic exposure (a prolonged period of the animal's life span) covering post-weaning maturation and growth well into adulthood, on target organs and on potential accumulation of the substance.

Chronic toxicity studies:

The chronic toxicity studies (OECD TG 452/EU B.30) provide information on the toxicological effects arising from repeated exposure over a prolonged period of time covering the major part of the animal's life span. The duration of the chronic toxicity studies should be at least 12 months.

The combined chronic toxicity / carcinogenicity studies (OECD TG 453/EU B.33) include an additional high-dose satellite group for evaluation of pathology other than neoplasia. The satellite group should be exposed for at least 12 months and the animals in the carcinogenicity part of the study should be retained in the study for the majority of the normal life span of the animals.

Ideally, the chronic studies should allow for the detection of general toxicity effects (physiological, biochemical and haematological effects etc.) but could also inform on neurotoxic, immunotoxic, reproductive and carcinogenic effects of the substance. However, in 12-month studies, non-specific life shortening effects, which require a long latent period or are

[36] All the test methods previously included in Annex V to Directive 67/548/EEC will be incorporated in a new Test Methods (TM) Regulation that is currently (February 2008) under adoption. The TM Regulation will be adapted to technical progress whenever a new test method has been developed, scientifically validated and accepted for regulatory use by the National Coordinators of the Member states

cumulative, may possibly not be detected in this study type. In addition, the combined study will allow for detection of neoplastic effects and a determination of a carcinogenic potential and the life-shortening effects.

The combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test:

The combined repeated dose toxicity / reproductive screening study (OECD TG 422[37]) provides information on the toxicological effects arising from repeated exposure (generally oral exposure) over a period of about 6 weeks for males and approximately 54 days for females (a relatively limited period of the animal's life span) as well as on reproductive toxicity. For the repeated dose toxicity part, the OECD TG 422 is in concordance with the OECD TG 407/EU B.7 except for use of pregnant females and longer exposure duration in the OECD TG 422 compared to the OECD TG 407/EU B.7.

Neurotoxicity studies:

The neurotoxicity study in rodents (OECD TG 424/EU B.43) has been designed to further characterise potential neurotoxicity observed in repeated dose systemic toxicity studies. The neurotoxicity study in rodents will provide detailed information on major neuro-behavioural and neuro-pathological effects in adult rodents.

Delayed neurotoxicity studies of organophosphorus substances:

The delayed neurotoxicity study (OECD TG 419/ EU Annex B.38) is specifically designed to be used in the assessment and evaluation of the neurotoxic effects of organophosphorus substances. This study provides information on the delayed neurotoxicity arising from repeated exposure over a relatively limited period of the animal's life span.

Other studies providing information on repeated dose toxicity:

Although not aiming at investigating repeated dose toxicity per se, other available OECD/EU test guideline studies involving repeated exposure of experimental animals may provide useful information on repeated dose toxicity. These studies are summarised in Table R.7.5-1.

It should be noted that the repeated dose toxicity studies, if carefully evaluated, may provide information on potential reproductive toxicity and on carcinogenicity (e.g., pre-neoplastic lesions).

The one- and two-generation studies (OECD TG 415/416/EU B.34/B.35) may provide information on the general toxicological effects arising from repeated exposure over a prolonged period of time (about 90 days for parental animals) as clinical signs of toxicity, body weight, selected organ weights, and gross and microscopic changes of selected organs are recorded.

The prenatal developmental toxicity study (OECD TG 414/EU B.31), the reproduction/developmental toxicity screening study (OECD TG 421[38]) and the developmental neurotoxicity study (draft OECD TG 426[38]) may give some indications of general toxicological effects arising from repeated exposure over a relatively limited period of the animals life span as clinical signs of toxicity and body weight are recorded.

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[37] To date there is no corresponding EU testing method available.

[38] To date there is no corresponding EU testing method available.

The carcinogenicity study (OECD TG 451/EU B.32) will, in addition to information on neoplastic lesions, also provide information on the general toxicological effects arising from repeated exposure over a major portion of the animal's life span as clinical signs of toxicity, body weight, and gross and microscopic changes of organs and tissues are recorded.

Table R.7.5-1 Overview of other *in vivo* test guideline studies giving information on repeated dose toxicity

Test	Design	Endpoints (general toxicity)
OECD TG 416 (EU B.35) Two-generation reproduction toxicity study	Exposure before mating for at least one spermatogenic cycle until weaning of 2nd generation At least 3 dose levels plus control At least 20 parental males and females per group	Clinical observations Body weight and food/water consumption Gross necropsy (all parental animals) Organ weights (reproductive organs, brain, liver, kidneys, spleen, pituitary, thyroid, adrenal glands, and known target organs) Histopathology (reproductive organs, previously identified target organ(s) - at least control and high-dose groups
OECD TG 415 (EU B.34) One-generation reproduction toxicity Study	Exposure before mating for at least one spermatogenic cycle until weaning of 1st generation At least 3 dose levels plus control At least 20 parental males and females per group	As in TG 416
OECD TG 414 (EU B.31) Prenatal developmental toxicity study	Exposure at least from implantation to one or two days before expected birth At least 3 dose levels plus control At least 20 pregnant females per group	Clinical observations Body weight and food/water consumption Macroscopical examination all dams for any structural abnormalities or pathological changes, which may have influenced the pregnancy
OECD TG 421[39] Reproduction/ developmental toxicity screening test	Exposure from 2 weeks prior to mating until at least post-natal day 4 At least 3 dose levels plus control At least 8-10 parental males and females per group	Clinical observations Body weight and food/water consumption Gross necropsy (adult animals, special attention to reproductive organs) Organ weights (all adult males: testes, epididymides) Histopathology (reproductive organs in at least control and high-dose groups)

[39] To date there is no corresponding EU testing method available.

OECD TG 426[39] Developmental neurotoxicity study (draft)	Exposure at least from implantation throughout lactation (PND 20) At least 3 dose levels plus control At least 20 pregnant females per group	Clinical observations Body weight and food/water consumption
OECD TG 451 (EU B.32) Carcinogenicity studies	Exposure for majority of normal life span At least 3 dose levels plus control At least 50 males and females per group	Clinical observations (special attention to tumour development) Body weight and food consumption Gross necropsy Histopathology (all groups - all grossly visible tumours or lesions suspected of being tumours; at least control and high-dose groups - brain, pituitary, thyroid, parathyroid, thymus, lungs, heart, salivary glands, liver, spleen, kidneys, adrenals, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, lymph nodes, pancreas, gonads, accessory sex organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord, sternum with bone marrow and femur, eyes)

R.7.5.3.2 Human data on repeated dose toxicity

Human data adequate to serve as the sole basis for the hazard and dose-response assessment are rare. When available, reliable and relevant human data are preferable over animal data and can contribute to the overall *Weight of Evidence*. However, human volunteer studies are not recommended due to practical and ethical considerations involved in deliberate exposure of individuals to chemicals.

The following types of human data may already be available, however:

- Analytical epidemiology studies on exposed populations. These data may be useful for identifying a relationship between human exposure and effects such as biological effect markers, early signs of chronic effects, disease occurrence, or long-term specific mortality risks. Study designs include case control studies, cohort studies and cross-sectional studies.

- Descriptive or correlation epidemiology studies. They examine differences in disease rates among human populations in relation to age, gender, race, and differences in temporal or environmental conditions. These studies may be useful for identifying priority areas for further research but not for dose-response information.

- Case reports describe a particular effect in an individual or a group of individuals exposed to a substance. Generally case reports are of limited value for hazard identification, especially if the exposure represents single exposures, abuse or misuse of certain substances.

- Controlled studies in human volunteers. These studies, including low exposure toxicokinetic studies, might also be of use in risk assessment.

Meta-analysis. In this type of study data from multiple studies are combined and analysed in one overall assessment of the relative risk or dose-response curve.

R.7.5.3.3 Exposure considerations on repeated dose toxicity

Information on exposure, use and risk management measures should be collected in accordance with Article 10 and Annex VI (Section 3) of REACH.

Such information may lead to adaptation of the extent and nature of information needed on repeated dose toxicity under REACH; three types of *adaptations* are possible due to exposure considerations: exposure-based waiving of a study, exposure-based triggering of further studies, or definition of appropriate exposure route.

More detailed guidance of exposure-based adaptations of the repeat dose toxicity information requirements is given in Sections R.7.5.4 (evaluation of available information) and R.7.5.6 (Integrated testing strategy).

R.7.5.4 Evaluation of available information on repeated dose toxicity

General guidance on how to evaluate the available information is given in Chapter R.4.

R.7.5.4.1 Non-human data on repeated dose toxicity

Non-testing data on repeated dose toxicity

Physico-chemical properties

The physico-chemical properties of a chemical substance under registration should always be considered before any new experimental *in vivo* repeated dose toxicity studies are undertaken.

The physico-chemical properties of a substance can indicate whether it is likely that the substance can be absorbed following exposure to a particular route and whether it (or an active metabolite) is likely to reach the target organ(s) and tissue(s). The physico-chemical properties are thus essential elements in deciding on the appropriate administration route to be applied in experimental *in vivo* repeated dose toxicity studies (see Section R.7.5.4.3).

The physico-chemical properties are also important in order to judge whether testing is technically possible. Testing for repeated dose toxicity may, as specified in Annex XI Section 2 of REACH, be omitted if it is technically not possible to conduct the study as a consequence of the properties of the substance, e.g. very volatile, highly reactive or unstable substances cannot be used, or mixing of the substance with water may cause danger of fire or explosion. The Annex further emphasises that the guidance given in the test methods referred to in REACH Article 13 (3), more specifically on the technical limitations of a specific method, shall always be respected.

Additional generic guidance on the use of physico-chemical properties is provided e.g. in Section R.7.12 on toxicokinetics.

Read-across to structurally or mechanistically similar substances (SAR)

The potential toxicity of a substance, for which no data are available on a specific endpoint can, in some cases, be evaluated by read-across from structurally or mechanistically related substances for which experimental data exists. The read-across approach is based on the principle that structurally and/or mechanistically related substances may have similar toxicological properties. Note that there are no formal criteria to identify structural alerts for repeated dose toxicity or for read-across to closely related substances.

Based on structural similarities between different substances, the repeated dose toxicity potential of one substance or a group of substances can be extended (read-across) to a substance, for which there are no or limited data on this endpoint.

A mechanism of toxicity or mode of action identified for a substance and/or group of substances and causally related to adverse effects in a target organ can be extended (read-across) to a substance for which a similar mechanism or mode of action has been identified, but where no or limited data on repeated dose toxicity are available. In such cases, the substance under evaluation may reasonably be expected to exhibit the same pattern of toxicity in the target organ(s) and tissue(s).

The chemical category concept has been developed under the OECD HPV programme (OECD 2004) as an approach to fill data gaps without the need for conduction of tests. A chemical category is a group of chemicals whose physico-chemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. In the category approach, not every substance needs to be tested for every endpoint. However, the information finally compiled for the category must prove adequate to support a hazard assessment, a risk assessment and a classification for the category and its members. That is, the final data set must allow one to assess the untested endpoints, ideally by interpolation between and among the category members.

When analogue data are used to fill the data gaps for repeated dose toxicity, the data for the analogues must be compared and discussed in relation to the substance under evaluation in order to shed light on the similarities and differences in the toxicological profile of the substance under evaluation and its analogue(s).

Specific guidance regarding use of analogues is available in Section R.6.2 in order to decide on when further *in vivo* repeated dose toxicity studies shall be proposed (Annex VIII) or may be proposed (Annex X) as well as to decide on when analogue data can replace *in vivo* testing (Annex XI Section 1.3).

(Q)SAR

A (Q)SAR analysis for a substance may give indications for a specific mechanism to occur and identify possible organ or systemic toxicity upon repeated exposure. The reliability, applicability and overall scope of (Q)SAR science to identify chemical hazard and assist in risk assessment have been evaluated by various groups and organizations. Guidance on this issue is presented in Section R.6.1 of this document and in OECD Monograph No. 69. (OECD 2007b).

Overall, (Q)SAR approaches are currently not well validated for repeated dose toxicity and consequently no firm recommendations can be made concerning their routine use in a testing strategy in this area. There are a large number of potential targets/mechanisms associated with repeated dose toxicity that today cannot be adequately covered by a battery of (Q)SAR models. Therefore, a negative result from current (Q)SAR models without other supporting evidence cannot be interpreted as demonstrating a lack of a toxicological hazard or a need for hazard classification. Another limitation of QSAR modelling is that dose-response information, including the N(L)OAEL, is not provided. Similarly, a validated QSAR model might identify a potential toxicological hazard, but because of limited confidence in this approach, such a result would not be adequate to support hazard classification.

In some cases, QSAR models could be used as part of a *Weight of Evidence* approach, when considered alongside other data, provided the applicability domain is appropriate. Also, QSAR's 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 QSAR modelling results can be of value in a read-across assessment and for classification purposes.

Testing data on repeated dose toxicity

In vitro data

As mentioned earlier in Section R.7.5.3.1 available *in vitro* data, at present, is not useful on its own for regulatory decisions such as risk assessment and C&L. However, such data may be helpful in the assessment of repeated dose toxicity, for instance to detect local target organ effects and/or to clarify the mechanisms of action. Since, at present, there are not validated and regulatory accepted *in vitro* methods, the quality of each of these studies and the adequacy of the data provided should be carefully evaluated.

Generic guidance is given in Chapters R.4 and R.5 for judging the applicability and validity of the outcome of various study methods, assessing the quality of the conduct of a study, reproducibility of data and aspects such as vehicle, number of replicates, exposure/incubation time, GLP-compliance or comparable quality description.

Animal data

The basic concept of repeated dose toxicity studies to generate data on target organ toxicity following sub-acute to chronic exposure is to treat experimental animals for 4 weeks, 13 weeks or longer. These studies are mentioned in Section R.7.5.3.1 and summarised in Table R.7.5-2. In addition, other studies performed in experimental animals may provide useful information on repeated dose toxicity. While at this time most alternative methods remain in the research and development stage and are not ready as surrogates for sub-chronic/chronic animal studies there are opportunities to improve data collection for risk assessment providing greater efficiency and use of fewer animals and better use of resources. Although not required by REACH, other opportunities include early development of kinetic data, in conjunction with early repeat dose toxicity testing thus ensuring that the maximum amount of information is drawn from the animal studies and for use in the risk assessment process.

The number of repeated dose toxicity studies available for a substance under registration is likely to be variable, ranging from none, a dose-range finding study, a 28-day repeated dose toxicity guideline study, to a series of guideline studies for some substances, including sub-chronic and/or chronic studies. There may also be studies employing different species and routes of exposure. In addition, special toxicity studies investigating further the nature, mechanism and/or dose-relationship of a critical effect in a target organ or tissue may also have been performed for some substances.

The following general guidance is provided for the evaluation of repeated dose toxicity data and the development of the *Weight of Evidence*:

- Studies on the most sensitive animal species should be selected as the significant ones, unless toxicokinetic and toxicodynamic data show that this species is less relevant for human risk assessment.

- Studies using an appropriate route, duration and frequency of exposure in relation to the expected route(s), frequency and duration of human exposure have greater weight.

- Studies enabling the identification of a NOAEL, and a robust hazard identification have a greater weight.

- Studies of a longer duration should be given greater weight than a repeated dose toxicity study of a shorter duration in the determination of the most relevant NOAEL.

- If sufficient evidence is available to identify the critical effect(s) (with regard to the dose-response relationship(s) and to the relevance for humans), and the target organ(s) and/or tissue(s), greater weight should be given to specific studies

investigating this effect in the identification of the NOAEL. The critical effect can be a local as well as a systemic effect.

While data available from repeated dose toxicity studies not performed according to conventional guidelines and/or GLP may still provide information of relevance for risk assessment and classification and labelling such data require extra careful evaluation. REACH Annex XI specifically identifies circumstances where use of existing studies not carried out according to GLP or test methods referred to in Article 13(3) (guideline studies) can replace *in vivo* testing performed in accordance with Article 13(3). Data from non-guideline studies shall be considered to be equivalent to data generated by corresponding test methods referred to in Article 13(3) if the following conditions are met:

- adequate for the purpose of classification and labelling and/or risk assessment,
- adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3),
- exposure duration comparable to or longer than the corresponding test methods referred to in REACH Article 13(3) if exposure duration is a relevant parameter, and
- adequate and reliable documentation of the study is provided.

In all other situations, non-guideline studies may contribute to the overall weight of the evidence but cannot stand alone for a hazard and risk assessment of a substance and thus, cannot serve as the sole basis for an assessment of repeated dose toxicity as well as for exempting from the standard information requirements for repeated dose toxicity at a given tonnage level, i.e. cannot be used to identify a substance as being adequately controlled in relation to repeated dose toxicity.

If sufficient information from existing studies is available on the repeated dose toxicity potential of a substance in order to perform a risk assessment as well as to conclude on classification and labelling for repeated dose toxicity (R48), no further *in vivo* testing is needed. The existing information is considered sufficient when, based on a *Weight of Evidence* analysis, the critical effect(s) and target organ(s) and tissue(s) can be identified, the dose-response relationship(s) and NOAEL(s) and/or LOAEL(s) for the critical effect(s) can be established, and the relevance for human beings can be assessed.

It should be noted that potential effects in certain target organs (e.g., the thyroid) following repeated exposure may not be observed within the span of the 28-day study. Attention is also drawn to the fact that the protocols for the oral 28-day and 90-day studies include additional parameters compared to those for the 28-day and 90-day dermal and inhalation protocols.

Where it is considered that the existing data as a whole is inadequate to provide a clear assessment of this endpoint, the need for further testing should be considered in view of all available relevant information on the substance, including use pattern, the potential for human exposure, physico-chemical properties, and structural alerts. The testing strategy is presented in Section R.7.5.6.3.

Specific investigations such as studies for neurotoxicity or immunotoxicity are also elements in the testing strategy presented in REACH.

Regarding neurotoxicity and immunotoxicity, standard oral 28-day and 90-day toxicity studies include endpoints capable of detecting such effects. Indicators of neurotoxicity include clinical observations, a functional observational battery, motor activity assessment and histopathological examination of spinal cord and sciatic nerve. Indicators of immunotoxicity include changes in haematological parameters, serum globulin levels, alterations in immune

system organ weights such as spleen and thymus, and histopathological changes in immune organs such as spleen, thymus, lymph nodes and bone marrow. Where data from standard oral 28-day and 90-day studies identify evidence of neurotoxicity or immunotoxicity other studies may be necessary to further investigate the effects. It should be noted that endpoints capable of detecting neurotoxicity and immunotoxicity are not examined in the standard 28-day and 90-day dermal or inhalation repeated dose toxicity studies.

More focus has also been put on endocrine disrupters during the latest decade. In relation to hazard and risk assessment, there are currently no test strategies or methods available, which specifically detect all effects, which have been linked to the endocrine disruption mechanism. It should be noted that work is on-going with the purpose of updating the present oral 28-days study (OECD TG 407/EU B.7) with more emphasis to be placed on detection of endocrine effects.

If data are not available from an oral standard 28-day repeated dose toxicity guideline study (OECD TG 407/EU B.7), the minimum repeated dose toxicity data requirement (28-day study) at tonnage levels from 10 t/y may in certain circumstances be met by results obtained from *the combined repeated dose toxicity study with the reproduction / developmental toxicity screening test* (OECD TG 422[40]). An advantage of this approach is obtaining information on repeated dose toxicity and reproductive toxicity in a single study providing an overall saving in the number of animals used for testing. In addition, the number of animals is higher (10 per sex compared to 5 per sex in the standard oral 28-day study) and the dosing period is longer in the combined study than in the standard oral 28-day study. Therefore, more information on repeated dose toxicity could be expected from the combined study. Potential complications in using the combined study include selecting adequate dose levels to examine adequately both repeated dose toxicity and reproductive toxicity. In addition, interpretation of the results may be complicated due to differences in sensitivity between pregnant and non-pregnant animals, and an assessment of the general toxicity may be more difficult especially when serum and histopathological parameters are not evaluated at the same time in the study. Consequently, where the combined study is used for the assessment of repeated dose toxicity, the use of data obtained from such a study should be clearly indicated. Despite such complications, the use of the combined study is recommended for the initial hazard assessment of the repeated dose toxicity potential of a substance when this study is relevant also for reproductive toxicity assessment.

In general, results from toxicological studies requiring repeated administration of a test substance (see also Section R.7.5.3.1) such as *reproduction and developmental toxicity studies* as well as *carcinogenicity studies* can contribute to the assessment of repeated dose toxicity. However, such toxicological studies rarely provide the information obtained from a standard repeated dose toxicity study and therefore, cannot stand alone as the sole basis for the assessment of repeated dose toxicity or for exempting from the standard information requirements for repeated dose toxicity at a given tonnage level.

Studies such as *acute toxicity and irritation studies* as well as *in vivo genotoxicity studies* contribute limited information to the overall assessment of the repeated dose toxicity. However, such studies may be useful in deciding on the dose levels for use in repeated dose toxicity.

Guidance on the dose selection for repeated dose toxicity testing (see also Table R.7.5-2) is provided in detail in the EU and OECD test guidelines. Unless limited by the physical-chemical nature or biological effects of the test substance, the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering.

[40] To date there is no corresponding EU testing method available.

Although not required by REACH, toxicokinetic studies may be helpful in the evaluation and interpretation of repeated dose toxicity data, for example in relation to accumulation of a substance or its metabolites in certain tissues or organs as well as in relation to mechanistic aspects of repeated dose toxicity and species differences. Toxicokinetic information can also assist in the selection of the dose levels. When conducting repeated dose toxicity studies it is necessary to ensure that the observed treatment-related toxicity is not associated with the administration of excessive high doses causing saturation of absorption and detoxification mechanisms. The results obtained from studies using excessive doses causing saturation of metabolism are often of limited value in defining the risk posed at more relevant and realistic exposures where a substance can be readily metabolised and cleared from the body. It is suggested that a key resource in designing better repeated dose toxicity studies is to select appropriate dose levels based on results from useful metabolic and toxicokinetic investigations. Further details on the application of toxicokinetic information in the design and evaluation of repeated dose toxicity studies is available in Section R.7.12 on toxicokinetics.

Table R.7.5-2 Overview of *in vivo* repeated dose toxicity test guideline studies

Test	Design	Endpoints
OECD TG 407 (EU B.7) Repeated dose 28-day oral toxicity study in rodents	Exposure for 28 days At least 3 dose levels plus control At least 5 males and females per group Preferred rodent species: rat	Clinical observations Functional observations (4th exposure week – sensory reactivity to stimuli of different types, grip strength, motor activity) Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain, heart) Histopathology (full, at least control and high-dose groups - all gross lesions, brain, spinal cord, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, gonads, accessory sex organs, urinary bladder, lymph nodes, peripheral nerve, a section of bone marrow)
OECD TG 410 (EU B.9) Repeated dose dermal toxicity: 21/28-day study	Exposure for 21/28 days At least 3 dose levels plus control At least 5 males and females per group Rat, rabbit or guinea pig	Clinical observations Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, normal and treated skin, liver, kidney)
OECD TG 412 (EU B.8) Repeated dose inhalation toxicity: 28-day or 14-day study	Exposure for 28 or 14 days At least 3 concentrations plus control At least 5 males and females per group Rodents: preferred species - rat	Clinical observations Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys,

Test	Design	Endpoints
		adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, lungs, liver, kidney, spleen, adrenals, heart)
OECD TG 408 (EU B.26) Repeated dose 90-day oral toxicity study in rodents	Exposure for 90 days At least 3 dose levels plus control At least 10 males and females per group Preferred rodent species: rat	Clinical observations Ophthalmological examination Functional observations (towards end of exposure period – sensory reactivity to stimuli of different types, grip strength, motor activity) Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart) Histopathology (full, at least control and high-dose groups - all gross lesions, brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea and lungs, aorta, gonads, uterus, accessory sex organs, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes, peripheral nerve, a section of bone marrow, and skin/eyes on indication)
OECD TG 409 (EU B.27) Repeated dose 90-day oral toxicity study in non-rodents	Exposure for 90 days At least 3 dose levels plus control At least 4 males and females per group Preferred species: dog	Clinical observations Ophthalmological examination Body weight and food/water consumption Haematology (as in TG 408) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (as in TG 408 - additional: gall bladder, thyroid, parathyroid) Histopathology (as in TG 408 – additional: gall bladder, eyes)
OECD TG 411 (EU B.28) Subchronic dermal toxicity: 90-day	Exposure for 90 days At least 3 dose levels plus control At least 10 males and	Clinical observations Ophthalmological examination Body weight and food/water consumption

Test	Design	Endpoints
study	females per group Rat, rabbit or guinea pig	Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, normal and treated skin, and essentially the same organs and tissues as in TG 408)
OECD TG 413 (EU B.29) Subchronic inhalation toxicity: 90-day study	Exposure for 90 days At least 3 concentrations plus control At least 10 males and females per group Rodents: preferred species - rat	Clinical observations Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, respiratory tract, and essentially the same organs and tissues as in TG 408)
OECD TG 452 (EU B.30) Chronic toxicity studies	Exposure for at least 12 months At least 3 dose levels plus control Rodents : At least 20 males and females per group Non-rodents: At least 4 males and females per group Preferred rodent species: rat Preferred non-rodent species: dog	Clinical observations, including neurological changes Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total leucocyte count, platelet count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - brain, liver, kidneys, adrenals, gonads, thyroid/parathyroid (non-rodents only)) Histopathology (full, at least control and high-dose groups - all grossly visible tumours and other lesions, as well as essentially the same organs and tissues as in the 90-day studies (TG 408/409))

Test	Design	Endpoints
OECD TG 453 (EU B.33) Combined chronic toxicity / carcinogenicity studies	Exposure for at least 12 months (satellite groups) or majority of normal life span (carcinogenicity part) At least 3 dose levels plus control At least 50 males and females per group Satellite group: At least 20 males and females per group Preferred species: rat	Essentially as in TG 452
OECD TG 422[41] Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test	Exposure for a minimum of 4 weeks (males) or from 2 weeks prior to mating until at least post-natal day 4 (females – at least 6 weeks of exposure) At least 3 dose levels plus control At least 10 males and females per group	Clinical observations as in TG 407 Functional observations as in TG 407 Body weight and food/water consumption Haematology as in TG 407 Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all adult animals) Organ weights (testes and epididymides - all males; liver, kidneys, adrenals, thymus, spleen, brain, heart - in 5 animals of each sex per group, i.e. as in TG 407) Histopathology (ovaries, testes, epididymides, accessory sex organs, all gross lesions - all animals in at least control and high-dose groups; brain, spinal cord, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, urinary bladder, lymph nodes, peripheral nerve, a section of bone marrow - in 5 animals of each sex in at least control and high-dose groups, i.e. as in TG 407)
OECD TG 424 (EU B.43) Neurotoxicity study in rodents	Exposure for at least 28 days Dose levels: not specified At least 10 males and females per group Preferred rodent species: rat Generally oral route of administration	Detailed clinical observations Functional observations (sensory reactivity to stimuli of different types, grip strength, motor activity, more specialized tests on indication) Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential)

[41] To date there is no corresponding EU testing method available

Test	Design	Endpoints
		Clinical biochemistry Histopathology: at least 5 animals/sex/ group) for neuropathological examinations (brain, spinal cord, and peripheral nerves); remaining animals to be used either for specific neurobehavioural, neuropathological, neurochemical or electrophysiological procedures that may supplement the histopathology or alternatively, for routine pathological evaluations according to the guidelines for standard repeated dose toxicity studies
OECD TG 419 (EU B.38) Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study	Exposure for 28 days At least 3 dose levels plus control At least 12 birds per group Species: domestic laying hen	Detailed clinical observations Body weight and food/water consumption Clinical biochemistry (NTE activity, acetylcholinesterase activity Gross necropsy (all animals) Histopathology (neural tissue)

R.7.5.4.2 Human data on repeated dose toxicity

Human data in the form of epidemiological studies or case reports can contribute to the hazard identification process as well as to the risk assessment process itself. Criteria for assessing the adequacy of epidemiology studies include an adequate research design, the proper selection and characterisation of the exposed and control groups, adequate characterisation of exposure, sufficient length of follow-up for the disease as an effect of the exposure to develop, valid ascertainment of effect, proper consideration of bias and confounding factors, proper statistical analysis and a reasonable statistical power to detect an effect. These types of criteria have been described in more detail (Swaen, 2006 and can be derived from Epidemiology Textbooks (Checkoway *et al*, 1989; Hernberg, 1991; Rothman, 1998).

The results from human experimental studies are often limited by a number of factors, such as a relatively small number of subjects, short duration of exposure, and low dose levels resulting in poor sensitivity in detecting effects.

In relation to hazard identification, the relative lack of sensitivity of human data may cause particular difficulty. Therefore, negative human data cannot be used to override the positive findings in animals, unless it has been demonstrated that the mode of action of a certain toxic response observed in animals is not relevant for humans. In such a case a full justification is required. It is emphasised that testing with human volunteers is strongly discouraged, but when there are good quality data already available they can be used in the overall *Weight of Evidence*.

R.7.5.4.3 Exposure considerations for repeated dose toxicity

Three types of *adaptations* from testing are possible due to exposure considerations: exposure-based waiving of a study, exposure-based triggering of further studies, or selection of appropriate exposure route. More information on exposure-based waiving is available in Section R.5.1. More detailed guidance of exposure-based adaptations of the testing for repeated dose toxicity is given below and in Section R.7.5.6 (Integrated Testing Strategy).

Comparison of exposure and effect data should consider the existing (or most likely expected) *exposure patterns* for humans (e.g. daily exposure during life-time or repeated short or medium periods of exposures) and the most adequate DNEL (Derived No Effect Level) that reflects the specific exposure route and time pattern for each human population group at exposure. For instance, short-term exposure estimates should be compared to a descriptor of short-term toxicity whereas repeated daily exposure estimates should be compared to a corresponding descriptor of chronic toxicity. In all cases actually experienced daily human exposures are to be used in this comparison instead of daily exposures obtained by averaging over exposed and non-exposed days.

Concerning repeated dose toxicity testing the oral route is the preferred one. However, dependent on the physico-chemical properties of a substance as well as on the most relevant route of human exposure, the dermal or the inhalation route could also be appropriate as specified in REACH Annex VIII and IX.

The dermal route is appropriate if the physico-chemical properties suggest potential for a significant rate of absorption through the skin. The inhalation route is appropriate if exposure of humans via inhalation is the most relevant route of human exposure taking into account the vapour pressure of the substance and/or the possibility of exposure to aerosols, particles or droplets of an inhalable size.

According to Annex VIII-X further studies shall be proposed by the registrant or may be required by the Agency for example if there is particular concern regarding exposure, e.g. use in consumer products leading to exposure levels which are:

- close to the dose levels at which toxicity to humans may be expected (Annex VIII) i.e. a dose lower than, but in the vicinity of, the dose levels at which toxicity to humans may be expected

- high relative to the dose levels at which toxicity to humans may be expected (Annex IX), i.e. exposure levels higher than the dose levels at which toxicity to humans may be expected

- close to the dose levels at which toxicity is observed (Annex X); i.e. a dose lower than, but in the vicinity of, the dose levels at which toxicity is observed from animal studies.

Any of the exposure-triggered studies proposed by the registrant or required by the Agency should be considered on a case-by-case basis.

Various types of exposure considerations are possible for *waiving* of repeated dose toxicity studies. For instance, it is stated in REACH Article 13 and Annex XI:3 that testing in accordance with Annex VIII, Sections 8.6 and 8.7 (i.e. repeated dose toxicity and reproductive toxicity), Annex IX and X may be omitted based on the exposure scenario(s) developed in the Chemical Safety Report. Adequate justification and documentation shall in all cases be provided (see Section R.5.1.).

Further, the sub-chronic toxicity study (90-days study) does not need to be conducted according to Annex IX of REACH if: “the substance is unreactive, insoluble and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-days *limit test*, particularly if such a pattern is *coupled with limited human exposure*. In order to omit the study the prerequisites interpreted above have to be considered jointly since the word “and” is used in between them. In addition, limited human exposure would strengthen the possibility for waiving.

The interpretation of *un-reactive* can be that it relates to the inherent chemical reactivity and as such, is an indicator of lack of local effects and mutagenicity, *insoluble and not inhalable*

can be interpreted as indicators of low exposure potential and should be further defined, and *no evidence of absorption* that there has to be evidence for lack of absorption in order to omit the study. Further *no evidence of toxicity in a 28-days limit test* can be interpreted as it has to be at least a 28-days limit test available in order to waive the 90-days study, and this 28-days study should not show any sign of toxicity at 1000 mg/kg.

Limited exposure should consider the level of exposure, the frequency and/or the duration of exposure. Therefore, limited exposure must be considered on a case-by-case basis.

Finally, according to REACH Annex VIII testing of repeated dose toxicity (28-days study) does not need to be conducted if: *relevant human exposure can be excluded*.

