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Guidance on Information Requirements and Chemical Safety Assessment

Chapter R.7a: Endpoint specific guidance

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NOTE

Please note that the present document is a proposed amendment to specific extracts **only** of the *Guidance on IR&CSA, Chapter R.7a*. This document was prepared by the ECHA Secretariat for the purpose of this consultation and includes only the parts open for the current consultation, i.e. Section R.7.2 only.

The full document (version before proposed amendments) is available on the ECHA website at http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf (version 3.0 published in August 2014).

The numbering and headings of the Sections that are displayed in the document for consultation correspond to those used in the currently published guidance document; this will enable the comparison of the draft revised Sections with the current text if necessary.

After conclusion of the consultation and before final publication the updated Sections will be implemented in the full document.

1 Document history

Version	Changes	Date
[…]	[…]	[…]
Version 4.0	Full revision addressing the content of Section R.7.2 related to *Skin corrosion/irritation, Serious eye damage / eye irritation, and Respiratory tract corrosion/irritation*. The update includes the following: • Modification of Section R.7.2 structure and subdivision by endpoint: Skin corrosion/irritation (Sections R.7.2.2 to R.7.2.6), Serious eye damage/eye irritation (Sections R.7.2.7 to R.7.2.11) and Respiratory tract corrosion/irritation (Sections R.7.2.12 to R.7.2.14). • Update of the information on new/revised EU test methods and OECD test guidelines for skin corrosion/irritation and serious eye damage/eye irritation; • Update of the information on respiratory tract corrosion/irritation assessment; • Replacement of the terms “eye corrosion” by “serious eye damage” and “respiratory irritation” by “respiratory tract corrosion/irritation”; • Update of the information on non-testing methods, in particular in Appendices R.7.2-2 *QSARs and expert systems for skin corrosion and irritation* and R.7.2-3 *QSARs and expert systems for serious eye damage and eye irritation*; • Update of the recommended testing and assessment strategy for skin corrosion/irritation and serious eye damage/eye irritation in Sections R.7.2.6 and R.7.2.11 respectively; • Replacement of the terms “Integrated Testing Strategy (ITS)” by “testing and assessment strategy” to account for the non-testing part of the evaluation strategy; • Update of the information on Classification and Labelling to reflect changes coming from the 2nd and 4th Adaptations to Technical and Scientific Progress of the CLP Regulation, and to align the text with the revised Sections 3.2 *Skin corrosion/irritation* and 3.3 *Serious Eye damage/Eye irritation* of the *Guidance on the Application of the CLP Criteria* (version 4.0, November 2013).	XX 2015

R.7.2 Skin corrosion / irritation, serious eye damage / eye irritation and respiratory tract corrosion / 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 by exposure to a substance. 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, dermal or inhalatory 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, dermal or inhalatory 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 (see Section R.7.2.1.1 for complete definitions). These tissues are in the present context skin, eye (cornea, iris and conjunctiva) and mucous epithelia such as the respiratory tract. Criteria for classification of irritant and corrosive substances are given in Annex I to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging (CLP) of substances and mixtures (CLP Regulation).

Certain substances may also cause irritant effects only after repeated exposure, for example organic solvents. This type of substance may have defatting properties (Ad-hoc Working group on Defatting substances, 1997). Substances that have a similar mode of action need to be considered for labelling with the supplemental statement EUH066 "*Repeated exposure may cause skin dryness or cracking*".

Information on the mechanisms underlying corrosion and irritation of skin, eye and respiratory tract is given in Appendix R.7.2-1 *Mechanisms of local toxicities: skin corrosion/irritation, serious eye damage/eye irritation and respiratory tract corrosion/irritation*.

R.7.2.1.1 Definitions of skin corrosion / irritation, serious eye damage / eye irritation and respiratory tract corrosion / irritation

Definitions of skin corrosion/irritation, serious eye damage/eye irritation and respiratory tract corrosion/irritation can be found in the CLP Regulation[1].

Skin irritation: Defined in Section 3.2.1.1 of Annex I to the CLP Regulation as *"[…] the production of reversible damage of the skin following the application of a test substance for up to 4 hours."*.

Dermal concern after repeated exposure: Used for a substance which may cause skin dryness, flaking or cracking upon repeated exposure but which cannot be considered as skin irritant (see Section 1.2.4 of Annex II to the CLP Regulation).

[1] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

Skin corrosion: Defined in Section 3.2.1.1 of Annex I to the CLP Regulation 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 Section 3.3.1.1 of Annex I to the CLP Regulation 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."*.

Serious eye damage: Defined in Section 3.3.1.1 of Annex I to the CLP Regulation 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 tract irritation and testing for respiratory tract irritation is not a standard information requirement under REACH. Respiratory tract irritation is considered under the CLP Regulation (Table 3.8.1 of Annex I) as a transient target organ effect, i.e. an *"[…] effect which adversely alter[s] human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. […]*". More specifically, respiratory tract irritation is often used to describe either or both of two different toxicological effects, *sensory irritation* and *local cytotoxic effects*. However, classification in STOT-SE Category 3 for respiratory tract irritation is generally limited to local cytotoxic effects. *"[…] Respiratory irritant effects [are] characterised [by] localised redness, oedema, pruritis and/or pain and they impair function with symptoms such as cough, pain, choking, and breathing difficulties […]"* (see Section 3.8.2.2.1 of Annex I to the CLP Regulation).

Respiratory tract corrosion: There is no EU or OECD TG for respiratory tract corrosion and testing for respiratory tract corrosion is not a standard information requirement under REACH. Respiratory tract corrosion is defined in Section 3.1.2.3.3 of Annex I to the CLP Regulation as *"[…] destruction of the respiratory tract tissue after a single, limited period of exposure analogous to skin corrosion; this includes destruction of the mucosa. […]".*

Classification and labelling under the CLP Regulation:

Substances and mixtures causing skin corrosion/irritation, serious eye damage/eye irritation and/or respiratory tract corrosion/irritation can be further characterised by their classification under the CLP Regulation[2].

Detailed information on the classification and labelling of substances and mixtures can be found in the *Guidance on the Application of the CLP criteria* (available at: http://echa.europa.eu/web/guest/guidance-documents/guidance-on-clp).

[2] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

a) For Skin effects

- **Skin corrosives** are classified in Category 1 with the Hazard statement H314 *"Causes severe skin burns and eye damage"*. Further subcategorisation is defined based on the Draize skin corrosion *in vivo* test:
  - Subcategory 1A: Destruction of skin tissue occurs after exposure times ≤ 3 minutes and is observed within a period ≤ 1 hour after exposure,
  - subcategory 1B: Destruction of skin tissue occurs after exposure times > 3 minutes and ≤ 1 hour and is observed within a period ≤ 14 days after exposure,
  - subcategory 1C: Destruction of skin tissue occurs after exposure times > 1 hour and ≤ 4 hours and is observed within a period ≤ 14 days after exposure.
- **Skin irritants** are classified in Category 2 with the Hazard statement H315 *"Causes skin irritation"*.

b) For Eye effects

- **Substances or mixtures causing serious eye damage** are classified in Category 1 with the Hazard statement H318 *"Causes serious eye damage"*.
- **Substances or mixtures causing eye irritation** are classified in Category 2 with the Hazard statement H319 *"Causes serious eye irritation"*.

c) For Specific Target Organ Toxicity with relevance to the respiratory tract

- **Substances or mixtures causing respiratory tract corrosion** are classified for Acute Toxicity by inhalation and labelled as EUH071 "*Corrosive to the respiratory tract*" if the corrosive effect causes the death of the animals within the criteria for Acute toxicity, or in Specific Target Organ Toxicity after Single Exposure (STOT-SE) Category 1 (with the Hazard statement H370 *"Causes damage to the respiratory tract"*) or Category 2 (with the Hazard statement H371 *"May cause damage to the respiratory tract"*), depending on the dose level required to cause the toxic effects.
- **Substances or mixtures causing respiratory tract irritation** via a local cytotoxic effect are classified in Specific Target Organ Toxicity after Single Exposure (STOT-SE) Category 3 with the Hazard statement H335 *"May cause respiratory irritation"*.

According to Section 1.2.6 of Annex II to the CLP Regulation, the Hazard statement EUH071 must also be applied to inhaled substances or mixtures classified for skin corrosion and not tested for acute inhalation toxicity.

Note that dermal and respiratory tract irritation following repeated exposure are not discussed in the present context, since this Guidance 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 Hazard Statement EUH066 (“Repeated exposure may cause skin dryness or cracking”) 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 corrosion / irritation, serious eye damage / eye irritation and respiratory tract corrosion / 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 provides 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 provides evidence of significant skin, eye or respiratory tract irritation.

c. to establish if possible the time of onset and the extent and severity of the responses and information on reversibility.

d. If possible 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 as 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 TGs available for acute respiratory tract irritation and there is no testing requirement for respiratory tract irritation under the REACH Regulation. Consequently no testing and assessment strategy for respiratory tract corrosion/irritation is included in this guidance. Nevertheless, account should be taken of any existing and available data that provide evidence of the respiratory tract corrosion/irritation potential of a substance. For instance, acute inhalation studies including histopathological evaluation of the respiratory tract and/or examinations of nasal or bronchioalveolar lavage as well as repeated inhalation studies may provide important information for classification and labelling (See Section R.7.2.12 for further details).

SKIN CORROSION / IRRITATION

R.7.2.2 Information requirements on skin corrosion / irritation

The information on skin corrosion/irritation that is required to be submitted for registration and evaluation purposes is specified in Annexes VI to XI to the REACH Regulation. According to Annex VI, the registrant should gather and evaluate all existing available information before considering further testing. This includes physico-chemical properties, (Q)SAR ((Quantitative) Structure-Activity Relationship), grouping, *in vitro* data, animal studies, and human data. For classified substances, information on exposure, use and risk management measures should also be collected and evaluated in order to ensure safe use of the substance.

If these data are inadequate for hazard and risk assessment, further testing should be carried out in accordance with the requirements of Annexes VII (≥1 tpa) and VIII (≥10 tpa) to the REACH Regulation.

R.7.2.2.1 Information requirements for quantities of ≥1 tpa (Annex VII to the REACH Regulation) [3]

If new testing data are necessary, these must be derived from *in vitro* methods only. Annex VII does not foresee *in vivo* testing for skin corrosion/irritation.

The standard information requirements at this tonnage level for skin corrosion/irritation (see Section 8.1 in Column 1 of Annex VII) can be fulfilled 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.

Section 8.1 in Column 2 of Annex VII lists specific rules for adaptation according to which steps 3 and 4 do not need to be conducted. These rules are applicable when:

1. the available information already indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes, or

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”), or

3. the substance is classified as very toxic in contact with skin (i.e. the “Substance is fatal in contact with skin” and classified in Category 1 for Acute toxicity according to current CLP terminology), or

4. an acute toxicity study via the dermal route does not indicate skin irritation up to the limit dose level (2000 mg / kg body weight) (Please see footnote d to Figure R.7.2-2 for further information).

[3] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

R.7.2.2.2 Information requirements for quantities of ≥10 tpa (Annex VIII to the REACH Regulation) [4]

For substances manufactured or imported in quantities of ≥10 tpa *in vivo* testing is the standard information requirement of Annex VIII (Column 1) for skin corrosion/irritation, in case the information requirement cannot be met with the information obtained as specified in section 8.1 of Annex VII.

Before new tests are carried out to determine the properties listed in Annex VIII, all available *in vitro* data, *in vivo* data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) must be assessed first. Due to the sequential nature of the REACH standard information requirements, it is reminded that at quantities of ≥10 tpa, the information requirements of Annex VII to the REACH Regulation also apply. This means that before a new *in vivo* test is performed, the appropriate *in vitro* testing must be undertaken according to the rules set out in section 8.1 of Annex VII and must be documented in the technical dossier (IUCLID). Finally, the information generated at Annex VII level must be taken into account in determining whether an *in vivo* test at Annex VIII level is really needed.

Column 2 of Annex VIII lists the following specific rules that allow deviating from the standard information required by Annex VIII for skin corrosion/irritation:

- the substance is classified as corrosive to the skin or as a skin 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"), or
- the substance is classified as very toxic in contact with skin (i.e. the "Substance is fatal in contact with skin" and classified in Category 1 for Acute toxicity according to current CLP terminology), 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) (Please see footnote d to Figure R.7.2-2 for further information).

The *in vitro* methods that can be used to adapt the standard information requirements are detailed in Sections R.7.2.3.1 and R.7.2.4.1 of this Guidance, under "*In vitro* data". In case results of the *in vitro* testing are used to adapt the standard information requirement of *in vivo* testing at Annex VIII level, an adaptation e.g. according to Annex XI to the REACH Regulation will need to be submitted in order to successfully submit a compliant dossier.[4]

Guidance on the application of these rules is given in the testing and assessment strategies described in Sections R.7.2.6 and R.7.2.11 of this Guidance.

[4] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

It should be noted that the conditions of acceptance by ECHA of implementation of any of the adaptation rules laid down in Annex XI are strict, and whenever an adaptation argument is being used (e.g. use of (Q)SARs, read-across or non-validated *in vitro* test methods), scientific justification, solid documentation and readiness for risk assessment and Classification and Labelling must be provided by registrants. For detailed information on these rules, see Annex XI to the REACH Regulation.

R.7.2.3 Information sources on skin corrosion / irritation

R.7.2.3.1 Non-human data on skin corrosion / irritation

Non-testing data on skin corrosion / irritation

Physico-chemical properties

Relevant information can be inferred from basic physico-chemical characteristics of a substance (e.g. extreme pH). Extreme pH values may indicate the potential of a substance to cause skin corrosion:

IF pH ≤ 2 or pH ≥ 11.5, THEN consider the substance to be corrosive to the skin (Category 1) when pH is used as the sole basis for classification decision (see also Section R.7.2.4.1 of this Guidance).

Grouping, (Q)SARs and expert systems [5]

In REACH Annex XI two types of non-testing methods are mentioned which can be used for adaptation of standard information requirements, either as standalone (where possible) or in concert with other information (in the context of a *Weight-of-Evidence* assessment):

- qualitative and quantitative Structure-Activity-Relationships (SARs/QSARs, section 1.2, including expert systems, generally incorporating multiple (Q)SARs, expert rules and data) on the one hand, and

- grouping of substances and read-across approaches. [6]

The adaptation of standard information requirements can be used for the assessment of skin corrosion/irritation, if it provides relevant and reliable data for the substance of interest. As specified in Annex XI of the REACH regulation, the use of non-testing methods needs to be justified and sufficiently documented. In the case of QSARs and expert systems, registrants need to prepare property predictions by completion of a QSAR Prediction Reporting Format (QPRF). The QPRF is a harmonised template for summarising and reporting substance-specific predictions generated by (Q)SAR models. For filling a data gap under REACH, it is also necessary to provide information on the prediction model employed following a QSAR Model

[5] Further information can be found in *Chapter R.6 QSAR and grouping of chemicals* of *the Guidance on IR&CSA* (available at: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment), the OECD Guidance on Grouping of Chemicals, Second Edition (OECD, 2014a), the new OECD Guidance on an Integrated Approach for Testing and Assessment (IATA) for skin corrosion and irritation (OECD, 2014b) and the JRC report on Alternative methods for regulatory toxicology (Worth, 2014).

[6] The relevant terminology is not always used consistently. With reference to the ECHA Guidance on QSAR and grouping, the terms category approach and analogue approach are used to describe techniques for grouping of substances, whilst the term read-across is reserved for a technique to fill data gaps, i.e. to transfer knowledge from one or more substances called source(s) to another substance with data gaps, named target substance.

Reporting Format (QMRF) document. The QMRF is a harmonised template for summarising and reporting key information on (Q)SAR model validity, including the results of any validation studies. The information is structured according to the OECD (Q)SAR validation principles (for further information see http://www.oecd.org/env/ehs/risk-assessment/validationofqsarmodels.htm). The JRC QSAR Model Database is an inventory of information on available QMRFs, freely accessible online (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/laboratories-research/predictive_toxicology/qsar_tools/QRF). More detailed guidance on QSAR models, their use and reporting formats, including the QMRF, is provided in Section R.6.1 of Chapter R.6 of the *Guidance on IR&CSA* (available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

In general, there are several different ways in which non-testing methods can be used in the context of an Integrated Approach to Testing and Assessment (IATA) (OECD, 2014b), e.g.:

- for direct prediction of corrosion/irritation potential or the absence thereof,
- as part of a *Weight-of-Evidence* scheme (where the information from non-testing methods alone is not sufficient for a decision), or
- in order to decide how best to proceed with further (*in vitro*) testing (i.e. via a top-down or bottom-up approach). For further information see Section R.7.2.6.2).

In the case of skin corrosion and irritation, many of the models have a mechanistic basis, which provides additional information on the relevance of the model.

- SAR and read-across for skin corrosion and irritation:

SARs and read-across are treated together in this section because the existence of a SAR (structural alert or set of fragments) provides one means of justifying read-across. In fact, structural alerts are substructures in the substance that are considered to reflect some kind of chemical or biochemical reactivity that underlies the toxicological effect. The occurence of a structural alert for a substance suggests the presence of effect, based on the notion that structural analogues that have exhibited corrosion (or irritation) potential can be used to predict a corrosive or irritant effect for the substance of interest, or to tailor further testing and assessment, as indicated in the OECD IATA for skin corrosion/irritation (OECD, 2014b).

The knowledge on structural alerts for skin irritation/corrosion is always evolving (in particular where new classes of substances are introduced into the market). Therefore predictions based on read-across may also be possible for chemically similar substances if it can be shown that their similarity reflects reactive substructures able to react with skin tissue, even if that substructure has so far not been coded into a structural alert in any of the available literature or software models.

Negative data from structural analogues may also be used to make predictions in certain cases. The absence of one of the known structural alerts for irritation and corrosion alone does not prove absence of effect, as knowledge of structural alerts for irritation and corrosion might be incomplete. For instance, other substructures (not yet identified as structural alerts) or other properties of the substance may be responsible for a corrosive or irritant effect. As an example, irritant contact dermatitis may occur indirectly, such as in the case of exposure to organic solvents with defatting properties. Substances that have a similar mechanism need to be considered for the supplemental labelling *'Repeated exposure may cause skin dryness or cracking'* (EUH066) (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 (R_1-O-O-R_2), based on the fact that peroxides decompose easily and thus have a low thermal stability. The radicals formed by breaking the O-O bond are reactive and may be the cause for irritation or corrosion.

A variety of SARs (including hydroperoxides) for predicting the presence of irritation or corrosion have been described by Hulzebos *et al*. (2001, 2003, 2005), and some of these have been incorporated into the BfR (German Federal Institute for Risk Assessment) rule-base, and the SICRET tool (Walker *et al*., 2005, see Appendix R.7.2-2). The BfR alerts ("inclusion rules") for corrosion and irritation have more recently been incorporated into the Toxtree software (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/laboratories-research/predictive_toxicology/qsar_tools/toxtree) and into the OECD QSAR Toolbox (http://www.qsartoolbox.org/).

- QSARs and expert systems on skin corrosion and irritation:

An overview of available (Q)SARs for skin corrosion and irritation is provided in Table R.7.2-1. QSARs and expert systems for skin corrosion and irritation have been described in several reviews (Hulzebos *et al*., 2001, 2003, 2005; Patlewicz *et al*., 2003; Gallegos Saliner *et al*., 2006, 2008). A comparison of the predictive capacities of three popular commercial tools is also available (Mombelli 2008). A few examples are presented in Appendix R.7.2-2, including literature-based QSAR models, and expert systems.

Most of the QSARs reported in the literature have been developed from small data sets of specific groups of substances, 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 substances. In contrast, models intended to predict the toxic potential of heterogeneous groups of substances emphasise the commonality of structural features.

A number of models are coded into 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 Nexus or the BfR- former DSS [7]). More details on available 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 to the regulatory endpoint of interest. The rule-base at the heart of the former BfR DSS has been developed to predict EU regulatory endpoints, however predictions refer to the former Dangerous Substance Directive (DSD) classification/labelling system used in the EU before the CLP regulation came into force, and in borderline cases the results of the prediction may not fully reflect the correct CLP classification. More details on this model are reported in Appendix R.7.2-2.

It should also be noted that the criteria for classification as skin irritant Category 2 based on the mean score for erythema/eschar or for oedema in the *in vivo* test have changed from ≥2 under DSD to ≥2.3 under CLP. Consequently predictions as skin irritant Cat 2 from models

[7] Distribution of the BfR expert system "Decision Support System for Local Lesions" (DSS) mentioned in previous versions of this guidance has been discontinued. However, the rule-base for skin and eye irritation/corrosion included in this system has been incorporated into software tools such as the OECD QSAR Toolbox or Toxtree (cf. below).

developed based on the DSD criteria should be interpreted with caution since they may lead to overprediction and should not be used for direct classification under CLP. These models can however be argued to be "conservative" and therefore acceptable for predicting no classification under CLP.

Based on the BfR rule-base, the freely downloadable OECD QSAR Toolbox software contains two profilers relevant for corrosion/irritation, which encode both the “inclusion rules” (structural alerts predicting corrosion/irritation potential) and the “exclusion rules” (“IF…THEN NOT…” rules predicting the absence of irritation/corrosion potential) due to certain physico-chemical properties. The use in combination with other profilers (e.g. for skin metabolism) and data for analogues allows for the prediction of skin corrosion/irritation for new chemicals through read-across or category approaches. More details on the Toolbox specific contents for skin corrosion and irritation are reported in Appendix R.7.2-2.

In the case of classification models for skin corrosion, where it is not indicated in the supporting documentation whether the predicted classification should be Skin Corrosive Category 1A, 1B or 1C, Category 1 prediction without further sub-categorisation should be used. Very few models are available (see Gallegos Saliner *et al*., 2006, 2008 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 classes. For classification and labelling, the BfR rule-base provides information that is the closest to the regulatory goal, since the system was designed to predict former EU Risk Phrases for skin irritation (R38) and corrosion (R34, R35) under the Dangerous Substance Directive (DSD). However, in borderline cases and as highlighted above, the prediction may not fully reflect the correct classification under CLP.

Table R.7.2-1 Overview of available (Q)SARs for skin corrosion / irritation. See Appendix R.7.2-2 for more information on these models.

Category of model or source	Reference or name of the model	Applicability domain
Literature models	Barratt (*et al.*) (1995a, 1996 a,b,c); Whittle *et al.* (1996)	Diverse local models for acids, bases , phenols, neutral organic and electrophiles
	Hayashi et al. (1999)	Phenols
	Kodithala *et al.* (1999)	Phenols, esters, and alcohols
	Nangia *et al*. (1996)	Bases
	Smith *et al*. (2000 a,b)	Esters
	Gerner *et al*. (2004); Hulzebos *et al.* (2005); Walker *et al*. (2004)	New Chemicals Database, organic chemicals with no significant hydrolysis potential and purity > 95%
	Golla *et al*. (2009)	Organic chemicals from diverse classes
Data repositories	Danish QSAR database (http://qsar.food.dtu.dk/, also included in the OECD QSAR Toolbox)	Industrial chemicals, pesticides, etc.

Computerised models	PaDEL-DDPredictor (http://padel.nus.edu.sg/software/padelddpredictor/) (Liew and Yap, 2013)	Calculated by the model based on the range of descriptors
	BfR rule-base, free (included in the OECD QSAR Toolbox and Toxmatch, Toxtree, ToxPredict and Ambit)	EU New chemicals (NONS) database, organic chemicals with no significant hydrolysis potential and purity > 95%
	ACD/Percepta	Organic chemicals
	Derek Nexus, commercial	Organic chemicals and some metals
	HazardExpert, commercial	Organic chemicals
	MolCode, commercial	Organic chemicals
	MultiCASE, commercial	Organic chemicals
	TOPKAT, commercial	Organic chemicals
Review papers	Hulzebos *et al*. (2001, 2003, 2005)	N.A.
	Patlewicz *et al*. (2003)	N.A.
	Gallegos Saliner *et al*. (2006, 2008)	N.A.
	Mombelli (2008)	N.A.

Abbreviation: N.A. = not applicable.

Testing data on skin corrosion / irritation

The internationally accepted testing methods for skin corrosion/irritation as described in the Annex to the EU Test Methods (TM) Regulation (Council Regulation (EC) No 440/2008) and in OECD TGs (available at http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm#Test_Guidelines) are: EU method B.4 (OECD TG 404), EU B.40 (OECD TG 430), EU B.40bis (OECD TG 431), OECD TG 435 and EU B.46 (OECD TG 439).

Please note that the latest version of an adopted test guideline should always be used when generating new data, independently from whether it is published by EU or OECD.

The testing strategy developed for skin corrosion/irritation (see Section R.7.2.6 of this Guidance) emphasises the need to evaluate all available information (including physico-chemical properties) before undertaking any *in vivo* testing. This strategy employs screening elements designed to avoid, as far as possible, *in vivo* testing of corrosive and severely irritating substances. In particular, *in vitro* tests should usually be performed first, and it should be assessed whether *in vivo* testing can be completely avoided.

In vitro data

Accepted *in vitro* test methods to detect skin corrosion/irritation (i.e. Category 1 and 2 under CLP) and/or absence of effects (i.e. not classified under CLP) are listed in Table R.7.2-2. More information on the specific scope and limitations of these tests is provided in Section R.7.2.4.1 of this Guidance, under "Testing data on skin corrosion/irritation".

In Table R.7.2-2, when the classification outcome in the column "Classification according to the CLP Regulation" is indicated as "Cat. 1B/1C" or "Cat. 1/Cat. 2", this means that the test method alone cannot differentiate between those (sub-)categories and more information is needed to conclude on the exact classification. For instance if the result of an *in vitro* skin irritation study according to B.46/OECD 439 is positive, it cannot be concluded whether the substance is either corrosive (Cat. 1) or irritant (Cat. 2) to the skin and therefore additional information on skin corrosion potential is needed e.g. by performing an *in vitro* skin corrosion study.

Table R.7.2-2: Accepted *in vitro* test methods for skin corrosion/irritation

	Test method	Validation status, regulatory acceptance	EU Test Methods / OECD test guideline	Classification according to CLP Regulation	EURL ECVAM DB-ALM protocol Nr.
Skin corrosion					
	TER	Validated and regulatory acceptance	B.40/TG 430	Cat. 1 or non corrosive	115
	EpiDerm ™ SCT	Validated and regulatory acceptance	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	119
	EpiSkin ™	Validated and regulatory acceptance	B.40 bis/TG 431	Cat. 1, 1A, 1B and 1C or non-corrosive[8]	118
	SkinEthic ™ RHE	Validated and regulatory acceptance	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	-
	epiCS®	Validated and regulatory acceptance	B.40 bis/TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	-
	Corrositex (*in vitro* membrane barrier test method)	Validated and regulatory acceptance	N.A./TG 435	Cat. 1, 1A, 1B and 1C or non-corrosive	116
Skin irritation					

[8] The EpiSkin SOP allows for differentiating between the 3 sub-categories and OECD GD 203 suggests the use of this method to distinguish 1B from 1C before *in vivo* testing is considered. However, OECD TG 431 currently only permits the use of EpiSkin to distinguish 1A from 1B/1C.

	EpiDerm ™ SIT	Validated and regulatory acceptance	B.46/TG 439	Cat. 1/Cat. 2 or NC	138
	EpiSkin ™	Validated and regulatory acceptance	B.46/TG 439	Cat. 1/Cat. 2 or NC	131
	SkinEthic ™ RHE	Validated and regulatory acceptance	B.46/TG 439	Cat. 1/Cat. 2 or NC	135
	LabCyte EPI-MODEL24 SIT	Validated and regulatory acceptance	B.46/TG 439	Cat. 1/Cat. 2 or NC	-

Abbreviations: N.A. = not available; NC = not classified; RHE = Reconstructed Human Epidermis; SCT = Skin Corrosion Test; SIT = Skin Irritation Test; TER=Transcutaneous electrical resistance.

Further test method developments may occur and the registrants are advised to follow the latest updates through e.g. EURL ECVAM website (https://eurl-ecvam.jrc.ec.europa.eu/) and ECHA's test methods site (Testing methods and alternatives) for potential new test guidelines and test guideline updates.

Animal data

Annex I to the CLP Regulation defines skin corrosion/irritation as local toxic effects, and, as such, an assessment of skin corrosion/irritation is normally part of the acute testing phase of a toxicity programme and it is an early requirement of all regulatory programmes. Testing for skin corrosion/irritation has, historically, used animal models and a variety of test methodologies depending upon, for example, the laboratory undertaking the test, the area and intended application. An IATA, which aims at minimisation of animal testing and instead largely relies on internationally approved *in vitro* tests, has been adopted by the OECD in 2014 as Guidance Document 203 (OECD, 2014b). Thereby, animal models have become unnecessary in most cases when testing for this endpoint. This is in line with one of the objectives of the REACH Regulation, as described in Articles 13(1) and 25(1), on that animal testing should be undertaken only as a last resort, i.e., where a substance falls outside of the applicability domain of the available *in vitro* methods or the results are not conclusive.

In cases in which *in vivo* testing may be necessary, current approaches for skin corrosion/irritation testing *in vivo* are covered by the Acute Dermal Irritation/Corrosion test method (EU B.4/OECD TG 404). This guideline requires a tiered approach, where existing and relevant data are evaluated first. The guideline also recommends that testing in animals should only be conducted if determined to be necessary after consideration of available alternative methods. The *in vivo* test uses one animal (the rabbit is the preferred species), which in the absence of severe effects is followed by a further testing of up to two animals (a total of maximum three animals).

Both EU and OECD 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. Skin irritants (Category 2) cause significant inflammation of the skin (erythema and/oedema) but this effect is transient, i.e. the affected sites are repaired within the observation period of the test.

A corrosive substance causes full thickness destruction of the skin tissue and is classified as Skin corrosive (Category 1) and sub-classified in subcategory 1A, 1B or 1C depending upon the exposure time (3 min, 1 hour, and 4 hours, respectively) and observation time (1 hour, 14 days, and 14 days, respectively).

For existing animal data, the use of methods other than those specified in the Annex to the EU Test Methods Regulation, or corresponding OECD methods may be accepted on a case-by-case basis.

In addition to the EU B.4/OECD TG 404 mentioned above, further animal data may be available e.g. from:

- Acute dermal toxicity test (EU B.3/OECD TG 402)
- Skin sensitisation tests (EU B.6/OECD TG 406, EU B.42/OECD TG 429, and OECD TG 442A and 442B)

Section R.7.2.6 of this Guidance provides comments on how to use information from these test in a testing and assessment strategy for skin corrosion/irritation. Additional *in vivo* tests may also provide relevant information (see paragraph 37 of the OECD Guidance Document 203 (OECD, 2014b)) although the reporting and scoring of the irritation in these tests may not be sufficient in all cases to allow final conclusion to be drawn.

R.7.2.3.2 Human data on skin corrosion / irritation

Existing human data include historical data that should be taken into account when evaluating intrinsic hazards of substances. *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, occupational experience, epidemiological studies and volunteer 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, absence of incidence in humans does not necessarily overrule *in vitro* data or existing animal data of good quality that are positive.

R.7.2.4 Evaluation of information on skin corrosion / irritation

R.7.2.4.1 Non-human data on skin corrosion / irritation

Non-testing data on skin corrosion / irritation

In 2014, the OECD approved an IATA for skin corrosion/irritation. The IATA includes description of various types of data that can be used in the assessement of these hazards, including the types of infomation presented below. The IATA has a modular approach, where the *domain, role in IATA, strengths, weaknesses and limitations* of each type of data are given in a tabular form. It is also explained with flow diagrams how the data can be then integrated. Detailed guidance is given on the *Weight-of-Evidence* approach and on how quality, adequacy and coverage and consistency of data is assessed within a *Weight-of-Evidence* approach (OECD, 2014b).

Physico-chemical properties

According to the current EU and OECD guidelines, substances should not be tested on animals for skin corrosion/irritation if they can be predicted to be corrosive to the skin (Category 1) from their physico-chemical properties. In particular, substances exhibiting strong acidity (pH ≤2.0) or alkalinity (pH ≥11.5) in solution are predicted to be corrosive to the skin and should

not be tested on animals. Testing with *in vitro* methods can nevertheless be performed, especially if skin corrosion sub-categorisation is required. It should also be noted that although prediction of skin corrosion based on pH extremes shows a very high specificity (> 90%), and therefore a low number of false positives (Worth *et al*., 1998), it cannot be ruled out that some substances may be overpredicted if classification is based solely on pH data. However, substances that have other pH values will need to be considered further for their potential for skin corrosion/irritation.This model is included in the OECD IATA for skin corrosion and irritation (OECD, 2014b). Several studies have investigated and confirmed the usefulness of pH as a predictor of corrosion (Worth and Cronin, 2001) and as an element in tiered testing strategies (Worth, 2004).

Where extreme pH is the only basis of classification as corrosive, it may also be important to take into consideration the acid/alkaline reserve, i.e. a measure of the buffering capacity of a substance (Young *et al*., 1988; Botham *et al*., 1998; Young and How, 1994). However, it should be noted that for pure substances the sensitivity of pH for identifying skin corrosivity may actually be significantly reduced when combined with acid/alkaline reserve information (Worth *et al*., 1998). 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 (see Section 3.2.2.2 of Annex I to the CLP Regulation).

Grouping, (Q)SARs and expert systems

Guidance has been developed by the former 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 of Chapter R.6 of the *Guidance on IR&CSA* and the OECD Guidance document on the validation of (Q)SAR models (OECD, 2007). Essentially, the determination of whether a (Q)SAR result may be used to replace a test result can be broken down into three main steps:

1. an evaluation of the scientific validity (relevance and reliability) of the model,

2. an assessment of the applicability of the model to the chemical of interest and the reliability of the individual model prediction,

3. an assessment of the adequacy of the information for making the regulatory decision, including an assessment of completeness, i.e. whether the information is sufficient to make the regulatory decision, and if not, what additional (experimental) information is needed.

Model validity assessment needs to be performed along the lines of the OECD principles for (Q)SAR validation (OECD, 2007), e.g. in terms of a defined endpoint, an unambiguous algorithm, a defined applicability domain, the statistical characteristics (“goodness-of-fit”), and mechanistic interpretation.

Inter alia the following questions should be addressed when assessing the reliability of an individual prediction:

1. Is the chemical of interest within the scope of the model, according to the defined applicability domain of the model?

2. Is the defined applicability domain suitable for the regulatory purpose?

3. How well does the model predict chemicals that are similar to the chemical of interest?

4. Is the model estimate reasonable, taking into account other information?

The mechanism of skin corrosion and irritation involves toxicodynamic and toxicokinetic parameters. Models that predict skin corrosion and irritation based on toxicodynamic properties only (e.g. acidity or basicity, electrophilicity, other reactivity, surfactant activity, solving membranes) have to be additionally evaluated for their consideration of toxicokinetic parameters (or have to be used in concert with data covering such parameters) related to the potential to cross relevant outer membranes of the skin (stratum corneum) to be active in the living tissue underneath. Conversely models that predict (the absence of) corrosion and irritation solely from e.g. physico-chemical properties considered to illustrate the toxicokinetic behaviour of a substance, should be evaluated for their consideration of its activity (toxicodynamics), in particular for potential corrosivity (where the corrosive action itself may lead to membrane destruction and subsequent tissue damage).