Relevant human exposure depends on the inherent properties of the substance, if the population comes into contact with the substance or not, and how the substance is used. Thus, waiving might be considered on a case-by-case basis.

The concept of the Threshold of Toxicological Concern (TTC) might be applied to reduce the use of animals and other evaluation resources (Kroes et al., 2004); Use of the TTC concept may also be seen as a driving force for deriving exposure information of adequate quality. However, there are a number of limitations or drawbacks that should be taken into consideration in deciding if the concept is to be applied for industrial chemicals and further discussions on the cut-off values are needed before integration into into the guidance (see Appendix R.7.1-1; TemaNord, 2005).

R.7.5.4.4 Remaining uncertainty on repeated dose toxicity

The key requirement for a CSA is the DNELs per exposure scenario (box 5 of Figure R.7.5-1). The DNEL for repeated dose toxicity is the threshold of the critical effect derived in a *Weight of Evidence* assessment of the available repeated dose toxicity data and an overall assessment factor (AF) that takes into account any uncertainty. The following elements contribute to the uncertainty in the determination of a threshold for the critical effects and the selection of the AF (further guidance on deriving a DNEL and application of AFs is provided in Chapter R.8).

Threshold of the critical effect

In the determination of the overall threshold for repeated dose toxicity all relevant information is evaluated to determine the lowest dose that induces an adverse effect (i.e. LOAEL or LOAEC) and the highest level with no biologically or statically significant adverse effects (i.e. NOAEL or NOAEC). In this assessment all toxicological responses are taken into account and the critical effect is identified. The uncertainty in the threshold depends on the strength of the data and is largely determined by the design of the underlying experimental data. Parameters such as group size, study type/duration or the methodology need to be taken into account in the assessment of the uncertainty in the threshold of the critical effect(s).

The NOAEL is typically used as the starting point for the derivation of the DNEL. In case a NOAEL has not been achieved, a LOAEL may be used, provided the available information is sufficient for a robust hazard assessment and for Classification and Labelling. The Bench Mark Dose (BMD) may also be used as the starting point for the derivation of the DNEL (Chapter R.8).

The selection of NOAEL or LOAEL is usually based on the dose levels used in the most relevant toxicity study, without considering the shape of the dose response curve. Therefore, the NOAEL/LOAEL may not reflect the true threshold for the adverse effect. On the other hand, the BMD is a statistical approach for the determination of the threshold and relies on the dose response curve. Alternatively, mathematical curve fitting techniques or statistical approaches exist to determine the threshold for an adverse effect. The use of such approaches (e.g.

Benchmark Dose) to estimate the threshold should be considered on a case-by-case basis. For further guidance see Chapter R.8

Overall AF

Variability in sensitivity across and within species is another source of uncertainty for repeated dose toxicity. These inter- and intraspecies differences, respectively, are linked with variations in the toxicokinetics and dynamics of a substance. Information derived from non-testing, *in vitro* or *in vivo* methods may lead to an improvement of the understanding of the relevance of animal data for human risk assessment and may lead to a replacement of adopted standard default AF for these differences.

The quality of the whole database should be assessed for reliability and consistency across different studies and endpoints and taking into account the quality of the testing method, size and power of the study design, biological plausibility, dose-response relationships and statistical association. Missing test data might be substituted by non-testing data obtained from physico-chemical properties, read-across to structurally or mechanistically related substances (SAR/chemical category) or by quantitative structure-activity relationships (QSARs). Also *in vitro* data might be used to fill in data gaps as well as *in vivo* non-standard animal experimental tests. Such data in combination with toxicity tests according to standard OECD/EU guidelines may in some cases lead to an improved understanding to the toxicological effect resulting in a reduction in the overall uncertainty. On the other hand information solely based on *in-vitro* and non-testing data are at present insufficient to act as a surrogate for repeated dose toxicity data and the uncertainty is sufficiently large that such information is unsuitable for use in a CSA and for classification and labelling. In the case of chemical categories information from non-testing methods or *in vitro* data may used to fulfil the data requirements on repeated dose toxicity and lead to improvement in the overall reliability and consistency for the read-across within a category of substances.

Since the adequacy and/or completeness of different data may vary, lack of quality and completeness of the overall database should be compensated for with an assessment factor for remaining uncertainty.

Besides AF addressing these differences (inter- and intraspecies, quality of the whole database), other uncertainties relating to differences between human and animal exposure conditions (e.g. route, and duration), and dose response characteristics are taken into account in the more extensive guidance on deriving a DNEL (see Section R.8.4.3).

Other considerations

Another situation may arise when testing is not technically possible, a waiving option indicated in Annex XI(2) (see also Chapter R.5). In such cases approaches such as QSAR, category formation and read-across may be helpful in the hazard characterisation; they should also be considered for information that might be suitable as a surrogate for a dose descriptor. Alternatively, generic threshold approaches, e.g. the Threshold of Toxicological Concern, TTC might be considered for the starting point of a risk characterisation (see Appendix R.7.1-1).

R.7.5.5 Conclusions on repeated dose toxicity

The evaluation of all available toxicological information for repeated dose toxicity (step 3 in Figure R.7.5-1 should include an assessment whether the available information as a whole (i.e. testing and non-testing, and relevant information from studies addressing other endpoints) meets the tonnage driven data requirements necessary to fulfil the REACH requirements. A *Weight of Evidence* approach should be used in assessing the database for a substance. This approach requires a critical evaluation of the entire body of available data for consistency and biological plausibility. Potentially relevant studies should be judged for quality and studies of high quality given more weight than those of lower quality. When both epidemiological and experimental data are available, similarity of effects between humans and animals is given more weight. If the mechanism or mode of action is well characterised, this information is used in the interpretation of observed effects in either human or animal studies. *Weight of Evidence* is not to be interpreted as simply tallying the number of positive and negative studies, nor does it imply an averaging of the doses or exposures identified in individual studies that may be suitable as starting points for risk assessment. The study or studies used for the starting point are identified by an informed and expert evaluation of all the available evidence.

The available repeated dose toxicity data should be evaluated in detail for a characterisation of the health hazards upon repeated exposure. In this process an assessment of all toxicological effect(s), their dose-response relationships and possible thresholds are taken into account. The evaluation should include an assessment of the severity of the effect, whether the observed effect(s) are adverse or adaptive, if the effect is irreversible or not or if it is a precursor to a more significant effect or secondary to general toxicity. Correlations between changes in several parameters, e.g. between clinical or biochemical measurements, organ weights and (histo)pathological effects, will be helpful in the evaluation of the nature of effects. Further guidance to this issue can be found in publications of the International Programme on Chemical Safety (IPCS 1994, 1999) and ECETOC (2002).

The effects data are also analysed for indications of potential serious toxicity of target organs or specific organ systems (e.g. neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity. Furthermore, the evaluation should take into account the study details and determine if the exposure conditions and duration and the parameters studied are appropriate for an adequate characterisation of the toxicological effect(s).

If an evaluation allows the conclusion that the information of the repeated dose toxicity is adequate for a robust characterisation of the toxicological hazards, including an estimate of a dose descriptor (NOAEL/LOAEL/BMD), and the data are adequate for risk assessment and classification and labelling, no further testing will be necessary unless there are indications for further risk, according to column 2 of Annexes VIII-X of REACH.

Another consideration to be taken into account is whether the study duration has been appropriate for an adequate expression of the toxicological effects. If the critical effect involves serious specific system or target organ toxicity (e.g. haemolytic anaemia, neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity and a threshold has **not** been established dose extrapolation may not be appropriate and further studies are required. In this case a specialised study is likely to be more appropriate for an improved hazard characterisation and should be considered instead of a standard short-term rodent or sub-chronic toxicity test at this stage.

In the identification of the NOAEL, other factors need to be considered such as the severity of the effect, the presence or absence of a dose- and time-effect relationship and/or a dose- and time-response relationship, the biological relevance, the reversibility, and the normal biological variation of an effect that may be shown by representative historical control values (IPCS, 1990).

R.7.5.5.1 Concluding on suitability for Classification and Labelling

In order to conclude on the suitability for classification and labelling (C&L), the data requirements in Annex VI of the dangerous substances Directive 67/548/EEC[42] have to be considered (box 4 in Figure R.7.5-1).

A decision on classification and labelling will affect downstream events/Directives under REACH. Therefore, it is important that the data are adequate for checking against the classification criteria in order to ensure safe use under REACH.

Basically the following conclusions can be obtained from the assessment of adequacy for C&L for repeated dose toxicity:

- Data are considered adequate for the purpose of C&L and can be checked against the criteria (boxes 6 and 11 in Figure R.7.5-1)[43].
- Data are considered as inadequate for the purpose of C&L and cannot be checked against the criteria (inconclusive or lacking data). In this case testing should be considered in relation to the risk management of the substance.

R.7.5.5.2 Concluding on suitability for Chemical Safety Assessment

In order to be suitable for CSA (box 5 of Figure R.7.5-1) appropriate DNELs 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 Chapter R.8).

Identification of the so-called dose descriptor: i.e. an appropriate threshold dose for the critical effect as the starting point for DNEL derivation, i.e. a NOAEL or BMD. If a NOAEL can not be identified, the LOAEL may be used instead provided the data are adequate for a robust hazard assessment.

It is to be noted that the dose descriptor should be route-specific. Thus, in case only animal data with oral exposure are available and humans are exposed mainly via skin and/or inhalation, a DNEL for dermal route and/or DNEL for inhalation route are needed: i.e. route-to-route extrapolation is needed, if allowed. Guidance for this route-to-route extrapolation is provided in Section R.8.4.2.

If this route-to-route extrapolation is not allowed, route-specific information is needed, possibly including testing, as a last resort (see Section R.7.5.6.3).

Derivation of a DNEL from this dose descriptor by applying AFs (to address uncertainty in the available data) is described elsewhere (see Section R.8.4.3; see also Section R.7.5.4.4).

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[42] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

[43] It should be noted that although the exposure assessment and risk characterisation need not to be performed, when a substance is not classified (see Part A, section A.1.2), for potency-based endpoints like repeated dose toxicity, there could still potentially be a risk. Therefore one might consider performing an exposure assessment and risk characterisation on voluntary basis, to ensure safe handling and use.

R.7.5.5.3 Information not adequate

A *Weight of Evidence* approach comparing available adequate information with the tonnage-triggered information requirements by REACH may result in the conclusion that the requirements are not fulfilled. In order to proceed in further information gathering the testing strategy described in Section R.7.5.6.3 can be adopted.

R.7.5.6 Integrated Testing Strategy (ITS) for repeated dose toxicity

R.7.5.6.1 Objective / General principles

The objective in this testing strategy is to give guidance on a stepwise approach to hazard identification with regard repeated dose toxicity. A principle of the strategy is that the results of one study are evaluated before another study is initiated. The strategy seeks to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

The core objectives of the Integrated Testing Strategy (ITS) for repeated dose toxicity are to generate sufficient information to allow:

- Characterisation of the hazard profile and the dose-response of a substance upon repeated exposure.
- Performance of a chemical safety assessment for repeated dose toxicity.

Information generated in this strategy should be suitable for Classification and Labelling according to the criteria given in Annex VI to Directive 67/548/EEC[44].

In addition, information from repeated dose toxicity studies can give valuable information to other endpoints based on repeated exposure (e.g. reproductive and developmental toxicity), and are valuable for other *in vivo* studies.

R.7.5.6.2 Preliminary considerations

On the basis of the objectives outlined above, a framework has been developed so that informed decisions can be made on the need for further testing. If generation of further data is deemed necessary, the information needs should be met efficiently in terms of resources and animal use. This means the use of the most appropriate study type in accordance with the tonnage-driven requirements stipulated by the REACH information requirements and taking into account modifications due to considerations of exposure, grouping and category formation. The data requirements may be increased or decreased taking into account exposure considerations or the level of concern noted during any of the stages in the testing strategy.

Testing for repeated dose toxicity is not required for chemicals produced at tonnage levels less than 10 tonne per annum (t/y). At higher production volumes, standard data requirements are, in general, increased with each tonnage band (see Section R.7.5.2); maintaining flexibility to adopt the most appropriate testing regime for any single chemical is a key component of the ITS. However, regardless of whether testing for repeated dose toxicity is required or not at a specific tonnage level, all existing test data, and all other available and relevant information on the substance should be collected.

[44] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

R.7.5.6.3 Testing strategy for repeated dose toxicity

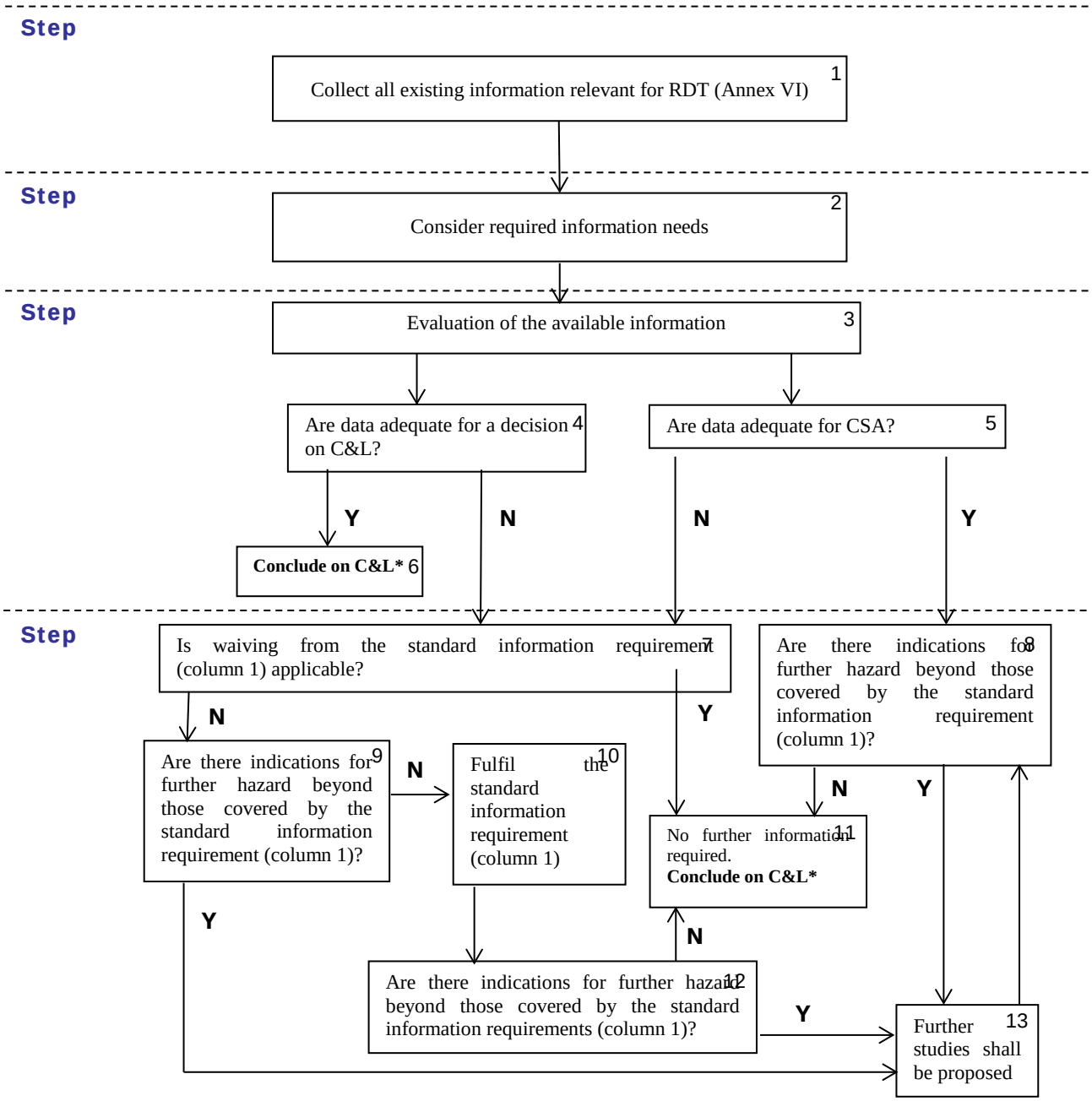
In order to proceed in further information gathering the following testing strategy is out-lined (step 4 in Figure R.7.5-1).

Before testing is initiated the available information should be scrutinised for evidence that may indicate severe effects, serious specific system or target organ toxicity (e.g. neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity (boxes 8, 9 and 12 in Figure R.7.5-1). These indications may provide a trigger for specialised study protocols instead of the standard protocols for the short-term and/or (sub)chronic toxicity (box 13 in Figure R.7.5-1). These specific protocols should be designed on a case-by-case basis, such that they enable an adequate characterisation of these hazards, including the dose-response, threshold for the toxic effect and an understanding of the nature of the toxic effects. An example of such an approach is given in Appendix R.7.5-1.

Annexes VII-X of the REACH regulation provide the standard information requirements in Column 1 (box 10 of Figure R.7.5-1) and specify triggering and waiving possibilities for the specific endpoints in Column 2. Different descriptors used for repeated dose toxicity in these annexes varying from *limited* (Annex IX) to *no relevant exposure* (Annex VIII). In addition, Annex XI of the REACH regulation contains basic approaches, or rules for adaptation of the standard testing regime, set out in Annexes VII-IX (see Chapter R.5; for waiving see box 7 in Figure R.7.5-1).

Exposure considerations at this stage may trigger a need for additional data if the applications include wide dispersive uses to a large population (e.g. consumer products) and if a particular concern exists for a low margin of exposure (box 13 in Figure R.7.5-1). The data to be generated at this stage should aim to improve the risk quotient and could therefore be a trigger for an improved exposure characterisation or an improved hazard characterisation. In the latter case the required information might include a special study leading to an improved characterisation of the critical toxic endpoint thereby decreasing the uncertainty in the NOAEL for repeated dose toxicity. An example of such a testing approach applied to neurotoxicity is given in Appendix R.7.5-1.

Figure R.7.5-1 Integrated Testing Strategy for repeated dose toxicity



Utilisation of the different tests at each of the different tonnage levels is summarised below:

10 t/y or more (Annex VIII)

At this tonnage level a short-term (28-day) toxicity test (OECD TG 407/EU B.7) is usually required. The use of a combined repeated dose toxicity study with the

reproduction/developmental toxicity screening test (OECD TG 422[45]) is recommended if an initial assessment of repeated dose toxicity and reproductive toxicity is required. The route of exposure in these tests is oral unless the predominant route of human exposure or the physico-chemical properties indicate that the dermal or inhalational route may be a more appropriate route of exposure to assess the repeated dose toxicity test (requiring OECD TG 410 or 412/EU B.9 or B.8).

If the results of a short-term rodent toxicity study (OECD TGs 407; 410, 412, 422) are adequate for a dose response characterisation and C&L and risk assessment, and if there are no indications for further risks, no further testing is required (see Section R.7.5.5.2 for a detailed discussion of the criteria for a robust hazard characterisation).

At this tonnage level the short-term toxicity study (28 days) does not need to be conducted if:

- a reliable sub-chronic (90 days) or chronic toxicity study is available, provided that an appropriate species, dosage, and route of administration were used; or
- where a substance undergoes immediate disintegration and there are sufficient data on the cleavage products; or
- relevant human exposure can be excluded in accordance with Annex XI Section 3.

It should be noted that any of the rules for adaptation according to Annex XI also apply (see Chapter R.5). For further details see this section under Annex XI (below).

According to REACH (Annex IX, 8.6.2), the sub-chronic toxicity study (90 days) shall be proposed by the registrant if:

- the frequency and duration of human exposure indicates that a longer term study is appropriate;

and one of the following conditions is met:

- other available data indicate that the substance may have a dangerous property that cannot be detected in a short-term toxicity study; or
- appropriately designed toxicokinetic studies reveal accumulation of the substance or its metabolites in certain tissues or organs which would possibly remain undetected in a short-term toxicity study but which are liable to result in adverse effects after prolonged exposure.

REACH also specifies that 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:

- failure to identify a NOAEL in the 28 or the 90 days study, unless the reason for the failure to identify a NOAEL is absence of adverse toxic effects; or
- toxicity of particular concern (e.g., serious/severe effects); or
- 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); or

[45] To date there is no corresponding EU testing method available.

- the route of exposure used in the initial repeated dose study was inappropriate in relation to the expected route of human exposure and route-to-route extrapolation cannot be made; or

- particular concern regarding exposure (e.g. use in consumer products leading to exposure levels which are close to the dose levels at which toxicity to humans may be expected); or

- effects shown in substances with a clear relationship in molecular structure with the substance being studied, were not detected in the 28 or the 90 days study.

It should be pointed out that a failure to identify a NOAEL does not lead to a data gap in every case and should not trigger additional studies by default. If the data are sufficient for a robust hazard assessment and for Classification and Labelling, the LOAEL may be used as the starting point for the CSA (see also Sections R.7.5.4.4 and R.7.5.5 and Chapter R.8).

A specialised study is likely to be more appropriate for an improved hazard characterisation and should be considered instead of a standard short-term rodent or sub-chronic toxicity test at this stage.

100 t/y or more (Annex IX)

At this tonnage level, the following information is required (REACH Annex IX, Sections 8.6.1 and 8.6.2):

- a short-term study (28 day) in a single rodent species is the minimum requirement. The default route of exposure in these tests is oral (OECD TG 407/EU B.7; TG 422[46]) unless the predominant route of human exposure or the physico-chemical properties indicates that the dermal or inhalational route (OECD TG 410, 412/EU B.9, B.8) is a more appropriate route of exposure in the repeated dose toxicity tests.

- a sub-chronic toxicity study (90-day) in a single rodent species is usually required. The default route of exposure in these tests is oral (OECD TG 408/EU B.26) unless the predominant route of human exposure or the physico-chemical properties indicates that the dermal or inhalational route (OECD TG 411, 413/EU B.28, B.29) is a more appropriate route of exposure in the repeated dose toxicity tests.

According to REACH, at this tonnage level the sub-chronic toxicity study (90 days) does not need to be conducted if:

- a reliable short-term toxicity study (28 days) is available showing severe toxicity effects according to the criteria for classifying the substance as R48, for which the observed NOAEL-28 days, with the application of an appropriate assessment factor, allows the extrapolation towards the NOAEL-90 days for the same route of exposure; or

- a reliable chronic toxicity study is available, provided that an appropriate species and route of administration were used; or

- a substance undergoes immediate disintegration and there are sufficient data on the cleavage products (both for systemic effects and effects at the site of uptake); or

- the substance is unreactive, insoluble and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-day limit test, particularly if such a pattern is coupled with limited human exposure;

[46] To date there is no corresponding EU testing method available.

It should be noted that any of the rules for adaptation according to Annex XI also apply. For further details see the section on Annex XI below.

In case human exposure is limited or different in frequency and duration from that used in the test protocol for repeated dose toxicity, the sub-chronic toxicity study may not be necessary if the data for the short-term toxicity study are adequate for a robust hazard characterisation, a risk assessment and classification and labelling. This adaptation requires full justification by the registrant.

In case the weight of the evidence indicates that the available information is adequate to characterise the short-term toxicity and sufficiently robust for proper dose-selection of the 90-day study, a dedicated 28-day study is not necessary at this stage.

No further testing is required if the available data, which may include a sub-chronic rodent toxicity study (OECD TG 408, 411, 413/EU B.26, B.28, B.29) are adequate for a dose response characterisation and C&L and risk assessment.

In case data are inadequate for hazard characterisation and risk assessment further studies shall be proposed by the registrant or may be required by the Agency in accordance with REACH Articles 40 or 41: According to REACH Annex IX Section 6.6.2 such a situation may arise if there is:

- failure to identify a NOAEL in the 90 days study unless the reason for the failure to identify a NOAEL is absence of adverse toxic effects; or
- toxicity of particular concern (e.g. serious/severe effects); or
- 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); or
- particular concern regarding exposure (e.g. use in consumer products leading to exposure levels which are high relative to the dose levels at which toxicity to humans occurs)

A specialised study is likely to be more appropriate for an improved hazard characterisation and should be considered instead of a standard short-term rodent or sub-chronic toxicity test. An example of such an approach given in Appendix R.7.5-1.

It should be pointed out that a failure to identify a NOAEL does not lead to a data gap in every case and should not be a default trigger for additional studies. If the data are sufficient for a robust hazard assessment or for Classification and Labelling, the LOAEL may be used as the starting point for the CSA (see also Sections R.7.5.4.4 and R.7.5.5 and Chapter R.8).

1000 t/y or more (Annex X)

There is no default testing requirement for repeated dose toxicity at this tonnage level beyond those recommended for the level 100 t/y or more (see above). However, in accordance with REACH Articles 40 and 41, if the frequency and duration of human exposure indicates that a long-term study is appropriate and one of the following conditions is met a long-term repeated toxicity test (≥12 months) may be proposed:

- serious or severe toxicity effects of particular concern were observed in the 28-days or 90-days study for which available evidence is inadequate for toxicological evaluation or risk characterisation; or

- effects shown in substances with clear relationship in molecular structure with the substance being studied were not detected in the 28-days or 90-days study; or
- the substance may have a dangerous property that cannot be detected in a 90-days study.

In addition, further studies shall be proposed by the registrant or may be required by the Agency in accordance with REACH Articles 40 or 41, in case of:

- toxicity of particular concern (e.g. serious/severe effects); or
- indications of an effect for which the available evidence is inadequate for toxicological evaluation 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); or
- particular concern regarding exposure (e.g. use in consumer products leading to exposure levels which are close to the dose levels at which toxicity is observed).

In some cases a specialised study might the most appropriate study in case an improved hazard characterisation is necessary and should be considered instead of a standard sub-chronic or chronic toxicity test. An example of such an approach given in Appendix R.7.5-1.

No further testing is required if the results of a sub-chronic rodent toxicity study (OECD TG 408, 410, 411, 412, 413 or EU B.26, B.9, B.28, B.8, B.29) are adequate for a robust hazard characterisation and suitable for risk assessment and classification and labelling (see step 3 Identify data gaps for a detailed discussion of the criteria for a robust hazard characterisation).

Also, the testing requirements can be adapted if any of the rules according to REACH Annex XI apply: For further details see this Section under *REACH Annex XI* (below).

As there is no standard test requirement at this tonnage level, column 2 also had no waiving options.

REACH Annex XI adaptations of the standard testing regime for repeated dose toxicity

General guidance on the application of the Annex XI adaptations to information requirements is given in Chapter R. 5. For repeated dose toxicity the following additional guidance applies.

Testing does not appear scientifically necessary

Some substances may be excluded from testing for repeated dose toxicity if it does not appear scientifically necessary (Annex XI Section 1). This might be the case for example if:

- a *Weight of Evidence* analysis demonstrates that the available information is sufficient for an adequate hazard characterisation, and a CSA where the exposure to the substance is adequately controlled;
- a substance is not bio-available via a specific route and possible local effects have been adequately characterised;
- the vapour pressure is sufficiently low that inhalational exposures are unlikely to be of significance, or if human exposure is limited to dusts or aerosols unlikely to be inhalable
- for substances belonging to a group or a category of substances that have a common functionality and/or breakdown products or sufficient information for a qualitative and quantitative understanding of the toxicological properties, testing of all individual

category members may not be necessary (Annex XI Section 1.5). The criteria for application of read-across for a category of substances and detailed guidance can be found in Sections R.4.3.2 and R.6.2.

Testing is technically not possible

There may also be cases where it is technically not possible to conduct a repeated dose toxicity test (Annex XI Section 2). This might be the case if

- The substance ignites in air at ambient conditions.
- The substance undergoes immediate disintegration. In such a case the information requirements for the cleavage products should be assessed following an approach similar to that outlined in this document.
- The substance is corrosive in the dose range of interest for the study. Also, for reasons of animal welfare such studies should be avoided.

Substance-tailored exposure-driven testing

Exposure considerations may also lead to adaptation of the testing requirements (Annex XI Section 3). This might be the case if:

Testing requirements may be adapted based on a substance-specific exposure-assessment according to Annex XI Section 3. In this case testing for short-term repeated dose toxicity (Annex VIII, 8.6.1) may be waived at the 10-100 tonnage level if relevant human exposure can be excluded (see Section R.7.5.4.3).

Human exposure is limited at the tonnage level of 100 t/y or more (Annexes IX and X). The need for a sub-chronic study should be considered if the substance is only handled in industrial or commercial installations using closed systems and/or handled only as preparations at low concentrations.

Appendix 1 to Section R.7.5

Appendix R.7.5-1 Testing strategy for specific system / organ toxicity.

Content of Appendix 7.5-1

1. General aspects
2. Structure-activity considerations
3. Assessment of available information or results from initial testing
4. Recommendations from the WHO/FAO Joint Meeting of Experts on Pesticide Residues (JMPR)
5. Further neurotoxicity testing

Mechanisms of respiratory irritation

1. GENERAL ASPECTS

For some specific system/organ effects the testing methods of EU Annex V or the OECD may not provide for adequate characterisation of the toxicity. There may be indications of such effects in the standard studies for systemic toxicity, or from SAR. For adequate characterisation of the toxicity and, hence, the risk to human health, it may be necessary to conduct studies using other published test methods, *in-house* methods or specially designed tests. Some references are given in Error! Reference source not found.. Before initiating a study to investigate specific organ/system toxicity, it is important that the study design is presented to the Agency, in order that the need for (and scope/size of) studies using live animals should be particularly carefully considered.

Specific investigation of organ/systemic toxicity is to some extent undertaken as part of the repeated dose toxicity tests conducted according to test guidelines of the OECD and Annex V to Directive 67/548/EEC[47]. Specific investigation (or further investigation) of any organ/system toxicity (e.g. immune, endocrine or nervous system) may sometimes be necessary and should be addressed on a case-by-case basis. As an example of a testing strategy the approach for neurotoxicity is given below.

Definition of neurotoxicity

Neurotoxicity is the induction by a chemical of adverse effects in the central or peripheral nervous system, or in sense organs. It is useful for the purpose of hazard and risk assessment to differentiate sense organ-specific effects from other effects which lie within the nervous system. A substance is considered *neurotoxic* if it induces a reproducible lesion in the nervous system or a reproducible pattern of neural dysfunction.

The starting point for the testing strategy are the REACH requirements specified in Annex VIII, IX and X and detailed in Section R.7.5.6.3 Depending on the tonnage level, these requirements may trigger a 28-day and/or a 90-day test (e.g. OECD TG 407, 408/EU B.7, B.26). These protocols include a number of nervous system endpoints (e.g. clinical observations of motor and autonomous nervous system activity, histopathology of nerve tissue), which should be regarded as the starting point for evaluation of a substance potential to cause neurotoxicity. It

[47] All the test methods previously included in Annex V to Directive 67/548/EEC will be incorporated in a new Test Methods (TM) Regulation that is currently (February 2008) under adoption. The TM Regulation will be adapted to technical progress whenever a new test method has been developed, scientifically validated and accepted for regulatory use by the National Coordinators of the Member states

should be recognised that the standard 28-/90-day tests only measure some aspects of nervous system structure and function e.g. Functional Observational Battery, while other aspects, e.g. learning and memory and sensory function is not or only superficially tested. SAR considerations may prompt the introduction of additional parameters to be tested in standard toxicity tests or the immediate request of studies such as delayed neurotoxicity (OECD TG 418 or 419/EU B.37 or B.38,; see below).

If there are no indications of neurotoxicity from available information i.e. adequately performed repeated dose toxicity tests, other testing systems (e.g. *in vitro*), non-testing systems ((Q)SAR and read-across) or human data, it will not be necessary to conduct any special tests for neurotoxicity.

The approach presented below is a hierarchical, step-wise strategy to investigate the potential neurotoxicity of a substance. It should be pointed out that the requirements outlined in steps 1 and 2 are met by the tonnage-based information requirements in Annex VIII, IX and X of REACH.

2. STRUCTURE-ACTIVITY CONSIDERATIONS

Structural alerts are only used as a positive indication of neurotoxic potential. Substance classes with an alert for neurotoxicity may include organic solvents (for chronic toxic encephalopathy); organophosphorus compounds (for delayed neurotoxicity), and carbamates (for cholinergic effects). Several estimation techniques are available, one of which is the rule-based DEREK (Deductive Estimation of Risk from Existing Knowledge) system. The rulebase comprises the following hazards and structural alerts: Organophosphate (for direct and indirect anticholinesterase activity); N-methyl or N,N-dimethyl carbamate (for direct anticholinesterase activity); gamma-diketones (for neurotoxicity).

3. ASSESSMENT OF AVAILABLE INFORMATION OR RESULTS FROM INITIAL TESTING

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.

Neurotoxicity may be indicated by the following signs: morphological (structural) changes in the central or peripheral nervous system or in special sense organs; neurophysiological changes (e.g. electroencephalographic changes); behavioural (functional) changes; neurochemical changes (e.g. neurotransmitter levels).