For example, the BfR rule-base implemented in Toxtree and the OECD QSAR Toolbox contains both physico-chemical exclusion rules and structure-based inclusion rules (structural alerts). Evaluations of these rules for the prediction/exclusion of skin corrosion/irritation (Rorije and Hulzebos, 2005, on the physico- chemical exclusion rules; Gallegos Saliner *et al.*, 2007, on the structural alerts) have been carried out in accordance with the OECD principles for (Q)SAR validation (see Appendix R.7.2-2). However, inclusion and exclusion rules were evaluated separately, and not used in concert in these works.

When applied, these two sets of rules might sometimes provide contradictory information, i.e. a structural alert might indicate corrosion/irritation potential, while at the same time, based on physico-chemical properties, absence of effect is predicted. In such cases, it is recommended to consider additional information (e.g. on skin permeability or on the behaviour of chemically similar substances). In other cases, applicability of one (or more) of the physico-chemical exclusion rules might indicate absence of a corrosion/irritation potential of the target substance, while no structural alert for corrosion/ irritation is triggered. Given that the absence of any known structural alert is not equivalent to the absence of a potential effect, in such a situation still the substance should be examined for potentially reactive substructures (and looking at the behaviour of chemical analogues still will be beneficial).

While these considerations apply to the use of the BfR rule-base for direct classification/non-classification, less certainty might be required e.g. for a decision on further *in vitro* testing: where the exclusion rules suggest the absence of an effect, a bottom-up approach might be followed, i.e. a test for irritation and not corrosion might be initiated (see Section R.7.2.6.2).

There is no other model available which sufficiently describes the absence of effects. Neutral organics[9] are expected not to be irritants, however their defatting potential should be discussed. Absence of reactivity needs to be described in sufficient detail or be substantiated with other information.

Testing data on skin corrosion / irritation

In vitro data

There are EU and OECD adopted test guidelines (see Section R.7.2.3.1), under which substances can be classified as skin corrosives, skin irritants, or not classified.

[9] By definition a neutral organic is a chemical which does not have potential reaction centres, even after skin metabolism.

Annex VII to the REACH Regulation requires information from the *in vitro* tests specified below for skin corrosion/irritation, not from animal tests. Guidance on how *in vitro* data can also be used to fulfil Annex VIII requirements, is given in Section R.7.2.6 of this document[10].

Data from the following types of test can be used for Annex VII requirements and may be accepted for Annex VIII requirements for skin corrosion/irritation when general rules for adaptation specified in Annex XI are used:

- For skin irritation:
  - **Reconstructed human epidermis (RHE) tests** (EU B.46/OECD TG 439): These tests are considered scientifically valid for the prediction of irritant (Category 2) and non-irritant (No category) substances for Annex VII purposes, and also Annex VIII according to the rules laid down in Annex XI (see Section R.7.2.6 of this Guidance).

  The specific scope and limitations of these tests are:

  - They discriminate skin irritants (Category 2) from substances not classified for skin irritation (No Category) under CLP. However, they cannot discriminate skin irritants (Category 2) from skin corrosives (Category 1). Such discrimination needs to be addressed with an *in vitro* skin corrosion test.
  - Cell viability in these models is measured by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue) assay. If a test substance acts directly on the MTT (e.g. is a direct MTT-reducer), is naturally coloured, or becomes coloured during tissue treatment, additional controls should be used to detect and correct for test substance interference with the viability measurement technique. Detailed description of how to correct for direct MTT reduction and interferences by colouring agents is available in the Standard Operating Procedures (SOPs) for the four validated test methods and referenced in the OECD and EU TGs.[11]
  - The use of this test method may not be applicable to all groups of chemical classes. For example metals or inorganic metal compounds were not included in the validation study and there is experience that some metals (e.g. cobalt) may give a false positive result.
  - They do not allow testing of gases and aerosols.

- For skin corrosion:
  - **Transcutaneous electrical resistance (TER) test method** (EU B.40/OECD TG 430)

[10] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

[11] A revision of OECD TG 439 including the use of HPLC/UPLC-spectrophotometry as an alternative way to measure MTT formazan is currently under discussion at the OECD with a high probability of adoption in April 2015. If this revision is accepted, it will reduce the limitation of these test methods towards strongly coloured substances.

- **Reconstructed Human Epidermis (RHE) test method** (includes more than one protocol) (EU B.40 bis/OECD TG 431)
- ***In vitro* membrane barrier test method** (OECD TG 435)

All the above-mentioned tests allow for the discrimination of skin corrosives (Category 1) from non-corrosive substances.

The specific scope and limitations of these tests are:

- None of them allows testing of gases and aerosols.
- Only the ***in vitro* Membrane Barrier** test method for skin corrosion is accepted to discriminate between skin corrosive subcategories 1A, 1B and 1C and non-corrosives.
- The ***in vitro* Membrane Barrier** test method has a limited applicability domain (only acids, bases and acid derivatives). In addition, test materials not causing detectable changes in the detection system (e.g. typically 4.5 < pH < 8.5) cannot be tested.
- The **RHE** test method can be used to distinguish subcategory 1A from subcategories 1B and 1C. The protocol of **EpiSkin,** which is one of the four validated methods included in the RHE test guideline, also allows for the discrimination of subcategory 1B from subcategory 1C and, according to the OECD IATA (OECD, 2014b), this information may be used in a *Weight-of-Evidence* assessment.
- TER cannot be used to subcategorise skin corrosive substances.
- The use of **RHE** test method may not be applicable to all groups of chemical classes. For example there is reasonable doubt on the adequacy of this model for certain groups of Fatty Amine Derivatives where RhE assays did not predict corrosivity, whereas these substances were corrosive in *in vivo* rabbit studies (Houthoff *et al.*, 2014). Furthermore, metals or inorganic metal compounds were not included in the validation study and there is experience that some metals (e.g. cobalt) may give a false positive result.
- In relation to cell viability measurement by the MTT assay in **RHE** models, the same limitations as those specified above for the *in vitro* skin irritation test (EU method B.46/OECD 439) apply.

- Quality aspects of existing *in vitro* data:

For quality assessment of existing *in vitro* data that will lay the basis for later possible *Weight-of-Evidence* considerations, see Section R.4.4 of Chapter R.4 of the *Guidance on IR&CSA*, and for aspects that need to be taken into account in such a *Weight of Evidence* see Section R.5.2.1.2 of Chapter R.5 of the *Guidance on IR&CSA*.

Animal data

Well-reported studies, particularly if conducted in accordance with the principles of GLP, can be used to identify substances which would be considered to cause, or not to cause, skin

corrosion or skin irritation. There may be a number of skin corrosion/irritation studies already available for an existing substance, none of which are fully equivalent to an OECD TG or an EU test method such as those in the Annex to the EU Test Methods Regulation. If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the skin corrosion/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 irritation 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.

Data from studies other than skin corrosion/irritation studies (e.g. other toxicological studies on the substance in which local responses of skin 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 irritation. More notably, skin reactions and symptoms are not systematically scored in e.g. acute and sub-acute dermal toxicity studies since these studies are not specifically designed to address skin corrosion/irritation.

- Quality aspects of existing *in vivo* data:

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 of Chapter R.4 of the *Guidance on IR&CSA*, and a judgment will need to be made as to whether any further testing is required. Some examples to note are:

i. Was the animal species used the rabbit or was it another species such as the rat or the mouse? The rat and the mouse are not as sensitive as the rabbit for irritation testing.

ii. How many animals were used? Current methodology requires a maximum of 3 animals tested in a sequential manner (with 1 or 2 animals being sufficient if skin corrosion effects are observed in the first or the second tested animal, respectively) but 6 were frequently used in the past (See sectionSection 3.2.2.3.2.2 of the *Guidance on the application of the CLP criteria* for the evaluation of results from tests that have been conducted with more than 3 animals).

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. Which exposure period was used? Single or repeated exposure?

v. The method used to apply the substance to the skin should be noted i.e. whether occluded or semi-occluded and 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.

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 expert judgment may be required to avoid repeat testing.

Observations such as those 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.

R.7.2.4.2 Human data on skin corrosion / irritation

Well-documented *existing* human data of different sources can often provide very useful information on skin corrosion/irritation , sometimes for a range of exposure levels. Often the only useful information available on irritation is obtained from human experience (e.g. 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.

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 skin corrosion or skin irritation 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 irritation are provided in an 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 and will present complete areas of alopecia and scars.

In addition to human data on local skin effects, which originate from clinical and occupational studies, poison information centres, case reports and retrospective epidemiological studies, existing human data from skin irritation human patch testing (HPT) might also be available. HPT is a controlled study involving the exposure of small patches of skin of human volunteers to substances for which skin corrosion and other unacceptable toxicological hazards can be excluded. HPT data have been compiled for example by Jírová *et al.* (2010), Basketter *et al.* (2012), as well as Ishii *et al.* (2013). Testing with human volunteers to obtain primary hazard data on skin corrosion/irritation for regulatory purposes is discouraged. Available good quality data should nevertheless be considered as appropriate and used for Classification and Labelling decision making. It should however be noted that the CLP Regulation does not contain clear criteria for classification for skin irritation based on human data.

R.7.2.4.3 Exposure considerations for skin corrosion / irritation

Exposure-based waiving from testing is not applicable to the endpoints of skin corrosion/irritation. Exposure-based waiving from testing as specified in Annex XI (3) of the

REACH Regulation only applies to tests listed in 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 skin corrosion / irritation

Usually it is possible to unequivocally 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 (e.g. because of poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, small numbers of subjects).

Data from studies in animals and from *in vitro* tests performed according to internationally accepted test methods will usually give relevant information on the skin corrosion/irritation potential of a substance. In general, it is assumed that substances which cause skin corrosion/irritation in EU or OECD TG-compliant studies in animals or *in vitro* will cause skin corrosion/irritation in humans, and those which are not irritant in EU or OECD TG-compliant studies will not be irritant in humans (Please note that in general test animals are considered to be more sensitive to skin corrosion/irritation effects than humans (e.g. OECD, 2014b)). It should be borne in mind that one of the limitations of the *in vivo* corrosion/irritation studies is the subjective grading of the lesions. Moreover, inconsistent results from a number of similar studies increase the uncertainty in deriving data from animal or *in vitro* studies.

The scope of the *in vitro* tests for corrosion/irritation has also some limitations, as explained in Section R.7.2.4.1 under “Testing data on corrosion/irritation”. In addition inconsistent results from two or more *in vitro* tests could add to the overall uncertainty in interpreting the data.

R.7.2.5 Conclusions on skin corrosion / irritation

R.7.2.5.1 Concluding on suitability for Classification and Labelling

In order to conclude on Classification and Labelling according to the CLP Regulation, all the available information needs to be taken into account, and consideration should be given to both the *Guidance on the application of the CLP criteria* and the various remarks (as they relate to Classification and Labelling) made throughout this guidance document[12].

R.7.2.5.2 Concluding on suitability for Chemical Safety Assessment

A dose-response assessment is difficult to make for skin corrosion/irritation simply because up to the present time most data have been produced with undiluted substances 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 and dose-response data and for local effects the concentrations is often the determinative dose metric. Consequently, if dose-response data are available, they must be taken into account (see Figure R.7.2-1). For instance, dose-response information might be available from sub-acute or sub-chronic dermal

[12] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

toxicity studies (as such studies require a determination of a non-irritant dose in the dose selection), from human experience, and may in certain cases be determined using *in vitro* studies. However, when information is used from existing dermal toxicity studies (e.g. repeated dose), it should be noted that the test conditions do not reflect the test conditions used in the *in vivo* skin corrosion/irritation study: e.g. test material is applied in dilution *vs.* neat, vehicles/solvents are often used, exposure duration is different and test material application areas differ (see Module 5 of the OECD IATA (OECD, 2014b)).

Guidance on the possibilities for derivation of DNELs for skin corrosion/irritation is given in Appendix R.8-9 of Chapter R.8 of the *Guidance on IR&CSA*.

R.7.2.5.3 Information not adequate

A *Weight-of-Evidence* approach comparing available adequate information with the tonnage-triggered information requirements under REACH may result in the conclusion that the requirements are not fulfilled. In order to proceed to further information gathering, the testing and assessment strategy described in Section R.7.2.6 below is recommended.

R.7.2.6 Testing and assessment strategy for skin corrosion / irritation

The OECD has approved an IATA for skin corrosion/irritation (OECD, 2014b), which includes a description of various types of data that can be used in the assessement of these hazards. The IATA has a modular approach, where the *domain, role in IATA, strengths, weaknesses and limitations* of each type of data are given in a tabular form. Some parts of the IATA provide more detailed scientific background than the present document. Furthermore, the IATA gives detailed guidance on the *Weight-of-Evidence* approach. At the *Weight-of-Evidence* step, all existing information is integrated and assessed in order to decide whether further *in vitro* testing of the substance (or *in vivo* testing as a last option if *in vitro* testing is not possible or not conclusive) is necessary. While the OECD IATA provides slightly more detailed guidance than the testing and assessment strategy below, there is no conceptual difference between these two.

R.7.2.6.1 Objective / General principles

The following testing and assessment strategy is recommended for developing adequate and scientifically sound data for assessment/evaluation and classification of the skin corrosive and skin irritating properties of substances. For existing substances with insufficient data, this strategy can also be used to decide which additional data, beside those already available, are needed. The testing and assessment strategy is aimed for the identification of skin corrosion/irritation by using different elements where appropriate depending on the information available. A principle of the strategy is that the results of one study or an information source 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 different elements provided in Figure R.7.2-1 describe information sources that can be used to conclude on a substance hazard potential towards skin. The elements described in Figure R.7.2-2 can be rearranged as appropriate, especially in Part 1. This may be particularly helpful in cases where a conclusion can be drawn from certain elements without having to consider all of them. If judged relevant, elements in Part 1 can be skipped and *in vitro* testing can be performed immediately.

Figure R.7.2-2 is divided into three parts where Part 1 aims at evaluating existing information that may be available on the substance. In Part 2 existing information and relevant data

should be assessed in order to consider if there is enough information available to conclude on the substance hazard properties within a *Weight-of-Evidence* analysis, in case it is not possible to make a conclusion based on single elements described in Part 1. In case no conclusion can be drawn in Parts 1 and 2, new data should be generated in Part 3 by first performing relevant *in vitro* testing. Only in case no conclusion can be drawn based on the *in vitro* testing, can *in vivo* testing be performed (for substances at or above 10 tonnes per annum only).

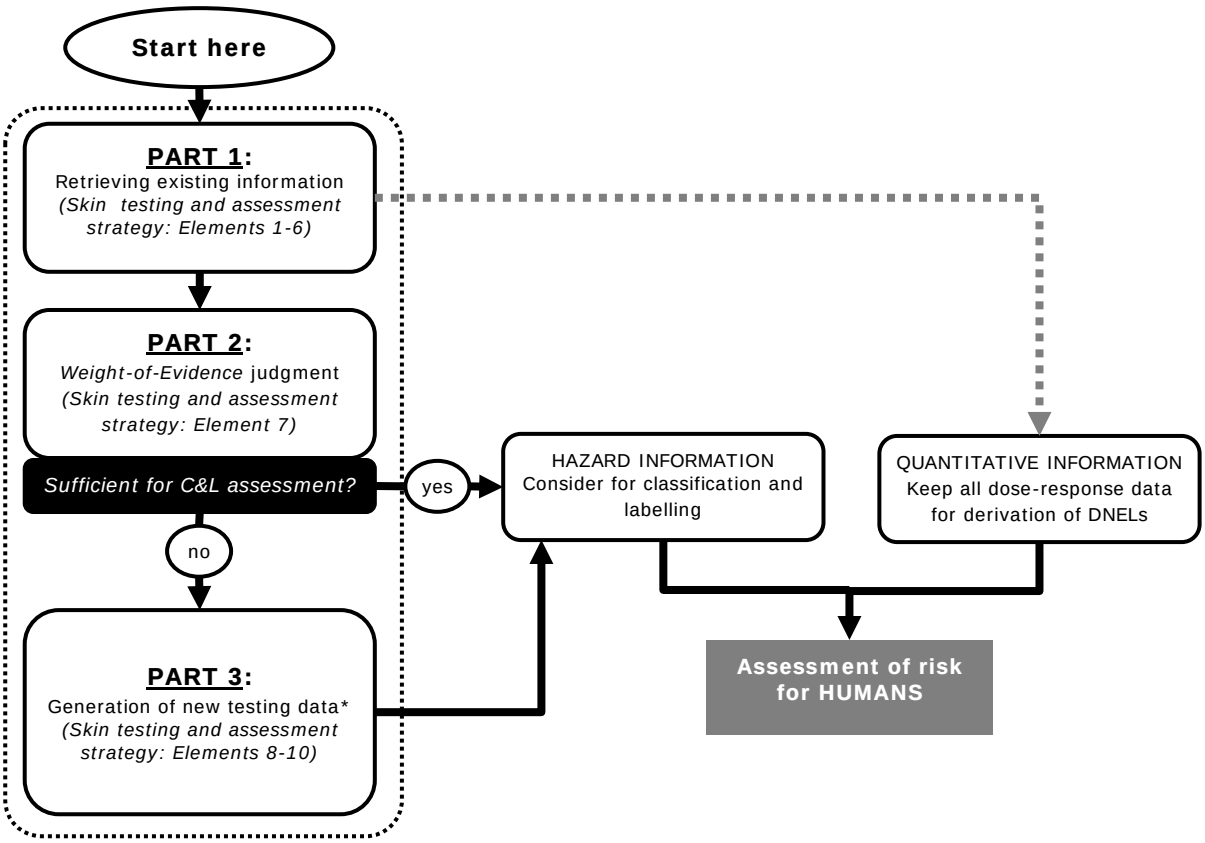
Some guidance for testing is provided by the specific rules for adaptation from standard information requirements, as described in column 2 of Annexes VII-X to the REACH Regulation, together with some general rules for adaptation from standard information requirements in Annex XI.

Risk assessment of the skin corrosion/irritation potential of a substance is normally made in a qualitative way provided that the substance has been classified as being corrosive or irritant to the 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 gathering 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 testing strategy and to perform a complete risk assessment, comprising qualitative hazard as well as quantitative information.

It is recommended that the testing and assessment strategy be followed until element 6 (Figure R.7.2-1 and Figure R.7.2-2) in all cases and thereafter the *Weight-of-Evidence* analysis be performed. Clearly, all information sources/elements can be rearranged as appropriate, i.e. not all elements will necessarily be accompanied by data but it is important that all potential data sources are explored prior to starting the *Weight-of-Evidence* analysis. While it is recommended that this approach be followed, other approaches may be more appropriate and efficient on a case-by-case basis. For example, in case there is no existing data and it is anticipated that generation of “pre-testing data” would be non-conclusive, it may be appropriate to directly proceed to the information generation part. Furthermore, prior to performing any new *in vivo* test, the use of *in vitro* methods should be fully exploited (see Articles 13(1) and 25(1) of the REACH Regulation) by using the general rules of Annex XI for adaptation of the standard testing regime set out in Annexes VII to X.

If the substance is not classified for skin corrosion/irritation, no risk assessment for this endpoint is performed, regardless of the exposure. Please note that there are no options for exposure-based waiving for these endpoints in the REACH Regulation.

The following flow chart (Figure R.7.2-1) gives an overview of a possible approach for defining a testing and assessment strategy for skin corrosion and irritation.



*Generation of new testing data according to Annex VII to VIII to the REACH Regulation and with due observation of the rules for adaptation of the standard testing regime laid down in Annex XI.[13]

Figure R.7.2-1 Overview of the testing and assessment strategy for skin corrosion / irritation

R.7.2.6.2 Testing and assesment strategy for skin corrosion / irritation

Recommended approach

The testing and assessment strategy presented here comprises three parts (see Figure R.7.2-2): Part 1 (elements 1 to 6) is about retrieving existing information, Part 2 (element 7) represents a *Weight-of-Evidence* analysis and expert judgment, and Part 3 (elements 8 to 10) is about the generation of new information by testing.

In Part 1, existing and available information from the literature and databases is gathered and considered in the strategy approach. The order of the different elements, i.e. 1 to 6, is only indicative and they may be arranged as appropriate. This may be especially helpful in cases where a reliable conclusion can be drawn from certain elements without having to consider all of them. For instance, if the substance has an extreme pH ($\leq$ 2.0 or $\geq$ 11.5) skin corrosivity is

[13] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

considered implicit (element 1c) and therefore the substance should be classified as skin corrosive (Category 1) according to CLP and further testing is not required. At the end of Part 1, and if no final conclusion could be derived directly from one or several of the available pieces of information, all the information collected should be analysed using a *Weight-of-Evidence* approach (element 7).

In the information generation part (elements 8 to 10), new information on the corrosion/irritation potential of substances is produced by means of *in vitro* (elements 8 and 9) or, as a last resort (see Articles 13(1) and 25(1) of the REACH Regulation), *in vivo* testing (element 10). Therefore, before concluding the *Weight-of-Evidence* analysis in element 7 and *in vitro* testing (elements 8 and 9), new *in vivo* tests should not be conducted. More information on how to use the *in vitro* methods for skin corrosion/irritation within the testing strategy can be found in the following paragraphs.

While it is recommended that this approach be followed, other approaches may be more appropriate and efficient on a case-by-case basis. For example, in case there is no existing data and it is anticipated that compilation of data at elements 1-7 would be non-conclusive, it may be appropriate to directly proceed to the information generation part.

Figure R.7.2-2 Testing and assessment strategy for evaluating the skin corrosion/irritation potential of substances.

Element	Information	Conclusion[14]
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable in contact with air (pyrophoric) or water at room temperature? →	**YES**: No testing required (Column 2 adaptation in section 8.1 of Annexes VII and VIII)
1b	Is the substance an organic hydroperoxide or an organic peroxide? →	**YES**: Consider classifying as: ■ corrosive (Skin Corrosive Cat. 1B)) if the substance is a hydroperoxide, or ■ irritant (Skin Irritant Cat. 2) if the substance is a peroxide. **OR** Provide evidence supporting deviating classification or non-classification[15].
1c	Is the pH of the substance ≤ 2.0 or ≥ 11.5? [a] →	**YES**: Consider classifying as corrosive (column 2, section 8.1. of Annex VIII) if pH is used as the sole basis for classification decision. Where classification is based upon consideration of pH alone, subcategorisation is not possible and therefore Skin Corrosive Cat.1 should be applied. Where consideration of alkali/acid reserve suggests that the substance is not corrosive, this has to be confirmed (preferably by using an appropriate *in vitro* test).
1d	Are there other physical or chemical properties that indicate that the substance is corrosive/irritant? →	**YES**: Use this information for *Weight-of-Evidence* analysis (Element 7).
Existing human data		
2	Are there adequate existing human data[b] which provide evidence that the substance is a corrosive or irritant? →	**YES**: Consider classifying accordingly.

[14] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

[15] Information on e.g. *in vitro* testing may provide evidence on a more suitable classification, if there is some doubt on the correct classification.

Existing animal data from corrosion/irritation studies		
3	Are there data from existing studies *on corrosion and irritation* in laboratory animals, which provide sound conclusive evidence that the substance is a corrosive, irritant or non-irritant? →	**YES**: Consider classifying accordingly (either Skin Corrosive Cat. 1, 1A, 1B, 1C or Skin Irritant Cat. 2) or consider no classification.
Existing data from general toxicity studies via the dermal route and from sensitisation studies		
4a	Is the substance classified as fatal in contact with skin ($LD_{50} \leq 50$ mg/kg bw, CLP hazard statement H310)? [c] →	**YES**: The substance will be classified for acute dermal toxicity (column 2 adaptation in section 8.1 of Annexes VII and VIII). No testing for skin irritation/corrosion is needed in this case.
4b	Has the substance proven to be a corrosive, irritant or non-irritant in a suitable acute dermal toxicity test? [d] →	**YES**: If test conditions are consistent with OECD TG 404, consider classifying accordingly (Skin Corrosive Cat. 1, 1A, 1B, 1C or Skin Irritant Cat. 2) or consider no classification.
4c	Has the substance proven to be a corrosive or an irritant in sensitisation studies or after repeated exposure? [e] →	**YES**: This information cannot be used for considering a concrete classification conclusion but must be used exclusively within the integrated *Weight-of-Evidence* judgment.
Existing/new (Q)SAR data and read-across		
5a	Are there structurally related substances (suitable "read-across" or grouping), which are classified as corrosive to the skin (Skin Corrosive Cat. 1), or do suitable (Q)SAR methods indicate corrosion potential of the substance? [f] →	**YES**: Consider classifying as Skin Corrosive Cat. 1.
5b	Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritant to the skin (Skin Irritant Cat. 2), or indicating that the substance is non-irritant, or do suitable (Q)SAR methods indicate irritant or non-irritant potential of the substance? [f] →	**YES**: Consider classifying accordingly.
Existing in vitro data		
6a	Has the substance demonstrated corrosive properties in an EU/OECD adopted *in vitro* test? Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions defined in Annex XI are met. →	**YES**: Consider classifying as corrosive. If discrimination between Skin Corrosive Cat. 1A, 1B and 1C is not possible, Cat. 1 must be chosen. If a negative result is obtained and there is no existing data from an *in vitro* skin irritation study(ies), the

		irritation potential must be determined, e.g. with an *in vitro* skin irritation test.
6b	Has the substance demonstrated irritant or non-irritant properties in an EU/OECD adopted *in vitro* test? Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions defined in Annex XI are met. →	**YES**: Consider classifying accordingly (Skin Irritant Cat. 2) or consider no classification. If a positive result is obtained and there is no exisiting data from an *in vitro* skin corrosion study(ies), the corrosion potential must be determined e.g. with an *in vitro* skin corrosion test (Element 8).
6c	Are there data from a non-validated suitable *in vitro* test(s), which provide sound conclusive evidence that the substance is corrosive/ irritant? [g] →	**YES**: Consider classifying accordingly (Skin Corrosive Cat 1, 1A, 1B, 1C or Skin Irritant Cat. 2).
Weight-of-Evidence analysis		
7	The "elements" described above may be arranged as appropriate. Taking all available existing and relevant data mentioned above (Elements 1-6) into account, is there sufficient information to make a decision on whether classification/labelling is necessary, and – if so – how to classify and label? →	**YES**: Classify accordingly (Skin Corrosive Cat. 1, 1A, 1B, 1C or Skin Irritant Cat. 2) or consider no classification. If discrimination between Skin Corrosive Cat 1A, 1B and 1C is not possible, Cat. 1 must be chosen.
New in vitro tests for corrosivity [g]		
8	Does the substance demonstrate corrosive properties in an EU/OECD adopted *in vitro* test(s) for skin corrosion? → Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions defined in Annex XI are met.	**YES**: Classify accordingly (Skin Corrosive Cat. 1A, 1B or 1C). If discrimination between Cat. 1A, 1B and 1C is not possible, Cat. 1 must be chosen. If a negative result is obtained, the irritation potential of the substance must be determined, e.g. with an *in vitro* skin irritation test (Element 9), in order to determine if the substance should be classified as Skin Irritant Cat. 2 or not classified.
New in vitro tests for irritation [g]		
9	Does the substance demonstrate irritating or non-irritating properties in an EU/OECD adopted *in vitro* test(s) for skin irritation? Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions defined in Annex XI are met. →	**YES**: Classify accordingly (Skin Irritant Cat. 2) or consider no classification. If a positive result is obtained and there is no existing data from an *in vitro* skin corrosion study(ies), the corrosion potential must be determined e.g. with an *in vitro* skin corrosion test (Element 8).

		If a conclusion on skin corrosion/irritation cannot be drawn by using *in vitro* testing, *in vivo* testing should be performed (at Annex VIII level only).
New in vivo test for corrosion/irritation as a last resort (Annex VIII to the REACH Regulation) [h]		
10	Does the substance demonstrate corrosive or irritant properties in an EU/OECD adopted *in vivo* test? →	**YES**: Classify accordingly (Skin Corrosive Cat. 1, 1A, 1B, 1C or Skin Irritant Cat. 2). **NO:** No classification needed.

Notes to the information scheme on skin corrosion/irritation:

[a)] Note that if the buffering capacity suggests that the substance may not be corrosive, further data are needed to confirm this, preferably using an appropriate *in vitro* test method.

[b)] Data from case reports, occupational experience, poison information centres, HPTs or from clinical studies.

[c)] If the substance is classified as fatal in contact with skin ($LD_{50} \leq 50$ mg/kg bw), further testing for skin corrosion/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 Hazard statement H310 *"Fatal in contact with skin"* and the GHS Pictogram GHS06 with the signal word "Danger". The classification as fatal in contact with skin requires strict risk management measures and hence, since all contact with the skin must be avoided, there is no need to investigate the skin corrosion/irritation potential further.

[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 Skin Corrosive (subcategorisation as 1A, 1B or 1C, where possible). 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 EU B.4/OECD TG 404 for dermal corrosion/irritation test with rabbits. If in the same range and adequate scoring of skin effects is provided, classify or not as Skin Irritant Category 2. In case conclusive negative data was obtained in rabbits, stop. If not in the same range and inadequate scoring of skin effects, use for *Weight-of-Evidence* analysis and proceed.

In case the test was performed in other species, which may be less sensitive (e.g. **rat**), 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 about 5x5 cm. Assuming a mean body weight of 250 g, 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 Skin Corrosive Category 1. All other data should be used for *Weight of Evidence*.

[e)] Regarding data from skin sensitisation 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

sensitisation test (Maximisation or Buhler) with the neat material or dilutions of solids in water, physiological saline or vegetable oil, the test substance should be classified as Skin Corrosive Category 1. 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 *Weight of Evidence* only. Information on irritant properties from skin sensitisation tests cannot be used to conclude a specific classification regarding acute skin irritation but may be used in a *Weight-of-Evidence* 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 to the REACH Regulation. Prediction of the absence of the classified effect can be made either by triggering an exclusion rule in the BfR system (to be checked on a case-by-case basis), or based on a negative prediction in a classification QSAR that was trained on both positive and negative substances. The suitability of the model (reliability, relevance) should be very carefully checked to make sure that the prediction is fit for purpose, and the applicability of the model to the substance should also be justified (e.g fulfilment of the conditions of Section 1.3 of Annex XI to the REACH Regulation should be checked). For read-across, generation of new *in vivo* data should be avoided.

g) New *in vitro* testing should be performed following a top-down or bottom-up approach. Please see the following paragraph “How to use the *in vitro* methods for skin corrosion/irritation within the strategy”. While it may be appropriate to use information from non-validated *in vitro* tests if already existing, it is highly recommended to adhere to the test protocols whose scientific validity has been established by formal validation and which, ideally, have been officially adopted by the European Commission and/or by the OECD. Data obtained from suitable non-validated suitable *in vitro* tests can only be used according to the criteria set out in Annex XI, section 1.4 of the REACH Regulation, i.e. only positive results can be accepted.

h) *In vivo* testing should not be conducted in case the substance falls under the scope of the specific *in vitro* tests performed, and there are no substance-specific limitations to use those tests, and the Registrant formulates an adaptation according to Annex XI to the REACH Regulation. Due to the current standard *in vivo* information requirement at Annex VIII level and above, an adaptation needs to be built up in a registration dossier in order to successfully submit a compliant dossier.[16]

How to use the *in vitro* methods for skin corrosion / irritation within the strategy

For skin corrosion and irritation no single *in vitro* test method can fully replace the *in vivo* test (EU TM B.4 / OECD TG 404) across the full range of skin responses. However, the *in vitro* methods specified in Section R.7.2.3.1 and R.7.2.4.1 may replace the *in vivo* test depending on the outcome of the study or when combined within a tiered testing strategy.

New *in vitro* testing should be performed following a top-down or bottom-up approach (Figure R.7.2-3). The top-down approach should be used when available information suggests that the substance may be irritant or corrosive to the skin. The bottom-up approach, on the other hand, should be followed only when available information suggests that the substance may not be irritant to the skin.

[16] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

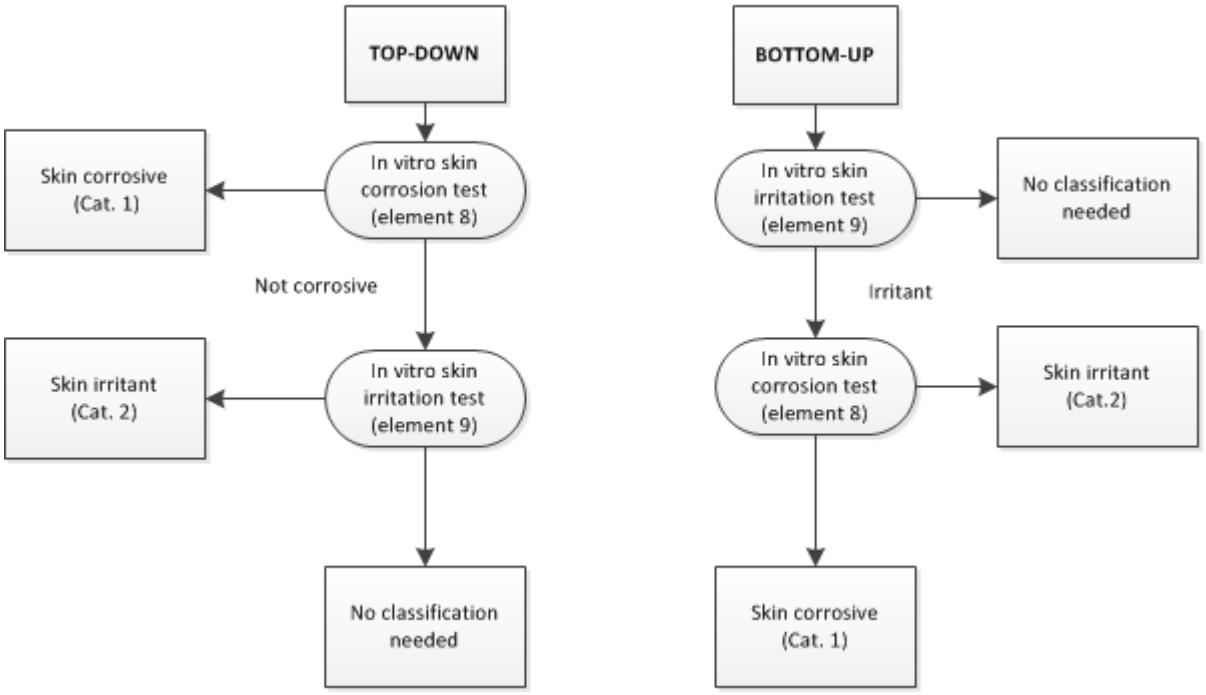


Figure R.7.2-3 Schematic presentation of Top-down and Bottom-up approaches for Skin Corrosion / irritation.