A *Weight of Evidence* approach should be taken into account for the assessment of the neurotoxicity and the type, severity, number and reversibility of the effect should be considered. A consistent pattern of neurotoxic findings rather than a single or a few unrelated effects should be taken as persuasive evidence of neurotoxicity.

It is important to ascertain whether the nervous system is the primary target organ. The reversibility of neurotoxic effects should also be considered. The potential for such effects to occur in exposed humans (i.e. the exposure pattern and estimated level of exposure are *acute*) should be considered in the risk characterisation. Reversible effects may be of high concern depending on the severity and nature of effect. In this context it should be kept in mind that effects observed in experimental animals that appear harmless might be of high concern in humans depending on the setting in which they occur (e.g. sleepiness in itself may not be harmful, but in relation to operation of machinery it is an effect of high concern). Furthermore

the possibility that a permanent lesion has occurred cannot be excluded, even if the overt effect is transient. The nervous system possesses reserve capacity, which may compensate for the damage, but the resulting reduction in the reserve capacity should be regarded as an adverse effect. Irreversible neurotoxic effects are of high concern and usually involve structural changes, though, at least in humans, lasting functional effects (e.g. depression, involuntary motor tremor) are suspected to occur as a result of neurotoxicant exposure, apparently without morphological abnormalities.

For the evaluation of organophosphate pesticides, the WHO/FAO Joint Meeting of Experts on Pesticide Residues (JMPR) has published recommendations on "Interpretation of Cholinesterase Inhibition" (FAO, 1998; 1999). The applicability of these recommendations, outlined below, could also be extended to other substances that inhibit cholinesterase. It should be pointed out that for substances that may have a structural alert for cholinesterase inhibition, the measurement of acetylcholinesterase activity as recommended by JMPR can be included in the list of parameters for the standard 28- or 90 day testing protocols required by REACH, irrespective of the route of exposure.

4. RECOMMENDATIONS FROM THE WHO/FAO JOINT MEETING OF EXPERTS ON PESTICIDE RESIDUES (JMPR)

The inhibition of brain acetylcholinesterase activity and clinical signs are considered to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterases. Inhibition of erythrocyte acetylcholinesterase is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. The use of erythrocyte acetylcholinesterase inhibition as a surrogate for peripheral effects is justified for acute exposures resulting in greater acetylcholinesterase inhibition in erythrocytes than in the brain. However, reliance on inhibition of erythrocytic enzyme in studies of repeated doses might result in an overestimate of inhibition on peripheral tissues, because of the lower rate of resynthesis of the enzyme in erythrocytes than in the nervous system. Plasma acetylcholinesterase inhibition is considered not relevant. Regarding brain and erythrocyte acetylcholinesterase inhibition, the experts defined that statistically significant inhibition by 20% or more represents a clear toxicological effect and any decision to dismiss such findings should be justified. JMPR also agreed on the convention that statistically significant inhibition of less than 20% or statistically insignificant inhibition above 20% indicate that a more detailed analysis of the data should be undertaken. The toxicological significance of these findings should be determined on a case-by-case basis. One of the aspects to consider is the dose-response characteristic.

5. FURTHER NEUROTOXICITY TESTING

If the data acquired from the standard systemic toxicity tests required by REACH provide indications of neurotoxicity which are not adequate for a hazard assessment, risk characterisation or classification and labelling, the nature of further investigation will need to be considered. If a 90-day study is triggered to meet the requirements of Annex IX following a standard 28-day study, a number of endpoints assessing the nervous system endpoints should be included,. irrespective of the administration route. In some cases, it may be necessary to conduct a specific study such as a neurotoxicity test using the OECD TG 424 with possible inclusion of a satellite group for assessment of reversibility of effects. The OECD TG 424 is intended for confirmation or further characterisation of potential neurotoxicity identified in previous studies. The OECD guideline allows for a flexible approach, in which the number of simple endpoints which duplicate those already examined during standard testing may be minimised, and where more effort is put into in-depth investigation of more specific endpoints by inclusion of more specialised tests. Adjustment of dose levels to avoid confounding by general toxicity should be considered.

If data from standard toxicity studies are clearly indicative of specific neurotoxicity, e.g. neurotoxicity occurring at lower dose levels than systemic toxicity, further specific neurotoxicity testing is required to confirm and extend the findings from the general toxicity studies and to establish an NOAEL for neurotoxicity. Again, the neurotoxicity test according to OECD TG 424 is considered appropriate for this situation.

Certain substances and/or certain effects are best investigated in particular species. Pyridine derivatives are neurotoxic to humans and primates but not to rats. Among other neurotoxic compounds, organophosphorus compounds are a group with known delayed neurotoxic properties, which need to be assessed in a specified test for delayed neurotoxicity, to be performed preferentially in the adult laying hen according to EU B.37 or OECD TG 418 (Delayed neurotoxicity of organophosphorus substances following acute exposure) and B.38 or OECD TG 419 (Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study). Such studies are specifically required for biocidal substances of similar or related structures to those capable of inducing delayed neurotoxicity. If anticholineesterase activity is detected, a test for response to reactivating agent may be required.

Standard exposure conditions may not always be adequate for neurotoxicity studies. The duration of exposure needed to induce specific neurotoxic effects in an animal experiment will depend on the underlying mechanism of action. Short-term peak exposures can be important for certain types of substance/effect. When the test compound is administered as a bolus via the intravenous, subcutaneous or oral route it is essential to determine the time-effect course, and to perform measurements of neurotoxicity parameters preferentially at the time of peak effect.

For example, the neurotoxicity associated with short-term exposure to some volatile organic solvents has largely been identified following human exposure - particularly occupational exposure. Acute inhalation studies, using protocols designed to detect the expected effects, are ideal for such substances/effects. For some neurotoxic substances a long exposure period is necessary to elicit neurotoxicity.

The most appropriate methods for further investigation of neurotoxicity should be determined on a case-by-case basis, guided by the effects seen in the standard systemic toxicity tests and/or from SAR-based predictions. Extensive coverage of methods which may be used is given in OECD (2004a), IPCS (1986) and ECETOC (1992), and some are summarised in the Table R.7.5-3.

Table R.7.5-3 Methods for investigation of neurotoxicity

Effect	Methods available	References*
Morphological changes	Neuropathology. Gross anatomical techniques. Immunocytochemistry. Special Stains	Krinke, 1989; Odonoghue, 1989; Mattson et al., 1990
Physiological changes	Electrophysiology (e.g. nerve conduction velocity (NCV), Electroencephalogram (EEG), evoked potentials	Fox et al., 1982; Rebert, 1983; Mattson and Albee, 1988
Behavioural changes	Functional observations. Sensory function tests. Motor function tests (e.g. locomotor activity). Cognitive function tests	Robbins, 1997; Tilson et al., 1980; Cabe and Eckerman, 1982; Pryor et al., 1983 Moser and McPhail, 1990; Moser 1995
Biochemical changes	Neurotoransmitter analysis. Enzyme/protein activity. Measures of cell integrity.	Dewar and Moffet, 1977; Damstra and Bondy, 1982; Cooper et al., 1986; Costa, 1998.

*Given in full in ECETOC (1982), IPCS (1986) or Mitchell (1982)

R.7.5.7 References on repeated dose toxicity

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R.7.6 Reproductive and developmental toxicity

R.7.6.1 Introduction

At the population level the property of reproductive toxicity is of obvious high concern because the continuance of the human species is dependent on the integrity of the reproductive cycle. Similarly, to the individual an impairment of the ability to reproduce and the occurrence of developmental disorders are self-evidently serious health conditions. Therefore it is important that the potential hazardous properties with respect to reproduction are established for chemicals with relevant human exposure that may be present in the environment, at the workplace and in consumer products.

R.7.6.1.1 Definition of reproductive toxicity

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, developmental toxicity in the offspring and effects on or mediated via lactation, as defined in Part 3 of the Globally Harmonised System of Classification and Labelling of Chemicals System (GHS) (United Nations 2005). 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*). Effects on male or female fertility include adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or hormonal or physiological response, which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation. Developmental toxicity includes any effect interfering with normal development, both before and after birth. It includes effects induced or manifested either pre- or postnatally. This includes embryotoxic/foetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional effects, peri- and postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

R.7.6.1.2 Objective of the guidance on reproductive toxicity

To provide guidance to all stakeholders, in order to establish:

- whether exposure of humans to the substance of interest has been associated with reproductive toxicity and/or
- whether, on the basis of information other than human data, it can be predicted that the substance will cause reproductive toxicity in humans.
- whether the pregnant female is potentially more susceptible to general toxicity;
- the dose-response relationship for any adverse effects on reproduction.

Substance-related adverse effects on reproduction are always of potential concern, but it is important, where possible, to distinguish between a specific effect on reproduction as a consequence of an intrinsic property of the substance and an adverse reproductive effect which is a non-specific consequence to general toxicity (e.g. marked changes in bodyweight, marked reductions in food or water intake, maternal stress, see Section R.7.6.4.1 for further discussion).

R.7.6.2 Information requirements for reproductive toxicity

The standard data requirements for reproductive toxicity under the REACH Regulations are as follows:

- A reproduction/developmental toxicity screening test (OECD TGs 421 or 422)[48], usually required at the Annex VIII tonnage.

- A prenatal developmental toxicity study (EU B.31, OECD TG 414) in one species, usually required at the REACH Annex IX level. A study in a second species should be considered at either Annex IX or at Annex X level.

- A two-generation reproduction toxicity study[49] (OECD TG 416, EU B.35) in one species, usually required at the Annex X level.

However, according to column 2 specific rules (see Annexes VIII-X of the REACH legislation) and to Annex XI these tonnage-related standard data requirements can be adapted, either as reduced (a data waiver) or deferred testing or as the need for extended testing, as detailed in the stepwise Integrated Testing Strategy presented in Section R.7.6.6. Factors that can influence the testing requirements include structural relationships with other chemicals, the results of other toxicity studies, presence of mutagenic and carcinogenic properties, available data from humans exposed to the substance, concerns for endocrine disruption and the use and human exposure patterns.

This guidance provides advice on how the registrant can meet the information requirements of REACH, thereby providing data on the hazardous properties that can be used for classification (include a PBT assessment) and in the risk assessment.

R.7.6.3 Information on reproductive toxicity and its sources

Relevant information on reproductive toxicity can be obtained from various of sources, which are indicated below.

R.7.6.3.1 Non-human data on reproductive toxicity

Non-testing data on reproductive toxicity

Information of relevance to reproductive toxicity can be inferred from the physico-chemical characteristics of a substance.

Information on SARs (chemical grouping or read-across) and (Q)SAR models may be available.

Testing data on reproductive toxicity

In vitro data

Currently there is no officially adopted EU or OECD test guideline for *in vitro* tests of relevance to reproductive toxicity. Three tests have recently been subjected to an extensive multicentre validation study in the EU (Genschow *et al.* 2002) and have been declared to be scientifically validated tests for use in assessing embryotoxic potential according to the European Centre for the Validation of Alternative Methods (ECVAM) procedures:

- embryonic stem cell test (EST, Genschow et al. 2004)

- limb bud micromass culture (Spielmann et al. 2004)

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

[49] A proposed *F1-extended one-generation study* may replace OECD TG 416 as a definitive study for reproductive toxicity in the near future, subject to gaining regulatory acceptance in the EU

- whole embryo culture (WEC, Piersma et al. 2004)

Recently, *in vitro* tests for detecting a potential to affect endocrine activity have become available (Nordic Chemicals Group, 2005). Most of the assays that are relevant to reproductive toxicity are designed to assess the ability of a chemical to bind and activate or block the androgen receptor (AR) or the oestrogen receptor (ER). These include cell-free or whole cell binding assays, cell proliferation assays and transcription assays. Also, tests for detecting the ability to interfere with steroidogenesis are currently being developed.

The latest information on the status of alternative methods that are under development can be obtained from the ECVAM website (current address: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam) and other international centres for validation of alternative methods.

Animal data

Data may be available from a wide variety of animal studies, which give different amounts of direct or indirect information on the potential reproductive toxicity of a substance; e.g.:

- screening studies (such as OECD TGs 421 or 422)[50]
- other short-term in vivo screening tests (e.g. Chernoff/Kavlock tests see Hardin et al. 1987, uterotrophic and Hershberger assays)
- one- or two- (or multi-) generation studies (such as B.35, OECD TGs 415 or 416,or EU B.34 or a 'F1-extended one-generation study, as proposed by the ILSI Agricultural Chemical Safety Assessment Project)
- prenatal developmental toxicity tests (such as EU B.31, OECD TG 414)
- developmental neurotoxicity studies (such as draft OECD TG 426)[51]
- peri-postnatal studies
- male or female fertility studies of non-standard design
- repeated-dose toxicity studies, if relevant parameters are included, for example semen analysis, oestrous cyclicity and/or reproductive organ histopathology
- dominant lethal assay (EU B.22, OECD TG 478)
- mechanistic and toxicokinetic studies
- studies in non-mammalian species

R.7.6.3.2 Human data on reproductive toxicity

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.

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

[51] To date there is no corresponding EU testing method available.

R.7.6.4 Evaluation of available information for reproductive toxicity

The generic guidance on the process of judging and ranking the available data for its adequacy (reliability and relevance) completeness and remaining uncertainty is provided in Chapter R.4. This generic guidance is relevant to reproductive toxicity.

R.7.6.4.1 Non-human data on reproductive toxicity

Non-testing data on reproductive toxicity

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 *Weight of Evidence* assessment.

Additional generic guidance on this topic is provided in Section R.7.6.6 (see also Section R.4.4).

Read-across to structurally or mechanistically similar substances (SAR)

The concept of structure-activity relationships (SAR) offers approaches for estimating the reproductive toxicity potential of a substance. By grouping substances with similar structures there is an opportunity for the toxicity potential of well-investigated substances to be extended to substances for which there are no or incomplete data. This is particularly the case where the toxicity profile (or lack thereof) can be associated with structural characteristics and reproductive toxicity potential may be extrapolated or interpolated across a homologous series or category. Such an approach has been endorsed under the chemical category concept, which has been developed under the OECD HPVC program (OECD 2004) and further elaborated for the context of REACH as an approach to fill data gaps with a reduced requirement for testing.

Another consideration relates to a substance for which a mechanism of toxicity has been identified that is causally related to reproductive toxicity. In such cases, substances with a similar mechanism identified in other screening tests (e.g. repeated-dose toxicity tests or screens for endocrine activity) may reasonably be expected to exhibit the same pattern of reproductive toxicity. Further testing may be required, on a case-by-case basis, to support a read-across proposal.

Additional generic guidance on this topic, including reporting formats, is provided in Section R.6.2.6.

(Q)SAR

There are a large number of potential targets/mechanisms associated with reproductive toxicity that, on the basis of current knowledge, cannot be adequately covered by a battery of QSAR models. Unlike some toxicological endpoints for which specific structural alerts have been identified (e.g. mutagenicity, sensitisation), there are currently no formal criteria to identify structural alerts for reproductive toxicity.

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. Therefore, a negative result from current QSAR models cannot be interpreted as demonstrating the absence of a reproductive hazard unless there is other supporting evidence.

Another limitation of QSAR modelling is that dose-response information, for example the N(L)OAEL, required for risk assessment is not provided.

However, a positive result in a validated QSAR model could provide a trigger (alert) for further testing but because of limited confidence in this approach such a result would not normally be adequate as a primary support for a hazard classification decision.

Additionally, QSAR models could be used as part of a *Weight of Evidence* approach, when considered alongside other data, provided the applicability domain is appropriate. Also, QSARs 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 QSAR modelling results can be of value in a read-across assessment.

Additional generic guidance on QSARs is provided in Section R.6.1.

Testing data on reproductive toxicity

In vitro data

In vitro testing is a rapidly developing field, with significant recent improvements particularly in developmental toxicity and the detection of a potential to affect endocrine activity, which holds much promise for the future. 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.

At the present time *in vitro* approaches have many limitations, for example the lack of capacity for biotransformation of the test substance (Coecke et al 2006). Consequently, no firm recommendations can be made for the exclusive use of *in vitro* methods in a testing strategy for reproductive 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 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 in a validated *in vitro* test could provide a justification for further testing, 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* assessment approach to gathering the information required to support a classification decision and risk assessment. *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 can be of value in a read-across assessment. Generic guidance is given in Chapters R.4 and R.5 for judging the applicability and validity of the outcome of various study methods.

Notably, the recent validation study of the three most promising tests for detection of developmental effects, the embryonic stem cell test, the limb bud micromass culture and the whole embryo culture, showed that these had high predictivity for the limited number of strongly embryotoxic chemicals included in the study (Genschow *et al*. 2002, Piersma 2006,

Spielmann *et al*. 2006). However, a number of weaknesses in the design of both the validation study and of the *in vitro* tests have been identified, such as the limited number and range of substances tested and absence of a biotransformation system, which have lead to the conclusion that the tests currently have limited value in a regulatory context. Nevertheless, as discussed above, the results of these tests can have a role, when considered alongside other data, in a *Weight of Evidence* assessment and in support of read-across approaches, and can serve as a trigger for further testing. The results of other *in vitro* tests for developmental toxicity should be assessed with reference to the generic guidance given in Section R.4.3.1.1.

The currently available *in vitro* testing approaches, focusing on the AR and ER binding and transcription have the following limitations. Endocrine disruption may occur via mechanisms other than through the AR or ER such as alterations in hormone synthesis or transport, actions on other receptors and altered metabolism, endpoints for which *in vitro* tests are not currently available. Furthermore, many *in vitro* test systems lack metabolic capability or the range of chemicals that can be tested is restricted due to of problems with solubility in the testing medium. Nevertheless, for certain classes of chemicals that do not require metabolic activation or deactivation, or the metabolites are known and tested, *in vitro* testing may offer practical advantages in terms of speed and cost over *in vivo* screening. Overall, positive *in vitro* test results may indicate a potential to affect endocrine activity *in vivo* by a mechanism relevant for humans, particularly if the *in vitro* activity is high, and may therefore provide a justification for *in vivo* testing. However, negative *in vitro* test results do not provide a reliable indication of a lack of potential to cause reproductive toxicity because of these limitations.

Animal data

Repeated-dose toxicity studies

Although not aimed directly at investigating reproductive toxicity, repeated-dose toxicity studies (e.g. EU B.7, OECD TG 407) may reveal clear effects on reproductive organs in adult animals. However, if these findings occur in the presence of marked systemic toxicity (up to the highest dose level tested in a repeated-dose study) may lower concerns for effects on fertility and can contribute to decisions on further testing requirements. However, this does not rule out the possibility that the substance may have the capacity to affect fertility.

The observation of effects on reproductive organs in repeated-dose toxicity studies may also be sufficient 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. In addition, a number of cases have demonstrated that effects on the reproductive system may occur at lower doses during the development of foetuses and young animals than in adults. Consequently, in cases where there are substantiated indications for adverse effects on the reproductive organs of adult animals the use of an increased assessment factor in the risk assessment process may be considered. Alternatively, further studies, for example a screening test (OECD TG 421)[52] or a two-generation study (EU B.35, OECD TG 416) may be triggered based on a *Weight of Evidence* assessment. Some effects seen in repeated-dose toxicity studies may be difficult to interpret, for example changes in sex hormone level, and should be investigated further as part of studies that may be required to meet standard REACH information requirements (for example EU B.26, OECD TG 408 or other repeated-dose toxicity studies), rather than serve as a trigger for the immediate conduct of a two-generation study.

Repeated-dose toxicity studies may also provide indications to evaluate the need to investigate developmental neurotoxicity endpoints.

[52] To date there is no corresponding EU testing method available.

In vivo assays for endocrine disruption

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.

A number of new *in vivo* assays are under development and may be available for a chemical (see Hass *et al*, 2004 for a detailed discussion). However, none of these assays are standard REACH information requirements and they do not have a role in the ITS (see Section R.7.6.6). The performance of these *single endpoint* assays is not favoured, unless there is strong scientific justification, as they provide only limited information in relation to the numbers of animals used.

The uterotrophic (OECD 2003a) and Hershberger (OECD 2003b) assays, presently being internationally evaluated under the OECD Test Guideline Program, appear reliable in identifying substances with oestrogenic or (anti)androgen modes of action. These studies involve dosing of immature or ovarectomised/castrated animals, and the weighing of oestrogen/ androgen dependent tissues (e.g. uterus or prostate).

A negative result in the uterotrophic assay, in a thorough dose-response study, indicates that the test substance is not an ER-ligand *in vivo*. Equally, a negative result in the Hershberger assay indicates that the test substance is neither an AR-ligand nor a 5-alpha reductase inhibitor *in vivo*. A test compound found negative in these assays may, however, still have endocrine disrupting properties as well as a potential for reproductive toxicity mediated through other mechanisms. Nevertheless, the uterotrophic and Hershberger assays provide *in vivo* NOEL/LOELs for the endpoints examined.

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 provide information about the potency of the compound *in vivo* (US-EPA 2002). Effects on the various endpoints included in these assays can be considered adverse and/or as representing an effect on a mechanism relevant for humans.

In summary, while these *in vivo* assays are considered predictive for hazard identification and risk assessment, and give indications of effects that may be seen in a more comprehensive study, they are not definitive studies. Positive and negative results in the uterotrophic or Hershberger assays, as well as pubertal assays, may be used in combination with other evidence to satisfy the data needs for the classification and risk assessment for effects on reproduction. Positive effects may also provide justification for the conduct of further higher tier testing, such as the two-generation study (EU B.35, OECD TG 416).

As part of the OECD test guideline development program, work is being conducted with the aim of updating the repeated-dose 28 day oral toxicity study (EU B.7, OECD TG 407, reviewed by Gelbke *et al* (2006) to ensure that chemicals acting through (anti)estrogenic, (anti)androgenic and (anti)thyroid mechanisms can be identified. The enhancements include additional parameters based on the respective target organs of the male and female reproductive tracts and the thyroid. Initial validation studies indicate that an enhanced design can reliably identify substances with a strong potential to act through endocrine modes of action on the gonads and thyroid. A negative result with respect to endocrine activity in such a study up to the highest dose tested provides some evidence of the absence of potent effects. However, effects of lower potency cannot be ruled out and therefore a negative result 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 endocrine disruption seen in a repeated-dose toxicity study provides a trigger for the conduct of a more comprehensive study, for example a two-generation study (EU B.35, OECD TG 416).

In vivo reproductive toxicity tests

The available OECD test guidelines (or drafts) specifically designed to investigate reproductive toxicity are shown in Table R.7.6-1.

The purpose of Reproduction/Developmental Toxicity Screening Test (OECD TGs 421 and 422) is to provide information of the effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of conceptus and parturition. 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 a classification and risk assessment (using an appropriate assessment factor), and providing a N(L)OAEL from which a DNEL can be identified. If so, there may be no requirement for the conduct of a two-generation study at higher tonnage levels (see the Testing Strategy in Section R.7.6.6 for more information). However, the results should be interpreted with caution because OECD TGs 421/422 are screening assays that were not designed as an alternative or a replacement of the definitive reproductive toxicity studies (OECD TGs 414 and 416, EU B.31 and B.35). These screening tests are not meant to provide complete information on all aspects of reproduction and development. In particular, the post-natal effects associated with prenatal exposure (such as undetected malformations affecting viability or functional effects) or effects resulting from post-natal or lactational exposure are not covered in these studies. Furthermore, the exposure duration in these studies may not be sufficient to detect all effects on the spermatogenic cycle, although it is likely that in practice the 2-week exposure period will be sufficient to detect the majority of testicular toxicants (Ulbrich and Palmer, 1995). However, the number of animals per dose group is limited which may affect the statistical power of the study to detect an effect. These screening tests may in some cases give indications for reproductive effects (e.g. fertility and post natal effects) that cannot be investigated in a prenatal developmental toxicity study (OECD TG 414, EU B.31). A negative result in a screening study may lower concerns for reproductive toxicity, but this will not provide reassurance of the absence of this hazardous property. However, a negative result can provide the basis for a DNEL in relation to reproductive toxicity derived from the highest dose level used in the study and using an assessment factor that takes account of the limitations of this study; but note that such a DNEL will be relevant only at the Annex VIII level. An evaluation of the 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).

The two-generation study (OECD TG 416, EU B.35) is a general test which allows evaluation of the effects of the test substance on the complete reproductive cycle including libido, fertility, development of the conceptus, parturition, post-natal effects in both dams (lactation) and offspring and the reproductive capacity of the offspring. The two-generation study has conventionally been preferred to the one-generation study (OECD TG 415, EU B.34) in the testing of chemicals because the latter does not test for potential effects on all phases of the reproductive cycle. Post weaning development, maturation and the reproductive capacity of the offspring are not assessed. Consequently some adverse effects, for example oestrogenic- or antiandrogenic-mediated alterations in testicular development, may not be detected. The ILSI Agricultural Chemical Safety Assessment Project has proposed a *F1-extended one-generation study* (as described by Cooper et al 2006). If properly validated and accepted in the EU this could be used in place of the two-generation study as the preferred definitive study to test for reproductive toxicity. This flexible study addresses the main limitation of OECD TG 415 (EU B.34) by incorporating additional post-natal evaluations, which include clinical pathology, a functional observation battery, immunotoxicity endpoints, oestrous cyclicity and semen analysis, and using an extended F1 generation dosing period (to PND day 70) endpoints addressing developmental neurotoxicity. The study has a shortened F0 male premating dosing period, justified by the observation of no differences in the detection rates for adverse effects on fertility between 4- and 9-week pre-mating dosing periods in a number of studies (reviewed by Ulbrich and Palmer 1995).

The prenatal developmental toxicity study (OECD TG 414, EU B.31) provides a focussed evaluation of potential effects on prenatal development, although only effects that are manifested before birth can be detected.
Positive results in these studies will be relevant to hazard classification and the human health risk assessment, unless there is information to show that effects seen in these studies could not occur in humans. N(L)OAELs can be identified from OECD TGs 414 (EU B.31), 415 (EU B.34), 416 (EU B.35), draft 426 and the F1-extended one-generation study.

Developmental neurotoxicity studies (e.g. draft OECD TG 426) are designed to provide information on the potential functional and morphological hazards to 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 test is able to reflect the entire complex and intricate function of behaviour. For testing behaviour, therefore, a range of parameters, a *test battery*, is used to identify changes in individual functions.

In exceptional cases when relevant triggers are met testing for developmental neurotoxicity effects should be considered. Relevant triggers could be if the substance has been shown to (1) cause structural abnormalities of the central nervous system, (2) cause clear signs of behavioural or functional adverse effects of nervous system involvement in adult studies e.g. repeated-dose toxicity studies or (3) have a mode of action that has been closely linked to neurotoxic or developmental neurotoxicity effects e.g. cholinesterase inhibition or thyroid effects. However, in the case of (3) targeted testing on the specific mode of action in developing animals may provide sufficient information for regulatory purposes.

The DNT test protocol (draft OECD TG 426, developmental neurotoxicity, not a REACH standard information requirement) is designed to be performed as an independent study. However, observations and measurements described in the protocol can also be added on to a two-generation reproduction study (EU B.35, OECD TG 416). An advantage of this approach is that fewer animals are needed compared to running both studies separately. However, when the developmental neurotoxicity study is incorporated within or attached to another study, it is imperative to preserve the integrity of both study types.

Positive results in a developmental 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.

See Nordic Chemicals Group (2005), ECETOC (2002) and WHO (2001) for more detailed reviews of how to interpret the test guidelines mentioned in this report, including a discussion of their strengths and limitations.

Table R.7.6-1 Overview of *in vivo* OECD test guidelines for reproductive toxicity

Test	Design	Endpoints
OECD TG 416 Two-Generation study	Exposure before mating for at least one spermatogenic cycle until weaning of 2nd generation 3 dose levels plus control N = 20 parental males and females	Fertility Oestrus cyclicity and sperm quality Pregnancy outcome, e.g. dystocia Growth, development and viability Anogenital distance if triggered Sexual maturation Histopathology and weight of reproductive organs, brain and target organs Recommended: motor activity, sensory function, reflex ontology in F_1 generation
OECD TG 415 One-Generation Study (not a standard REACH information requirement)	Exposure before mating for at least one spermatogenic cycle until weaning of 1st generation 3 dose levels plus control N = 20 parental males and females	Fertility Growth, development and viability Histopathology and weight of reproductive organs, brain and target organs
OECD TG 414 Prenatal Developmental Toxicity Study (Teratology study)	At least from implantation to one or two days before expected birth 3 dose levels plus control N = 20 pregnant females	Implantation, resorptions Foetal growth Morphological variations and malformations
OECD TG 426 Developmental Neurotoxicity Study (draft, not a standard REACH information requirement)	At least from implantation throughout lactation (PND 20) 3 dose levels plus control N = 20 pregnant females	Birth and pregnancy length Growth, development and viability Physical and functional maturation Behavioural changes due to CNS and PNS effects Brain weights and neuropathology
OECD TG 421 and 422 Reproduction/ Developmental toxicity screening test	From 2 weeks prior to mating until at least day 4 postnatally 3 dose levels plus control N = 8-10 parental males and females	Fertility Pregnancy length and birth Foetal and pup growth and survival until day 4 OECD TG 422 combines reproduction/developmental screen with repeated-dose toxicity investigations that are in concordance with the requirements of OECD TG 407

Developmental effects should be considered in relation to adverse effects occurring in the parents. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of

maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections etc., it is important that the effects observed should be interpreted in conjunction with possible concomitant maternal toxicity (ECB 2004, Fleeman *et al*. 2005, Cappon *et al*. 2005). 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.

R.7.6.4.2 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 1 and for risk assessment, including the identification of a N(L)OAEL. In such cases, there will normally not 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 for study that is 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.7.6.4.3 Exposure considerations for reproductive toxicity

General information on the pattern and extent of human exposure to the substance must be considered, as this may influence the data requirements with respect to reproductive toxicity. Generic aspects of data waivers based on exposure considerations are presented in Section R.5.1.3. There are rules for waiving certain reproductive information requirements that include criteria relating to human exposure levels in REACH Annexes IX and X. Furthermore, all the reproductive toxicity tests (and also most other *in vivo* toxicity) may be omitted at any of the tonnage levels based on exposure scenarios developed in the Chemical Safety Report according to REACH Annex XI Section 3. The influence of human exposure on the reproductive toxicity ITS is discussed in more detail in Section R.7.6.6.

R.7.6.4.4 Remaining uncertainty on reproductive toxicity

The adequacy and reliability of the various types of data that may be available, or could be generated using the Integrated Testing Strategy (see Section R.7.6.6), as a basis for a decision on classification and for a risk assessment are described in Sections R.7.6.4.1 and R.7.6.4.2.

R.7.6.5 Conclusions on reproductive toxicity

Reproductive toxicity endpoints should be considered collectively, using a *Weight of Evidence* approach to establish the most relevant endpoint and its NOAEL or Critical Effect Dose to be used in risk assessment.

A *Weight of Evidence* assessment involves the consideration of all data that is available and may be relevant to reproductive toxicity, as listed in Section R.7.6.3. There can be no firm rules to the conduct of a *Weight of Evidence* assessment as this process involves expert judgment and because the mix and reliability of information available for a particular substance will probably be unique. Also, the *Weight of Evidence* assessment should consider all toxicity endpoints together, and not look at reproductive toxicity in isolation.

One example of a *Weight of Evidence* assessment is the pooling of information from several *in vivo* reproductive toxicity studies. Individually, these studies may have deficiencies, such as brief reporting, small group size, limited range of endpoints evaluated, the dose levels or the dosing schedule was not appropriate for a comprehensive evaluation of potential effects on the reproductive cycle, the study was not in compliance with GLP. However, taking account of their reliability and relevance and consistency of findings, collectively these studies could provide a level of information similar to that of the EU or OECD test guideline studies, and therefore meet the tonnage-related information requirements needed for the classification decision and risk assessment.

R.7.6.5.1 Concluding on Classification and Labelling

In order to conclude on a proper C&L, all the available information needs to be taken into account, and considerations should be given to both Annex VI of the Directive 67/548/EEC[53] and the various remarks (as they relate to classification and labelling) made throughout this guidance document.

R.7.6.5.2 Concluding on suitability for Chemical Safety Assessment

In order to be suitable for CSA appropriate DNELs 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 Chapter R.8 and Appendix R.8-12.

R.7.6.6 Integrated Testing Strategy (ITS) for reproductive toxicity

R.7.6.6.1 Objective / General principles

Fundamentally based on a *Weight of Evidence* approach, the Integrated Testing Strategy (ITS) has been developed around two core objectives:

- to have sufficient information to support risk assessment.
- to have adequate information to consider whether classification as a reproductive toxicant is warranted.

With these objectives underpinning each stage of the process, the ITS was designed to permit informed decisions on reproductive toxicity potential in a step-by-step tiered manner, within the production tonnage related data requirements framework of REACH Annexes VII to X and influenced by toxicological factors (termed *alerts*, see Section R.7.6.6.4) or exposure considerations that may increase or decrease concerns for reproductive toxicity.