There are steps to be considered before any testing (*in vitro* or *in vivo*) is conducted. These steps are specified in Section 8.1 in column 1 of Annexes VII and VIII to the REACH Regulation and also in Figure R.7.2-2.

After these steps, no *in vivo* testing, as specified in section 8.1 of Annex VIII, is necessary in the case where:

a) the substance falls under the scope of the specific *in vitro* tests performed, and there are no substance-specific limitations to using those tests, and

b) the Registrant formulates an adaptation according to Annex XI to the REACH Regulation.

If an *in vivo* study for skin irritation is a standard information requirement (i.e. for substances registered at or above 10 tonnes per annum) and the steps above have been followed, the Registrant should choose to adapt the standard information requirement for the *in vivo* study by using Annex XI adaptation possibilities. Due to the current standard *in vivo* information requirement at Annex VIII level and above, an adaptation needs to be built up in a registration dossier in order to successfully submit it.[17]

[17] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

Instructions on how to submit *in vitro* information instead of *in vivo* can be found e.g. in Section 3.7 of *Practical Guide 1: How to report in vitro data* (available at http://echa.europa.eu/practical-guides).

It is important to note that it is the responsibility of the registrant to ensure that the chosen test method is suitable for the substance in order to obtain adequate information from the *in vitro* studies. For most substances, the use of adopted EU or OECD TGs for skin corrosion/irritation purposes will provide results that will have regulatory acceptance under REACH.

SERIOUS EYE DAMAGE/EYE IRRITATION

R.7.2.7 Information requirements for serious eye damage/eye irritation

The information on serious eye damage/eye irritation that is required to be submitted for registration and evaluation purposes is specified in Annexes VI to XI to the REACH Regulation. According to Annex VI, the registrant should gather and evaluate all existing available information before considering further testing. This includes physico-chemical properties, (Q)SAR ((Quantitative) Structure-Activity Relationship), grouping, *in vitro* data, animal studies, and human data. For classified substances, information on exposure, use and risk management measures should also be collected and evaluated in order to ensure safe use of the substance.

If these data are inadequate for hazard and risk assessment, further testing should be carried out in accordance with the requirements of Annexes VII (≥1 tpa) and VIII (≥10 tpa) to the REACH Regulation.

R.7.2.7.1 Information requirements for quantities of ≥1 tpa (Annex VII to the REACH Regulation) [18]

If new testing data are necessary, these must be derived from *in vitro* methods only. Annex VII does not foresee *in vivo* testing for for serious eye damage/eye irritation.

The standard information requirements at this tonnage level for serious eye damage/eye irritation (see Section 8.2 in Column 1 of Annex VII) can be satisfied by following three steps: (1) assessment of the available human and animal data, (2) assessment of the acid or alkaline reserve, (3) *in vitro* eye irritation study (Please note that when the REACH Regulation refers to the "eye irritation" endpoint, this covers both serious eye damage and eye irritation).

Section 8.2 in Column 2 of Annex VII lists specific rules for adaptation according to which step 3 is not necessary. These rules are applicable when:

1. the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes (Please note that when a substance is classified as Skin corrosive Category 1 under the CLP Regulation, the risk of severe damage to eyes is considered implicit and the substance is also classified in Category 1 for Serious eye damage), or

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").

[18] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

R.7.2.7.2 Information requirements for quantities of ≥10 tpa (Annex VIII to the REACH Regulation) [19]

For substances manufactured or imported in quantities of ≥10 tpa *in vivo* testing is the standard information requirements of Annex VIII (Column 1) for serious eye damage/eye irritation, in case the information requirement cannot be met with the information obtained as specified in section 8.2 of Annex VII.

Before new tests are carried out to determine the properties listed in Annex VIII, all available *in vitro* data, *in vivo* data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) must be assessed first. Due to the sequential nature of the REACH standard information requirements, it is reminded that at quantities of ≥10 tpa, the information requirements of Annex VII to the REACH Regulation also apply. This means that before a new *in vivo* test is performed, the appropriate *in vitro* testing must be undertaken according to the rules set out in section 8.2 of Annex VII and must be documented in the technical dossier (IUCLID). Finally, the information generated at Annex VII level must be taken into account in determining whether an *in vivo* test at Annex VIII level is really needed.

Column 2 of Annex VIII lists the following specific rules that allow deviating from the standard information required by Annex VIII for serious eye damage/eye irritation:

- the substance is classified as irritating to eyes with risk of serious damage to eyes, or
- the substance is classified as corrosive to the skin and provided that the registrant classified the substance as eye irritant (Please note that the reference to eye irritation here means Category 1 for Serious eye damage, according to the CLP Regulation), 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").

The *in vitro* methods that can be used to adapt the standard information requirements are detailed in Sections R.7.2.8.1 and R.7.2.9.1 of this Guidance, under "*In vitro* data". It should be noted that the use of an EU or OECD adopted *in vitro* test methods (one or several in combination) on serious eye damage/eye irritation may provide adequate information for the **replacement** of the regulatory *in vivo* test (EU TM B.5 / OECD TG 405). The standard testing requirement of Annex VIII should be adapted according to the general rules laid down in Annex XI, allowing to avoid unnecessary animal testing as required in Annex VIII by the use of non-testing data or *in vitro* testing (see Section R.7.2.8.1 of this Guidance for possible alternatives to animal testing), and in order to successfully submit a compliant dossier.[19]

Guidance on the application of these rules is given in the testing and assessment strategy for serious eye damage/eye irritation described in Section R.7.2.11 of this Guidance.

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[19] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

It should be noted that the conditions of acceptance by ECHA of implementation of any of the adaptation rules laid down in Annex XI are strict, and whenever an adaptation argument is being used (e.g. use of (Q)SARsSARS, read-across or non-validated *in vitro* test methods), scientific justification, solid documentation and readiness for risk assessment and Classification and Labelling must be provided by registrants. For detailed information on these rules, see Annex XI to the REACH Regulation.

R.7.2.8 Information sources on serious eye damage / eye irritation

R.7.2.8.1 Non-human data on serious eye damage / eye irritation

Non-testing data on serious eye damage / eye irritation

Physico-chemical properties

Relevant information can be inferred from basic physico-chemical characteristics of a substance (e.g. extreme pH). *Extreme* pH values may indicate the potential of a substance to cause skin corrosion or serious eye damage:

IF pH ≤ 2 or pH ≥ 11.5, THEN consider the substance to be corrosive to the skin (Category 1) and to cause serious eye damage (Category 1) when pH is used as the sole basis for classification decision (See also Sections R.7.2.4.1 and R.7.2.9.1 of this Guidance).

Grouping, (Q)SARs and expert systems [20]

In REACH Annex XI two types of non-testing methods are mentioned which can be used for adaptation of standard information requirements, either as standalone (where possible) or in concert with other information (in the context of a *Weight-of-Evidence* assessment):

- qualitative and quantitative Structure-Activity-Relationships (SARs/QSARs, section 1.2, including expert systems, generally incorporating multiple (Q)SARs, expert rules and data) on the one hand, and

- grouping of substances and read-across approaches. [21]

The adaptation of standard information requirements can be used for the assessment of serious eye damage/eye irritation, if it provides relevant and reliable data for the substance of interest. As specified in Annex XI of the REACH regulation, the use of non-testing methods needs to be justified and sufficiently documented. In the case of QSARs and expert systems, registrants need to prepare property predictions by completion of a QSAR Prediction Reporting Format (QPRF). The QPRF is a harmonised template for summarising and reporting substance-

[20] Further information can be found in *Chapter R.6 QSAR and grouping of chemicals* of *the Guidance on IR&CSA* (available at: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment), the OECD Guidance on Grouping of Chemicals, Second Edition (OECD, 2014a), the new OECD Guidance on an Integrated Approach for Testing and Assessment (IATA) for skin corrosion and irritation (OECD, 2014b) and the JRC report on Alternative methods for regulatory toxicology (Worth, 2014).

[21] The relevant terminology is not always used consistently. With reference to the ECHA Guidance on QSAR and grouping, the terms category approach and analogue approach are used to describe techniques for grouping of substances, whilst the term read-across is reserved for a technique to fill data gaps, i.e. to transfer knowledge from one or more substances called source(s) to another substance with data gap, named target substance.

specific predictions generated by (Q)SAR models. For filling a data gap under REACH, it is also necessary to provide information on the prediction model employed following a QSAR Model Reporting Format (QMRF) document. The QMRF is a harmonised template for summarising and reporting key information on (Q)SAR model validity, including the results of any validation studies. The information is structured according to the OECD (Q)SAR validation principles (for further information see http://www.oecd.org/env/ehs/risk-assessment/validationofqsarmodels.htm). The JRC QSAR Model Database is an inventory of information on available QMRFs, freely accessible online (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/laboratories-research/predictive_toxicology/qsar_tools/QRF). More detailed guidance on QSAR models, their use and reporting formats, including the QMRF, is provided in Section R.6.1 of Chapter R.6 of the *Guidance on IR&CSA* (available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment).

In general, there are several different ways in which non-testing methods can be used in the context of an IATA (an IATA for serious eye damage and eye irritation is currently under development by the OECD), e.g.:

- for direct prediction of serious eye damage/eye irritation potential or the absence thereof,
- as part of a *Weight-of-Evidence* scheme (where the information from non-testing methods alone is not sufficient for a decision), or
- in order to decide how best to proceed with further (*in vitro*) testing (i.e. via a top-down or bottom-up approach). For further information see Section R.7.2.11.2.

- SARs and read-across for serious eye damage and eye irritation:

In principle, the same considerations apply as with the use of SARs and read-across for skin corrosion/irritation (see Section R.7.2.3.1). Structural alerts for serious eye damage/eye irritation have been described in the literature, e.g. in Gerner *et al.* (2005).

The occurrence of structural analogues that exhibit serious eye damage (or eye irritation) potential can also be used to predict the effect in the substance of interest and adapt the respective information requirements. Negative data from structural analogues may also be used to make predictions in certain cases, however, absence of one of the known structural alerts for irritation and corrosion alone does not prove absence of effect, as knowledge of structural alerts for irritation and corrosion might be incomplete. For instance, other substructures (not yet identified as structural alerts) or other properties of the substance may be responsible for a corrosive or irritant effect.

- QSARs and expert systems for serious eye damage and eye irritation:

An overview of available (Q)SARs for serious eye damage/eye irritation is provided in Table R.7.2-3. An extensive review of the state-of-the-art was published by the former ECB (Gallegos Saliner *et al*. 2006, 2008). 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. 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 expert systems for assessing eye irritation are the same as those used for assessing skin corrosion and irritation. Details on automated rule-induction systems (e.g. TOPKAT and MultiCASE), and on knowledge-based systems (e.g. DEREK Nexus, and the BfR rule-base) are reported in Appendix R.7.2-3.

The freely downloadable OECD QSAR Toolbox software contains two profilers relevant for serious eye damage/eye irritation based on the BfR rule-base, which encode "inclusion rules" (structural alerts predicting serious eye damage/eye irritation potential) with a suggestion that exclusion of serious eye damage/eye irritation potential might be possible based on certain physico-chemical properties. The use in combination of profilers and data for analogues could allow for the prediction of serious eye damage/eye irritation for new substances through a read-across or category approach. More details on the OECD QSAR Toolbox specific contents for skin irritation and corrosion 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 to the regulatory endpoint of interest. The BfR model for the prediction of serious eye damage/eye irritation has been developed to predict EU regulatory endpoints, however predictions refer to the former DSD classification/labelling system used in the EU before the CLP Regulation came into force, and in borderline cases the results of the prediction may not fully reflect the correct CLP classification. More details on this model are reported in Appendix R.7.2-3.

It should also be noted that the criteria for classification as eye irritant Category 2 based on the mean score for corneal opacity and conjunctival redness in the *in vivo* test have changed from ≥2 and ≥2.5, respectively, under DSD to ≥1 and ≥2.0, respectively, under CLP. Consequently predictions as eye irritant Cat 2 from models developed based on the DSD criteria should be interpreted with caution since they may lead to underprediction and should not be used for direct classification under CLP.

In the case of classification models for serious eye damage/eye irritation, the classification criteria used in model development 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 Category 1 (Serious eye damage) or Category 2 (Eye irritation), the category chosen should be supported with expert judgment.

Table R.7.2-3 Overview of available (Q)SARs for serious eye damage/eye irritation. See Appendix R.7.2-3 for more information on these models.

Category of model or source	Reference or name of the model	Applicability domain
Literature models	Solimeo *et al.* (2012)	Not available
	Abraham *et al.* (2003)	Pure bulk liquids
	Gerner *et al.* (2005)	Based on Physico-chemical values
	Barratt (1995b, 1997)	Neutral organic chemicals
Computerised models	PaDEL-DDPredictor (http://padel.nus.edu.sg/software/padelddpredictor/) (Liew and Yap, 2013)	Calculated by the model based on the range of descriptors
	BfR rule-base, free (included in the OECD QSAR Toolbox and Toxmatch, Toxtree, ToxPredict and Ambit)	EU New chemicals (NONS) database, organic chemicals with no significant hydrolysis potential and purity >95%
	ACD/Percepta, commercial	Organic chemicals

	Derek Nexus, commercial	Organic chemicals and some metals
	HazardExpert, commercial	Organic chemicals
	MolCode, commercial	Organic chemicals
	MultiCASE, commercial	Organic chemicals
	TOPKAT, commercial	Organic chemicals
Review papers	Patlewicz *et al.*, 2003	N.A.
	Gallegos Saliner *et al.* (2006, 2008)	N.A.

Abbreviation: N.A. = not applicable.

Testing data on serious eye damage / eye irritation

The internationally accepted testing methods for serious eye damage/eye irritation as described in the Annex to the EU Test Methods (TM) Regulation (Council Regulation (EC) No 440/2008) and in OECD TGs (available at http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm#Test_Guidelines) are: EU B.5 (OECD TG 405), EU B.47 (OECD TG 437), EU B.48 (OECD TG 438) and OECD TG 460.

At the OECD there are currently three additional draft TGs under discussion regarding the eye hazard, i.e. EpiOcular™ EIT, Short-time exposure (STE) test method and Cytosensor® microphysiometer (CM) test method. Additional test methods may become available for addressing the eye hazard, therefore the reader is advised to check the OECD webpage and ECHA test methods website to check the current status of these test methods.

Please note that the latest version of an adopted test guideline should always be used when generating new data, independently from whether it is published by EU or OECD.

The testing and assessment strategy developed for serious eye damage/eye irritation (see Section R.7.2.11 of this Guidance) emphasises the need to evaluate all available information (including physico-chemical properties) before undertaking any *in vivo* testing. This strategy employs screening elements designed to avoid, as far as possible, *in vivo* testing of corrosive and severely irritating substances. In particular, *in vitro* tests should usually be performed first, and it should be assessed whether *in vivo* testing can be completely avoided.

In vitro data

Accepted *in vitro* test methods to detect serious eye damage (Category 1 under CLP) and/or absence of effects requiring classification for serious eye damage/eye irritation (i.e. not classified under CLP) are listed in Table R.7.2-4. More information on the specific scope and limitations of these tests is provided in Section R.7.2.9.1 under “Testing data on serious eye damage/eye irritation”.

Table R.7.2-4: Accepted *in vitro* test methods for serious eye damage/eye irritation

	Test method	Validation status, regulatory acceptance	EU Test Method /OECD test guideline	Classification according to CLP Regulation	EURL ECVAM DB-ALM protocol Nr.
Serious eye damage / eye irritation					
	BCOP	Validated and regulatory acceptance	B.47 / OECD TG 437	Cat 1 or NC	98, 124
	ICE	Validated and regulatory acceptance	B.48 / OECD TG 438	Cat 1 or NC	80
	FL	Validated and regulatory acceptance	N.A. / OECD TG 460	Cat 1	71
	CM [22]	Validated and considered to be scientifically valid	N.A. / OECD draft TG available and being considered for adoption	Cat 1 or NC	130
	STE [23]	Validated and considered to be scientifically valid	N.A. / OECD draft TG available and being considered for adoption	Cat 1 or NC	N.A.
	EpiOcular ™ EIT [24]	Validated and considered to be scientifically valid	N.A. / OECD draft TG available and being considered for adoption	NC	N.A.
	Ocular Irritection ® Assay [25]	Validated	N.A. / N.A.	Cat 1	157

[22] The CM test method was validated by EURL ECVAM and considered to be scientifically valid (https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/topical-toxicity/eye-irritation; section 1.2) and was also reviewed by ICCVAM (http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFE8725DF1F9F5D); A draft OECD Test Guideline is available at: http://www.oecd.org/env/ehs/testing/section4healtheffects.htm.

[23] The STE test method was validated by JaCVAM and peer-reviewed by ICCVAM and considered to be scientifically valid (http://ntp.niehs.nih.gov/?objectid=2D70C7A2-CCDB-D782-06CB38302BD7D10E); A draft OECD Test Guideline is available at: http://www.oecd.org/env/ehs/testing/section4healtheffects.htm.

[24] The EpiOcular ™ EIT test method was validated by EURL ECVAM and considered to be scientifically valid (PLACEHOLDER for the link to EURL ECVAM recommendation); A draft OECD Test Guideline is available at: http://www.oecd.org/env/ehs/testing/section4healtheffects.htm.

[25] The Ocular Irritection® Assay has undergone an external prospective and retrospective validation study co-sponsored by In Vitro International (the method developer) and INT.E.G.RA (Eskes *et al.*, 2014) and appears to be a suitable test method for the identification of substances causing serious eye damage (CLP Category 1) and not

Test methods currently with limited application under REACH					
	IRE [26]	validated	N.A. / N.A.	Cat 1	85
	HET-CAM [26]	Validated	N.A. / N.A.	Cat 1	47, 96

NOTE: During the validation exercise EURL ECVAM concluded that the SkinEthic ™ Human Corneal Epithelium (HCE) is not sufficiently sensitive for identifying substances not classified for serious eye damage/eye irritation (the test method produced an unacceptable number of false negative results in the validation study) and recommended optimisation and further validation of the test method by the developer (EURL ECVAM, 2014).

Abbreviations: BCOP = Bovine Corneal Opacity and Permeability; CM = Cytosensor Microphysiometer; EpiOcular ™ EIT = EpiOcular™ Eye Irritation Test; FL = Fluorescein Leakage; HET-CAM = Hen's Egg Test on Chorioallantoic Membrane; ICE = Isolated Chicken Eye; IRE = Isolated Rabbit Eye; N.A. = not available; STE = Short-Time Exposure.

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requiring classification for the eye hazard. The test method is also proposed by the developer to be suitable for the identification of substances not classified for serious eye damage/eye irritation based on the outcome of a validation study. However, an independent peer-review of the validation study is still pending and therefore the final applicability of the test method still needs to be confirmed. Therefore conclusions on classification cannot be drawn from negative results before the scientific validity of the test method to correctly identify substance not requiring classification for serious eye damage/eye irritation has been confirmed.

[26] Concerning the IRE and HET-CAM test methods, ICCVAM validation assessments in 2007 and 2010 that these test methods were not sufficiently accurate for regulatory use or that there was not sufficient data, especially for Category 2 chemicals, to make a final conclusion on their validity and recommended additional studies (http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/ocular/in-vitro/index.html & http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/ocular/in-vitro-test-methods/index.html). The Manual of Decisions of the Competent Authorities (EC, 2009) concluded that there is enough evidence available to conclude that the test methods are able to detect substances causing severe damage to eyes. Positive results can therefore be used for classification purposes i.e. leading to a classification of Category 1 for serious eye damage and labelling with H318 *"Causes serious eye damage"* according to CLP.

The test methods indicated in Table R.7.2-4 above are either organotypic assays (BCOP, ICE, IRE and HET-CAM), cytotoxicity and cell function based assays (CM, FL and STE), reconstructed human cornea-like epithelium assays (EpiOcular™ EIT), or *in chemico* assays (Ocular Irritection®). These test methods are mainly concerned with modelling the immediate effects of substances on the cornea**.** *In vivo* eye irritation endpoints which may not be covered by the above-mentioned optimised protocols are the following:

i. persistence/reversibility of effects

ii. discolouration on the cornea[27]

Concerning persistence and reversibility of effects, the OECD TGs for BCOP (OECD TG 437) and ICE (OECD TG 438) and the OECD GD 160 (OECD, 2011) state that histopathological examination of the corneas may be potentially useful when a more complete characterization of corneal damage is needed. Some evidence has been published showing that histopathology may support the identification of irreversible effects produced by non-extreme pH detergent and cleaning products when used in combination with the ICE test method (Cazelle *et al.*, 2014). However, more work is still needed to assess the usefulness of the histopathological evaluation concerning identification of irreversible effects.

There are currently no validated *in vitro* eye irritation test methods available that could be used for the direct identification of Eye irritants Category 2 under CLP.

Additional test methods currently under development to assess different ranges of eye irritation potential are e.g. the *Ex Vivo* Eye Irritation Test (EVEIT) and the Porcine Cornea Reversibility Assay (PorCORA). The EVEIT and PorCORA test methods are organotypic assays which use either isolated rabbit or porcine corneas, respectively, and have been proposed to be able to discriminate between reversible and irreversible (persistent) effects by directly monitoring the recovery process in excised corneas kept in culture for several days following chemical exposure (Frentz *et al.*, 2008; Spöler *et al.,* 2010; Piehl *et al*., 2010, 2011).

Testing and Assessment strategies combining different test methods according to their applicability domain and capacity to classify in the different ranges of serious eye damage/eye irritation (from those listed in table R.7.2-4 and those mentioned in the previous paragraphs) still need to be developed to facilitate the identification of Category 2 substances on the basis of methods that currently can only be used to directly identify Category 1 and/or not classified substances.

Further test method developments may occur and the registrants are advised to follow the latest updates through e.g. EURL ECVAM website (https://eurl-ecvam.jrc.ec.europa.eu/) and ECHA's test methods site (Testing methods and alternatives) for potential new test guidelines and test guideline updates.

Animal data

Annex I to the CLP Regulation defines serious eye damage/eye irritation as local toxic effects, and, as such, an assessment of serious eye damage/eye irritation is normally part of the acute testing phase of a toxicity programme and it is an early requirement of all regulatory programmes. Testing for serious eye damage/eye irritation has, historically, used animal

[27] Current *in vitro* TGs (listed in table R.7.2-4 above) do no cover discoloration of the cornea, but some test methods may give indications about this effect.

models and a variety of test methodologies depending upon, for example, the laboratory undertaking the test, the area and intended application. However, in line with one of the objectives of the REACH Regulation, as described in Articles 13(1) and 25(1) and Annex VI, animal testing should be undertaken only as a last resort after i) considering all existing available test data and ii) generating information whenever possible by means of alternative methods to animal testing such as *in vitro* methods, QSAR models, grouping or read-across.

In cases in which *in vivo* testing is necessary, current approaches for serious eye damage/eye irritation testing *in vivo* are covered by the Acute Eye Irritation/Corrosion test method (EU B.5/OECD TG 405). This guideline recommends a tiered approach, where existing and relevant data are evaluated first. The guideline also recommends that testing in animals should only be conducted if determined to be necessary after consideration of available alternative methods. The *in vivo* test uses one animal (the rabbit is the preferred species), which in the absence of severe effects is followed by a further testing of up to two animals (a total maximum of three animals).

Both EU and OECD 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, *irritants* (Category 2 Eye irritants) cause significant inflammation of the eye (conjunctiva redness/oedema, cornea and/or iris) but this effect is transient, i.e. the affected sites are repaired within the observation period of the test. A substance causing considerable damage to the cornea and/or iris is classified in Category 1 for Serious Eye Damage. The criteria for classification in Category 1 for Serious Eye Damage include persistence of effects (effects on the cornea, iris or conjunctiva that are not expected to be reversed or have not fully reversed within an observation period of normally 21 days, i.e. with a score >0), irreversible staining of the eye and/or criteria for the degree of severity.

For existing data, the use of methods other than those specified in the Annex to the EU Test Methods Regulation, or corresponding OECD methods, such as the rabbit Low Volume Eye Test (LVET) (Griffith *et al*., 1980) may be accepted on a case-by-case basis (see also ESAC, 2009).

R.7.2.8.2 Human data on serious eye damage / eye irritation

Existing human data include historical data that should be taken into account when evaluating intrinsic hazards of substances. *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, occupational experience, epidemiological studies and volunteer 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, absence of incidence in humans does not necessarily overrule *in vitro* data or existing animal data of good quality that are positive.

R.7.2.9 Evaluation of information on serious eye damage / eye irritation

R.7.2.9.1 Non-human data on serious eye damage / eye irritation

Non-testing data on serious eye damage / eye irritation

Physico-chemical properties

According to the current EU and OECD guidelines, substances should not be tested on animals for serious eye damage/eye irritation if they can be predicted to be corrosive to the skin (Category 1 of CLP) or cause serious eye damage (Category 1 of CLP) from their physico-chemical properties. In particular, substances exhibiting strong acidity (pH ≤2.0) or alkalinity (pH ≥11.5) in solution are predicted to be corrosive to the skin or cause serious eye damage, and should not be tested on animals. Testing with *in vitro* methods can nevertheless be performed to confirm classification decisions (see Section 3.3.2.3 of Annex I to the CLP Regulation).

A substance known or predicted to be corrosive to the skin can be considered to cause Serious Eye Damage (Category 1). However, no conclusion can be made regarding serious eye damage/eye irritation potential when the pH has an intermediate value (when 2.0< pH <11.5). Where extreme pH is the only basis for classification as serious eye damage, it may also be important to take into consideration the acid/alkaline reserve, i.e. a measure of the buffering capacity (Young *et al.*, 1988,; Young and How, 1994). 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 may not cause serious eye damage, further *in vitro* testing should be considered (see Section 3.3.2.3 of Annex I to the CLP Regulation).

Grouping, (Q)SARs and expert systems

Guidance has been developed by the former 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 of Chapter R.6 of the *Guidance on IR&CSA*. Essentially, the determination of whether a (Q)SAR result may be used to replace a test result can be broken down into three main steps:

1. an evaluation of the scientific validity (relevance and reliability) of the model,
2. an assessment of the applicability of the model to the chemical of interest and the reliability of the individual model prediction,
3. an assessment of the adequacy of the information for making the regulatory decision, including an assessment of completeness, i.e. whether the information is sufficient to make the regulatory decision, and if not, what additional (experimental) information is needed.

Model validity assessment needs to be performed along the lines of the OECD principles for (Q)SAR validation (OECD, 2007), e.g. in terms of a defined endpoint, an unambiguous algorithm, a defined applicability domain, the statistical characteristics (“goodness-of-fit”), and mechanistic interpretation.

Inter alia the following questions should be addressed when assessing the reliability of an individual prediction:

i. Is the chemical of interest within the scope of the model, according to the defined applicability domain of the model?

ii. Is the defined applicability domain suitable for the regulatory purpose?

iii. How well does the model predict chemicals that are similar to the chemical of interest?

iv. Is the model estimate reasonable, taking into account other information?

The mechanism of serious eye damage/eye irritation involves toxicodynamic and toxicokinetic parameters. Models that predict serious eye damage and eye irritation based on toxicodynamic properties only (e.g. acidity or basicity, electrophilicity, other reactivity, surfactant activity, solving membranes) have to be additionally evaluated for their consideration of toxicokinetic parameters (or have to be used in concert with data covering such parameters) related to the potential to cross relevant outer membranes of the eye (cornea) to be active in the living tissue underneath. Conversely models that predict (the absence of) serious eye damage/eye irritation solely from e.g. physico-chemical properties considered to illustrate the toxicokinetic behaviour of a substance, should be evaluated for their consideration of its activity (toxicodynamics), in particular for potential serious eye damage (where the corrosive action itself may lead to membrane destruction and subsequent tissue damage).

For example, the BfR rule-base implemented in Toxtree and the OECD QSAR Toolbox contains both physico-chemical exclusion rules and structure-based inclusion rules (structural alerts). Evaluations of these rules for the prediction/exclusion of eye irritation (Tsakovska *et al.*, 2005, on structural alerts; Tsakovska *et al.*, 2007, on physico-chemical exclusion rules) have been carried out in accordance with the OECD principles for (Q)SAR validation (see Appendix R.7.2-3). However, inclusion and exclusion rules were evaluated separately, and not used in concert in these works.

When applied, these two sets of rules might sometimes provide contradictory information, i.e. a structural alert might indicate serious eye damage/eye irritation potential, while at the same time, based on physico-chemical properties, absence of effect is predicted. In such cases, it is recommended to consider additional information (e.g. on the behaviour of chemically similar substances). In other cases, applicability of one (or more) of the physico-chemical exclusion rules might indicate absence of serious eye damage/eye irritation potential of the target substance, while no structural alert for serious eye damage/eye irritation is triggered. Given that the absence of any known structural alert is not equivalent to the absence of a potential effect, in such a situation still the substance should be examined for potentially reactive substructures (and looking at the behaviour of chemical analogues still will be beneficial).

While these considerations apply to the use of the BfR rule-base for direct classification/non-classification, less certainty might be required e.g. for a decision on further *in vitro* testing: where the exclusion rules suggest the absence of an effect, a bottom-up approach might be followed, i.e. a test for eye irritation irritation and not serious eye damage might be initiated (see Section R.7.11.2).

There is no other model available which sufficiently describes the absence of effects. Neutral organics[28] are expected not to be irritants. Absence of reactivity needs to be described in sufficient detail or be substantiated with other information.

[28] By definition a neutral organic is a chemical which does not have potential reaction centres, even after skin metabolism.

Testing data on serious eye damage / eye irritation

In vitro data

There are EU and OECD adopted test guidelines (see Section R.7.2.8.1), under which substances can be classified as causing serious eye damage or not classified.

Annex VII to the REACH Regulation requires information from *in vitro* tests for serious eye damage/eye irritation, not from animal tests. Guidance on how *in vitro* data can also be used to fulfil Annex VIII requirements, is given in Section R.7.2.11 of this document[29].

Data from the following types of tests can be used for Annex VII requirements and may be accepted for Annex VIII requirements for serious eye damage/eye irritation when the general rules for adaptation specified in Annex XI are used:

- **Bovine Corneal Opacity and Permeability (BCOP) test method** (EU B.47/OECD TG 437): The specific scope and limitations are:
  - This test is recommended to identify substances inducing serious eye damage, i.e. substances to be classified in Eye Damage Category 1 under CLP, without further testing, and also recommended to identify substances that do not require classification for eye irritation or serious eye damage i.e. leading to non-classification under CLP, without further testing.
  - If, as a result of testing, the substance is neither classified as Eye Damage Category 1 nor identified as not requiring classification under CLP, further testing/evaluation is required.
  - This test may result in false positive Category 1 predictions (serious eye damage) for alcohols and ketones and false negative predictions (underpredicted Category 1 substances) for substances that would be classified as Category 1 (serious eye damage) *in vivo* based on persistence of effects only (i.e., that do not meet Category 1 classification criteria based on the mean scores obtained from the first 3 observation days but show persistent effects at the 21st observation day) (Adriaens *et al.*, 2014; OCED, 2013). See also Section R.7.2.8.1 for "*In vitro* test methods for serious eye damage/eye irritation".
  - This test does not allow testing of gases and aerosols.

- **Isolated Chicken Eye (ICE) test method** (EU B.48/OECD TG 438): The specific scope and limitations are:
  - This test is recommended to identify substances inducing serious eye damage, i.e. substances to be classified in Eye Damage Category 1 under CLP, without further testing, and also recommended to identify substances that do not require classification for eye irritation or serious eye damage i.e. leading to non-classification under CLP, without further testing.

[29] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

- If, as a result of testing, the substance is neither classified as Eye Damage Category 1 nor identified as not requiring classification under CLP, further testing/evaluation is required.
- Similar limitations in relation to false positive and false negative predictions, as specified for the BCOP assay above, apply to this test method as well.
- This test does not allow testing of gases and aerosols.

o **Fluorescein leakage (FL) test method** (OECD TG 460): The specific scope and limitations are:

- This test is recommended to identify substances inducing serious eye damage, i.e. substances to be classified in Eye Damage Category 1 under CLP, without further testing.
- This test is not recommended for the identification of substances which should be classified as Eye irritants Category 2 or of substances which should not be classified for serious eye damage and eye irritation.
- This test is only applicable to water soluble substances and/or where the toxic effect is not affected by dilution.
- Its applicability domain does not include strong acids and bases, cell fixatives and highly volatile substances.
- If, as a result of testing, the substance is not classified as Eye Damage Category 1 under CLP, further testing/evaluation is required.

In case of REACH Annex VIII information requirement, a positive outcome (Serious Eye Damage Category 1) from one of five *in vitro* assays (i.e. the IRE, HET-CAM, CM, STE, Ocular Irritection™ assay) is accepted in the EU to classify a substance as Eye Damage Category 1 under CLP using the adaptations of the standard testing regime specified in REACH Annex XI. A negative outcome, i.e. leading to non-classification according to CLP, can also be accepted for fulfilling Annex VIII information requirement on the basis of test data obtained with the CM, EpiOcular EIT and STE test methods, in case the substance falls into the applicability domain of the test method(s) and Annex XI adaptations are used.

Currently, there are no validated *in vitro* methods available for the direct identification of Category 2 Eye irritants.

- Quality Aspects of exisiting *in vitro* data:

For quality assessment of existing *in vitro* data that will lay the basis for later possible *Weight-of-Evidence* considerations, see Section R.4.4 of Chapter R.4 of the *Guidance on IR&CSA,* and for aspects that need to be taken into account in such a *Weight of Evidence* see Section R.5.2.1.2 of Chapter R.5 of the *Guidance on IR&CSA*.

Animal data

Well-reported studies, particularly if conducted in accordance with the principles of GLP, can be used to identify substances which would be considered to cause, or not to cause serious eye damage or eye irritation. There may be a number of serious eye damage/eye irritation studies already available for an existing substance, none of which are fully equivalent to an OECD TG

or an EU test method such as those in the Annex to the EU Test Methods Regulation. If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the serious eye damage/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 irritation effects, even those which do not lead to classification. Effects such as persistent corneal opacity, discolouration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight which do not reverse within the test period may indicate that a substance will cause persistent damage to the human eye.

Data from studies other than skin corrosion/irritation studies (e.g. other toxicological studies on the substance in which local responses of skin 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 irritation.

Data from studies other than serious eye damage/eye irritation studies (e.g. other toxicological studies on the substance in which local responses of the eye 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 eye irritation. More notably, eye reactions and symptoms are not systematically scored in studies not specifically designed to address serious eye damage/eye irritation.

- Quality Aspects of existing *in vivo* data:

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 of Chapter R.4 of the *Guidance on IR&CSA*, and a judgment will need to be made as to whether any further testing is required. Some examples to note are:

i. Was the animal species used the rabbit or was it another species such as the rat or the mouse? Normally the rabbit is used for eye irritation testing.

ii. How many animals were used? Current methodology requires a maximum of 3 animals tested in a sequential manner (with 1 or 2 animals being sufficient if serious eye damage/irreversible effects are observed in the first or second tested animal, respectively) but 6 were frequently used in the past (see Section 3.3.2.3.2.2 of the *Guidance on the application of the CLP criteria* for the evaluation of results from tests that have been conducted with more than 3 animals)..