By adhering to the criteria outlined above, the ITS will enable decisions to be made at the relevant tonnage level on the need for further testing or whether sufficient information already

[53] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

exist to meet the agreed objectives. Furthermore, if further testing is deemed necessary, the use of the most appropriate study in accordance with the REACH proposal is considered rather than a *one study fits all* approach. An overarching principle is that all data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

R.7.6.6.2 Preliminary considerations

Consistent with the parameters defined within the REACH programme (Annex VII-XI), testing for reproductive toxicity is not required for chemicals produced at tonnage levels <10 tonnes per annum (t/y), although all available information relevant to reproductive toxicity must be evaluated, and classification for this area of toxicity should be considered. At higher production volumes, standard data requirements are, in general, proportional to the tonnage level (≥10 t/y, ≥100 t/y or ≥1000 t/y) although maintaining flexibility to adopt the most appropriate testing regime for any single chemical is a key component of the ITS.

However, regardless of tonnage level, before any testing is triggered, careful consideration of all the available toxicological data, exposure characteristics and current risk management procedures is necessary to ascertain whether the fundamental objectives of the ITS (see above) have already been met. This consideration should take account of discussions that have taken place under other regulatory schemes, such as the EU Existing Substances Regulation (ESR), pesticides and the EU hazard classification scheme. If it is concluded that further testing is required, then a series of decision points are defined to help shape the scope of an appropriate testing programme. To satisfy these multiple objectives, the ITS provides a three-stage process for clear decision-making, relevant for all tonnage levels ≥10 t/y.

Stage 1. a series of preliminary questions to consider before deciding on the scope of further reproductive toxicity testing that may be required. Therefore, dependent on the outcome of this analysis, it is possible that some chemicals may not progress beyond Stage 1.

Stage 2. evaluation of the available toxicology database and consideration of reproductive toxicity alerts. This evaluation should consider data for substances with a similar structure or causing toxicity via a similar mode of action. The aim of this stage is to determine the scope of reproductive and/or developmental toxicity testing necessary to satisfy the REACH information requirements. It is possible that, following this review coupled to a Weight of Evidence analysis in Stage 1 or if sufficient data for risk assessment/risk management and classification purposes already are available, no further testing may be necessary.

Stage 3. describes the relevant reproductive and developmental toxicity tests upon which classification, labelling and risk assessment decisions will be based for chemicals progressing beyond Stages 1 and 2.

R.7.6.6.3 Testing strategy for reproductive toxicity

Stage 1. Questions to consider before deciding whether any testing for reproductive toxicity potential is required (relevant for <u>all</u> tonnage levels ≥10 t/y)

Stage 1.1. Has the substance already been classified for effects on fertility as Reproductive Toxicity Category 1 or Cat 2: R60 <u>and</u> development as Reproductive Toxicity Category 1 or Category 2: R61?
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 for reproductive toxicity will be necessary. If the available data are not adequate to support a robust risk assessment then proceed to Stage 2.

Stage 1.2. Is the substance classified as a genotoxic carcinogen (Carcinogen Category 1 and Mutagen Category 3 or Carcinogen Category 2 and Mutagen Category 3) or a germ cell mutagen (Mut. Cat. 1 or Cat. 2)?

If the answer is no, proceed to Stage 1.3. 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. Exceptionally, appropriate risk management measures may not be in place and a Stage 2 review of the available data should be considered.

Stage 1.3. Does the substance exhibit (a) low toxicological activity and (b) negligible systemic absorption and (c) no or no significant human exposure?
At the ≥100 and ≥1000 t/y levels, no further testing for reproductive toxicity will be required if all three criteria (a, b and c, above) are met; otherwise proceed to the stage 2 analysis. In addition, testing will not be required if the application of a parallel exposure-based information waiving provision in Annex XI Section 3 of REACH (Substance-tailored exposure-driven testing) is justified.
However, these three criteria do not apply at the >10 t/y level. At this level, no further testing for reproductive toxicity will be necessary only if the application of the exposure-based information waiving provision in Annex XI Section 3 of REACH is justified; otherwise proceed to the stage 2 analysis.

For further discussion see Section R.7.6.6.5.

Stage 2. Conduct a detailed review of all existing toxicological data to identify any specific alerts and testing requirements for reproductive and/or developmental toxicity

Substances may be excluded from further testing at Stage 3 (for more details on criteria for decision making, see Section R.7.6.6.4), this can only be achieved if sufficient data exist to conclude[54] that the substance does not present a reproductive toxicity hazard or that further data are unlikely to change a classification in Reproductive Toxicity Category 3. In the latter case, a thorough scientific justification is needed.

≥10 t/y

Before any testing is conducted, all substances at this tonnage level will be subject to a thorough data review. If sufficient data are available to permit a conclusion on reproductive and developmental toxicity potential, then no further testing is required. If there is insufficient data or alerts exist, then a testing strategy for reproductive and/or developmental toxicity in Stage 3 will be recommended. It should be pointed out that the observation of no adverse effects on the reproductive organs in a repeated-dose toxicity, such as a 28- or 90-day toxicity study, may justify a lower priority for further testing for effects on fertility. However, this would not provide sufficient data to justify a lower priority for testing for effects on development.

≥100 and ≥1000 t/y

For substances at these tonnage levels progressing beyond Stage 1, the standard data requirements include the definitive OECD tests for reproductive toxicity (for details see Stage 3 below). However, before any specific reproductive toxicity testing is undertaken, all substances at these tonnage levels will be subject to a thorough Stage 2 data review. If sufficient data exist to permit a robust conclusion on reproductive toxicity potential, then no further testing is required. If there is insufficient data or alerts exist, then a reproductive toxicity testing strategy for Stage 3 will be recommended.

[54] Data adequate for Classification and Labelling and risk assessment

Stage 3. Reproductive toxicity tests triggered by tonnage level or alerts identified in Stage 1 and 2

Four internationally harmonised guideline studies are listed in the REACH Annexes that can be used at Stage 3 to provide the necessary information to support a robust classification and risk assessment and to identify N(L)OAELs. However, it will not usually be necessary to assess all chemicals reaching Stage 3 in all four tests. Instead, individual chemical testing requirements will be customised based on the nature of alerts identified in Stages 1 and 2 and the tonnage level of that substance.

The tests listed in the REACH annexes are:

- Reproduction/developmental toxicity screening test (OECD TG 421) OR the combined repeat dose toxicity study with the reproduction/ developmental toxicity screening test (OECD TG 422)
- Prenatal developmental toxicity study (OECD TG 414, EU B.31) in a first species and possibly second species
- Two-generation reproduction study (OECD TG 416, EU B.35)[55]

A brief description of the study protocols considered in the ITS is presented in Table R.7.6-1 in Section R.7.6.4.1. Utilisation of these tests at each of the three tonnage levels is summarised below.

≥ 10 t/y

At this tonnage level, progression beyond Stages 1 and 2 will trigger a reproduction/ developmental toxicity screening test (OECD TG 421/422) as the standard information requirement, if there is no evidence from available information on structurally related substances, QSAR estimates or from in vitro methods that the substance may be a developmental toxicant. If this test provides no alerts for reproductive and developmental toxicity, then dependent on the *Weight of Evidence* from Stages 1 and 2, further testing for reproductive toxicity will not be required at this tonnage level. Similarly, if a clear and unequivocal reproductive and/or developmental toxicity effect is observed in these tests which is deemed sufficient to enable a scientifically robust decision on classification and risk assessment, then no further testing beyond the OECD TG 421 or 422 is recommended at this tonnage level. If a 28-day study (EU B.7, OECD TG 407) is not already available, the conduct of the OECD TG 422 is preferred to TG 421 for animal welfare reasons, as the former also includes an investigation of repeated-dose toxicity equivalent to that of the 28-day toxicity study, thus eliminating the need to conduct the 28-day study (see Section R.7.5). If an alert for reproductive and/or developmental toxicity is generated from an OECD TG 421 or OECD TG 422 study but is deemed insufficient for a classification assessment then the regulatory actions should take account of the additional uncertainty. For example, the DNEL identification may require the application of a larger Assessment Factor; exceptionally, further testing may be required on a case-by-case basis. The specific testing requirement will be dependent on the nature of the alerts.

However, dependent on the nature of the alert(s) observed in Stages 1 and 2, it may be more appropriate to conduct a two-generation reproduction study[5] (EU B.35, OECD TG 416) or a prenatal developmental toxicity study (EU B.31, OECD TG 414) instead of the screening study. In general, it should be noted that the OECD TG 414 (EU B.31) study does not incorporate post-natal parameters and therefore it is advisable not to bypass the screening study when

[55] As discussed earlier (Section R.7.6.4.1), a proposed *F1-extended one-generation study* may replace OECD TG 416 as a definitive study for reproductive toxicity in the near future, subject to gaining regulatory acceptance in the EU.

data of a prenatal developmental toxicity study is either available or a respective study is triggered. This is because the screening study will provide information on the viability and postnatal development of the offspring which can be important to the developmental toxicity assessment, as well as information on many other aspects of reproduction that would not otherwise be available. However, if the prenatal developmental toxicity test was positive, there would be less need for the screening test. If an OECD TG 414 (EU B.31) study has been performed, it will be important to establish whether these data are sufficient to enable a clear regulatory decision and to assess whether the results of further testing for developmental toxicity in a second species are likely to influence regulatory decisions. Testing in a second species will not normally be required at this tonnage level if the study is negative. Additional guidance on the acquisition of information on potential developmental toxicity from two animal species is provided in Section R.7.6.6.4 It should be noted that although the OECD TG 414 study does not incorporate post-natal parameters, some findings might raise concerns for post-natal effects such as pup survival and in such cases follow-up testing in a two-generation reproductive toxicity study (EU B.35, OECD TG 416) in the most relevant species (usually the rat) may be appropriate. Alternatively, such effects could initially be investigated in an OECD TG 421/422 test that has been modified to include an extended postnatal observation period.

≥ 100 t/y

At this tonnage level, progression beyond Stage 1 and 2 will trigger a prenatal developmental toxicity study (OECD TG 414), conducted in the most relevant species (see Section R.7.6.6), as a standard data requirement and, in case of an alert for this test, a two-generation reproduction study (EU B.35, OECD TG 416). Additionally, the ≥10 t/y standard data requirement for a reproduction/developmental toxicity screening test (OECD TG 421/422) will need to be met. However, this screening test will not be necessary if a two-generation study is proposed at the >100 t/y level; also this test will not be required if adverse effects on reproductive organs have been observed in existing repeated-dose studies and these findings are sufficient to support classification for effects on fertility and the risk assessment.

As for ≥10 t/y substances, following completion of the OECD TG 414 (EU B.31) study it will be important to establish whether these data are sufficient to enable a clear regulatory decision and to assess whether the results of further testing for developmental toxicity are likely to influence regulatory decisions. Guidance on the investigation of developmental toxicity in a second species is presented in Section R.7.6.6. As outlined above in Stage 2 for this tonnage level, a detailed review of the available data will be conducted to identify any reproductive toxicity alerts. This review coupled to the data emerging from the OECD TG 414 (EU B.31) study will form the basis of a Weight of Evidence assessment of the requirement for a two-generation reproductive toxicity study (EU B.35, OECD TG 416). If specific triggers are present as discussed in Section R.7.6.4.1 the need for inclusion of the optional developmental neurotoxicity endpoints should be evaluated. The conduct of OECD TG 416 (EU B.35) should also be considered if it is anticipated that the ≥1000 t/y supply tonnage threshold will be reached in the near future.

REACH Annex IX specific rules for adaptation states that the need to perform a OECD TG 416 (EU B.35) study in a second species, either at this tonnage level or the next, should be considered, based on the outcome of the first test and any other data. However, the two-generation study is very rarely conducted in a species other than the rat, and it is envisaged that a second species study could not be justified.

≥ 1000 t/y

At this tonnage level, progression beyond Stage 1 and 2 will trigger a prenatal developmental toxicity study (EU B.31, OECD TG 414) and a two-generation reproductive toxicity study (EU B.35, OECD TG 416) in the most relevant species as a standard data requirement. The need for a developmental toxicity study (EU B.31, OECD TG 414) in a second species should be

evaluated, following the guidance presented above in Section R.7.6.6.3.
If specific triggers are present as discussed in Section R.7.6.4.1, inclusion of optional developmental neurotoxicity endpoints should be considered. The reproduction/ developmental toxicity screening test (OECD TG 421/422) , a standard data requirement at the ≥10 t/y level, will not be needed if a two-generation is conducted because this study provides a superior level of information.

R.7.6.6.4 Elements of the ITS

Alerts from existing toxicological database and their implications for further testing, classification / labelling and risk assessment

Challenging the existing toxicity database from a reproductive toxicity perspective.

An *alert* is any factor, with the exclusion of convincing evidence derived from the definitive reproductive toxicity studies (i.e. OECD TG 414 and 416), that is 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 reproductive toxicant.

As part of the Stage 2 data review the following questions should be asked:

- are there alerts for reproductive toxicity?
- are the data sufficient/adequate for assessing the classification and labelling and risk assessment without further testing, irrespective of the presence or absence of alerts?
- if the data are insufficient, what study (or studies) is most appropriate? This decision must take account of both the standard tonnage related information requirements of REACH, the nature of the alert(s) and *Weight of Evidence* as well as human exposure considerations.
- is there any knowledge of the chemical, chemical groups or categories, that would indicate special features to be included in the study design? If so, what?

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. Instead, alerts mentioned in this report should be viewed as a helpful guide of indicators that would provide input to the regulatory decision-making process – in other words, contribute to a *Weight of Evidence* analysis requiring expert judgement, that leads to the most appropriate testing and regulatory outcome.

Section R.7.6.4, which discusses the information that may be available for a substance, provides many examples of alerts and their implications for testing, classification and risk assessment.

Consideration of existing reproductive studies not required under REACH

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. These could include one-generation studies (for example EU B.34, OECD 415 or the previously discussed *F_1-extended one-generation study*), non-GLP studies, or non-guideline investigations such as the NTP continuous breeding study (Chapin and Sloane, 1997). The available data should be evaluated to assess their suitability for use, taking account of the robustness of design, and quality. As an example, a one-generation study (EU B.34, OECD TG 415) and repeated-dose toxicity study that includes oestrous cycle monitoring and semen analysis may already have been performed. In this case, the level of information

available, though not equivalent to that provided by a two-generation study, could be sufficient using a *Weight of Evidence* analysis for classification and risk assessment.

In summary, the information requirements set out in the REACH annexes should be treated as endpoints to be evaluated rather than studies to be conducted. Thus, relevant existing studies that do not conform to the OECD test guidelines referred to the REACH Annexes but nevertheless provide an equivalent level of information can be used to meet the REACH information requirements.

Selection of Species for Assessment of Prenatal Developmental Toxicity

The purpose of the prenatal developmental toxicity study (EU B.31, OECD TG 414) is to identify effects upon organogenesis and foetal growth prior to parturition. For a comprehensive assessment of developmental toxicity according to Annexes IX and X information from two species, one rodent (usually the rat) and one non-rodent (usually the rabbit) should be considered. When considering the use of two species, care should be given to deciding the order in which these studies are performed. Since most acute, repeated-dose, and toxicokinetic studies are conventionally conducted in the rat, it is advisable that the first developmental toxicity study should also be conducted in this species. Findings from previous studies may be useful in dose selection, or the identification of additional endpoints for evaluation. In addition, the outcome of the prenatal developmental toxicity study may be helpful in the interpretation of other reproductive toxicity studies (e.g. OECD TG 421/422), for which the rat is generally the favoured species.

Although the OECD TG 414 (EU B.31) is designed specifically to identify developmental toxicity, information on this endpoint can also be obtained from observations of the offspring in a one- or two-generation study, which will almost always have been conducted in the rat. So, if a generation study is available, a prenatal developmental toxicity study (EU B.31, OECD TG 414) in the rat may not provide any additional information that would have an influence on the classification decision or risk assessment, and therefore the conduct of this study in the rat may not always be necessary.

If the outcome of this first developmental toxicity study is positive, this may be enough for classification and risk assessment; if this is so, a study in a second species will not be required. Further investigations may be warranted, on a case-by-case basis, if the outcome of the first study is equivocal or if the relevance of the findings to humans is unclear. At ≥1000 t/y, a study in a second species will normally be required when the first study is negative, unless *Weight of Evidence* assessment or specific data e.g. toxicokinetic data provide scientific justification not to conduct the study in a second species. This could be the case if available data demonstrate that for example the rat is the most relevant species for extrapolating to humans or if the rabbit is not a suitable model for testing for developmental toxicity.

R.7.6.6.5 Exposure considerations (and substances of low toxicological activity and with negligible systemic absorption) for reproductive toxicity

Exposure considerations may be used to justify the waiver of certain data requirements or, exceptionally, the conduct of reproductive toxicity testing that is additional to the REACH Annex VIII, IX and X information requirements.

Upgraded testing requirements

The use pattern or the exposures to a substance may indicate a need for additional information requirements, on a case-by-case basis. For example, there may be serious concerns that human exposures, particularly to consumers, are close to the levels at which toxicity might be expected. Such concerns for human health may be satisfactorily addressed by improved risk

management measures and therefore additional information on hazard would be of limited value. Thus, proposals to refine a risk assessment with the use of information obtained from new *in vivo* testing that is in excess of the REACH tonnage-related information requirements can be justified only in exceptional circumstances.

Reduced testing requirements: ≥ 10 t/y

As stated in REACH Annex VIII specific rules for adaptation the OECD TG 421/422 study listed as a standard information requirement does not need to be conduced *if relevant human exposure can be excluded* in accordance with Annex XI Section 3. This clause states that tests may be omitted based on exposure scenarios developed in the Chemical Safety Report. The criteria defining what constitutes adequate justification for omitting these tests under Annex XI Section 3 are not currently available, but will be adopted by the Commission within 18 months of REACH coming into force.

Reduced testing requirements: ≥ 100 t/y and ≥ 1000 t/y

According the REACH Annex IX and X specific rules for adaptation (mainly column 2), the reproductive toxicity studies listed as standard information requirements do not need to be conducted if the three 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.

At least two cases pertain, the first being no human exposure (e.g., substances only produced and used in closed systems) and the second being no significant human exposure. Whether a human exposure is significant depends on the reproductive toxicity potency of the substance relative to exposure (consequence of a risk) and might be decided on the basis of other information indicating e.g. the probability of a risk. E.g.: At least substances used in closed systems fall under this criterion, but other possibilities may be identified as well e.g. industrial and commercial uses for substances exclusively used in preparations in very low concentrations or substances, uses of substances in consumer products which are completely chemically reacted during manufacturing, integrated in a matrix and characterised by very low migration.

In addition to the REACH Annex IX and X specific rules for adaptation, there is the parallel exposure-based provision in Annex XI Section 3 of the REACH Regulation (*Substance-tailored exposure-driven testing); all the reproductive toxicity tests (and also most other in vivo toxicity*) may be omitted at any of the tonnage levels based on exposure scenarios developed in the Chemical Safety Report. As stated above, the criteria defining what constitutes adequate justification for omitting these tests under Section 3 are not currently available.

R.7.6.7 References on reproductive toxicity

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R.7.7 Mutagenicity and carcinogenicity

R.7.7.1 Mutagenicity

R.7.7.1.1 Definition of mutagenicity

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes or chromosomes. The term clastogenicity is used for agents giving rise to structural chromosome aberrations. A clastogen can cause breaks in chromosomes that result in the loss or rearrangements of chromosome segments. Aneugenicity (aneuploidy induction) refers to the effects of agents that give rise to a change (gain or loss) in chromosome number in cells. An aneugen can cause loss or gain of chromosomes resulting in cells that have not an exact multiple of the haploid number. For example, three number 21 chromosomes or trisomy 21 (characteristic of Down syndrome) is a form of aneuploidy.

Genotoxicity is a broader term and refers to processes which alter the structure, information content or segregation of DNA and are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as DNA strandbreaks, unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), DNA adduct formation or mitotic recombination, as well as tests for mutagenicity.

The chemical and structural complexity of the chromosomal DNA and associated proteins of mammalian cells, and the multiplicity of ways in which changes to the genetic material can be effected make it difficult to give more precise, discrete definitions.

In the risk assessment of substances it is necessary to address the potential effect of *mutagenicity*. It can be expected that some of the available data will have been derived from tests conducted to investigate potentially harmful effects on genetic material (*genotoxicity*). Hence, both the terms *mutagenicity* and *genotoxicity* are used in this document.

R.7.7.1.2 Objective of the guidance on mutagenicity

The aims of testing for genotoxicity are to assess the potential of substances to induce genotoxic effects which may lead to cancer or cause heritable damage in humans. Genotoxicity data are used in risk characterisation and classification of substances. Genotoxicity data are useful for the determination of the general mode of action of a substance (*i.e.* type(s) of genotoxic damage induced) and can provide some indication on the dose (concentration)-response relationship and on whether the observed effect can be reasonably assumed to have a threshold or not. Genotoxicity data are thus useful in deciding the best approach to use for the risk assessement. Expert judgement is necessary at each stage of the testing strategy to decide on the relevance of a result based on the data available for each endpoint.

Alterations to the genetic material of cells may occur spontaneously endogenously or be induced as a result of exposure to ionising or ultraviolet radiation, or genotoxic substances. In principle, human exposure to substances that are mutagens may result in increased frequencies of mutations above background.

Mutations in somatic cells may be lethal or may be transferred to daughter cells with deleterious consequences for the affected organism (*e.g.* cancer may result when they occur in proto-oncogenes, tumour suppressor genes and/or DNA repair genes) ranging from trivial to detrimental or lethal.

Heritable damage to the offspring, and possibly to subsequent generations, of parents exposed to substances that are mutagens may follow if mutations are induced in parental germ cells. To

date, all known germ cell mutagens are also mutagenic in somatic cells *in vivo.* Substances that are mutagenic in somatic cells may produce heritable effects if they, or their active metabolites, have the ability to interact with the genetic material of germ cells. Conversely, substances that do not induce mutations in somatic cells *in vivo* would not be expected to be germ cell mutagens.

There is considerable evidence of a positive correlation between the mutagenicity of substances *in vivo* and their carcinogenicity in long-term studies with animals. Genotoxic carcinogens are substances for which the most plausible mechanism of carcinogenic action involves genotoxicity.

R.7.7.2 Information requirements on mutagenicity

The information requirements on mutagenicity are described by REACH Annexes VI-XI, that specify the information that must be submitted for registration and evaluation purposes. The information is thus required for substances produced or imported in quantities of >1 t/y (tons per annum). When a higher tonnage level is reached, the requirements of the corresponding Annex have to be considered. However, factors including not only production volume but also pre-existing toxicity data, information about the identified use of the substance and exposure of humans to the substance will influence the precise information requirements. The REACH Annexes must thus be considered as a whole, and in conjunction with the overall requirements of registration, evaluation and the duty of care.

Column 1 of REACH Annexes VII-X informs on the standard information requirements for substances produced or imported in quantities of >1 t/y, >10 t/y, >100 t/y, and >1000 t/y, respectively.

Column 2 of REACH Annexes VII-X lists specific rules according to which the required standard information may be omitted, replaced by other information, provided at a different stage or adapted in another way. If the conditions are met under which column 2 of these Annexes allows adaptations, the fact and the reasons for each adaptation should be clearly indicated in the registration dossier.

The standard information requirements for mutagenicity and the specific rules for adaptation of these requirements are presented in Table R.7.7-1.

Table R.7.7-1 REACH information requirements for mutagenicity

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
Annex VII: 1. *In vitro* gene mutation study in bacteria.	Further mutagenicity studies shall be considered in case of a positive result.
Annex VIII: 1. *In vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study.	1. The study does not usually need to be conducted · if adequate data from an *in vivo* cytogenicity test are available or · the substance is known to be carcinogenic category 1A or 1B or germ cell mutagenic category 1A, 1B or 2.
2. *In vitro* gene mutation study in mammalian cells, if a negative result in Annex VII, 1 and Annex VIII, 1.	2. The study does not usually need to be conducted if adequate data from a reliable *in vivo* mammalian gene mutation test are available. Appropriate *in vivo* mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII.
Annex IX:	If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the registrant. If there is a positive result from an *in vivo* somatic cell study available, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.
Annex X:	If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data. If there is a positive result from an *in vivo* somatic cell study available, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.

In addition to these specific rules, the required standard information set may be adapted according to the general rules contained in Annex XI. In this case as well, the fact and the reasons for each adaptation should be clearly indicated in the registration.

In some cases, the rules set out in Annex VII to XI may require certain tests to be undertaken earlier than or in addition to the tonnage-triggered requirements. Registrants should note that a testing proposal must be submitted for a test mentioned in Annex IX or X, independently from the registered tonnage. Following examination of such a testing proposal ECHA has to approve the test in its evaluation decision before it can be undertaken. See Section R.7.7.6 for further guidance on testing requirements.

R.7.7.3 Information and its sources on mutagenicity

To be able to evaluate the mutagenic potential of a substance in a comprehensive way, information is required on its capability to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity). Many test methods are available by which such information can be obtained. Non-testing methods, such as SAR, QSAR and read-across approaches, may also provide information on the mutagenic potential of a substance.

Typically, *in vitro* tests are performed with cultured bacterial cells, human or other mammalian cells. The sensitivity and specificity of tests will vary with different classes of substances and, if adequate data are available for the class of substance to be tested, these data can guide the selection of the most appropriate test systems to be used. In order to detect mutagenic effects also of substances that need to be metabolically activated to become mutagenic, an exogenous metabolic activation system is usually added in *in vitro* tests. For this purpose the post-mitochondrial 9000 x *g* supernatant (S-9 fraction) of whole liver tissue homogenate containing a high concentration of metabolising enzymes and extracted from animals that have been induced to raise the oxidative P450 levels is most commonly employed. In the case when information is required on the mutagenic potential of a substance *in vivo,* several test methods are available. In *in vivo* tests whole animals are used, in which metabolism and toxicokinetic mechanisms in general exist as natural components of the test animal. It should be noted that species-specific differences in metabolism are known. Therefore, different genotoxic responses may be obtained. Some *in vivo* genotoxicity tests such as the Transgenic rodent (TGR) somatic and germ cell gene mutation assays and the comet assay employ methods by which any tissue (containing nucleated cells) of an animal can in theory be examined for effects on the genetic material. This gives the possibility to examine target tissues (including germ cells) and site-of-contact tissues (*i.e.* skin, epithelium of the respiratory or gastro-intestinal tract). However differences can exist regarding the number and type of tissues for which the use a specific test has been scientifically validated. For instance, the TGR assays can be used to examine germ cells whereas the comet assay as described in the OECD test guideline (TG) is, at present, not recommended for that purpose.

Some test methods, but not all, have an officially adopted EU and/or OECD TG for the testing procedure. In cases where no adopted EU or OECD TG is available for a test method, rigorous and robust protocols should be followed, such as those defined by internationally recognised groups of experts like the International Workshop on Genotoxicity Testing (IWGT) under the umbrella of the International Association of Environmental Mutagen Societies. Furthermore, modifications to OECD TGs have been developed for some classes of substances and may serve to enhance the accuracy of test results. Use of such modified protocols is a matter of expert judgement and will vary as a function of the chemical and physical properties of the substance to be evaluated. Similarly, use of standard test methods for the testing of tissue(s) not covered by those standard test methods should be scientifically justified and validity of the results will depend on the appropriateness of the acceptability criteria, which should have been specifically developed for this (these) tissue(s) based on sufficient experience and historical data.

R.7.7.3.1 Non-human data on mutagenicity

Non-testing data on mutagenicity

Non-test information about the mutagenicity of a substance can be derived in a variety of ways, ranging from simple inspection of the chemical structure through various read-across techniques, the use of expert systems, metabolic simulators, to *global* or *local* (Q)SARs. The usefulness of such techniques varies with the amount and nature of information available, as well as with the specific regulatory questions under consideration.

Regarding substances for which testing data exist, non-test information can be used in the *Weight of Evidence* approach, to help confirm results obtained in specific tests, or to help

develop a better understanding of mutagenicity mechanisms. The information may be useful in deciding if, or what, additional testing is required. At the other extreme, where no testing data are available, similar alternative sources of information may assist in setting test priorities. In cases where no testing is likely to be done (low exposure, <1 t/y) they may be the only options available to establish a hazard profile.

Weight of Evidence approaches that use expert judgement to include test results for close chemical analogues are ways of strengthening regulatory positions on the mutagenicity of a substance. Methods that identify general *structural alerts* for genotoxicity such as the Ashby-Tennant super-mutagen molecule (Ashby and Tennant, 1988) may also be useful.

Prediction models for mutagenicity

There are hundreds of (Q)SAR models available in the literature for predicting test results for genotoxic endpoints for closely related structures (Naven *et al.*, 2012; Bakhtyari *et al.*, 2013). These are known as *local* (Q)SARs. When essential features of the information domain are clearly represented, these models may constitute the best predictive tools for estimating a number of mutagenic/genotoxic endpoints. However, quality of reporting varies from model to model and predictivity must be assessed case-by-case on the basis of clear documentation. Use of harmonised templates, such as the QSAR Model Reporting Format (QMRF) and the QSAR Prediction Reporting Format (QPRF) developed by the Joint Research Centre (JRC) of the European Commission (http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools/QRF), can help ensure consistency in summarising and reporting key information on (Q)SAR models and substance-specific predictions generated by (Q)SAR models. The JRC website also hosts the JRC (Q)SAR Model Inventory, which is an inventory of information on the validity of (Q)SAR models that have been submitted to the JRC (http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsar-inventory).

Generally, (Q)SAR models that contain putative mechanistic descriptors are preferred; however many models use purely structural descriptors. While such models may be highly predictive, they rely on statistical methods and the toxicological significance of the descriptors may be obscure.

(Q)SAR models for mutagenicity can apply to a limited set of congeneric substances (local models) or to a wide variety of non-congeneric substances (global models). Global (Q)SARs are usually implemented in computer programs and may comprise a set of local models; these global models first categorise the input molecule into the chemical domain it belongs to, and then apply the corresponding local prediction model. These are known as expert systems. Other global models apply the same mathematical algorithm on all input molecules without prior separation. It is generally observed that the concept of applicability domain is a useful one and the endpoints for substances inside the applicability domains of the models are better predicted than for substances falling outside.

Many global models for mutagenicity are commercial and some of the suppliers of these global models consider the data in their modelling sets to be proprietary. Proprietary means that the training set data used to develop the (Q)SAR model is hidden from the user. In other cases it means that it may not be distributed beyond use by regulatory authorities. The models do not always equal the software incorporating them, and the software often has flexible options for expert uses. Thus, the level of information available, from both (Q)SAR models and compiled databases, should be adequate for the intended purpose.

A list of the available (free and commercial) predictive software for ecotoxicological, toxicological and environmental endpoints, including mutagenicity models, has been compiled within the frame of the EU project Antares (http://www.antares-life.eu/).

The most common genotoxicity endpoint for global models has been to predict results of the Ames test. Some models for this endpoint include a metabolic simulator.

There are models for many other mutagenicity endpoints. For example, the Danish EPA and the Danish QSAR group at DTU Food (National Food Institute at the Technical University of Denmark) have developed a (Q)SAR database that contains predictions from a number of mutagenicity models. In addition to assorted Ames models, the database contains predictions of the following *in vitro* endpoints: chromosomal aberrations (CHO and CHL cells), mouse lymphoma/*tk*, CHO/hprt gene-mutation assays and UDS (rat hepatocytes); and the following *in vivo* endpoints: *Drosophila* SLRL, mouse micronucleus, rodent dominant lethal, mouse SCE in bone marrow and mouse comet assay data. The database is freely accessible via http://qsar.food.dtu.dk. The online database contains predictions for over 166,000 substances and includes a flexible system for chemical structure and parameter searching. A user manual with information on the individual models including training set information and validation results is available at the website. The database is also integrated into the OECD (Q)SAR Toolbox. A major update of the database with consensus predictions by use of different QSAR models for each of the modelled endpoints for more than 600,000 structures, including over 70,000 REACH pre-registered substances, and with an improved user interface is scheduled for the beginning of 2015.

Another example of a database with predictions on mutagenicity is the Enhanced NCI Database Browser (http://cactus.nci.nih.gov) sponsored by the U.S. National Cancer Institute. It contains predictions for over 250,000 substances for mutagenicity as well as other non-mutagenic endpoints, some of which may provide valuable mechanistic information (for example alkylating ability or microtubule formation inhibition). It is also searchable by a wide range of parameters and structure combinations.

Neither of these two examples is perfect, but they illustrate a trend towards predictions of multiple endpoints and may assist those making *Weight of Evidence* decisions regarding the mutagenic potential of untested substances. More detailed information on the strengths and limitations of the different (Q)SAR models can be found elsewhere (Serafimova *et al.*, 2010).

OECD QSAR Toolbox

To increase the regulatory acceptance of (Q)SAR models, the OECD has started the development of a QSAR Toolbox to make (Q)SAR technology readily accessible, transparent and less demanding in terms of infrastructure costs (http://www.qsartoolbox.org/). The OECD QSAR Toolbox facilitates the practical application of grouping and read-across approaches to fill gaps in (eco-)toxicity data, including genotoxicity and genotoxic carcinogenicity, for chemical hazard assessment. In particular, the OECD QSAR Toolbox covers the *in vitro* gene mutation (Ames test), *in vitro* chromosomal aberration, *in vivo* chromosomal aberration (micronucleus test), and genotoxic carcinogenicity endpoints. The predictions are based on the implementation of a range of profilers connected with genotoxicity and carcinogenicity (to quickly evaluate substances for common mechanisms or modes of action), and the incorporation of numerous databases with results from experimental studies (to support read-across and trend analysis) into a logical workflow. The Toolbox and guidance on its use are freely available. A user manual “Strategies for chemicals to fill data gaps to assess genetic toxicity and genotoxic carcinogenicity” and various tutorials for categorisation of substances by use of the Toolbox in relation to protein- and DNA- binding and Ames test mutagenicity are also available on the OECD QSAR Toolbox web site.

The *Guidance on IR&CSA Chapter R.6: QSARs and grouping of chemicals* (available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) explains basic concepts of (Q)SARs and gives generic guidance on validation, adequacy and documentation for regulatory purposes. It also describes a stepwise approach for the use of read-across/grouping and (Q)SARs. Further

information on the category formation and read-across approach for the prediction of toxicity can be found in Enoch (2010).

Testing data on mutagenicity

Test methods preferred for use are listed in Table R.7.7-2, Table R.7.7-3 and Table R.7.7-4. The introduction to the OECD TGs on genetic toxicity testing as well as some of the related OECD TGs are currently being revised under the OECD Test Guidelines Programme (TGP). In addition, an OECD Guidance Document on the selection and application of the assays for genetic toxicity is being developed. For further information, please see http://www.oecd.org/env/testguidelines.

In vitro data

Table R.7.7-2 *In vitro* test methods

Test method	GENOTOXIC ENDPOINTS measured / PRINCIPLE OF THE TEST METHOD	EU / OECD guideline[a]
Bacterial reverse mutation test	Gene mutations / The test uses amino-acid requiring strains of bacteria to detect (reverse) gene mutations (point mutations and frameshifts).	EU: B.13/14 OECD: 471
In vitro mammalian cell gene mutation test – *hprt* test	Gene mutations / The test identifies substances that induce gene mutations in the *hprt* gene of established cell lines.	EU: B.17 OECD: 476[b]
In vitro mammalian cell gene mutation test – Mouse lymphoma assay	Gene mutations and structural chromosome aberrations / The test identifies substances that induce gene mutations in the *tk* gene of the L5178Y mouse lymphoma cell line. If colonies in a *tk* mutation test are scored using the criteria of normal growth (large) and slow growth (small) colonies, gross structural chromosome aberrations (*i.e.* clastogenic effect) may be measured, since mutant cells that have suffered damage to both the *tk* gene and growth genes situated close to the *tk* gene have prolonged doubling times and are more likely to form small colonies.	EU: B.17 OECD: 476[b]
In vitro mammalian chromosome aberration test	Structural and numerical chromosome aberrations / The test identifies substances that induce structural chromosome aberrations in cultured mammalian established cell lines, cell strains or primary cell cultures. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not routinely used for that purpose. Accordingly, this test guideline is not designed to measure numerical aberrations.	EU: B.10 OECD: 473[b]
In vitro micronucleus test	Structural and numerical chromosome aberrations / The test identifies substances that induce micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic substances.	EU: B.49 OECD: 487[b]

[a] For EU guidelines, see Regulation (EC) No 440/2008 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32008R0440:en:NOT) / for OECD guidelines see http://www.oecd.org/env/testguidelines

[b] OECD TGs 473, 476 and 487 are currently being revised (see http://www.oecd.org/env/testguidelines)

As noted earlier, accepted modifications to the standard test guidelines/methods have been developed to enhance test sensitivity to specific classes of substances. Expert judgement should be applied to judge whether any of these are appropriate for a given substance being registered. For example, protocol modifications for the Ames test might be appropriate for

substances such as gases, volatile liquids, azo-dyes, diazo compounds, glycosides, and petroleum oil derived products, which should be regarded as special cases.

Animal data

- Somatic cells

Table R.7.7-3 *In vivo* test methods, somatic cells

Test method	GENOTOXIC ENDPOINTS measured / PRINCIPLE OF THE TEST METHOD	EU / OECD guideline[a]
In vivo mammalian bone marrow chromosome aberration test	Structural and numerical chromosome aberrations / The test identifies substances that induce structural chromosome aberrations in the bone-marrow cells of animals, usually rodents. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not routinely used for that purpose. Accordingly, this test guideline is not designed to measure numerical aberrations.	EU: B.11 OECD: 475[b]
In vivo mammalian erythrocyte micronucleus test	Structural and numerical chromosome aberrations / The test identifies substances that cause micronuclei in erythroblasts sampled from bone marrow and/or peripheral blood cells of animals, usually rodents. These micronuclei originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic substances.	EU: B.12 OECD: 474[b]
Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*	DNA repair / The test identifies substances that induce DNA damage followed by DNA repair (measured as unscheduled "DNA" synthesis) in liver cells of animals, commonly rats. The test is usually based on the incorporation of tritium labelled thymidine into the DNA by repair synthesis after excision and removal of a stretch of DNA containing a region of damage.	EU: B.39 OECD: 486
Transgenic rodent (TGR) somatic and germ cell gene mutation assays	Gene mutations and chromosomal rearrangements (the latter specifically in the plasmid and Spi- assay models) / Since the transgenes are transmitted by the germ cells, they are present in every cell. Therefore, gene mutations and/or chromosomal rearrangements can be detected in virtually all tissues of an animal, including target tissues and specific site of contact tissues.	EU: B.58 OECD: 488
In vivo alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay)	DNA strand breaks / The DNA strand breaks may result from direct interactions with DNA, alkali labile sites or as a consequence of incomplete excision repair. Therefore, the alkaline comet assay recognises primary DNA damage that would lead to gene mutations and/or chromosome aberrations, but will also detect DNA damage that may be effectively repaired or lead to cell death. The comet assay can be applied to almost every tissue of an animal from which single cell or nuclei suspensions can be made, including specific site of contact tissues.	EU: none OECD: 489

[a] For EU guidelines, see Regulation (EC) No 440/2008 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32008R0440:en:NOT) / for OECD guidelines see http://www.oecd.org/env/testguidelines
[b] OECD TGs 474 and 475 are currently being revised (see http://www.oecd.org/env/testguidelines)

A detailed review of transgenic animal model assays, including recommendations on how to perform such assays in somatic cells, has been produced for the OECD (Lambert *et al.,* 2005; OECD, 2009).

Validation studies and recommendations have been published in recent years, identifying experimental factors which are of importance for improved harmonisation of data obtained in the alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (Ersson

et al., 2013; Azqueta *et al.*, 2013; Forchhammer *et al*., 2012; Azqueta *et al*., 2011a; Azqueta *et al*., 2011b; Forchhammer *et al*., 2009; Collins *et al*., 2008). Specifically, various international groups have proposed protocols and recommendations for performing the *in vivo* alkaline comet assay (Tice *et al.,* 2000; Hartmann *et al.,* 2003; McKelvey-Martin *et al.*, 1993; Brendler-Schwaab *et al.*, 2005; Burlinson et al., 2007; Smith *et al.*, 2008; Rothfuss *et al.*, 2010; Burlinson, 2012; Vasquez, 2012; Johansson *et al.*, 2010; Kirkland and Speit, 2008; EFSA, 2012). An international validation study on the *in vivo* alkaline single-cell gel electrophoresis assay was coordinated by the Japanese Centre for the Validation of Alternative Methods (JaCVAM) from 2006 to 2012. The validation study report was peer reviewed by the OECD and an OECD expert group drafted the comet OECD TG, which was approved by the OECD Working Group of National Coordinators of the Test Guidelines Programme (WNT) in April 2014. While awaiting the adoption of the comet OECD TG 489, the minimum criteria for acceptance of the comet assay published by EFSA (2012) can be used.

- Germ cells

Testing in germ cells has in the past been conducted only on very rare occasions (see Section R.7.7.6).

Table R.7.7-4 *In vivo* test methods, germ cells

Test method	GENOTOXIC ENDPOINTS measured / PRINCIPLE OF THE TEST METHOD	EU / OECD guideline[a]
Mammalian spermatogonial chromosome aberration test	Structural and numerical chromosome aberrations / The test identifies substances that induce structural chromosome aberrations in mammalian, usually rodent, spermatogonial cells and is, therefore, expected to be predictive of induction of heritable mutations in germ cells. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not routinely used for that purpose. Accordingly, this test guideline is not designed to measure numerical aberrations.	EU: B.23 OECD: 483[b]
Rodent dominant lethal test	Structural and numerical chromosome aberrations / The test identifies substances that induce dominant lethal effects causing embryonic or foetal death resulting from inherited dominant lethal mutations induced in germ cells of an exposed parent, usually the male. It is generally accepted that dominant lethals are due to structural and numerical chromosome aberrations. Rats or mice are recommended as the test species.	EU: B.22 OECD: 478[b]
Transgenic rodent (TGR) somatic and germ cell gene mutation assays	Gene mutations and chromosomal rearrangements (the latter specifically in the plasmid and Spi- assay models) / Since the transgenes are transmitted by the germ cells, they are present in every cell. Therefore, gene mutations and/or chromosomal rearrangements can be detected in virtually all tissues of an animal including specific site of contact tissues and germ cells. Delayed sampling times may need to be considered in order to detect mutations in different stages of spermatogenesis.	EU: none OECD: 488

[a] For EU guidelines, see Regulation (EC) No 440/2008 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32008R0440:en:NOT) / for OECD guidelines see http://www.oecd.org/env/testguidelines
[b] OECD TGs 478 and 483 are currently being revised (see http://www.oecd.org/env/testguidelines)

A detailed review of transgenic animal model assays, including recommendations on how to perform such assays in germ cells, has been produced for the OECD (Lambert *et al.,* 2005; OECD, 2009). The ability to include sampling of somatic and germ cells in a single study significantly reduces the need to perform additional studies to obtain such information, thereby conforming to the 3Rs principles. As specified in the OECD TG 488, additional sampling times

may be needed to cover for the all the stages of spermatogenesis. The test can also be used to investigate transmission of mutations to the offspring since treatment of transgenic male mice can result in offspring carrying mutations (Barnett *et al*., 2002). An example of mutagenicity investigation in epididymal spermatozoa using a transgenic mouse model has been published (Olsen *et al.,* 2010).

The applicability of the standard alkaline comet assay to germ cells has been discussed by the OECD. The assay as described in the OECD TG 489 (see http://www.oecd.org/env/testguidelines) is not considered appropriate to measure DNA strand breaks in mature germ cells. Since high and variable background levels in DNA damage were reported in a literature review on the use of the comet assay for germ cell genotoxicity (Speit *et al.*, 2009), protocol modifications together with improved standardization and validation trials are deemed necessary before the comet assay on mature germ cells (*e.g.* sperm) can be included in the test guideline. In addition, the recommended exposure regimen described in this guideline is not optimal and longer exposures or sampling times would be necessary for a meaningful analysis of DNA strand breaks in mature sperm. Genotoxic effects as measured by the comet assay in testicular cells at different stages of differentiation have been described in the literature (Zheng *et al.*, 1997; Cordelli *et al.*, 2003). However, it should be noted that gonads contain a mixture of somatic and germ cells. For this reason, positive results in whole gonad (testis) are not necessarily reflective of germ cell damage, nevertheless, they suggest that tested chemicals have reached the gonad.

Databases with experimental data

There are several open-source databases with experimental information on mutagenicity and carcinogenicity (the two endpoints can often not easily be separated). A review of these databases can be found in Serafimova *et al.* (2010).

R.7.7.3.2 Human data on mutagenicity

Occasionally, studies of genotoxic effects in humans exposed by, for example, accident, occupation or participation in clinical studies (*e.g.* from case reports or epidemiological studies) may be available. Generally, cells circulating in blood are investigated for the occurrence of various types of genetic alterations.

R.7.7.4 Evaluation of available information on mutagenicity

Genotoxicity is a complex endpoint and requires evaluation by expert judgement. For both steps of the effects assessment, *i.e.* hazard identification and dose (concentration)-response (effect) assessment, it is very important to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy should address the reliability and relevance of the data in a way as outlined in the introductory chapter. The completeness of the data refers to the conclusion on the comparison between the available adequate information and the information that is required under the REACH provisions for the applicable tonnage level of the substance. Such a conclusion relies on *Weight of Evidence* approaches, which categorise available information based on the methods used: *guideline tests*, *non-guideline tests*, and other types of information which may justify adaptation of the standard testing regime. Such a *Weight of Evidence* approach also includes an evaluation of the available data as a whole, *i.e.* both *over and across* toxicological endpoints (for example, consideration of existing carcinogenicity data, repeated dose toxicity data and genotoxicity data all together can help understand whether a substance could be a genotoxic or non-genotoxic carcinogen).

This approach provides a basis to decide whether further information is needed on endpoints for which specific data appear inadequate or not available, or whether the requirements are fulfilled.

R.7.7.4.1 Non-human data on mutagenicity

Non-testing data for mutagenicity

In a more formal approach, documentation can include reference to a related substance or group of substances that leads to the conclusion of concern or lack of concern. This can either be presented according to scientific logic (read-across) or sometimes as a mathematical relationship of chemical similarity.

If well-documented and applicable (Q)SAR data are available, they should be used to help reach the decision points described in the section below. In many cases the accuracy of such methods will be sufficient to help, or allow either a testing or a specific regulatory decision to be made. In other cases the uncertainty may be unacceptable due to the severe consequences of a possible error. This may be driven by many factors including high exposure potential or toxicological concerns.

Substances for which no test-data exist or for which testing is technically not possible represent a special case in which reliance on non-testing data may be absolute. Many factors will dictate the acceptability of non-testing methods in reaching a conclusion based on no tests at all. It may be discussed whether *Weight of Evidence* decisions based on multiple genotoxicity and carcinogenicity estimates can equal or exceed those obtained by one or two *in vitro* tests, and whether general rules for adaptation of the standard testing regime as described in Annex XI to REACH may be invoked based on such estimates. This must be considered on a case-by-case basis.

Testing data on mutagenicity

Evaluation of genotoxicity test data should be made with care.

Regarding ***positive* findings**, particular points should be taken into account:

- are the testing conditions (*e.g.* pH, osmolality, precipitates) in *in vitro* mammalian cell assays relevant to the conditions *in vivo*?
- for studies *in vitro*, factors known to influence the specificity of mammalian cell assays such as the cell line used, the top concentration tested, the toxicity measure used or the metabolic activation system used, should be taken into consideration
- responses generated only at highly toxic/cytotoxic doses or concentrations should be interpreted with caution (*i.e.* taking into account the criteria defined in OECD guidelines)
- the presence or absence of a dose (concentration)-response relationship should be considered

Particular points to take into account when evaluating ***negative* test results** include:

- the doses or concentrations of test substance used (were they high enough? For studies *in vivo*, was a sufficienlty high dose level inducing signs of toxicity used? For studies *in vitro*, was a sufficient level of cytotoxicity reached?)
- was the test system used sensitive to the nature of the genotoxic changes that might have been expected? For example, some *in vitro* test systems will be sensitive to point mutations and small deletions but not to mutagenic events that create large deletions
- the volatility of the test substance (were concentrations maintained in tests conducted *in vitro*?)

- for studies *in vitro*, the possibility of metabolism not being appropriate in the test system including studies in extra-hepatic organs
- was the test substance taken up by the test system used for *in vitro* studies?
- were sufficient cells scored/sampled for studies *in vitro*? Has the appropriate number of samples/technical replicates been scored to support statistical significance of the putative negative result?
- for studies *in vivo*, did the substance reach the target organ? Or was the substance only in a position to act at the site of contact due to its high reactivity or insufficient systemic availability (taking also toxicokinetic data into consideration, *e.g.* rate of hydrolysis and electrophilicity may be factors that need to be considered)?
- for studies *in vivo*, was sampling appropriate? (Was a sufficient number of animals used? Were sufficient sampling times used? Was a sufficient number of cells scored/sampled?)

Different results between different test systems should be evaluated with respect to their individual significance. Examples of points to be considered are as follows:

- different results obtained in non-mammalian systems and in mammalian cell tests may be addressed by considering possible differences in substance uptake and metabolism, or in genetic material organisation and ability to repair. Although the results of mammalian tests may be considered of higher significance, additional data may be needed to explain differences
- if the results of indicator tests detecting putative DNA lesions (*e.g.* DNA binding, DNA damage, DNA repair; SCE) are not in agreement with results obtained in tests for mutagenicity, the results of mutagenicity tests are generally of higher significance provided that appropriate mutagenicity tests have been conducted. This is subject to expert judgement.
- if different findings are obtained *in vitro* and *in vivo*, in general, the results of *in vivo* tests indicate a higher degree of reliability. However, for evaluation of *negative* results *in vivo*, it should be considered whether the most appropriate tissues were sampled and whether there is adequate evidence of target tissue exposure
- the sensitivity and specificity of different test systems vary for different classes of substances. If available testing data for other related substances permit assessment of the performance of different assays for the class of substance under evaluation, the result from the test system known to produce more accurate responses would be given higher priority

Different results may also be available from the same test, performed by different laboratories or on different occasions. In this case, expert judgement should be used to evaluate the data and reach an overall conclusion. In particular, the quality of each of the studies and of the data provided should be evaluated, with special consideration of the study design, reproducibility of data, dose (concentration)-effect relationships, and biological relevance of the findings. The identity and purity of the test substance may also be a factor to take into account. In the case where an EU/OECD guideline is available for a test method, the quality of a study using the method is regarded as being higher if it was conducted in compliance with the requirements stated in the guideline, unless convincing scientific evidence can be provided to justify certain deviations from the standard test guideline for the specific substance evaluated. Furthermore, compared to non GLP-studies, studies compliant with GLP for the same assay generally provide more documentation and details of the study, which are important factors to consider when assessing study reliability/quality.

When making an assessment of the potential mutagenicity of a substance, or considering the need for further testing, data from various tests and genotoxic endpoints may be found. Both the strength and the weight of the evidence should be taken into account. The strongest evidence will be provided by modern, well-conducted studies with internationally established test guidelines/methods. For each test type and each genotoxic endpoint, there should be a separate *Weight of Evidence* analysis. It is not unusual for positive evidence of mutagenicity to be found in just one test type or for only one endpoint. In such cases the positive and negative results for different endpoints are not conflicting, but illustrate the advantage of using test methods for a variety of genetic alterations to increase the probability of identifying substances with mutagenic potential. Hence, results from methods testing different genotoxic endpoints should not be combined in an overall *Weight of Evidence* analysis, but should be subjected to such analysis separately for each endpoint. Based on the whole data set one has to consider whether there are data gaps: if there are data gaps further testing should be considered, otherwise an appropriate conclusion/assessment can be made.

R.7.7.4.2 Human data on mutagenicity

Human data have to be assessed carefully on a case-by-case basis. The interpretation of such data requires considerable expertise. Attention should be paid especially to the adequacy of the exposure information, confounding factors, co-exposures and to sources of bias in the study design or incident. The statistical power of the test may also be considered. It may be mentioned that, to date, no germ cell mutagen has been identified based on human data.

R.7.7.4.3 Remaining uncertainty on mutagenicity

Reliable data can be generated from well-designed and conducted studies *in vitro* and *in vivo*. However, due to the lack of human data available and the degree of uncertainty which is always inherent in testing, a certain level of uncertainty remains when extrapolating these testing data to the effect in humans.

R.7.7.5 Conclusions on mutagenicity

R.7.7.5.1 Concluding on Classification and Labelling

In order to conclude on an appropriate classification and labelling position with regard to mutagenicity, the available data should be considered using the criteria according to Annex I to the CLP Regulation (EC) No 1272/2008 (See also Section 3.5 of the *Guidance on the application of the CLP criteria*, available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-clp).

R.7.7.5.2 Concluding on suitability for Chemical Safety Assessment

Considerations on dose (concentration)-response shapes and mode of action of mutagenic substances in test systems

Considerations on the dose (concentration)-response relationship and on possible mechanisms of action are important components of a risk assessment. The default assumption for genotoxic substances has for long been that they have a linear dose (concentration)-response relationship. However, this assumption has recently been challenged by experimental evidence showing that both direct and indirect acting genotoxins can possess non-linear or thresholded dose (concentration)-response curves.

Examples of non-DNA reactive mechanisms that may be demonstrated to lead to genotoxicity via non-linear or thresholded dose (concentration)-response relationships include inhibition of

DNA synthesis, alterations in DNA repair, overloading of defence mechanisms (anti-oxidants or metal homeostatic controls), interaction with microtubule assembly leading to aneuploidy, topoisomerase inhibition, high cytotoxicity, metabolic overload and physiological perturbations (*e.g.* induction of erythropoeisis). The mechanisms underlying non-linear or thresholded dose (concentration)-response relationships for some DNA reactive genotoxic substances like alkylating agents seem linked to DNA repair capacity.

Assessment of the significance to be assigned to genotoxic responses mediated by such mechanisms would include an assessment of whether the underlying mechanism can be induced at substance concentrations that can be expected to occur under relevant *in vivo* conditions.

In general, several concentrations/doses are tested in genotoxicity assays. At least three experimental concentrations/doses have to be tested as recommended in the OECD test guidelines for genotoxicity. Determination of experimental dose (concentration)-effect relationships is one of several pieces of experimental information that are important to assess the genotoxic potential of a substance, and may be used as indicated below. It should be recognised that not all of these considerations may be applicable to *in vivo* data.

- the OECD introduction to the genotoxicity test guidelines lists the relevant criteria for identification of clear positive findings: (i) the increase in genotoxic response is concentration- or dose-related, (ii) at least one of the data points exhibits a statistically significant increase compared to the concurrent negative control, and (iii) the statistically significant result is outside the distribution of the historical negative control data (*e.g.* 95% confidence interval). In practice, the criterion for dose (concentration)-related increase in genotoxicity will be most helpful for *in vitro* tests, but care is needed to check for cytotoxicity or cell cycle delay which may cause deviations from a dose (concentration)-response related effect in some experimental systems

- genotoxicity tests are not designed in order to derive no effect levels. However, the magnitude of the lowest dose with an observed effect (*i.e.* the Lowest Observed Effect Dose or LOED) may, on certain occasions, be a helpful tool in risk assessment. This is true specifically for genotoxic effects caused by thresholded mechanisms, like, *e.g.* aneugenicity. Further, it can give an indication of the mutagenic potency of the substance in the test at issue. Modified studies, with additional dose or concentration points and improved statistical power may be useful in this regard. The Benchmark dose (BMD) approach presents several advantages over the NOED/LOED approach and can be used as an alternative strategy for dose (concentration)-response assessment (see *Guidance on IR&CSA, Chapter R.8*)

- unusual shapes of dose (concentration)-response curves may contribute to the identification of specific mechanisms of genotoxicity. For example, extremely steep increases suggest an indirect mode of action or metabolic switching which could be confirmed by further investigation.

Considerations on genetic risks associated with human exposure to mutagenic substances

There are no officially adopted methods for estimating health risks associated with (low) exposures of humans to mutagens. In fact, most – if not all tests used today – are developed and applied to identify mutagenic properties of the substance, *i.e.* identification of the mutagenic hazard *per se*. In today's regulatory practice, the assessment of human health risks from exposure to mutagenic substances is considered to be covered by assessing and regulating the carcinogenic risks of these agents. The reason for this is that mutagenic events underlie these carcinogenic effects. Therefore, mutagenicity data is not used for deriving dose descriptors for risk assessment purposes and the reader is referred to this aspect in Section R.7.7.8 (Carcinogenicity) for guidance on how to assess the chemical safety for mutagenic substances.

R.7.7.5.3 Information not adequate

A *Weight of Evidence* approach, comparing available adequate information with the tonnage-triggered information requirements by REACH, may result in the conclusion that the requirements are not fulfilled. In order to proceed in gathering further information, the following testing strategy can be adopted:

R.7.7.6 Integrated Testing Strategy (ITS) for mutagenicity

R.7.7.6.1 Objective / General principles

This testing strategy describes a flexible, stepwise approach for hazard identification with regard to the mutagenic potential of substances, so that sufficient data may be obtained for adequate risk characterisation including classification and labelling. It serves to help minimise the use of animals and costs as far as it is consistent with scientific rigour. A flow chart of the testing strategy is presented in Figure R.7.7-1 and recommendations on follow up procedures based on different testing data sets are given in Table R.7.7-5.As noted later in this section, deviations from this strategy may be considered if existing data for related substances indicate that alternate testing strategies yield results with greater sensitivity and specificity for mutagenicity *in vivo*.

The strategy defines a level of information that is considered sufficient to provide adequate reassurance about the potential mutagenicity of most substances. As described below, this level of information will be required for most substances at the Annex VIII tonnage level specified in REACH, although circumstances are described when the data may be required for substances at Annex VII.

For some substances, relevant data from other sources/tests may also be available (*e.g.* physico-chemical, toxicokinetic, and toxicodynamic parameters and other toxicity data; data on well-investigated, structurally similar, substances). These should be reviewed because, sometimes, they may indicate that either more or less genotoxicity studies are needed on the substance than defined by standard information requirements; *i.e.* they may allow tailored testing/selection of test systems. For example, bacterial mutagenesis assays of inorganic metal compounds are frequently negative due to limited capacity for uptake of metal ions and/or the induction of large DNA deletions by metals in bacteria potentially leading to an increased death rate in mutants. The high prevalence of false negatives for metal compounds might suggest that mutagenesis assays with mammalian cells, as opposed to bacterial cells, would be the preferred starting point for testing for this class of Annex VII substances.

In summary, a key concept of the strategy is that initial genotoxicity tests and testing guidelines/methods should be selected with due consideration to existing data that has established the most accurate testing strategy for the class of compound under evaluation. Even then, initial testing may not always give adequate information and further testing may sometimes be considered necessary in the light of all available relevant information on the substance, including its use pattern. Further testing will normally be required for substances which give rise to positive results in any of the *in vitro* tests.

If negative results are available from an adequate evaluation of genotoxicity from existing data in appropriate test systems, there may be no requirement to conduct additional genotoxicity tests.

Substances for which there is a harmonised classification in category 1A, 1B or 2 for germ cell mutagenicity and/or category 1A or 1B for carcinogenicity according to Annex VI to the CLP Regulation (EC) No 1272/2008 will usually not require additional testing in order to meet the requirements of Annex VIII for the *in vitro* cytogenicity study in mammalian cells. Provided that appropriate risk management measures are implemented, the carcinogenicity study to meet the requirements of Annex X (see Section R.7.7.2 of this Guidance) and the reproductive

toxicity studies to meet the requirements of Annexes VIII to X (see Section R.7.7.6 of this Guidance) may also be omitted for substances classified in category 1A or 1B for germ cell mutagenicity. In cases where a registrant is unsure of the formal position on the classification of a substance, or wishes to make a classification proposal himself, advice should be sought from an appropriate regulatory body before proceeding with any further testing.

In case additional testing is needed to meet the requirements of Annexes IX or X, the registrant must first submit a testing proposal to the European Chemicals Agency (ECHA) and obtain prior authorisation before any testing can be initiated.

It should also be noted that recommendations on a strategy for genotoxicity testing have also recently been published by other authoritative organisations (EFSA, 2011; EMA, 2012; UK COM, 2011). These strategies are based either on a step-wise approach or on a test-battery approach. Their principle is basically similar to the one detailed in this Guidance, *i.e.* the use of different pieces of information, including non-testing data and results from *in vitro* and *in vivo* testing, for a comprehensive assessment of the genotoxic potential a substance since no single test is capable of detecting all genotoxic mechanisms. However, as these strategies aim at serving different regulations and purposes, some differences can exist between them, in particular regarding the list of *in vitro* and *in vivo* tests recommended and the way to use them. For instance, while the UK COM and EFSA now both recommend the use of a core two-test battery (*i.e.* a bacterial reverse mutation test combined with an *in vitro* micronucleus test) for *in vitro* genotoxicity assessment, the REACH Regulation and this Guidance state the *in vitro* mammalian cell gene mutation test as a legal requirement in addition to the Ames test and the *in vitro* cytogenicity test if both are negative. Moreover, the *in vitro* chromosome aberration test is considered as a possible alternative option to the *in vitro* micronucleus test under REACH while it is now generally agreed that these tests are not equivalent since the *in vitro* chromosome aberration test is not optimal to measure numerical chromosome aberrations. Although this guidance aims at implementing the latest scientific developments in the field of genotoxicity testing, its main goal is to provide advice and support to the registrant in complying with the legal requirements under REACH and is thus in line with this Regulation.

R.7.7.6.2 Preliminary considerations

For a comprehensive coverage of the potential mutagenicity of a substance, information on gene mutations (base substitutions and deletions/additions), structural chromosome aberrations (breaks and rearrangements) and numerical chromosome aberrations (loss or gain of chromosomes, defined as aneuploidy) is required. This may be obtained from available data or tests on the substance itself or, sometimes, by prediction using appropriate *in silico* techniques (*e.g.* chemical grouping, read-across or (Q)SAR approaches).

It is important that whatever is known of the physico-chemical properties of the test substance is taken into account before devising an appropriate testing strategy. Such information may impact upon both the selection of test systems to be employed and/or modifications to the test protocols used. The chemical structure of a substance can provide information for an initial assessment of mutagenic potential. The need for special testing in relation to photomutagenicity may be indicated in some specific cases by the structure of a molecule, its light absorbing potential or its potential to be photoactivated. By using expert judgement, it may be possible to identify whether a substance, or a potential metabolite of a substance, shares or does not share structural characteristics with known mutagens. This can be used to justify a higher or lower level of priority for the characterisation of the mutagenic potential of a substance. Where the level of evidence for mutagenicity is particularly strong, it may be possible to make a conclusive hazard assessment in accordance with Annex I to REACH without additional testing on the basis of structure-activity relationships alone: in this case, the registrant still has to provide sufficient information to meet the requirements of Annexes VII to X but he may, if scientifically justified and duly documented in the registration dossier, invoke the general rules of Annex XI for adaptation of the standard testing regime by demonstrating, *inter alia*, that the results he wishes to use instead of testing in that context are adequate for the purpose of classification and labelling and/or risk assessment.

In vitro tests are particularly useful for gaining an understanding of the potential mutagenicity of a substance and they have a critical role in this testing strategy. They are not, however, without their limitations. Animal tests will, in general, be needed for the clarification of the relevance of positive findings and in case of specific metabolic pathways that cannot be simulated adequately *in vitro*.

The toxicokinetic and toxicodynamic properties of the test substance should be considered before undertaking, or appraising, animal tests. Understanding these properties will enable appropriate protocols for the standard tests to be developed, especially with respect to tissue(s) to be investigated, the route of substance administration and the highest dose tested. If little is understood about the systemic availability of a test substance at this stage, toxicokinetic investigations or modelling may be necessary.

Certain substances in addition to those already noted may need special consideration, such as highly electrophilic substances that give positive results *in vitro*, particularly in the absence of metabolic activation. Although these substances may react with proteins and water *in vivo* and thus be rendered inactive towards many tissues, they may be able to express their mutagenic potential at the initial site of contact with the body. Consequently, the use of test methods such as the comet assay or the gene mutation assays using transgenic animals that can be applied to the respiratory tract, upper gastrointestinal tract and skin may be appropriate. It is possible that specialised test methods will need to be applied in these circumstances, and that these may not have recognised, internationally valid, test guidelines. The validity and utility of such tests and the selection of protocols should be assessed by appropriate experts or authorities on a case-by-case basis.

Criteria for the evaluation and interpretation of results (e.g. how to define clear positive and clear negative results) are normally defined in the testing guidelines/methods. There is no requirement for verification of a clear positive or clear negative result. In cases where the response is neither clearly negative nor clearly positive and in order to assist in establishing the biological relevance of a result (e.g. a weak or borderline increase), the data should be evaluated by expert judgement and/or further investigations. A substance giving such a response should be reinvestigated immediately, normally using the same test method, but varying the conditions to obtain conclusive results. Only if, even after further investigations, the data set precludes coming to a conclusion of a positive or negative result, will the result be concluded as equivocal. Wherever possible, clear results should be obtained for one step in the strategic procedure before going on to the next. In cases where this does not prove to be possible and the study is inconclusive as a consequence of *e.g.* some limitation of the test or procedure, a further test should be conducted in accordance with the strategy.