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. 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.

v. Was initial pain noted after instillation of the test substance onto the eye? Was the substance washed out from the eye? Was fluorescent staining used?

vi. How was the test material applied onto the eye?

Irritation scores from old reports, reports produced for regulatory submission in the USA or in publications may be expressed as a Maximum Average Score (MAS). 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 expert judgment may be required to avoid repeat testing.

Observations such as those 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:

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, 10 µl 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 was reviewed by EURL ECVAM between 2006 and 2009 via retrospective validation for the detergent and cleaning products applicability domain (for further details, see http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/validation-regulatory-acceptance/topical-toxicity/eye-irritation). Anatomical and physiological considerations for rabbit and human eyes indicate that a dose volume of 10 µl is appropriate (A.I.S.E. 2006): the tear volume in both rabbit and man is approximately the same (~ 7-8 µl), and after blinking, the volume capacity in the human eye is ~10 µl after blinking. Furthermore the use of direct cornea exposure mimics human exposure scenarios that can be reasonably expected (e.g. accidental ocular exposure during household use) and for the specific use domain of household detergents and cleaning products as well as their main ingredients (i.e. surfactants) as used in these products. 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 for the safety evaluation of single substances (Griffith *et al*., 1980) and detergent and cleaning products (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 in man. It still overpredicts, but less than the classical Draize test of OECD TG 405.

After peer review, the LVET was not recommended for prospective use, i.e. to generate new data but it was acknowledged that existing LVET data of the limited use domain mentioned above may be used for purposes of classification and labeling decisions. Moreover, it was recognised that existing LVET data of this limited use domain may be used as supplementary data for future validation studies. No additional testing should be however performed to further develop or validate the LVET test. It was also pointed out that LVET has a tendency to classify in lower hazard categories when compared to OECD TG 405. Nevertheless, it was acknowledged that these data may still be useful on a case-by-case basis, with respect to test data for household detergents, cleaning products and surfactants used in such products (ESAC, 2009).

In summary, available data from the LVET on substances should be considered and must be carefully evaluated. For the classification of substances it must be taken into account that the test has a limited applicability domain (detergent and cleaning products). Consequently, within the applicability domain of household detergents, cleaning products and their main ingredients, positive LVET data (be it Category 2 or Category 1) can be used for the appropriate classification for either serious eye damage or eye irritation, but negative data from LVET as a *stand alone method* (in the absence of any other information) are not conclusive for *no classification*.

R.7.2.9.2 Human data on serious eye damage / eye irritation

Well-documented *existing* human data of different sources can often provide very useful information on serious eye damage/eye irritation, sometimes for a range of exposure levels. Often the only useful information available on irritation is obtained from human experience (e.g. 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 mixtures (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 serious eye damage or eye irritation 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 irritation are provided in an ECETOC monograph (ECETOC, 2002).

Substances causing Serious eye damage Category 1 give more severe corneal opacity and iritis than Eye irritants Category 2. Category 1 substances induce considerable tissue damage which can result in serious physical decay of vision. It is recognised that such severe lesions usually do not reverse within 21 days (relates to animals) (see Section 3.3 of Annex I to the CLP Regulation). In contrast, the effects of Category 2 substances are reversible within 21 days. In humans, an ophthalmic examination by a physician would reveal a decay of vision. If it is not transient but persistent it implies classification in Category 1. If the discrimination between Category 1 and Category 2 is not obvious, then Category 1 might be chosen, however other types of information may be generated e.g. by performing *in vitro* testing, to support the conclusion (for further information, see Section 3.3 of the *Guidance on the application of the CLP criteria*).

R.7.2.9.3 Exposure considerations for serious eye damage / eye irritation

Exposure-based waiving from testing is not applicable to the endpoint of serious eye damage/eye irritation. Exposure-based waiving from testing as specified in Annex XI (3) of the REACH Regulation only applies to tests listed in Sections 8.6 and 8.7 of Annex VIII, Annex IX and Annex X according to the REACH text.

R.7.2.9.4 Remaining uncertainty on serious eye damage / eye irritation

Usually it is possible to unequivocally identify (or accept) a substance as causing serious eye damage, whatever type of study provides the information.

There may be a significant level of uncertainty in human data on irritant effects (e.g. because of poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, small numbers of subjects).

Data from studies in animals and from *in vitro* tests performed according to internationally accepted test methods will usually give relevant information on the serious eye damage/eye irritation potential of a substance. In general, it is assumed that substances which cause serious eye damage/eye irritation in EU or OECD TG-compliant studies in animals or *in vitro* will cause serious eye damage/eye irritation in humans, and those which are not irritant in EU or OECD TG-compliant studies will not be irritant in humans (Please note that in general test

animals are considered to be more sensitive to serious eye damage/eye irritation than humans (e.g. Adriaens *et al.*, 2014)). It should be borne in mind that some of the limitations of the *in vivo* serious eye damage/eye irritation study include its high variability, the variable exposure being dependent on the physico-chemical properties of the test substance, and the subjective grading of the lesions (Adriaens *et al.*, 2014; Cormier *et al.*, 1996; Prinsen, 2006; Marzulli and Ruggles, 1973; Weil and Scala, 1971). Moreover, inconsistent results from a number of similar studies increases the uncertainty in deriving data from animal or *in vitro* studies.

The scope of the *in vitro* tests for serious eye damage/eye irritation has also some limitations, as explained in Section R.7.2.9.1 under "Testing data on serious eye damage/eye irritation". In addition inconsistent results from two or more *in vitro* tests could add to the overall uncertainty in interpreting the data.

R.7.2.10 Conclusions on serious eye damage / eye irritation

R.7.2.10.1 Concluding on suitability for Classification and Labelling

In order to conclude on Classification and Labelling according to the CLP Regulation, all the available information needs to be taken into account, and consideration should be given to both the *Guidance on the application of the CLP criteria* and the various remarks (as they relate to Classification and Labelling) made throughout this guidance document [30].

R.7.2.10.2 Concluding on suitability for Chemical Safety Assessment

A dose-response assessment is difficult to make for serious eye damage/eye irritation simply because up to the present time most data have been produced with undiluted substances 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 and dose-response data and for local effects the concentrations is often the determinative dose metric. Consequently, if dose-response data are available, they must be taken into account (see Figure R.7.2-4).

Guidance on the possibilities for derivation of DNELs for serious eye damage/eye irritation is given in Appendix R.8-9 of Chapter R.8 of the *Guidance on IR&CSA*.

R.7.2.10.3 Information not adequate

A *Weight-of-Evidence* approach comparing available adequate information with the tonnage-triggered information requirements under REACH may result in the conclusion that the requirements are not fulfilled. In order to proceed to further information gathering the testing and assessment strategy described in Section R.7.2.11 below is recommended.

[30] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

R.7.2.11 Testing and assessment strategy for serious eye damage / eye irritation

R.7.2.11.1 Objective / General principles

The following testing and assessment strategy is recommended for developing adequate and scientifically sound data for assessment/evaluation and classification of the serious eye damage and eye irritation properties of substances. For existing substances with insufficient data, this strategy can also be used to decide which additional data, beside those already available, are needed. The testing and assessment strategy is aimed for the identification of serious eye damage/eye irritation by using different elements where appropriate depending on the information available. A principle of the strategy is that the results of one study or an information source 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 different elements provided in the Figure R.7.2-4 describe information sources that can be used to conclude on a substance hazard potential towards the eye. The elements described in Figure R.7.2-5 can be rearranged as appropriate, especially in Part 1. This may be particularly helpful in cases where a conclusion can be drawn from certain elements without having to consider all of them. If judged relevant, elements in Part 1 can be skipped and *in vitro* testing can be performed immediately.

Figure R.7.2-5 is divided into three parts where Part 1 aims at evaluating existing information that may be available on the substance. In Part 2 existing information and relevant data should be assessed in order to consider if there is enough information available to conclude on the substance hazard properties within a *Weight-of-Evidence* analysis, in case it is not possible to make a conclusion based on single elements described in Part 1. In case no conclusion can be drawn in Parts 1 and 2, new data should be generated in Part 3 by first performing relevant *in vitro* testing. Only in case no conclusion can be drawn based on the *in vitro* testing, can *in vivo* testing be performed (for substances at or above 10 tonnes per annum only).

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 to the REACH Regulation, together with some general rules for adaptation from standard information requirements in Annex XI.

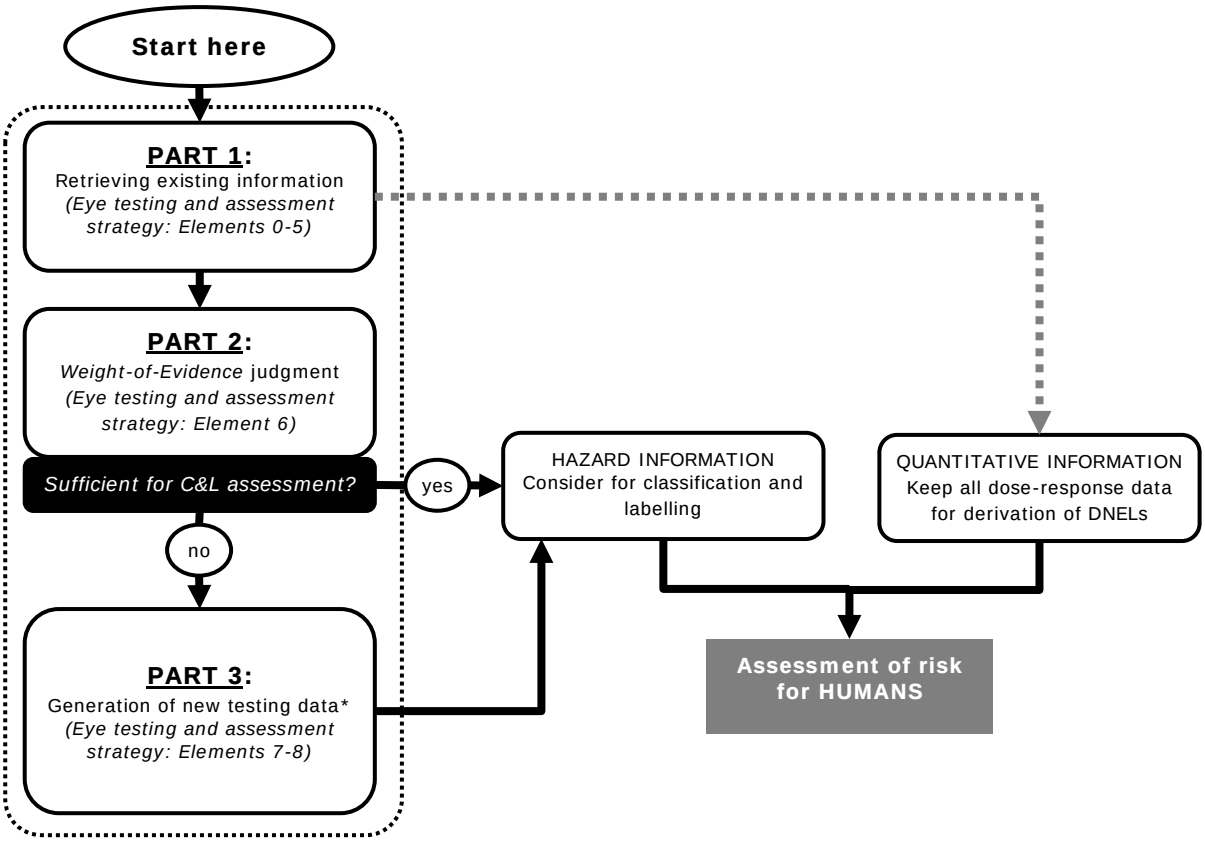
Risk assessment of the serious eye damage/eye irritation potential of a substance is normally made in a qualitative way provided that the substance has been classified as causing serious eye damage/eye irritation. Existing test guidelines do not contain dose-response assessment, so that a quantitative analysis will often not be possible. Therefore, hazard identification and appropriate classification is the key determinant in the information gathering 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 testing strategy and to perform a complete risk assessment, comprising qualitative hazard as well as quantitative information.

It is recommended that the testing and assessment strategy be followed until element 5 (Figure R.7.2-4 and Figure R.7.2-5) in all cases and thereafter the *Weight-of-Evidence* analysis be performed. Clearly, all information sources/elements can be rearranged as appropriate, i.e. not all elements will necessarily be accompanied by data but it is important that all potential data sources are explored prior to starting the *Weight-of-Evidence* analysis. While it is recommended that this approach be followed, other approaches may be more appropriate and efficient on a case-by-case basis. For example, in case there is no existing data and it is anticipated that generation of “pre-testing data” would be non-conclusive, it may be appropriate to directly proceed to the information generation part. Furthermore, prior to performing any new *in vivo* test, the use of *in vitro* methods should be fully exploited (see

Articles 13(1) and 25(1) of the REACH Regulation) by using the general rules of Annex XI for adaptation of the standard testing regime set out in Annexes VII to X.

If the substance is not classified for serious eye damage/eye irritation, no risk assessment for this endpoint is performed, regardless of the exposure. Please note that there are no options for exposure-based waiving for these endpoints in the REACH Regulation.

The following flow chart (Figure R.7.2-4) gives an overview of a possible approach for defining a testing and assessment strategy for serious eye damage/eye irritation.



*Generation of new testing data according to Annex VII to VIII to the REACH Regulation and with due observation of the rules for adaptation of the standard testing regime laid down in Annex XI.

Figure R.7.2-4 Overview of the testing and assessment strategy for serious eye damage / eye irritation

R.7.2.11.2 Testing and assessment strategy for serious eye damage / eye irritation

Recommended approach

The testing and assessment strategy for serious eye damage/eye irritation (see Figure R.7.2-5) is completely analogous in structure to that for skin corrosion/irritation. The testing and assessment strategy consists of three parts: Part 1 (elements 0 to 5) is about retrieving exisiting information, Part 2 (element 6) represents a *Weight-of-Evidence* analysis and expert judgement (element 6), and Part 3 is about generation of new information by testing (elements 7 to 8).

In Part 1, existing and available information from the literature and databases is gathered and considered in the strategy approach. The order of the different elements, i.e. 0 to 5, is only indicative and they may be arranged as appropriate. This may be particularly helpful in cases where a reliable conclusion can be drawn from certain elements without having to consider all of them. For instance, if the substance is classified as corrosive to the skin or has an extreme pH ($\leq$ 2.0 or $\geq$ 11.5) serious eye damage is considered implicit (element 1c) and therefore the substance should be classified as causing serious eye damage (Category 1) according to CLP and further testing is not required. At the end of Part 1 and if no final conclusion could be derived directly from one or several of the available pieces of information, all the information collected should be analysed using a *Weight-of-Evidence* approach (element 6).

In the information generation part (elements 7 to 8), new information on the serious eye damage/eye irritation potential of substances is generated by means of *in vitro* (element 7) or, as a last resort (see Articles 13(1) and 25(1) of the REACH Regulation), *in vivo* testing (element 9). Therefore, before concluding the *Weight-of-Evidence* analysis in element 6 *and in vitro* testing (elements 7a and 7b), new *in vivo* tests should not be conducted. More information on how to use the *in vitro* methods for serious eye damage/eye irritation within the testing strategy can be found in the following paragraphs.

While it is recommended that this approach be followed, other approaches may be more appropriate and efficient on a case-by-case basis. For example, in case there is no existing data and it is anticipated that compilation of data at elements 0-6 would be non-conclusive, it may be appropriate to directly proceed to the information generation part.

Figure R.7.2-5 Testing and assessment strategy for evaluating the serious eye damage / eye irritation potential of substances.

Element	Information	Conclusion [31]
Conclusion of the information strategy on skin corrosion / irritation		
0	Is the substance classified as a skin corrosive? →	**YES**: When assigned Skin Corrosive Cat. 1, 1A, 1B or 1C, the risk of severe damage to eyes is considered implicit (Serious Eye Damage Cat. 1) (Column 2 adaptation of Annexes VII and VIII).
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable in contact with air (pyrophoric) or water at room temperature? →	**YES**: No testing required (Column 2 adaptation of Annexes VII and VIII).
1b	Is the substance an organic hydroperoxide or an organic peroxide? →	**YES**: Consider classifying for: ■ When assigning a Skin Corrosive Cat. 1B classification for a hydroperoxide, the risk of serious eye damage is considered implicit. Consider classifying as Serious Eye Damage Cat. 1, or ■ When assigning a Skin Irritant Cat. 2 classification for a peroxide, the risk of eye irritation is considered implicit. Consider classifying as Eye Irritant Cat. 2. **OR** Provide evidence supporting deviating classification or non-classification [32].
1c	Is the pH of the substance ≤ 2.0 or ≥ 11.5? [a] →	**YES**: Consider classifying as Serious Eye Damage Cat. 1 (column 2 adaptation in section 8.2 of Annex VIII) if pH is used as the sole basis for classification decision. Where consideration of the acid/alkaline reserve suggests that the substance is not corrosive, this has to be confirmed (preferably using an appropriate *in vitro* test).

[31] Please note that the 8th Adaptation to Technical and Scientific Progress (ATP) of the CLP Regulation is currently under discussion. The 8th ATP will take into account the 5th Revision of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which was adopted in 2012 and contains in particular refined criteria for skin corrosion/irritation and serious eye damage/eye irritation.

[32] Information on e.g. *in vitro* testing may provide evidence on more suitable classification, if there is some doubt on the correct classification.