Tests need not be performed if it is not technically possible to do so, or if they are not considered necessary in the light of current scientific knowledge. Scientific justifications for not performing tests required by the strategy should always be documented. It is preferred that tests as described in OECD Guidelines or Regulation (EC) No 440/2008 are used where possible. Alternatively, for other tests, up-to-date protocols defined by internationally recognised groups of experts, *e.g.* International Workshop on Genotoxicity Testing (IWGT, under the umbrella of the International Association of Environmental Mutagen Societies), may be used provided that the tests are scientifically justified. It is essential that all tests be conducted according to rigorous protocols in order to maximise the potential for detecting a mutagenic response, to ensure that negative results can be accepted with confidence and that results are comparable when tests are conducted in different laboratories. At the time of writing this guidance, a standard test guideline/method is still to be established for the *in vivo* comet assay described below. So if this test is to be conducted, and in waiting for the adoption of the comet OECD TG 489, consultation on the protocol with an appropriate expert or authority is advisable.

If a registrant wishes to undertake any tests for substances at the Annex IX or X tonnage levels that require the use of vertebrate animals, then there is a need to make a testing

proposal to ECHA first. Testing may only be undertaken after ECHA has accepted the testing proposal in a formal decision.

R.7.7.6.3 Testing strategy for mutagenicity

Standard information requirement at Annex VII

A preliminary assessment of mutagenicity is required for substances at the REACH Annex VII tonnage level. All available information should be included but, as a minimum, there should normally be data from a gene mutation test in bacteria unless existing data for analogous substances indicates this would be inappropriate. For substances with significant toxicity to bacteria, not taken up by bacteria, or for which the gene mutation test in bacteria cannot be performed adequately, an *in vitro* mammalian cell gene mutation test may be used as an alternative test.

When the result of the bacterial test is positive, it is important to consider the possibility of the substance being genotoxic in mammalian cells. The need for further test data to clarify this possibility at the Annex VII tonnage level will depend on an evaluation of all the available information relating to the genotoxicity of the substance.

Standard information requirement at Annex VIII

For a comprehensive coverage of the potential mutagenicity of a substance, information on gene mutations, and structural and numerical chromosome aberrations is required for substances at the Annex VIII tonnage level of REACH.

In order to ensure the necessary minimum level of information is provided, at least one further test is required in addition to the gene mutation test in bacteria. This should be an *in vitro* mammalian cell test capable of detecting both structural and numerical chromosome aberrations.

There are essentially two different methods that can be viewed as alternative options according to REACH for this first mammalian cell test:

- An *in vitro* chromosome aberration test (OECD TG 473), *i.e.* a cytogenetic assay for structural chromosome aberrations using metaphase analysis. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not routinely used for that purpose. Accordingly, this test guideline is not designed to measure numerical aberrations.
- An *in vitro* micronucleus test (OECD TG 487). This is a cytogenetic assay that has the advantage of detecting not only structural chromosomal aberrations but also aneuploidy. Use of a cytokinesis block, fluorescence *in situ* hybridisation with probes for centromeric DNA, or immunochemical labelling of kinetochore proteins can provide information on the mechanisms of chromosome damage and micronucleus formation. The labelling and hybridisation procedures can enable aneugens to be distinguished from clastogens. This may sometimes be useful for risk characterisation. If a substance is demonstrated to be an aneugen, it is assumed that its genotoxicity is thresholded, in contrast to non-thresholded genotoxicity. Both types of genotoxicity mechanisms trigger different ways to perform risk assessment.

Other *in vitro* tests may be acceptable as the first mammalian cell test, but care should be taken to evaluate their suitability for the substance being registered and their reliability as a screen for substances that cause structural and/or numerical chromosome aberrations. A supporting rationale should be presented for a registration with any of these other tests.

It is possible to present existing data from an *in vivo* cytogenetic test (*i.e.* a study or studies conducted previously) as an alternative to the first *in vitro* mammalian cell test. For instance, if an adequately performed *in vivo* micronucleus test is available already it may be presented as an alternative. There may however be specific cases where the *in vitro* mammalian cell test can still be justified even though *in vivo* cytogeneticity data exist. For example, in the *in vivo* micronucleus test, certain substances may not reach the bone marrow due to low bioavailability or specific tissue/organ distribution and would result negative. In addition, even if bioavailability of the parent compound in the bone marrow can be demonstrated, a clastogen requiring liver metabolism and for which the reactive metabolites formed are too short-lived to reach the bone marrow could give a negative result in the *in vivo* micronucleus test. In this case, *in vitro* testing could provide useful information on the mode of action of the substance, *e.g.* to understand whether the substance is clastogenic (or aneugenic) *in vitro*, and whether it requires a specific metabolism to be genotoxic. Justification of *in vitro* testing when *in vivo* data already exist should be considered on a case-by-case basis.

An *in vitro* gene mutation study in mammalian cells (OECD TG 476) is the second part of the standard information set required for registration at the Annex VIII tonnage level. For substances that have been tested already, this information should always be presented as part of the overall *Weight of Evidence* for mutagenicity with reference to induction of gene mutations in mammalian cells. For other substances, this second *in vitro* mammalian cell test will normally only be required when the results of the bacterial gene mutation test and the first study in mammalian cells (*i.e.* an *in vitro* chromosome aberration test or an *in vitro* micronucleus test) are negative. This is to detect *in vitro* mutagens that give negative results in the other two tests.

Under specific circumstances it may be possible to omit the second *in vitro* study in mammalian cells, *i.e.* if it can be demonstrated that this mammalian cell test will not provide any further useful information about the potential *in vivo* mutagenicity of a substance, then it does not need to be conducted. This should be evaluated on a case-by-case basis as there may be classes of compound for which conclusive data can be provided to show that the sensitivity of the first two *in vitro* tests cannot be improved by the conduct of the third test.

The *in vitro* mammalian cell gene mutation test will not usually be required if adequate information is available from a reliable *in vivo* study capable of detecting gene mutations. Such information may come from a TGR gene mutation assay. A comet assay or a liver UDS test may also be adequate. However, these two tests being indicator assays detecting putative DNA lesions, their use should be justified on a case-by-case basis, *e.g.* the UDS should be used only when it can be reasonably assumed that the liver is a target organ, since the UDS is restricted to the detection of primary DNA repair in liver cells.

Provided the *in vitro* tests have given negative results, normally, no *in vivo* tests will be required to fulfil the standard information requirements at Annex VIII. However, there may be rare occasions when it is appropriate to conduct testing *in vivo*, for example when it is not possible technically to perform satisfactory tests *in vitro*. Substances which, by virtue of, for example, their physico-chemical characteristics, chemical reactivity or toxicity cannot be tested in one or more of the *in vitro* tests should be considered on a case-by-case basis. In the same way, it may not always be possible with the S9 fraction used *in vitro* to mimic the *in vivo* metabolism of some substances, and the relevance of the *in vitro* negative results for those substances should be evaluated case by case. In addition, equivocal *in vitro* results or different results from different *in vitro* studies may require the consideration of further testing to reach a clear conclusion on mutagenicity. For those types of cases, expert judgement would be needed to determine whether *in vivo* testing is appropriate.

Requirement for testing beyond the standard levels specified for Annexes VII and VIII

Introductory comments

Concerns raised by positive results from *in vitro* tests usually require the consideration of further testing. The chemistry of the substance, data on analogous substances, toxicokinetic and toxicodynamic data, and other toxicity data will also influence the timing and pattern of further testing.

Unless there are appropriate results from an *in vivo* study already, testing beyond the standard set of *in vitro* tests is normally first directed towards investigating the potential for mutagenicity in somatic cells *in vivo*. Positive results in somatic cells *in vivo* constitute the trigger for consideration of investigation of potential expression of genotoxicity in germ cells. However, to avoid unnecessary testing of vertebrate animals and for cost reasons, as the TGR assays give the possibility to include sampling of somatic and male germ cells in a single study providing adapted sampling times (see OECD TG 488 for details), it is recommended to include such samples in the testing proposal for the TGR assays and to appropriately store the germ cell samples for later analysis in case there is a positive result in any of the somatic tissues tested.

Substances that are negative in the standard set of *in vitro* tests

In general, substances that are negative in the full set of *in vitro* tests specified in REACH Annexes VII and VIII are considered to be non-genotoxic. There are only a very limited number of substances that have been found to be genotoxic *in vivo*, but not in the standard *in vitro* tests. Most of these are pharmaceuticals designed to affect pathways of cellular regulation, including cell cycle regulation, and this evidence is judged insufficient to justify routine *in vivo* testing of industrial chemicals. However, occasionally, knowledge about the metabolic profile of a substance may indicate that the standard *in vitro* tests are not sufficiently reassuring and a further *in vitro* test, or an *in vivo* test, may be needed in order to ensure mutagenicity potential is adequately explored (*e.g.* use of an alternative to rat liver S9 mix, a reducing system, a metabolically active cell line, or genetically engineered cell lines might be judged appropriate).

Substances for which an *in vitro* test is positive

REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII.

Regarding Annex VIII, when both the mammalian cell tests are negative but there was a positive result in the bacterial test, it will be necessary to decide whether any further testing is needed on a case-by-case basis. For example, suspicion that a unique positive response observed in the bacterial test was due to a specific bacterial metabolism of the test substance could be explored further by investigation *in vitro.* Alternatively, an *in vivo* test may be required (see below).

In REACH Annex VIII, following a positive result in an *in vitro* mammalian cell mutagenicity test, adequately conducted somatic cell *in vivo* testing is required to ascertain if this potential can be expressed *in vivo*. In cases where it can be sufficiently deduced that a positive *in vitro* finding is not relevant for *in vivo* situations (*e.g.* due to the effect of the test substances on pH or cell viability, *in vitro*-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached *in vivo* has been identified (*e.g.* damage to non-DNA targets at high concentrations), *in vivo* testing will not be necessary.

Annex VIII, Column 2 requires the registrant to consider appropriate mutagenicity *in vivo* studies already at the Annex VIII tonnage level, in cases where positive results in genotoxicity studies have been obtained. It should be noted that where this involves tests mentioned in Annexes IX or X, such as *in vivo* somatic cell genotoxicity studies, testing proposals must be submitted by the registrant and accepted by ECHA in a formal decision before testing can be initiated.

Standard information requirement according to Annexes IX and X

According to the requirements of Annexes IX and X, if there is a positive result in any of the *in vitro* studies from Annex VII or VIII and there are no appropriate results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study should be proposed.

Before any decisions are made about the need for *in vivo* testing, a review of the *in vitro* test results and all available information on the toxicokinetic and toxicodynamic profile of the test substance is needed. A particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. If necessary, a targeted investigation of toxicokinetics should be conducted before progressing to *in vivo* testing (*e.g*. a preliminary toxicity test to confirm that absorption occurs and that an appropriate dose route is used).

In the interest of ensuring that the number of animals used in genotoxicity tests is kept to a minimum, both males and females should not automatically be used. In accord with standard guidelines, testing in one sex only is possible when the substance has been investigated for general toxicity and no sex-specific differences in toxicity have been observed. If the test is performed in a laboratory with substantial experience and historical data, it should be considered whether a concurrent positive control and a concurrent negative control for all time points (*e.g*. for both the 24h and 48h time point in the micronucleus assay) will really be necessary (Hayashi *et al*., 2000).

For test substances with adequate systemic availability (*i.e*. evidence for adequate availability to the target cells) there are several options for the *in vivo* testing:

- A rodent bone marrow or mouse peripheral blood micronucleus test (OECD TG 474) or a rodent bone marrow chromosome aberration test (OECD TG 475). The micronucleus test has the advantage of detecting not only structural chromosomal aberrations (clastogenicity) but also numerical chromosomal aberrations (aneuploidy). Potential species-specific effects may also influence the choice of species and test method used.
- A transgenic rodent (TGR) mutation assay (OECD TG 488). TGR assays measure gene mutations and chromosomal rearrangements (the latter specifically in the plasmid and Spi- assay models) using reporter genes present in every tissue. In principle every tissue can be sampled, including target tissues and specific site of contact tissues.
- A comet (single cell gel electrophoresis) assay (OECD TG 489), which detects DNA strand breaks and alkali labile DNA lesions. In contrast to the above-mentioned *in vivo* micronucleus test and *in vivo* chromosome aberration test, this assay has the advantage of not being restricted to bone marrow cells. In principle every tissue from which single cell or nuclei suspensions can be prepared can be sampled, including specific site of contact tissues.
- Other DNA strand breakage assays may be presented as alternatives to the comet assay. All DNA strand break assays should be considered as surrogate tests, they do not necessarily detect permanent changes to DNA.
- A rat liver Unscheduled DNA synthesis (UDS) test (OECD TG 486). The UDS test is an indicator test measuring DNA repair of primary damage in liver cells but not a surrogate

test for gene mutations *per se*. The UDS test can detect some substances that induce *in vivo* gene mutation because this assay is sensitive to some (but not all) DNA repair mechanisms. However not all gene mutagens are positive in the UDS test and it is thus useful only for some classes of substances. A positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage by the substance under investigation but it is not sufficient information to conclude on the induction of gene mutation by the substance. A negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation.

Only the first two options for testing mentioned above can be used directly for providing evidence of *in vivo* chromosomal and gene mutagenicity, respectively. The other test methods require specific supporting information, for example results from *in vitro* mutagenicity studies, to be used for making definitive conclusions about *in vivo* mutagenicity and lack thereof.

In the framework of the 3Rs principles, the combination of *in vivo* genotoxicity studies or integration of *in vivo* genotoxicity studies into repeated dose toxicity studies, whenever possible and when scientifically justified, is strongly encouraged if this is to be performed to meet the requirements of the REACH Annex VIII tonnage level. All the above-mentioned *in vivo* tests for somatic cells are in principle amenable to such integration although sufficient experience is not yet available for all of the tests. It is possible for two or more endpoints to be combined into a single *in vivo* study, and thereby save on resources and numbers of animals used. The comet assay and the *in vivo* micronucleus test can be combined into a single acute study, although some modification of treatment and sampling times is needed (Hamada *et al*., 2001; Madrigal-Bujaidar *et al*., 2008; Pfuhler *et al.*, 2009; Bowen *et al*., 2011,). These same endpoints can be integrated into repeated dose (*e.g.* 28-day) toxicity studies (Pfuhler *et al*., 2009; Rothfuss *et al.*, 2011; EFSA, 2011).

Any one of these tests may be conducted, but this has to be decided using expert judgement on a case-by-case basis. The nature of the original *in vitro* response(s) (*i.e.* gene mutation, structural or numerical chromosome aberration) should be considered when selecting the *in vivo* study. For example, if the test substance showed evidence of *in vitro* clastogenicity, then it would be appropriate to follow this up with either a micronucleus test or chromosomal aberration test or a comet assay. However, if a positive result were obtained in the *in vitro* micronucleus test, the rodent micronucleus test would be appropriate to best address clastogenic and aneugenic potential.

For substances that appear preferentially to induce gene mutations, the TGR assays are the most appropriate and usually preferred tests to follow-up an *in vitro* gene mutation positive result and detect, *in vivo*, substances that induce gene mutation. With respect to the 3Rs principle and taking into account that a positive result in somatic cells triggers the need to consider the potential for germ cell testing, germ cells should always be collected, if possible, when a TGR study is performed. The rat liver UDS test has a long history of use and may in some specific cases be adequate to follow-up an *in vitro* gene mutation positive result, but not for tissues other than the liver. The sensitivity of the UDS test has been questioned (Kirkland and Speit, 2008) and the use of this test should be justified on a case-by-case basis, and take account of substance-specific considerations. The recommended use of the comet assay has been discussed at the OECD level and is indicated in the corresponding OECD TG (see http://www.oecd.org/env/testguidelines). The choice of any of these three assays can be justified only if it can be demonstrated that the tissue(s) studied in the assay is (are) sufficiently exposed to the test substance (or its metabolites). This information can be derived from toxicokinetic data or, in case no toxicokinetic data are available, from the observation of treatment-related effects in the organ of interest. Another type of data that can support evidence of organ exposure is knowledge on the target organ(s) of specific classes of substances (*e.g.* the liver for aromatic amines). In case the *in vivo* comet assay is used or proposed by the registrant, the test protocol followed or suggested should be described in detail and be in accordance with current scientific best practice, so as to ensure acceptability of the generated data. In waiting for the adoption of the comet OECD TG 489 the registrant should follow the EFSA guidance indicating the minimum criteria for acceptance of the comet

assay (2012), as well as, for the combined comet-micronucleus test, the 3-day treatment schedule described by *e.g.* Bowen *et al.* (2011). The TGR and comet assays offer greater flexibility than the UDS test, most notably with regard to the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. It should be realised that the UDS and comet tests are indicator assays: the comet assay detects DNA lesions whereas the UDS assay detects DNA repair patches (which depend on the DNA repair pathway involved and the proficiency of the cell type investigated), indirectly showing DNA lesions. In contrast, the TGR gene mutation assays measure mutations, *i.e.* permanent transmissible changes in the DNA.

Additionally, evidence for *in vivo* DNA adduct formation in somatic cells together with positive results from *in vitro* mutagenicity tests are sufficient to conclude that a substance is an *in vivo* somatic cell mutagen. In such cases, positive results from *in vitro* mutagenicity tests may not trigger further *in vivo* somatic tissue testing, and the substance would be classified at least as a category 2 mutagen. The possibility for effects in germ cells would need further investigation (see Section R.7.7.6.3, *Substances that give positive results in an in vivo test for genotoxic effects in somatic cells*).

Non-standard studies supported by published literature may sometimes be more appropriate and informative than established assays. Guidance from an appropriate expert or authority should be sought before undertaking novel studies. Furthermore, additional data that support or clarify the mechanism of action may justify a decision not to test further.

For substances inducing gene mutation or chromosomal aberration *in vitro*, and for which no indication of sufficient systemic availablity has been presented, or that are short-lived or reactive, an alternative strategy involving studies to focus on tissues at initial sites of contact with the body should be considered. Expert judgement should be used on a case-by-case basis to decide which tests are the most appropriate. The main options are the *in vivo* comet assay, TGR gene mutation assays, and DNA adduct studies. For any given substance, expert judgement, based on all the available toxicological information, will indicate which of these tests are the most appropriate. The route of exposure should be selected that best allows assessment of the hazard posed to humans. For insoluble substances, the possibility of release of active molecules in the gastrointestinal tract may indicate that a test involving the oral route of administration is particularly appropriate.

If the testing strategy described above has been followed and the first *in vivo* test is negative, the need for a further *in vivo* somatic cell test should be considered. The second *in vivo* test should only then be proposed if it is required to make a conclusion on the genotoxic potential of the substance under investigation; *i.e.* if the *in vitro* data show the substance to have potential to induce both gene and chromosome mutations and the first *in vivo* test has not addressed this comprehensively. In this regard, on a case-by-case basis, attention should be paid to the quality and relevance of all the available toxicological data, including the adequacy of target tissue exposure.

For a substance giving negative results in adequately conducted, appropriate *in vivo* test(s), as defined by this strategy, it will normally be possible to conclude that the substance is not an *in vivo* mutagen.

Substances that give positive results in an *in vivo* test for genotoxic effects in somatic cells

Substances that have given positive results in cytogenetic tests both *in vitro* and *in vivo* can be studied further to establish whether they specifically act as aneugens, and therefore whether thresholds for their genotoxic activity can be identified, if this has not been established adequately already. This should be done using *in vitro* methods and will be helpful in risk evaluation.

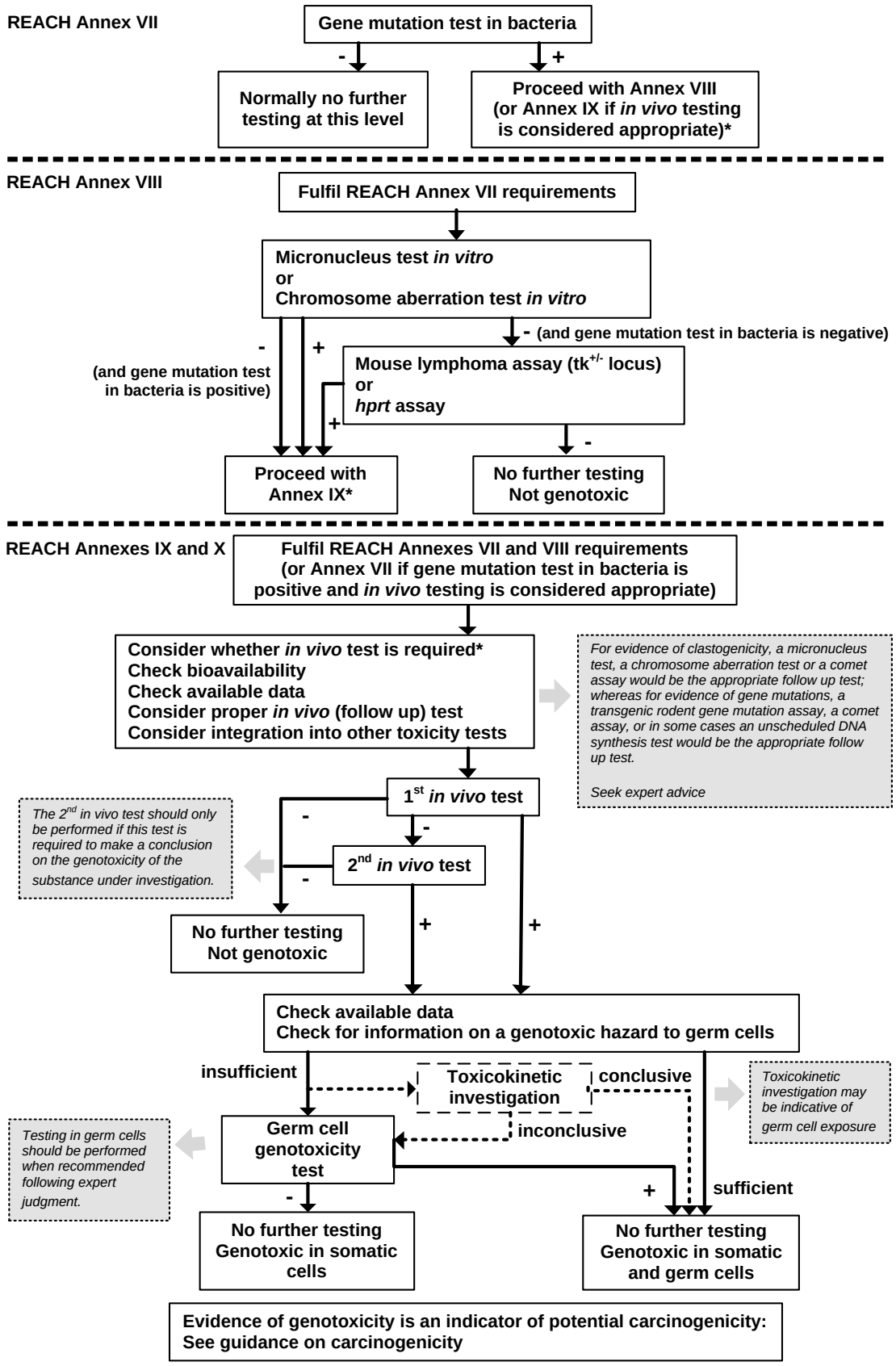
The potential for substances that give positive results in *in vivo* tests for genotoxic effects in somatic cells to affect germ cells should always be considered. The same is true for substances otherwise classified as category 2 mutagens under the CLP Regulation (EC) No 1272/2008 (for detailed information on the criteria for classification of substances for germ cell mutagenicity under the CLP Regulation (EC) No 1272/2008, see Section 3.5 of the *Guidance on the application of the CLP criteria*, available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-clp). The first step is to make an appraisal of all the available toxicokinetic and toxicodynamic properties of the test substance. Expert judgement is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified. Consequently, the substance is classified as a category 1B mutagen. If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation will be necessary. In the event that additional information about the toxicokinetics of the substance would resolve the problem, toxicokinetic investigation (*i.e.* not a full toxicokinetic study) tailored to address this should be performed. Although the hazard class for mutagenicity primarily refers to germ cells, the induction of genotoxic effects at site of contact tissues by substances for which no indication of sufficient systemic availability or presence in germ cells has been presented are also relevant and considered for classification. For such substances, at least one positive *in vivo* genotoxicity test in somatic cells can lead to classification in Category 2 germ cell mutagens and to the labelling as 'suspected of causing genetic defects' if the positive effect *in vivo* is supported by positive results of *in vitro* mutagenicity tests. Classification as Category 2 germ cell mutagen may also have implications for potential carcinogenicity classification.

If specific germ cell testing is to be undertaken, expert judgement should be used to select the most appropriate test strategy. Internationally recognised guidelines are available for investigating clastogenicity in rodent spermatogonial cells and for the dominant lethal test. Dominant lethal mutations are believed to be primarily due to structural or numerical chromosome aberrations.

Alternatively, other methods can be used if deemed appropriate by expert judgement. These may include the TGR gene mutation assays (with modified sampling times as indicated in the OECD TG 488 to detect effects at the different stages of spermatogenesis), or DNA adduct analysis. In principle, it is the potential for effects that can be transmitted to the progeny that should be investigated, but tests used historically to investigate transmitted effects (the heritable translocation test and the specific locus test) use very large numbers of animals. They are rarely used and should normally not be proposed for substances registered under REACH.

In order to minimise animal use, it is recommended to include cell samples from both relevant somatic and germ cell tissues (*e.g.* testes) in *in vivo* mutagenicity studies: the somatic cell samples can be investigated first and, if they are positive, germ cell tissues can then also be analysed. Finally, the possibility to combine reproductive toxicity testing with *in vivo* mutagenicity testing could be considered.

Figure R.7.7-1 Flow chart of the mutagenicity testing strategy



* Registrants should note that a testing proposal must be submitted for a test mentioned in Annex IX or X, independently from the registered tonnage. Following examination of such testing proposal ECHA has to approve the test in its evaluation decision before it can be undertaken.

Table R.7.7-5 Examples of different testing data sets and follow-up procedures to conclude on genotoxicity/mutagenicity according to the mutagenicity testing strategy.

Depending on the *in vitro* and *in vivo* test results available and the REACH Annex(es) of interest, further testing may be required to meet the standard information requirements for mutagenicity and allow for a conclusion on genotoxicity/mutagenicity to be reached. Recommendations on what should be done or particurlarly looked at in those different cases are mentioned in the table, together with specific rules for adaptation when applicable (for detailed guidance see also main text).

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
1	neg					Annex VII: no further tests are required. Annexes VIII, IX & X: conduct a CAbvitro or preferably a MNTvitro, and if this is negative, a GMvitro.	Annex VII: not genotoxic		Annexes VIII, IX & X: Select further tests in such a way that all the tests, together with other available information, enable thorough assessment for gene mutations and effects on chromosome structure and number.
2	neg	neg				Annex VII: no further tests are required. Annexes VIII, IX & X: conduct a GMvitro.	Annex VII: not genotoxic		Annexes VIII, IX & X: Select tests in such a way that all the tests, together with other available information, enable a thorough assessment for gene mutations and effects on chromosome structure and number.
3	neg		neg			Annex VII: no further tests are required. Annexes VIII, IX & X: conduct a CAbvitro or preferably a MNTvitro	Annex VII: not genotoxic		Annexes VIII, IX & X: Select tests in such a way that all the tests, together with other available information, enable a thorough assessment for gene mutations and effects on chromosome structure and number.

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
4	neg	neg	neg			Annexes VII, VIII, IX & X: no further tests are required.	not genotoxic		The available metabolic evidence may, on rare occasions, indicate that *in vitro* testing is inadequate*; in vivo* testing is needed. Seek expert advice. Annexes VIII, IX & X: Select tests in such a way that all the tests, together with other available information, enable a thorough assessment for gene mutations and effects on chromosome structure and number.
5	pos					Annexes VII, VIII, IX & X: Complete *in vitro* testing with a CAbvitro or preferably a MNTvitro.			Consider need for further tests to understand the *in vivo* mutagenicity hazard, to make a risk assessment, and to determine whether C&L is justified.

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
6	pos	neg				Annexes VII & VIII: *Complete in vitro* testing by conducting a GMvitro only under special conditions (see column 'Specific rules for adaption') Annexes IX & X: If systemic availability cannot be ascertained reliably, it should be investigated before progressing to *in vivo* tests. Select adequate somatic cell i*n vivo* test to investigate gene mutations *in vivo* (TGR, comet or if justified UDSvivo). If the TGR is to be conducted on somatic tissues, germ cell samples should be collected if possible, frozen and analysed for mutagenicity only in case of a positive result in somatic cells. If necessary seek expert advice.		Suspicion that a positive response observed in the GMbact was due to a specific bacterial metabolism of the test substance could be explored further by investigation *in vitro*.	Ensure that all tests together with other available information enable thorough assessment for gene mutations and effects on chromosome structure and number. Consider on a case-by-case basis need for further tests to understand the *in vivo* mutagenicity hazard, to make a risk assessment, and to determine whether C&L is justified.
7	neg	pos				Annexes VII, VIII, IX & X: If systemic availability cannot be ascertained reliably, it should be investigated before progressing to *in vivo* tests. Select adequate somatic cell *in vivo* test to investigate structural or numerical chromosome aberrations (MNTvivo or comet for *in vitro* clastogens and/or aneugens or CAbvivo for *in vitro*-clastogens) If necessary seek expert advice.			Ensure that all tests together with other available information enable thorough assessment for gene mutations and effects on chromosome structure and number. Consider need for further tests to understand the *in vivo* mutagenicity hazard, to make a risk assessment and to determine whether C&L is justified.

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
8	pos	pos				Annexes VII, VIII, IX & X: If systemic availability cannot be ascertained with acceptable reliability, it should be investigated before progressing to *in vivo* tests. Select adequate somatic cell *in vivo* tests to investigate both structural or numerical chromosome aberrations and gene mutations. If necessary seek expert advice.		Generally, both genotoxic endpoints should be investigated. If the first *in vivo* test is positive, a second *in vivo* test to confirm the other genotoxic endpoint need not be conducted. If the first *in vivo* test is negative, a second *in vivo* test is required if the first test did not address the endpoints comprehensively.	Ensure that all tests together with other available information enable thorough assessment for gene mutations and effects on chromosome structure and number. Consider need for further tests to understand the *in vivo* mutagenicity hazard, to make a risk assessment, and to determine whether C&L is justified.
9	neg	neg	pos			Annexes VII, VIII, IX & X: If systemic availability cannot be ascertained reliably, it should be investigated before progressing to *in vivo* tests. Select adequate somatic cell i*n vivo* test to investigate gene mutations *in vivo* (TGR, comet or if justified UDSvivo). If the TGR is to be conducted on somatic tissues, germ cell samples should be collected if possible, frozen and analysed for mutagenicity only in case of a positive result in somatic cells. If necessary seek expert advice.			Ensure that all tests together with other available information enable thorough assessment for gene mutations and effects on chromosome structure and number. Consider on a case-by-case basis need for further tests to understand the *in vivo* mutagenicity hazard, to make a risk assessment, and to determine whether C&L is justified.
10	pos	neg			neg	Annexes VII, VIII, IX & X: no further tests are required.	not genotoxic		Further *in vivo* test may be necessary depending on the quality and relevance of available data.
	neg	pos		neg					

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
11	pos	neg			pos	Annexes VII, VIII, IX & X: No further testing in somatic cells is needed. Germ cell mutagenicity tests should be considered. If necessary seek expert advice on implications of all available data on toxicokinetics and toxicodynamics and on the choice of the proper germ cell mutagenicity test.	genotoxic	Expert judgement is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified.	If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation may be necessary. Risk assessment and C&L can be completed.
	neg	pos		pos					
	neg	neg	pos		pos				
12	pos	pos	(pos)	pos		Annexes VII, VIII, IX & X: No further testing in somatic cells is needed. Germ cell mutagenicity tests should be considered. If necessary seek expert advice on implications of all available data on toxicokinetics and toxicodynamics and on the choice of the proper germ cell mutagenicity test.	genotoxic	Expert judgement is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified.	If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation may be necessary. Risk assessment and C&L can be completed.
	pos	pos	(pos)		pos				
13	pos	pos	(pos)	neg		Annexes VII, VIII, IX & X: Select adequate somatic cell *in vivo* tests to investigate both structural or numerical chromosome aberrations and gene mutations. If necessary seek expert advice.			
	pos	pos	(pos)		neg				
14	pos	pos	(pos)	neg	neg	Annexes VII, VIII, IX & X: no further tests are required.	not genotoxic	Further *in vivo* test may be necessary pending on the quality and relevance of available data.	Risk assessment and C&L can be completed.

	GM bact	Cyt vitro	GM vitro	Cyt vivo	GM vivo	Standard information required General follow up procedure	Conclusion	Specific rules for adaptation *[for detailed guidance, incl. timing of the tests, see main text]*	Comments
15	pos	pos	(pos)	neg	pos	Annexes VII, VIII, IX & X: No further testing in somatic cells is needed. Germ cell mutagenicity tests should be considered.	genotoxic	Expert judgement is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified.	If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation will be necessary. Risk assessment and C&L can be completed.
	pos	pos	(pos)	pos	neg	If necessary seek expert advice on implications of all available data on toxicokinetics and toxicodynamics and on the choice of the proper germ cell mutagenicity test.			

Abbreviations: pos: positive; neg: negative; (pos): the follow up is independent from the result of this test; GM_{bact}: gene mutation test in bacteria (Ames test); Cyt_{vitro}: cytogenetic assay in mammalian cells; CAb_{vitro}: *in vitro* chromosome aberration test; MNT_{vitro}: *in vitro* micronucleus test; GM_{vitro}: gene mutation assay in mammalian cells; Cyt_{vivo}: cytogenetic assay in experimental animals; GM_{vivo}: gene mutation assay in experimental animals; CAb_{vivo}: *in vivo* chromosome aberration test (bone marrow); MNT_{vivo}: *in vivo* micronucleus test (erythrocytes); UDS_{vivo}: *in vivo* unscheduled DNA synthesis test; TGR: *in vivo* gene mutation test with transgenic rodent; comet: comet assay.

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R.7.7.8 Carcinogenicity

R.7.7.8.1 Definition of carcinogenicity

Chemicals are defined as carcinogenic if they induce tumours, increase tumour incidence and/or malignancy or shorten the time to tumour occurrence. Benign tumours that are considered to have the potential to progress to malignant tumours are generally considered along with malignant tumours. Chemicals can induce cancer by any route of exposure (e.g., when inhaled, ingested, applied to the skin or injected), but carcinogenic potential and potency may depend on the conditions of exposure (e.g., route, level, pattern and duration of exposure). Carcinogens may be identified from epidemiological studies, from animal experiments and/or other appropriate means that may include (Quantitative) Structure-Activity Relationships ((Q)SAR) analyses and/or extrapolation from structurally similar substances (read-across). Each strategy for the identification of potential carcinogens is discussed in detail later in this report. The determination of the carcinogenic potential of a chemical is based on a *Weight of Evidence* approach. Classification criteria are given in the (EU Directive 67/548/EEC).[56]

The process of carcinogenesis involves the transition of normal cells into cancer cells via a sequence of stages that entail both genetic alterations (i.e. mutations[57]) and non-genetic events. Non-genetic events are defined as those alterations/processes that are mediated by mechanisms that do not affect the primary sequence of DNA and yet increase the incidence of tumours or decrease the latency time for the appearance of tumours. For example; altered growth and death rates, (de)differentiation of the altered or target cells and modulation of the expression of specific genes associated with the expression of neoplastic potential (e.g. tumour suppressor genes or angiogenesis factors) are recognised to play an important role in the process of carcinogenesis and can be modulated by a chemical agent in the absence of genetic change to increase the incidence of cancer.

Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action: genotoxic or non-genotoxic[57]. Genotoxic modes of action involve genetic alterations caused by the chemical interacting directly with DNA to result in a change in the primary sequence of DNA. A chemical can also cause genetic alterations indirectly following interaction with other cellular processes (e.g., secondary to the induction of oxidative stress). Non-genotoxic modes of action include epigenetic changes, i.e., effects that do not involve alterations in DNA but that may influence gene expression, altered cell-cell communication, or other factors involved in the carcinogenic process. For example, chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced: the induction of urinary bladder tumours in rats may, in certain cases, be due to persistent irritation/inflammation, tissue erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones. Other modes of non-gentoxic action can involve specific receptors (e.g., PPARα, which is associated with liver tumours in rodents; or tumours induced by various hormonal mechanisms). As with other nongenotoxic modes of action, these can all be presumed to have a threshold.

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[56] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

[57] For a definition and for background information on the terms mutagnicity and genotoxicity see Section R.7.7.1.1.

R.7.7.8.2 Objective of the guidance on carcinogenicity

The objective of investigating the carcinogenicity of chemicals is to identify potential human carcinogens, their mode(s) of action, and their potency.

With respect to carcinogenic potential and potency the most appropriate source of information is directly from human epidemiology studies (e.g. cohort, case control studies). In the absence of human data, animal carcinogenicity tests may be used to differentiate carcinogens from non-carcinogens. However, the results of these studies subsequently have to be extrapolated to humans, both in qualitative as well as quantitative terms. This introduces uncertainty, both with regard to potency for as well as relevance to humans, due to species specific factors such as differences in chemical metabolism and toxicokinetics and difficulties inherent in extrapolating from the high doses used in animal bioassays to those normally experienced by humans.

Once a chemical has been identified as a carcinogen, there is a need to elucidate the underlying mode of action, i.e. whether the chemical is directly genotoxic or not. In risk assessment a distinction is made between different types of carcinogens (see above).

For genotoxic carcinogens exhibiting direct interaction with DNA it is not generally possible to infer the position of the threshold from the *no-observed-effect level* on a dose-response curve, even though a biological threshold below which cancer is not induced may exist.

For non-genotoxic carcinogens, *no-effect-thresholds* are assumed to exist and to be discernable (e.g. if appropriately designed studies of the dose response for critical non-genotoxic effects are conducted). No effect thresholds may also be present for certain carcinogens that cause genetic alterations via indirect effects on DNA following interaction with other cellular processes (e.g. carcinogenic risk would manifest only after chemically induced alterations of cellular processes had exceeded the compensatory capacity of physiological or homeostatic controls). However, in the latter situation the scientific evidence needed to convincingly underpin this indirect mode of genotoxic action may be more difficult to achieve. Human studies are generally not available for making a distinction between the above mentioned modes of action; and a conclusion on this, in fact, depends on the outcome of mutagenicity/genotoxicity testing and other mechanistic studies. In addition to this, animal studies (e.g. the carcinogenicity study, repeated dose studies, and experimental studies with initiation-promotion protocols) may also inform on the underlying mode of carcinogenic action.

The cancer hazard and mode of action may also be highly dependent on exposure conditions such as the route of exposure. A pulmonary carcinogen, for example, can cause lung tumours in rats following chronic inhalation exposure, but there may be no cancer hazard associated with dermal exposure. Therefore, all relevant effect data and information on human exposure conditions are evaluated in a *Weight of Evidence* approach to provide the basis for regulatory decisions.

R.7.7.9 Information requirements on carcinogenicity

For the endpoint of carcinogenicity, standard information requirements are specifically described for substances produced or imported in quantities of ≥1000 t/y (Annex X). The precise information requirements will differ from substance to substance, according to the toxicity information already available and details of use and human exposure for the substance in question. The REACH Annexes VI to XI should be considered as a whole and in conjunction with the overall requirements of registration and evaluation.

Column 2 of Annex X lists specific rules according to which the required standard information may be omitted, replaced by other information, provided at a different stage

or adapted in another way. If the conditions are met for adaptations under column 2 of this Annex, the fact and the reasons for each adaptation should be clearly indicated in the registration.

The standard information requirements for carcinogenicity and the specific rules for adaptation of these requirements are presented in Table R.7.7-6.

Table R.7.7-6 Standard information requirements for carcinogenicity and the specific rules for adaptation of these requirements

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
Annexes VII-IX	
Annex X: 1. Carcinogenicity study.	1. A carcinogenicity study may be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 if: - the substance has a widespread dispersive use or there is evidence of frequent or long-term human exposure; and - the substance is classified as mutagen category 3 or there is evidence from the repeated dose study(ies) that the substance is able to induce hyperplasia and/or pre-neoplastic lesions. If the substance is classified as mutagen category 1 or 2, the default presumption would be that a genotoxic mechanism for carcinogenicity is likely. In these cases, a carcinogenicity test will normally not be required.

R.7.7.10 Information and its sources on carcinogenicity

There are many different sources of information that may permit inferences to be drawn regarding the potential of chemicals to be carcinogenic to humans. Clearly, these sources not only allow the identification of potential carcinogenic activity, but in case a substance is identified as a likely carcinogen they should also be informative with respect to the underlying mode of action as well as probable carcinogenic potency. The requirements of REACH call for proper classification and labelling, as well as for a quantitative assessment of risk that permits conclusions to be drawn regarding conditions under which safe use of the chemical may occur: i.e. the data should allow concluding on threshold or non-threshold mode of action, and on some dose descriptor (characterising the dose-response), preferably in quantitative terms.

It is noted (and indicated below), that the various sources inform differently on the aspects of hazard identification, mode of action, or carcinogenic potency.

R.7.7.10.1 Non-human data on carcinogenicity

Non-testing data on carcinogenicity

The capacity for performing the standard rodent cancer bioassay is limited by economic, technical and animal welfare considerations, such that an increased emphasis is being placed on the development of alternative, non-animal testing methods. However, carcinogenicity predictions through use of non-testing data currently represent an

extreme challenge due to the multitude of possible mechanisms. Prediction of carcinogenicity in humans is especially problematic.

Although significant challenges remain, a broad spectrum of non-testing techniques exist for elucidating mechanistic, toxicokinetic or toxicodynamic factors important in understanding the carcinogenic process. These range from expert judgement, to the evaluation of structural similarities and analogues (i.e. read-across and grouping), to the use of (Q)SAR models for carcinogenicity. Such information may assist with priority setting, hazard identification, elucidation of the mode of action, potency estimation and/or with making decisions about testing strategies based on a *Weight of Evidence* evaluation.

Genotoxicity remains an important mechanism for chemical carcinogenesis and its definitive demonstration for a chemical is often decisive for the choice of risk assessment methodology. A commentary about non-testing options for genotoxicity is provided in Section R.7.7.1. It has long been known that certain chemical structures or fragments can be associated with carcinogenicity, often through DNA-reactive mechanisms. Useful guidance for structures and fragments that are associated with carcinogenicity via DNA reactive mechanisms has been provided by the US Food and Drug Administration's "Guideline for Threshold Assessment, Appendix I, Carcinogen Structure Guide" (US FDA, 1986); the Ashby-Tennant "super-mutagen model" (*e.g.*, Ashby and Tennant, 1988); and subsequent builds on this model (*e.g.*, Ashby and Paton, 1993; Munro *et al*., 1996). Additional information on structural categories can be found in the "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man" (IARC, 2006).

Models predicting test results for genotoxic endpoints for closely related structures are known as *local* or congeneric (Q)SARs. These congeneric models are less common for carcinogenicity than for mutagenicity. Franke *et al.* (2001) provide an example of such a model for a set of genotoxic carcinogens.

The situation is far more complex for non-genotoxic carcinogenicity due to the large number of different mechanisms that may be involved. However, progress is being made in predicting activity for classes of compounds that exert effect via binding to oestrogen receptors, induction of peroxisomal proliferation, and binding to tubulin proteins. Although many potentially useful models exist, their applicability will be highly dependant on the proposed mechanism and chemical class.

Several *global* (non-congeneric) models exist which attempt to predict (within their domain) the carcinogenic hazard of diverse (non-congeneric) groups of substances (*e.g.* Matthews and Contrera, 1998). These models may also assist in screening, priority-setting, deciding on testing strategies and/or the assessment of hazard or risk based on *Weight of Evidence*. Most are commercial and include expert systems such as Onco-Logic® (currently made available by US-EPA) and DEREK, artificial intelligence systems from MULTICASE, and the TOPKAT program. Historically, the performance of such models has been mixed and is highly dependent on the precise definition of carcinogenicity among those substances used to develop and test the model. These have been reviewed by ECETOC (2003) and Cronin *et al*. (2003).

Free sources of carcinogenicity predictions include the Danish EPA (Q)SAR database (accessible through the European Commission's Chemicals Bureau: ECB http://ecbqsar.jrc.it/). Predictions in this database for 166,000 compounds include eight MULTICASE FDA cancer models, a number of genotoxicity predictions, rodent carcinogenic potency, hepatospecificity, oestrogenicity and aryl hydrocarbon (AH) receptor binding. Another source of carcinogenicity predictions is the Enhanced NCI database "*Browser*", which is sponsored by the US National Cancer Institute. This has 250,000 chemical predictions within it (http://cactus.nci.nih.gov), including general

carcinogenicity, mutagenicity and additional endpoints, which may be of potential mechanistic interest in specific cases.

Further information on carcinogenicity models is available in the OECD Database on Chemical Risk Assessment Models where they are listed in an effort to identify tools for research and development of chemical substances. (http://www.olis.oecd.org/comnet/env/models.nsf/MainMenu?OpenForm).

The guidance on the Grouping of Chemicals and on (Q)SARs (see Sections R.6.2 and R.6.1, respectively) explains basic concepts of grouping and (Q)SARs and gives generic guidance on validation, adequacy and documentation for regulatory purposes. The guidance also describes a stepwise approach for the use of read-across/grouping and (Q)SARs.

It is noted that all the above mentioned sources may potentially inform on possible carcinogenic hazard and on the underlying mode of action, as well as on carcinogenic potency.

Testing data on carcinogenicity

In vitro data

The following *in vitro* data, which provide direct or indirect information useful in assessing the carcinogenic potential of a substance and (potentially) on the underlying mode(s) of action, may be available. No single endpoint or effect in and of itself possesses unusual significance for assessing carcinogenic potential but must be evaluated within the context of the overall toxicological effects of a substance under evaluation as described in Section R.7.7.11.1. Except as noted, standardised protocols do not exist for most of the *in vitro* endpoints noted. Rather, studies are conducted in accordance with expert judgement using protocols tailored to the specific substance, target tissue and cell type or animal species under evaluation.

genotoxicity studies: the ability of substances to induce mutations or genotoxicity (as defined in Section R.7.7.1) can be indicative of carcinogenic potential. However, correlations between mutagenicity/genotoxicity and carcinogenesis are stronger when effects are observed in appropriately designed *in vivo* as opposed to *in vitro* studies.

***in vitro* cell transformation assay results:** such assays assess the ability of chemicals to induce changes in the morphological and growth properties of cultured mammalian cells that are presumed to be similar to phenotypic changes that accompany the development of neoplastic or pre-neoplastic lesions *in vivo* (OECD, 2006). The altered cells detected by such assays may other targeted mechanisms of action

possess, or can subsequently acquire, the ability to grow as tumours when injected into appropriate host animals. As in vitro assays, cell transformation assays are restricted to the detection of effects of chemicals at the cellular level and will not be sensitive to carcinogenic activity mediated by effects exerted at the level of intact tissues or organisms.

mechanistic studies, e.g. on:

possess, or can subsequently acquire, the ability to grow as tumours when injected into appropriate host animals. As in vitro assays, cell transformation assays are restricted to the detection of effects of chemicals at the cellular level and will not be sensitive to carcinogenic activity mediated by effects exerted at the level of intact tissues or organisms.

mechanistic studies, e.g. on:

- cell proliferation: sustained cell proliferation can facilitate the growth of neoplastic/pre-neoplastic cells and/or create conditions conducive to spontaneous changes that promote neoplastic development.

- altered intercellular gap junction communication: exchange of growth suppressive or other small regulatory molecules between normal and neoplastic/pre-neoplastic cells through gap junctions is suspected to suppress phenotypic expression of neoplastic potential. Disruption of gap junction function, as assessed by a diverse array of assays for fluorescent dye transfer or the exchange of small molecules between cells, may attenuate the suppression of neoplastic potential by normal cells.

- hormone- or other receptor binding; a number of agents may act through binding to hormone receptors or sites for regulatory substances that modulate the growth of cells and/or control the expression of genes that facilitate the growth of neoplastic cells. Interactions of this nature are diverse and generally very compound specific.

other targeted mechanisms of action

- immunosuppressive activity: neoplastic cells frequently have antigenic properties that permit their detection and elimination by normal immune system function. Suppression of normal immune function can reduce the effectiveness of this *immune surveillance* function and permit the growth of neoplastic cells induced by exogenous factors or spontaneous changes.

- ability to inhibit or induce apoptosis: apoptosis, or programmed cell death, constitutes a sequence of molecular events that results in the death of cells, most often by the release of specific enzymes that result in the degradation of DNA in the cell nucleus. Apoptosis is integral to the control of cell growth and differentiation in many tissues. Induction of apoptosis can eliminate cells that might otherwise suppress the growth of neoplastic cells; inhibition of apoptosis can permit pre-neoplastic/neoplastic cells to escape regulatory controls that might otherwise result in their elimination.

- ability to stimulate angiogenesis or the secretion of angiogenesis factors: the growth of pre-neoplastic/neoplastic cells in solid tumours will be constrained in the absence of vascularisation to support the nutritional requirements of tumour growth. Secretion of angiogenesis factors stimulates the vascularisation of solid tumour tissue and enables continued tumour growth.

Animal data

A wide variety of study categories may be available, which may provide direct or indirect information useful in assessing the carcinogenic potential of a substance to humans. They include:

carcinogenicity studies (conventional long-term or life-time studies in experimental animals): Carcinogenicity testing is typically conducted using rats and mice, and less commonly in animals such as the Guinea pig, Syrian hamster and occasionally mini-pigs, dogs and primates. The standard rodent carcinogenicity bioassay would be conducted using rats or mice randomly assigned to treatment groups. Exposures to test substances may be via oral, inhalation or dermal exposure routes. The selection of exposure route is often dictated by *a priori* assumptions regarding the routes of exposure relevant to humans and/or other data sources (e.g. epidemiology studies or

repeated dose toxicity studies in animals) that may indicate relevance of a given exposure route. Standardised protocols for such studies have been developed and are well validated (*e.g.* OECD TGs 451, 453 or US-EPA 870.4200).

short and medium term bioassay data (e.g., mouse skin tumour, rat liver foci model, neonatal mouse model): multiple assays have been developed that permit the detection and quantitation of putative pre-neoplastic changes in specific tissues. The induction of such *pre-neoplastic foci* may be indicative of carcinogenic potential. Such studies are generally regarded as adjuncts to conventional cancer bioassays, and while less validated and standardised, are applicable on a case-by-case basis for obtaining supplemental mechanistic and dose response information that may be useful for risk assessment (Enzmann et al, 1998).

genetically engineered (transgenic) rodent models (*e.g.*, *Xpa*$^{-/-}$, *p53*$^{+/-}$, *ras*H2 or Tg.AC): animals can be genetically engineered such that one or more of the molecular changes required for the multi-step process of carcinogenesis has been accomplished (Tennant et al., 1999). This can increase the sensitivity of the animals to carcinogens and/or decrease the latency with which spontaneous or induced tumours are observed. The genetic changes in a given strain of engineered animals can increase sensitivity to carcinogenesis in a broad range of tissues or can be specific to the changes requisite for neoplastic development in one or only a limited number of tissues (Jacobson-Kram, 2004; Pritchard et al., 2003; ILSI/HESI 2001). Data from these models may be used in a *Weight of Evidence* analysis of a chemical's carcinogenicity.

genotoxicity studies *in vivo*: the ability of substances to induce mutations or genotoxicity (as defined in Section R.7.7.1.1) can be indicative of carcinogenic potential. There is, in general, a good correlation between positive genotoxicity findings *in vivo* and animal carcinogenicity bioassay results

repeated dose toxicity tests: can identify tissues that may be specific targets for toxicity and subsequent carcinogenic effects. Particular significance can be attached to the observation of pre-neoplastic changes (e.g. hyperplasia or metaplasia) suspected to be conducive to tumour development and may assist in the development of dose-effect relationships (Elcombe *et al*, 2002).

studies on the induction of sustained cell proliferation: substances can induce sustained cell proliferation via compensatory processes that continuously regenerate tissues damaged by toxicity. Some substances can also be tissue-specific mitogens, stimulating cell proliferation in the absence of overt toxic effects. Mitogenic effects are often associated with the action of tumour promoters. Both regenerative cell proliferation and mitogenic effects can be necessary, but not sufficient, for tumour development but have sufficiently different mechanistic basis that care should be exercised in assessing which is occurring (Cohen and Ellwein, 1991; Cohen et al., 1991).

studies on immunosuppressive activity: as noted earlier, suppression of normal immune surveillance functions can interfere with normal immune system functions that serve to identify and eliminate neoplastic cells.

studies on toxicokinetics: can identify tissues or treatment routes that might be the targets for toxicity and can deliver data on exposure and metabolism in specific organs. Linkages to subsequent carcinogenic impacts may or may not exist, but such data can serve to focus carcinogenesis studies upon specific tissue types or animal species.

other studies on mechanisms/modes of action, e.g. OMICs studies (toxicogenomics, proteomics, metabonomics and metabolomics): carcinogenesis is associated with multiple changes in gene expression, transcriptional regulation, protein

synthesis and other metabolic changes. Specific changes diagnostic of carcinogenic potential have yet to be validated, but these rapidly advancing fields of study may one day permit assessment of a broad array of molecular changes that might be useful in the identification of potential carcinogens.

It is noted that the above tests differently inform on hazard identification, mode of action or carcinogenic potency. For example, conventional bioassays are used for hazard identification and potency estimation (i.e. derivation of a dose descriptor), whereas studies using genetically engineered animals are informative on potential hazard and possibly mode of action, but less on carcinogenic potency as they are considered to be highly sensitive to tumour induction.

R.7.7.10.2 Human data on carcinogenicity

Human data may provide direct information on the potential carcinogenicity of the substance. Relevant human data of sufficient quality, if available, are preferable to animal data as no extrapolations between species, or from high to low dose are necessary. Epidemiological data will not normally be available for new substances but may well be available for substances that have been in use for many decades. For substances in common use prior to the implementation of modern occupational hygiene measures, the intensity of human exposures to some carcinogens was sufficient to produce highly significant, dose-dependent increases in cancer incidence.

A number of basic epidemiological study designs exist and include cohort, case-control and registry based correlational (e.g. ecological) studies. The most definitive epidemiological studies on chemical carcinogenesis are generally cohort studies of occupationally exposed populations, and less frequently the general population. Cohort studies evaluate groups of initially healthy individuals with known exposure to a given substance and follow the development of cancer incidence or mortality over time. With adequate information regarding the intensity of exposure experienced by individuals, dose dependent relationships with cancer incidence or mortality in the overall cohort can be established. Case-control studies retrospectively investigate individuals who develop a certain type of cancer and compare their chemical exposure to that of individuals who did not develop disease. Case control studies are frequently nested within the conduct of cohort studies and can help increase the precision with which excess cancer can be associated with a given substance. Correlational or ecological studies evaluate cancer incidence/mortality in groups of individuals presumed to have exposure to a given substance but are generally less precise since measures of the exposure experienced by individuals are not available. Observations of cancer clusters and case reports of rare tumours may also provide useful supporting information in some instances but are more often the impetus for the conduct of more formal and rigorous cohort studies.

Besides the identification of carcinogens, epidemiological studies may also provide information on actual exposures in representative (or historical) workplaces and/or the environment and the associated dose-response for cancer induction. Such information can be of much value for risk characterisation.

Although instrumental in the identification of known human carcinogens, epidemiology studies are often limited in their sensitivity by a number of technical factors. The extent and/or quality of information that is available regarding exposure history (e.g. measurements of individual exposure) or other determinants of health status within a cohort is often limited. Given the long latency between exposure to a carcinogen and the onset of clinical disease, robust estimates of carcinogenic potency can be difficult to generate. Similarly, occupational and environmentally exposed cohorts often have co-exposures to carcinogenic substances that have not been documented (or are incompletely documented). This can be particularly problematic in the study of long established industry sectors (e.g. base metal production) now known to entail co-

exposures to known carcinogens (e.g. arsenic) present as trace contaminants in the raw materials being processed.. Retrospective hygiene and exposure analyses for such sectors are often capable of estimating exposure to the principle materials being produced, but data documenting critical co-exposures to trace contaminants may not be available. Increased cancer risk may be observed in such settings, but the source of the increased risk can be difficult to determine. Finally, a variety of lifestyle confounders (smoking and drinking habits, dietary patterns and ethnicity) influence the incidence of cancer but are often inadequately documented for purposes of adequate confounder control. Thus, modest increases in cancer at tissue sites known to be impacted by confounders (e.g. lung and stomach) can be difficult to interpret.

Techniques for biomonitoring and molecular epidemiology are developing rapidly. These newly developed tools promise to provide information on biomarkers of individual susceptibility, critical target organ exposures and whether effects occur at low exposure levels. Such ancillary information may begin to assist in the interpretation of epidemiology study outcomes and the definition of dose response relationships. For example, monitoring the formation of chemical adducts in haemoglobin molecules (Birner et al., 1990; Albertini et al., 2006), the urinary excretion of damaged DNA bases (Chen, H.J. and Chiu, W.L. (2005), and the induction of genotoxicity biomarkers (micronuclei or chromosome aberrations; Boffetta et al., 2007) are presently being evaluated and/or validated for use in conjunction with classical epidemiological study designs. Such data are usually restricted in their application to specific chemical substances but such techniques may ultimately become more widely used, particularly when combined with animal data that defines potential mechanisms of action and associated biomarkers that may be indicative of carcinogenic risk. Monitoring of the molecular events that underlie the carcinogenic process may also facilitate the refinement of dose response relationships and may ultimately serve as early indicators of potential cancer risk. However, as a generalisation, such biomonitoring tools have yet to demonstrate the sensitivity requisite for routine use.

R.7.7.10.3 Exposure considerations for carcinogenicity

Information on exposure, use and risk management measures should be collected in accordance with Article 9 and Annex VI of REACH.

It is indicated in REACH Annex X a carcinogenicity study may be required by the European Chemicals Agency (or proposed by the registrant) when the substance has a widespread dispersive use or there is evidence of frequent or long-term human exposure. Preliminary toxicokinetic studies may be required first to address specific questions regarding potential target tissues and relevant exposure routes relevant for the chemical of concern.

On the other hand, investigations on the carcinogenic properties of a chemical can be deferred, if it can be demonstrated to the satisfaction of the Agency that the chemical is used only in a closed system and that human exposures are negligible, (i.e. risk reduction measures on the substance are already equivalent to those applied to high potency carcinogenic substances of category 1 and 2.Reasons for this could include the presence of other substances for which strict exposure regimes are implemented or enforced). The rationale for exemption from testing, of course, needs to be clearly documented upon registration.

Also, considerations on exposure may influence the search for information, e.g. applicable to the actual route of exposure. For example, if from exposure scenarios it is clear that only a single specific route is involved, toxicity data for this route is of higher relevance in data gathering and evaluation than for the other routes. Also, the

involvement of inhalation exposure to particles will prioritise toxicity information needs in order to allow a proper hazard evaluation and risk assessment.

R.7.7.11 Evaluation of available information on carcinogenicity

This particular endpoint is complex and requires evaluation by expert judgement.

Note that the objective of this evaluation is to acquire information on the carcinogenic potential of the substance: i.e. is the substance carcinogenic or not, and, if so, what is the underlying mode of action (thresholded or not), and what is its carcinogenic potency (i.e. there is a need to define a dose descriptor).

An evaluation on the above mentioned properties requires a combining of various types of information, as indicated in Section R.7.7.10 (and below). Such an evaluation needs a *Weight of Evidence* approach for arriving at conclusions, i.e. a careful gathering, sorting and weighing of the various pieces of information available. This exercise is particularly complex and, therefore, requires expert judgement input.

R.7.7.11.1 Non-human data on carcinogenicity

Non-testing data for carcinogenicity

To date little experience is available for the evaluation of substances on non-testing data, since the use of non-testing data for regulatory decisions is rather new. Therefore, at every stage in the assessment for potential chemical toxicity, specialist judgement is essential. It is recognised though, that non-testing data may potentially inform on all carcinogenic properties, i.e. including mode of action and potency.

Documentation should include reference to a related chemical or groups of chemicals that give rise to concern or lack of concern. This can either be presented according to scientific logic (read-across) or as a mathematical relationship of chemical similarity.

In some cases, the carcinogenic potential posed by a substance can be assessed based upon analysis of the relative concentrations of constituents believed to present a risk in a complex mixture. For example, the classification of certain complex coal- and oil-derived substances as carcinogens can varies as a function of the content of marker carcinogens (benzene, 1,3-butadiene and benzene), whereas for others it depends on the level of polycyclic aromatic hydrocarbons measured following DMSO solvent extraction. (see Annex I of EU Directive 67/548/EEC). When properly validated, such chemical extraction and analysis techniques are highly predictive of the outcomes that would be obtained in animal carcinogenicity studies.

If well documented and applicable, (Q)SARs can be used to help reach the decision points described in the section below. The accuracy of such methods may be sufficient to help or allow either a testing or a specific regulatory decision to be made. Expert judgment is needed to make this determination.

Chemicals for which no test-data exist present a special case in which reliance on non-testing methods may be absolute. Many factors will dictate the acceptability of non-testing methods in reaching a conclusion based on no tests at all. A *Weight of Evidence* evaluation of carcinogenicity based on multiple genotoxicity and carcinogenicity estimates (*e.g.* from (Q)SAR models) may in some cases equal or exceed the decision basis which could be obtained by experimentally testing a chemical in one or two *in vitro* tests. This must be considered on a case-by-case basis by the registrant.

Further guidance on the use of Grouping of Chemicals and on (Q)SARs both for a qualitative (i.e. classification and labelling) as well as a quantitative assessment (i.e.

identifying some dose descriptor value) is provided in Sections R.4.3.2 and R.6.2, respectively, and also includes basic concepts used, validation status, adequacy and documentation needs for regulatory purposes.

Testing data on carcinogenicity

In vitro data

In vitro data can only give preliminary information about the carcinogenic potential of a substance and possible underlying mode(s) of action. For example, *in vitro* genotoxicity studies may provide information about whether or not the substance is likely to be genotoxic *in vivo*, and thus a potential genotoxic carcinogen (see Section R.7.7.1), and herewith on the potential mode of action underlying carcinogenicity: with or without a threshold.

Besides genotoxicity data other *in vitro* data (described in Section R.7.7.10.1) such as *in vitro* cell transformation can help to decide, in a *Weight of Evidence* evaluation, whether a chemical possesses a carcinogenic potential. Cell transformation results in and of themselves do not inform as to the actual underlying mode(s) of action, since they are restricted to the detection of effects exerted at the level of the single cell and may be produced by mechanistically distinct processes.

Studies can also be conducted to evaluate the ability of substances to influence processes thought to facilitate carcinogenesis. Many of these endpoints are assessed by experimental systems that have yet to be formally validated and/or are the products of continually evolving basic research. Formalised and validated protocols are thus lacking for the conduct of these tests and their interpretation. Although it is difficult to give general guidance on each test due to the variety and evolving nature of tests available, it is important to consider them on a case-by-case basis and to carefully consider the context on how the test was conducted.

A number of the test endpoints evaluate mechanisms that may contribute to neoplastic development, but the relative importance of each endpoint will vary as a function of the overall toxicological profile of the substance being evaluated. It should further be noted that there are significant uncertainties associated with extrapolating *in vitro* data to an *in vivo* situation. Such *in vitro* data will, in many instances, provide insights into the nature of the *in vivo* studies that might be conducted to define carcinogenic potential and/or mechanisms.

Animal data

In vivo data can give direct information about the carcinogenic potential of a substance, possible underlying mode(s) of action, and its potency.

Testing for carcinogenicity is conventionally carried out in groups of rats or mice according to standard test protocols or guidelines (e.g. OECD TGs 451, 453 or US-EPA 870.4200) and a conclusion is based on a comparison of the incidence, nature and time of occurrence of neoplasms in treated animals and controls.

Knowledge of the historic tumour incidence for the strain of animal used is important (laboratory specific data are preferable). Also attention to the study design used is essential because of the requirement for statistical analyses. The quality, integrity and thoroughness of the reported data from carcinogenicity studies are essential to the subsequent analysis and evaluation of studies. A qualitative assessment of the acceptability of study reports is therefore an important part of the process of independent evaluation. Sources of guidance in this respect can be found in IEH (2002),

CCCF (2004) and OECD (2002). If the available study report does not include all the information required by the standard test guideline, judgement is required to decide if the experimental procedure is or is not acceptable and if essential information is lacking.

The final design of a carcinogenicity bioassay may deviate from OECD guidelines if expert judgement and experience in the testing of analogous substances supports the modification of protocols. Such modifications to standard protocols can be considered as a function of the specific properties of the material under evaluation.

Carcinogenicity data may sometimes be available in species other than those specified in standard test guidelines (e.g., Guinea pig, Syrian hamster and occasionally mini-pigs, dogs and primates). Such studies may be in addition to, or instead of, studies in rats and mice and they should be considered in any evaluation.

Data from non-conventional carcinogenicity studies, such as short- and medium-term carcinogenicity assays with neonatal or genetically engineered (transgenic) animals, may also be available (CCCF, 2004; OECD, 2002). Genetically engineered animals possess mutations in genes that are believed to be altered in the multi-step process of carcinogenesis, thereby enhancing animal sensitivity to chemically induced tumours. A variety of transgenic animal models exist and new models are continually being development. The genetic alteration(s) in a specific animal model can be those suspected to facilitate neoplastic development in a wide range of tissue types or the alterations can be in genes suspected to be involved in tissue specific aspects of carcinogenesis. The latter must be applied with recognition of both their experimental nature and the specific mechanistic pathways they are designed to evaluate. For example, a transgenic animal model sensitive to mesothelioma induction would be of limited value in the study of a suspected liver carcinogen. While such animal model systems hold promise for the detection of carcinogens in a shorter period of time and using fewer animals, their sensitivity and specificity remains to be determined. Due to a relative lack of validation, such assays have not yet been accepted as alternatives to the conventional lifetime carcinogenicity studies, but may be useful for screening purposes or to determine the need for a rodent 2-year bioassay. Several evaluations of these types of study have been published (e.g., Jacobson-Kram, 2004; Pritchard et al., 2003; ILSI/HESI (2001).

When data are available from more than one study of acceptable quality, consistency of the findings should be established. When consistent, it is usually straightforward to arrive at a conclusion, particularly if the studies were in more than one species or if there is a clear treatment-related incidence of malignant tumours in a single study. If a single study only is available and the test substance is not carcinogenic, scientific judgement is needed to decide on whether (a) this study is relevant or (b) additional information is required to provide confidence that it should not be considered to be carcinogenic.

Study findings also may not clearly demonstrate a carcinogenic potential, even when approved study guidelines have been followed. For example, there may only be an increase in the incidence of benign tumours or of tumours that have a high background incidence in control animals. Although less convincing than an increase in malignant and rare tumours, and recognising the potential over-sensitivity of this model (Haseman, 1983; Ames and Gold, 1990), a detailed and substantiated rationale should be given before such positive findings can be dismissed as not relevant.

Repeated dose toxicity studies may provide helpful additional information to the *Weight of Evidence* gathered to determine whether a substance has the potential to induce cancer, and for potential underlying modes of action (Elcombe *et al*., 2002). For example, the induction of hyperplasia (either through cytotoxicity and regenerative cell proliferation, mitogenicity or interference with cellular control mechanisms) and/or the induction of pre-neoplastic lesions may contribute to the *Weight of Evidence* for

carcinogenic potential. Toxicity studies may also provide evidence for immunosuppressive activity, a condition favouring tumour development under conditions of chronic exposure.

Finally, toxicokinetic data may reveal the generation of metabolites with relevant structural alerts. It may also give important information as to the potency and relevance of carcinogenicity and related data collected in one species and its extrapolation to another, based upon differences in absorption, distribution, metabolism and or excretion of the substance. Species specific differences mediated by such factors may be demonstrated through experimental studies or by the application of toxicokinetic modelling.

Positive carcinogenic findings in animals require careful evaluation and this should be done with reference to other toxicological data (e.g. *in vitro* and/or *in vivo* genotoxicity studies, toxicokinetic data, mechanistic studies, (Q)SAR evaluations) and the exposure conditions (e.g., route). Such comparisons may provide evidence for (a) specific mechanism(s) of action, a significant factor to take into account whenever possible, that may then be evaluated with respect to relevance for humans.

A conceptual framework that provides a structured and transparent approach to the *Weight of Evidence* assessment of the mode of action of carcinogens has been developed (see Sonich-Mullin *et al.*, 2001; Boobis et al., 2006). This framework should be followed when the mechanism of action is key to the risk assessment being developed for a carcinogenic substance and can be particularly critical in a determination of whether a substance induces cancer via genotoxic or nongenotoxic mechanisms.

For example, a substance may exhibit limited genotoxicity *in vivo* but the relevance of this property to carcinogenicity is uncertain if genotoxicity is not observed in tissues that are the targets of carcinogenesis, or if genotoxicity is observed via routes not relevant to exposure conditions (e.g. intravenous injection) but not when the substance is administered via routes of administration known to induce cancer. In such instances, the apparent genotoxic properties of the substance may not be related to the mechanism(s) believed to underlie tumour induction. For example, oral administration of some inorganic metal compounds will induce renal tumours via a mechanism believed to involve organ specific toxicity and forced cell proliferation. Although genotoxic responses can be induced in non-target tissues for carcinogenesis via intravenous injection, there is only limited evidence to suggest that this renal carcinogenesis entails a genotoxic mechanism (IARC, 2006). The *burden of proof* in drawing such mechanistic inferences can be high but can have a significant impact upon underlying assumptions made in risk assessment.

In general, tumours induced by a genotoxic mechanism (known or presumed) are, in the absence of further information, considered to be of relevance to humans even when observed in tissues with no direct human equivalent. Tumours shown to be induced by a non-genotoxic mechanism are, in principle, also considered relevant to humans but there is a recognition that some non-genotoxic modes of action do not occur in humans (see OECD 2002). This includes, for example, some specific types of rodent kidney, thyroid, urinary bladder, forestomach and glandular stomach tumours induced by rodent-specific modes of action, i.e., by mechanisms/modes of action not operating in humans or operative in humans under extreme and unrealistic conditions. Reviews are available for some of these tumour types providing a detailed characterisation that includes the key biochemical and histopathological events that are needed to establish these rodent-specific mechanisms that are not relevant for human health (see Technical Publication Series by IARC). Recently, the IPCS has developed a framework and provided some examples on how to evaluate the relevance to humans of a postulated mode of action in animals (ILSI RSI, 2003; Boobis *et al.*, 2006).

The information available for substances identified as carcinogenic based on testing and/or non-testing data should be further evaluated in an effort to identify underlying mode(s) of action and potency in order to subsequently allow a proper quantitative assessment of risk (see Section R.7.7.12.2). As already pointed out, the use of non-standard animal models (e.g. transgenic or neonatal animals) needs careful evaluation by expert judgement as to how to apply the results obtained for hazard and risk assessment purposes; it is not possible to provide guidance for such evaluations.

R.7.7.11.2 Human data on carcinogenicity

Epidemiological data may potentially be used for hazard identification, exposure estimation, dose response analysis, and risk assessment. The degree of reliability for each study on the carcinogenic potential of a substance should be evaluated using accepted causality criteria, such as that of Hill (1965). Particular attention should be given to exposure data in a study and to the choice of the control population. Often a significant level of uncertainty exists around identifying a substance unequivocally as being carcinogenic because of inadequate reporting of exposure data. Chance, bias and confounding factors can frequently not be ruled out. A clear identification of the substance, the presence or absence of concurrent exposures to other substances and the methods used for assessing the relevant dose levels should be explicitly documented. A series of studies revealing similar excesses of the same tumour type, even if not statistically significant, may suggest a positive association, and an appropriate joint evaluation (meta-analysis) may be used in order to increase the sensitivity, provided the studies are sufficiently similar for such an evaluation. When the results of different studies are inconsistent, possible explanations should be sought and the various studies judged on the basis of the methods employed.

Interpretation of epidemiology studies must be undertaken with care and include an assessment of the adequacy of exposure classification, the size of the study cohort relative to the expected frequency of tumours at tissue sites of special concern and whether basic elements of study design are appropriate (e.g. a mortality study will have limited sensitivity if the cancer induced has a high rate of successful treatment). A number of such factors can limit the sensitivity of a given study – unequivocal demonstration that a substance is not a human carcinogen is difficult and requires detailed and exact measurements of exposure, appropriate cohort size, adequate intensity and duration of exposure, sufficient follow-up time and sound procedures for detection and diagnosis of cancers of potential concern. Conversely, excess cancer risk in a given study can also be difficult to interpret if relevant co-exposures and confounders have not been adequately documented. Efforts are ongoing to improve the sensitivity and specificity of traditional epidemiological methods by combining cancer endpoints with data on established pre-neoplastic lesions or molecular indicators (biomarkers) of cancer risk.

Once identified as a carcinogenic substance on the basis of human data, well-performed epidemiology studies may be valuable for providing information on the relative sensitivity of humans as compared to animals, and/or may be useful in demonstrating an upper bound on the human cancer risk. Identification of the underlying mode(s) of action – needed for the subsequent risk assessment (see Section R.7.7.12.2) – quite often depends critically on available testing and/or non-testing information.

R.7.7.11.3 Exposure considerations for carcinogenicity

Exposure considerations may lead to adaptation of the evaluation of available information, and / or of the testing strategy.

As indicated before, waiving of carcinogenicity studies may apply, e.g. when it can be demonstrated that the substance is only produced and used in closed systems, which among other reasons may be due to the presence of other substances for which strict exposure regimes are implemented or enforced. On the other hand, a carcinogenicity study may be required (by the Agency or proposed by the registrant) when the substance has a widespread dispersive use or there is evidence of frequent or long-term human exposure, and information on its carcinogenic properties cannot be obtained by others means (from available effect information). Preliminary toxicokinetic studies may be required first to identify the potential target tissues and exposure routes that would guide the design of appropriate studies for the chemical of concern.

In the former case, i.e. when the substance is produced and used in closed systems only, conclusions on safe use and handling can be verified by use of read-across to risk assessments of structurally related carcinogens or to the so-called Threshold of Toxicological Concern (TTC) concept (see Appendix R.7-1): this concept identifies a *de minimis* exposure value for all chemicals, including genotoxic carcinogens, below which there is no appreciable risk to human health for any chemical. If it can be demonstrated that exposures are below these values, there is good reason for not performing the required tests. Clearly, good quality exposure information is essential in all these cases.

R.7.7.11.4 Remaining uncertainty on carcinogenicity

As indicated in the previous sections, adequate human data for evaluating the carcinogenic properties of a chemical are most often not available, and alternative approaches have to be used.

As also indicated in the previous sections and the Section R.7.7.1, test systems for identifying genotoxic carcinogens are reasonably well developed and adequately cover this property. There is also agreement that animal carcinogens which act by a genotoxic mode of action may reasonably be regarded as human carcinogens unless there is convincing evidence that the mechanisms by which mutagenicity and carcinogenicity are induced in animals are not relevant to humans. Unclear, however, and herewith introducing some uncertainty, is the relationship between carcinogenic potency in animals and in humans.

There is, on the other hand, a shortage of sensitive and selective test systems to identify non-genotoxic carcinogens, apart from the carcinogenicity bioassay. In the absence of non-testing information on the carcinogenicity of structurally related chemicals, indications for possible carcinogenic properties may come from existing repeated dose toxicity data, or from *in vitro* cell transformation assays. However, whereas the former source of data will have a low sensitivity (*e.g.* in case of a 28-day study), there is a possibility that the latter may lead to an over-prediction of carcinogenic potential. Insufficient data are available to provide further general guidance in this regard.

Non-genotoxic carcinogens may be difficult to identify in the absence of animal carcinogenicity test data. However, it could be argued that current conservative (cautious) risk assessment methodology should cover the risk for carcinogenic effects via this mode of action as well: i.e. current risk assessments for many non-genotoxic carcinogens are based on NOAELs for precursor effects or target organ toxicity with the application of conservative assessment factors to address uncertainty. For example, see the risk assessment for coumarin (EFSA, 2004; Felter *et al.*, 2006). Such a risk

assessment is not performed, though, in case this substance is not classified as dangerous for any other properties.

Once identified as a non-genotoxic carcinogen (from testing or non-testing data) there may be uncertainty as to the human relevance of this observation, i.e. to the human relevance of the underlying mode of action. In the absence of specific data on this, observations in the animal are taken as relevant to humans. However, additional uncertainty will exist for the relationship between carcinogenic potency in animals and in humans; this uncertainty, though, will be addressed in the procedure for deriving human standards (ILSI RSI 2003).

Finally, conventional assays of carcinogenicity in animals have been found to be insensitive for some well-established human carcinogenic substances (e.g. asbestos and arsenic compounds). These substances can be shown to be carcinogenic when the test conditions are modified, thus illustrating that there will always be a possibility that a chemical could pose a carcinogenic hazard in humans but be missed in conventional animal studies. This is also true for other toxicological endpoints and should be taken into account by risk managers, especially when making decisions about the acceptability of scenarios showing particularly high exposures to workers and/or consumers.

R.7.7.12 Conclusions on carcinogenicity

R.7.7.12.1 Concluding on suitability for Classification and Labelling

In order to conclude on an appropriate classification and labelling position with regard to carcinogenicity, the available data should be considered using the criteria and guidance associated with the (EU Directive 67/548/EEC)[58].

R.7.7.12.2 Concluding on suitability for Chemical Safety Assessment

Besides the identification of a chemical as a carcinogenic agent from either animal data, epidemiological data or both, dose response assessment is an essential further step in order to characterise carcinogenic risks for certain exposure conditions or scenarios. A critical element in this assessment is the identification of the mode of action underlying the observed tumour-formation, as already explained in Section R.7.7.11.1: i.e. whether this induction of tumours is thought to be via a genotoxic mechanism or not.

In regulatory work, it is generally assumed that in the absence of data to the contrary an effect-threshold cannot be identified for genotoxic carcinogens exhibiting direct interaction with DNA, i.e., it is not possible to define a *no-effect level* for carcinogenicity induced by such agents. However, in certain cases even for these compounds a threshold for carcinogenicity may be identified in the low-dose region: e.g. it has in certain cases been clearly demonstrated that an increase in tumours did not occur at exposures below those associated with local chronic cytotoxicity and regenerative hyperplasia. It is also recognised that for certain genotoxic carcinogens causing genetic alterations, a practical threshold may exist for the underlying genotoxic effect. For example, this has been shown to be the case for aneugens (agents that induce aneuploidy – the gain or loss of entire chromosomes to result in changes in chromosome number), or for chemicals that cause indirect effects on DNA that are secondary to another effect (e.g., through oxidative stress that overwhelms natural antioxidant defence mechanisms).

[58] Directive 67/548/EEC will be repealed and replaced with the EU Regulation on classification, labelling and packaging of substances and mixtures, implementing the Globally Harmonized System (GHS).

Non-genotoxic carcinogens exert their effects through mechanisms that do not involve direct DNA-reactivity. It is generally assumed that these modes of actions are associated with threshold doses, and it may be possible to define no-effect levels for the underlying toxic effects of concern. There are many different modes of action thought to be involved in non-genotoxic carcinogenicity. Some appear to involve direct interaction with specific receptors (e.g. oestrogen receptors), whereas appear to be non-receptor mediated. Chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be induced: the induction of urinary bladder tumours in rats, for example, may, in certain cases, be due to persistent irritation/inflammation/erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones which eventually results in tumour formation. Specific cellular effects, such as inhibition of intercellular communication, have also been proposed to facilitate the clonal growth of neoplastic/pre-neoplastic cells.

The identification of the mode of action of a carcinogen is based on a combination of results in genotoxicity tests (both *in vitro* and *in vivo*) and observations in animal experiments, e.g. site and type of tumour and parallel observations from pathological and microscopic analysis. Epidemiological data seldom contribute to this.

Once the mode of action of tumour-formation is identified as having a threshold or not, a dose descriptor has to be derived for the purpose of allowing to conclude on chemical safety assessment. For threshold mechanisms the No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed-Adverse-Effect-Level (LOAEL) (see general introduction for definition and derivation of these descriptors) for tumour-formation or for the underlying (toxic) effect should be established to allow the derivation of a so-called Derived-No-Effect-Level (DNEL) (Chapter R.8), that subsequently is used in the safety assessment to establish safe exposure levels.

If the mode of action of tumour formation is identified as non-thresholded, dose descriptors such as T25, BMD10 or BMDL10 (general introduction for definition and derivation these descriptors) are to be established, that allow the derivation of a so-called Derived-Minimal-Effect-Level (DMEL; for guidance see Section R.8.5), that subsequently is used in the safety assessment to establish exposure levels of minimal concern.

Though mainly derived from animal data, epidemiological data may also occasionally provide dose descriptors that allow derivation of a DNEL or DMEL, e.g. Relative Risk (RR) or Odds Ratio (OR).

Substance-specific data for carcinogenicity normally will be absent, especially for the lower tonnage level substances. As indicated in Section R.7.7.11.1, non-testing data (read-across, grouping and/or (Q)SAR) may occasionally be considered sufficient to conclude on this endpoint, i.e. for classification, but also for establishing the underlying mode of action and for estimating the carcinogenic potency. This may introduce some additional uncertainty, especially with respect to the dose descriptor value, the addressing of which requires expert judgement; it is noted that experience to date on this is extremely limited. Guidance on read-across and/or grouping, and the use of (Q)SAR is provided in Sections R.6.2 and R.6.1.respectively.

R.7.7.12.3 Information not adequate

A *Weight of Evidence* approach comparing available adequate information with the tonnage-tiered information requirements by REACH may result in the conclusion that the information/data requirements are not fulfilled. In order to proceed in further information gathering, the following testing strategy can be adopted.

R.7.7.13 Integrated Testing Strategy (ITS) for carcinogenicity

R.7.7.13.1 Objective / General principles

The objective of this strategy is to describe where required how carcinogenicity should be assessed for all substances subject to registration under REACH: i.e. to identify substances with carcinogenic properties, their associated underlying mode of action, and their potency. Guidance is provided especially for those substances lacking pre-existing epidemiological or toxicological data on carcinogenicity.

The strategy provides the rationale for deciding whether or not a standard animal carcinogenicity study or any other further testing is required. It is recognised that standard carcinogenicity tests take considerable time to conduct and report, are expensive, and involve the use of a large number of animals. Consequently, it is preferable that decisions about the potential carcinogenicity of substances under REACH be taken as frequently as possible without the conduct of such tests.

The strategy recognises that the available information will differ from substance to substance. This may include various different types of toxicity information for the substance in question and/or for its analogues/structurally related chemicals. Details about the use and human exposure potential of the substance will also be available. All this will have an impact on the need for further data acquisition. Proposals for conducting a carcinogenicity test should be made with regard to the potential risk to human health and with consideration of the actual or intended production and/or use pattern.

REACH only specifies a carcinogenicity test for substances at the Annex X tonnage level (≥1000 t/y; see Section R.7.7.9). However, REACH also requires that carcinogenic substances at all tonnage levels be identified as substances of high concern, taking into account information from all available relevant sources (see Section 0).

At the tonnage levels below 1000 t/y, the main concern is for those chemicals that are genotoxic. Chemicals may cause cancer secondary to other forms of toxicity, but protection of human health against the underlying toxicity (e.g., as identified from a repeat-dose toxicity study) will also protect against cancer that is secondary to that toxicity. It is noted, though, that some of these non-genotoxic carcinogens, when not classified for any other property and not identified as such in (limited) repeated dose toxicity studies will go unidentified; this also regards the risks associated with human exposures.

Finally, the strategy recognises that the carcinogenic process is a complex multi-step process. Chemically-induced cancer may be induced by any number of different pathways or modes of action and this allows for a variety of different approaches to carcinogenicity assessment. Substances that have the potential to act as genotoxic carcinogens can be identified by *in vitro* and *in vivo* mutagenicity tests, as described in Section R.7.7.1. Carcinogens that act by non-genotoxic modes of action are more difficult to identify because comparable, well-validated, short-term tests for the potentially numerous modes of actions involved are generally not available, and those tests that are available are not required as part of the standard information requirements of REACH.

A flow chart of the strategy is presented in Figure R.7.7-2.

R.7.7.13.2 Preliminary considerations

As a starting point, there will be the information collected with respect to mutagenicity. If they are available, test and non-test data from a literature search and, if possible, from members of an applicable chemical category or (Q)SAR analysis should be taken into account.

For substances for which there is no concern for mutagenic activity, and no other toxicological indicators of concern for carcinogenicity (i.e. for the substance itself or for structurally-related substances), there is no need for further consideration of its carcinogenic potential. This applies equally to those substances at the Annex X tonnage level as to those at lower tonnage levels.

If, however, for non-genotoxic substances toxicological indicators of concern are available (e.g. hyperplastic or pre-neoplastic lesions in repeated dose toxicity studies of the substance itself and/or of closely related substances), they should be investigated further on a case-by-case basis. Any decision on further testing is dependent upon the type and strength of the indications for carcinogenicity, the potential mechanism of action and their relevance to humans, and the type and level of human exposure (see Section R.7.7.10.2).

If no conclusion can be drawn regarding the potential genotoxicity of the substance then, in general, it will be determined on a case-by-case basis when and how the carcinogenic potential should be explored further. Again, this will then depend on the type and strength of the indications for carcinogenicity, the potential mechanism(s) of action, and the type and level of human exposure.

At least for substances at the higher tonnage levels, subchronic and/or chronic studies may provide additional important information on possible carcinogenic effects. There may, for example, be indications of peroxisomal proliferation or of hyperplastic or pre-neoplastic responses, including dose-response characteristics. These should be investigated further on the already indicated case-by-case basis, depending on the type and strength of the indications for carcinogenicity, the potential mechanism of action and relevance to humans, and the type and level of human exposure.

It may be appropriate on occasions to propose other tests to be undertaken, e.g. to test a read-across option with available non-testing data. These could include short-term tests, such as those for *in vitro* cell transformation or cell proliferation, or medium-term tests, like genetically engineered (transgenic) or neonatal models. It may well be that data generated in this way supports this read-across to available non-testing data, and herewith provides sufficient confidence in a read-across derived estimate of the carcinogenic potency for the substance and also for the magnitude of the risks associated with experienced exposure levels. The data generated may also weaken or even disprove the basis for read-across. It is noted that experience to date on this is very limited (as indicated in Section R.7.7.11.1). Guidance on read-across and/or grouping is provided in Section R.6.2.

As validated testing procedures are not yet available and published in the OECD test guideline programme, it is essential that appropriate expert advice is sought regarding the application and suitability of any of these other tests.

Substances for which concern for carcinogenicity is solely based on positive genotoxicity data will, in a first step, be evaluated according to the approach outlined for identification of the genotoxicity hazard (see Section R.7.7.5).

Formally, for a substance classified as a category 1 or 2 mutagen, a carcinogenicity study will not normally be required (see Section R.7.7.9); *i.e.* it will be regarded as a genotoxic carcinogen. In order to allow an assessment of the magnitude of potential cancer risks associated with the prevailing human exposures, it may well be that available non-testing data (read-across, grouping, (Q)SAR) provide a sufficiently helpful estimate of the carcinogenic potency of the substance (i.e. by read-across) from which risks can be assessed. Guidance on read-across and/or grouping, and the use of (Q)SAR is provided in Sections R.6.2 and R.6.1, respectively.

In case such an approach is not possible, an estimate of acceptable exposure conditions may alternatively be obtained by use of the available data from animal toxicity studies: i.e. by identifying the minimal toxic dose in sub-chronic studies (if available, as some surrogate value for the dose descriptor) and by applying a large assessment factor; see for further guidance Gold *et al*. (2003). It is stressed that expert judgement is definitively needed here.

On very rare occasions, a case may be made to perform a carcinogenicity study in animals for substances that have been classified for mutagenicity in categories 1 or 2. Such a case would have to explain why the study was critically important; e.g. in the context of the clarification of carcinogenic risk associated with human exposures.

For substances classified as category 3 mutagens, and for which there is no carcinogenicity study, there should first be an evaluation of whether classification in category 2 for mutagenicity is possible. If such a classification is made, then the approach described above can be followed with regards to carcinogenicity. Occasionally, it may be established that classification as a category 2 mutagen is not appropriate. In such instances, it should not be assumed automatically that the substance has carcinogenic potential. However, unless there is clear evidence to indicate the contrary, it is expected that these substances will be regarded as genotoxic carcinogens.

As the previous paragraph implies, mutagenic potential *in vivo* is not always a reliable indicator of carcinogenic potential. If repeated dose toxicity studies indicate that pre-neoplastic changes (e.g. hyperplasia, precancerous lesions) occur, then the probability that carcinogenic activity will be expressed is increased. Non-testing data such as read-across and (Q)SAR may also contribute to this evaluation.

For substances at the REACH Annex X tonnage level, the need for or waiving of a standard animal test should be clearly explained, taking into account all the available toxicological and hygiene information on the substance and/or other relevant substances. For example, if it can be demonstrated that the substance is used only in a closed system and that human exposures are negligible, it is possible to propose no further testing for carcinogenicity.

It is recommended that when a carcinogenicity bioassay is required, study design and test protocol are well considered prior to delivering the test-proposal (e.g. OECD TG 453). Particular consideration, based on all the available data, should be given to the selection of the species and strain to be used in the carcinogenicity test, the route of exposure and dose level selection. It is also recommended that when a carcinogenicity test is to be conducted, an investigation of chronic toxicity should, whenever possible, form part of the study protocol. Finally, the limited value of a mouse assay as second species should be considered in this (Doe *et al.*, 2006).

The approaches outlined below may be used in the assessment of the potential carcinogenic risk of a substance to humans, and to help decide whether or not a carcinogenicity test will be required and, if so, when.

R.7.7.13.3 Testing strategy for carcinogenicity

As for other endpoints, the following three steps apply for the assessment of carcinogenicity (i.e. the hazard, underlying mode of action, and potency) for substances at each of the tonnage levels specified in Annexes VII to X of REACH.

i. Gather and assess all available test and non-test data from read-across/proper chemical category and suitable predictive models. Examine the Weight of Evidence that relates to carcinogenicity.

ii. Consider whether the standard information requirements are met.

iii. Ensure that the information requirements of Annexes VII and VIII are met; make proposals to conform with Annexes IX and X.

Further details about the procedures to follow at each of the different tonnage levels are described below.

Substances at Annexes VII, VIII and IX

A definitive assessment of carcinogenicity is usually not possible from the data available at the Annex VII, VIII and IX tonnage levels. However, for all substances, any relevant test data that are already available, together with information from predictive techniques such as read-across or chemical grouping, should be used to form a judgement about this important hazard endpoint.

The minimum information to be provided at the Annex VII, VIII and IX tonnage levels in relation to this endpoint is equivalent to that required for the mutagenicity endpoint (see Section R.7.7.2): positive results from *in vitro* mutagenicity studies provide an alert for possible carcinogenicity, and need confirmation via further testing *in vitro* and/or *in vivo* mutagenicity testing. As such, this will not lead to classification of a substance as a carcinogen, but this evidence should be taken into account in risk assessment: substances shown to be *in vivo* mutagens should be assumed to be potentially carcinogenic.

Furthermore, the results of repeated dose toxicity studies and /or reproductive/ developmental toxicity tests may be informative about a possible carcinogenic potential: hyperplasia or other pre-neoplastic effects may be observed in these studies. These observations may also be informative on potential mode(s) of action underlying the carcinogenic effect.

Although the criteria for carcinogenicity classification may not be met in the absence of substance-specific carcinogenicity data, the evidence from the available information alerting to possible carcinogenicity should be taken into account in the risk assessment for this endpoint: ways that allow an assessment of the magnitude of potential cancer risks associated with human exposures without performing the assay are indicated in indicated in Section R.7.7.13.2. (see Section for derivation of DMEL and DNEL values Chapter R.8).

It is important to note that at the tonnage levels below 1000 t/y, the main concern is for those chemicals that are genotoxic. The repeated dose toxicity studies mentioned above may indicate cancers which are secondary to other forms of toxicity. For those the protection of human health against the underlying toxicity will also protect against cancer that is secondary to the toxicity. It is noted, though, that some of these non-genotoxic carcinogens, when not classified for any other property and not identified as such in (limited) repeated dose toxicity studies will go unidentified; this also regards the risks associated with human exposures.

Substances at Annex X

All substances at this tonnage should be evaluated for carcinogenicity.

All relevant data from all toxicity studies should be assessed to see whether a sufficiently reliable assessment about the carcinogenicity of the substance is possible, including alternative means, if needed: i.e. predictive techniques such as chemical grouping and read-across, and the use of (Q)SARs. On some occasions, it may be proposed to supplement these predictive approaches with *in vitro* or alternative shorter-term *in vivo* investigations in order to circumvent the need for a carcinogenicity study. This should usually be in the context of adding to the *Weight of Evidence* that a substance may be carcinogenic.

Formally, if the substance is classified as a category 1 or 2 mutagen (GHS category 1), a carcinogenicity study will not normally be required. For a substance classified as a category 3 mutagen (GHS category 2) it should first be established whether a case should be made for a higher level of classification.

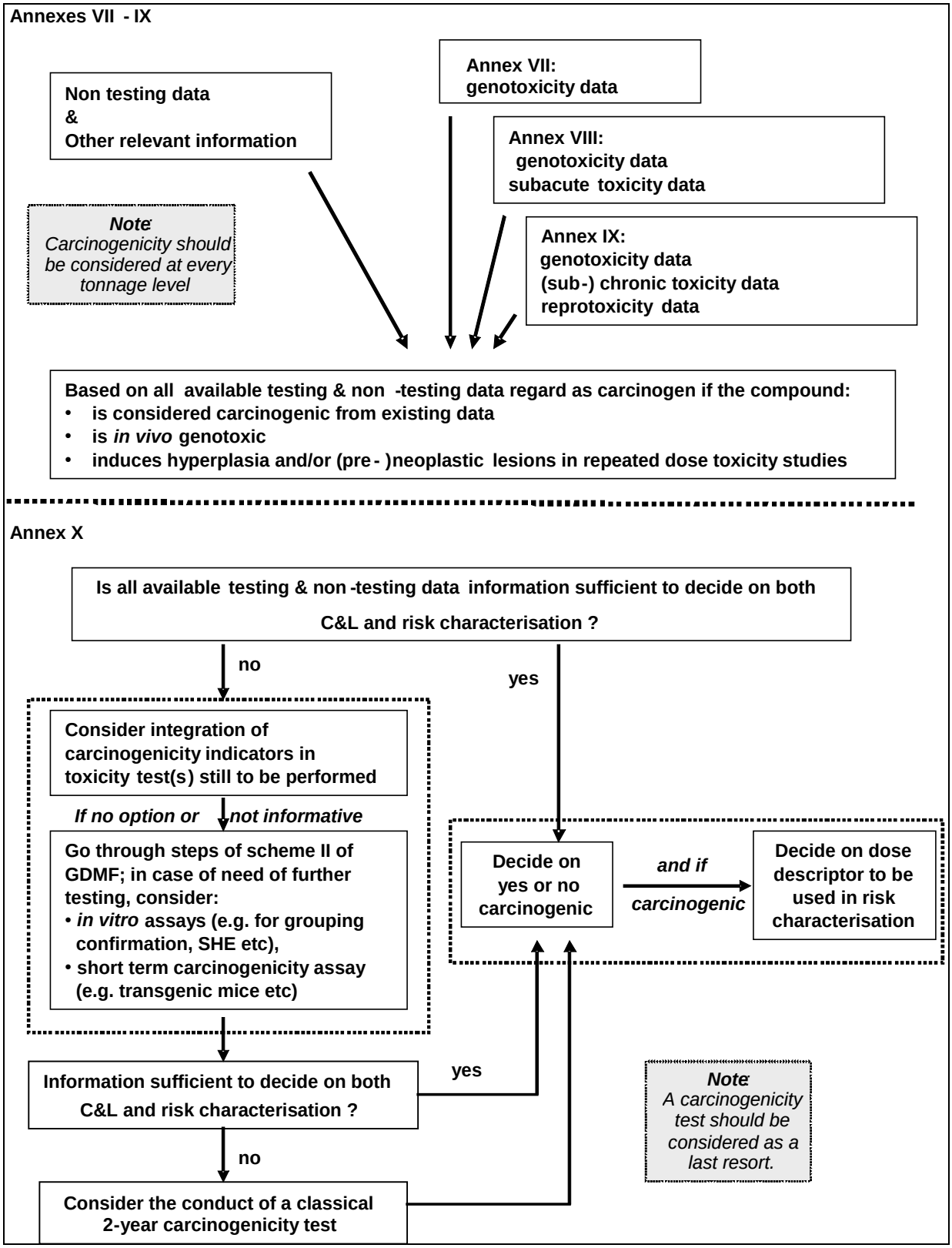
For risk assessment, all the substances are then regarded as genotoxic carcinogens unless there is scientific evidence to the contrary. Ways that allow an assessment of the magnitude of potential cancer risks associated with human exposures without performing the assay are indicated in Section R.7.7.13.2. (see Chapter R.8 for derivation of DMEL and DNEL values).

A carcinogenicity study may, on occasion, be justified. If there are clear suspicions that the substance may be carcinogenic, and available information (from both testing and non-testing data) are not conclusive in this, both in terms of hazard and potency, then the need for a carcinogenicity study should be explored. In particular, such a study may be required for substances with a widespread, dispersive use or for substances producing frequent or long-term human exposures. However, it should be considered only as a last resort.

It is noted, though, that some of non-genotoxic carcinogens, i.e. when not classified for any other property and not identified as potential carcinogens in (limited) repeated dose toxicity studies will go unidentified; this also regards the risks associated with human exposures.

If, in any case there is a need for further testing, the registrant must prepare and submit a well-considered test proposal (see Section R.7.7.6.2), and a time schedule for fulfilling the information requirements.

Figure R.7.7-2 Integrated Testing Strategy for carcinogenicity



R.7.7.14 References on carcinogenicity

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