1d	Are there other physical or chemical properties that indicate that the substance is causing serious eye damage or eye irritation? →	**YES**: Use this information for *Weight-of-Evidence* analysis (Element 6).
Existing human data		
2	Are there adequate existing human data [b] which provide evidence that the substance has the potential to cause serious eye damage or eye irritation? →	**YES**: Consider classifying accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2).
Existing animal data from serious eye damage/eye irritation studies		
3	Are there data from existing studies on serious eye damage/eye irritation in laboratory animals, which provide sound conclusive evidence that the substance is seriously damaging to the eye, eye irritant or non-irritant? →	**YES**: Consider classifying accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2) or consider no classification.
Existing/new (Q)SAR data and read-across		
4	Are there structurally related substances (suitable "read-across" or grouping), which are classified as causing serious eye damage/eye irritation, or indicating that the substance is non-irritant, or do valid (Q)SAR methods indicate serious eye damage/eye irritation or non-irritation of the substance? [c] →	**YES**: Consider classifying accordingly.
Existing in vitro data		
5a	Has the substance demonstrated serious eye damage, eye irritation or non-irritating properties in an EU/OECD adopted *in vitro* test? Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions defined in Annex XI are met. →	**YES**: Consider classifying accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2) or consider no classification. If discrimination between Serious Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Serious Eye Damage Cat. 1 must be chosen.
5b	Are there acceptable data from a non-validated suitable *in vitro* test(s), which provide sound evidence that the substance is causing serious eye damage/eye irritation?[d] →	**YES**: Consider classifying accordingly (SeriousEye Damage Cat. 1 or Eye Irritant Cat. 2). If discrimination between Serious Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Serious Eye Damage Cat. 1 must be chosen.
Weight-of-evidence analysis		
6	The "elements" described above may be	**YES**:

	arranged as appropriate. Taking all available existing and relevant data mentioned above (Elements 0 – 5) into account, is there sufficient information to make a decision on whether classification/labelling is necessary, and – if so – how to classify and label? →	Classify accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2) or consider no classification.
New in vitro tests for serious eye damage / eye irritation (Annex VII to the REACH Regulation) [e]		
7a	Does the substance demonstrate serious eye damage, eye irritation or non-irritant properties in an EU/OECD adopted *in vitro* test(s) for the eye hazard charaterisation? [e] → Data from *in vitro* test methods that have been validated and are considered scientifically valid but are not yet adopted by EU and/or OECD may also be used if the provisions of Annex XI are met.	**YES**: Classify accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2) or consider no classification). If discrimination between Serious Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Serious Eye Damage Cat. 1 must be chosen. If a conclusion on the eye hazard cannot be drawn by using *in vitro* testing, *in vivo* testing should be performed (at Annex VIII level only).
7b	Does the substance demonstrate serious eye damage or eye irritant properties in a non-validated suitable *in vitro* test(s) for serious eye damage/eye irritation? [d] →	**YES**: Classify as Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2. If a conclusion on the eye hazard cannot be drawn by using *in vitro* testing, *in vivo* testing should be performed (at Annex VIII level only).
New in vivo test for serious eye damage / eye irritation as a last resort (Annex VIII to the REACH Regulation) [f]		
8	Does the substance demonstrate serious eye damage or eye irritation in an OECD adopted *in vivo* test? →	**YES**: Classify accordingly (Serious Eye Damage Cat. 1 or Eye Irritant Cat. 2). **NO:** No classification needed.

Notes to the information scheme on serious eye damage / eye irritation:

[a)] Note that if the buffering capacity suggests the substance may not cause serious eye damage, further data are needed to confirm this, preferably using an appropriate in vitro test method.

[b)] Data from case reports, occupational experience, poison information centres or from clinical studies.

[c)] 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 to the REACH Regulation. Prediction of the absence of the classified effect can be made either by triggering an exclusion rule in the BfR system (to be checked on a case-by-case basis), or based on a negative prediction in a classification QSAR that was trained on both positive and negative substances. The suitability of the model (reliability, relevance) should be very carefully checked to make sure that the prediction is fit for purpose, and the applicability of the model to the substance should also be justified (e.g fulfilment of the conditions of

Section 1.3 of Annex XI to the REACH Regulation should be checked). For read-across, generation of new *in vivo* data should be avoided.

[d] Data obtained from non-validated suitable *in vitro* tests can only be used according to the criteria set out in section 1.4 of Annex XI to the REACH Regulation, i.e. only positive results can be accepted. However, there are already several EU/OECD adopted test methods which should be primarily used (see table 7.2-4).

[e] New *in vitro* testing should be performed following a top-down or bottom-up approach. Please see the following paragraph "How to use the *in vitro* methods serious eye damage/eye irritation within the strategy". It is highly recommended to adhere to the test protocols whose scientific validity has been established by validation and which, ideally, have been officially adopted by the European Commission and/or by the OECD.

[f] *In vivo* testing should not be conducted in case the substance falls under the scope of the specific *in vitro* test(s) performed, and there are no substance-specific limitations to using those tests, and the Registrant formulates an adaptation according to Annex XI to the REACH Regulation. Due to the current standard *in vivo* information requirement at Annex VIII level and above, an adaptation needs to be built up in the registration dossier in order to successfully submit a compliant dossier. [33]

How to use the *in vitro* methods for serious eye damage/eye irritation within the strategy

For serious eye damage/eye irritation no single *in vitro* test method is currently able to fully replace the regulatory *in vivo* test, known as the Draize eye test (EU B.5/OECD TG 405) across the full range of ocular responses for different chemical classes. However, the *in vitro* test methods specified in Sections R.7.2.8.1 and R.7.2.9.1 may be used for partial replacement within a tiered testing strategy or as stand-alone test methods depending on the outcome of the study. Moreover, combinations of several alternative test methods may be able to fully replace the Draize eye test. Testing strategies such as the top-down or bottom-up approaches provide a means of incorporating existing information, QSAR predictions, read-across and grouping and *in vitro* test results.

New *in vitro* testing should be performed following a top-down or bottom-up approach (Scott *et al.*, 2010). The top-down approach (start with an *in vitro* test able to identify substances that are seriously damaging to the eye, i.e. classified as Serious eye damage Cat. 1) should be used when all available collected information and the *Weight-of-Evidence* assessment result in a high a-priori probability of the substance being seriously damaging to the eye. The bottom-up approach, on the other hand (start with an *in vitro* test able to identify substances not requiring classification for serious eye damage/eye irritation, i.e. not classified) should be followed when all available collected information and the *Weight-of-Evidence* assessment result in a high a-priori probability of the substance being non-irritant to the eyes..

There are steps to be considered before any testing (*in vitro* or *in vivo*) is conducted. These steps are specified in Section 8.2 in column 1 of Annexes VII and VIII to the REACH Regulation and include:

[33] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

1. Assessment of all available human and animal data (e.g. animal data may be available from acute dermal toxicity studies or a substance may already be classified as a skin corrosive or as causing serious eye damage);

2. Assessment of acid and alkaline reserve. It should be noted that other substance properties e.g. pH or others could indicate that the substance is seriously damaging or irritant to eyes. Consideration of information obtained from the use of (Q)SARs or from similar substances may be useful in predicting the serious eye damage/eye irritation potential of the substance (see Figure R.7.2-5);

3. Assessment of existing data on physico-chemical properties. No further testing is required if the substance is spontaneously flammable in air (pyrophoric) or water at room temperature.

   After following steps 1, 2 and 3, if a conclusion on classification cannot be drawn, *in vitro* studies for serious eye damage/eye irritation should be conducted.

4. One or more *in vitro* studies for serious eye damage/eye irritation should be performed, and the outcome can be:

   a) In the case of a positive and definitive result from e.g. the BCOP, ICE, FL or other scientifically valid *in vitro* test methods, the substance can be classified as inducing "serious eye damage" (Serious Eye Damage Category 1 under CLP), and no further test *in vivo* is necessary.

   b) In addition, the BCOP and ICE, or other scientifically valid *in vitro* test methods can also provide information on whether the substance does not require any classification for the eye hazard. If no classification is needed, no further testing *in vivo* is necessary.

   c) For Annex VIII information requirements, if a definitive conclusion on the serious eye damage/eye irritation potential of the substance cannot be reached from the use of one or several *in vitro* methods used in a testing strategy, a further test conducted *in vivo* to assess the eye hazard potential of the substance is needed.

Note: Registrants must make sure that the substance falls under the scope and applicability domain of the specific *in vitro* tests performed, and there are no substance-specific limitations to using those tests (see *in vitro* tests for serious eye damage/eye irritation and sections R.7.2.8.1 and R.7.2.9.1).)

Registrants who must fulfil the Annex VIII information requirement for an *in vivo* eye irritation study and have completed the above steps, may be able to do so by using an adaptation according to Annex XI to the REACH Regulation and without testing on animals. Due to the current standard *in vivo* information requirement at Annex VIII level and above, an adaptation needs to be built up in a registration dossier in order to successfully submit it. However, an *in vivo* eye irritation test may still be necessary depending on the assessment of the available information and outcomes of *in vitro* studies.[34]

[34] Please note that the information requirements in REACH Annexes VII and VIII in relation to skin corrosion/irritation and serious eye damage/eye irritation are currently under revision. This revision is expected to strengthen the role of *in vitro* methods and to remove the standard information requirement for an *in vivo* study at the Annex VIII level. As a consequence, once the new REACH Annexes come into force, an *in vivo* study would only be required where a substance falls outside of the applicability domain of the available *in vitro* methods or the results obtained from such methods would not allow a conclusive decision on (non-)classification and risk assessment.

Instruction on how to submit *in vitro* information instead of *in vivo* can be found e.g. in section 3.7 of *Practical Guide 1: How to report in vitro data* (available at http://echa.europa.eu/practical-guides).

It is important to note that it is the responsibility of the registrant to ensure that the chosen test method(s) is (are) suitable for the substance in order to obtain adequate information from the *in vitro* studies. For most substances, the use of EU- or OECD-adopted test methods for the eye hazard characterisation will provide results that will have regulatory acceptance under REACH.

RESPIRATORY TRACT CORROSION / IRRITATION

R.7.2.12 Information sources on respiratory tract corrosion / irritation

The evaluation of respiratory tract corrosion/irritation can be based on expert judgment using evidence such as: human and animal experience, existing (*in vitro*) data, substance properties like pH values, volatility (Saturated Vapour Concentration (SVC)) or dustiness, information from similar substances or any other pertinent data.

R.7.2.12.1 Animal data

There are currently no EU or OECD adopted test guidelines that deal specifically with respiratory tract corrosion or irritation. Studies that could inform on the respiratory tract corrosion/irritation potential of the substance concerned are single or repeated inhalation exposure studies (information on (histo-)pathological changes).

Single inhalation exposure studies *in vivo* 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.

It is noteworthy that, while histopathology is not a standard element of the OECD TG 436 for Acute Inhalation Toxicity, TG 436 specifies that "*Additional examinations included a priori by design may be considered to extend the interpretive value of the study, such as… providing evidence of irritation by microscope examination of the respiratory tract. Examined organs may include those showing evidence of gross pathology in animals surviving 24 or more hours, and organs known or expected to be affected. Microscopic examination of the entire respiratory tract may provide useful information for test articles that are reactive with water, such as acids and hygroscopic test articles*".

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 tract irritation or even corrosion when vaporised or in the form of an aerosol. 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 tract corrosion/irritation potency of a substance. Existing and available information from acute and repeated dose inhalation toxicity studies may also be considered sufficient to show that the substance causes respiratory tract corrosion/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.7.2.12.2 Human data

Existing human data include historical data that should be taken into account when evaluating intrinsic hazards of substances. *New* testing in humans for hazard identification purposes is not acceptable for ethical reasons.

Existing human data can be obtained from case reports, poison information centres, medical clinics, and occupational experience or from epidemiological studies or volunteer 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, absence of incidence in humans does not necessarily overrule existing good quality animal data that are positive.

Specifically with regard to respiratory tract 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.7.2.13 Evaluation of information on respiratory tract corrosion / irritation

All data available should be evaluated to estimate a substance's potential to induce respiratory tract corrosion or irritation.

R.7.2.13.1 Animal data

The evaluation is based on data from inhalation studies (acute, repeated exposure):

- Clinical symptoms of dyspnoea or breathing difficulties,
- Histomorphology of the respiratory tract,
- Lavage examination (nasal, bronchoalveolar).

Useful information may be obtained from the single and repeated inhalation toxicity studies for classification and labelling as well as for DNEL derivation.

For derivation of a DNEL (acute - inhalation, local effects) information from animal studies with acute and/or repeated inhalation exposure may be used. This usually requires that in the study several exposure concentrations were used that allow derivation of a No Observed Adverse Effect Concentration (NOAEC) and/or a Low Observed Adverse Effect Concentration (LOAEC) or a benchmark concentration (BMC) as starting points for DNEL derivation (Section R.8.2.1 and Appendix R.8-8 of Chapter R.8 of the *Guidance on IR&CSA*). In case such information is only available from repeated dose inhalation studies, derivation of a long-term DNEL (long-term - inhalation, local effects) might be more appropriate.

For classification and labelling purposes, the severity of the effects (reversible versus irreversible) and the target within the respiratory tract (upper versus lower respiratory tract) need to be considered.

In case animal studies show reversible effects (usually in the upper respiratory tract), the studies can be used as part of a *Weight-of-Evidence* evaluation for classification for STOT-SE Category 3. Reversible respiratory tract effects may be clinical signs of toxicity like dyspnoea or rhinitis and histopathological effects like hyperemia, oedema, minimal inflammation or thickened mucous layer which may be reflective of the characteristic clinical symptoms described above.

In case the studies show significant changes, more than transient in nature, especially in the lower respiratory tract (bronchiolar and alveolar region), classification for STOT-SE Category 1 or 2 might be considered, depending on the concentration at which the effects occur. Significant changes to the respiratory tract may include necrosis, or other morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction. However, if such effects were only observed in inhalation studies with repeated exposure and the mode of action indicates that the significant damage to the respiratory tract is due to repeated exposure, classification for "Specific Target Organ Toxicity after Repeated Exposure (STOT-RE), Category 1 or 2 might be more appropriate (see Section 3.9 of the *Guidance on the application of the CLP criteria*).

For corrosive substances that may be acutely toxic, the additional labelling with EUH071 *"Corrosive to the respiratory tract"* should be considered (see Section 3.1 of the *Guidance on the application of the CLP criteria*). It is presumed that corrosive substances will cause toxicity by inhalation exposure. The Hazard statement EUH071 must be assigned for substances that may be inhaled in addition to classification for acute inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity. In cases where no acute inhalation test has been performed and the substance may be inhaled, this hazard statement must also be assigned. However, if corrosive substances are used in mixtures in sub-corrosive concentrations, it needs to be ensured that an appropriate classification for potential respiratory tract irritation is applied. For liquids the volatiliy/SVC, and for solids dustiness, if applicable, should be taken into consideration.

R.7.2.13.2 Human data

The evaluation is based on:

- Experience from occupational exposure;
- Published data on volunteers (objective measurements, psychophysical methods, and subjective reporting);
- Other data (e.g. from nasal lavage).

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 tract 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 odour as a bias. 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.

Human data demonstrating respiratory tract irritation are used primarily for classification for Specific Target Organ Toxicity after Single Exposure (STOT-SE), Category 3 (H335: *"May cause respiratory irritation"*) under CLP (see Section 3.8 of the *Guidance on the application of the CLP criteria,* available at http://echa.europa.eu/web/guest/guidance-documents/guidance-on-clp).

Such effects are characterised by localised redness, oedema, pruritis and/or pain and they impair function with symptoms such as cough, pain, choking, and breathing difficulties. Subjective human observations could be supported by objective measurements of clear respiratory tract irritation (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids). Furthermore, the symptoms observed in humans should also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of 'irritation' must be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory tract irritation.

R.7.2.14 Conclusions on respiratory tract corrosion / irritation

R.7.2.14.1 Concluding on suitability for Classification and Labelling

In order to conclude on Classification and Labelling according to the CLP Regulation, all the available information needs to be taken into account, and consideration should be given to both the *Guidance on the application of the CLP criteria* and the various remarks (as they relate to Classification and Labelling) made throughout this guidance document.

R.7.2.14.2 Concluding on suitability for Chemical Safety Assessment

A dose-response assessment might be possible. Animal studies, especially those with repeated inhalation exposure and several exposure concentrations, may be available that allow derivation of a NOAEC and/or a LOAEC as starting points for DNEL derivation.

Human data indicative of respiratory tract irritation that provide reliable quantitative information on the threshold for the irritative effects may also be used to derive DNEL (acute - inhalation, local effects) (see Section R.8.2.1 and Appendix R.8-8 of Chapter R.8 of the *Guidance on IR&CSA*).

R.7.2.15 References

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Appendices R.7.2-1 to 3 to Section R.7.2

Appendix R.7.2-1 Mechanisms of local toxicities: skin corrosion/irritation, serious eye damage/eye irritation and respiratory tract corrosion/irritation

Content of Appendix R.7.2-1:

- Mechanisms of skin corrosion and irritation
- Mechanisms of serious eye damage/eye irritation
- Mechanisms of respiratory tract corrosion and 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 and 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 and 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 (1995a) and further elaborated by Walker *et al*. (2004), for organic substances, the mechanisms leading to skin irritation are normally described by a two-stage process where a substance 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 substances are binding to macromolecules in the 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 substances.

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 substances with sufficient hydrophobic and hydrophilic properties, and

   - Elicitation of an inflammatory and/or cytotoxic response in the epidermis or dermis.

   The severity of these responses may determine whether irritation or corrosion occurs.

MECHANISMS OF SERIOUS EYE DAMAGE AND EYE IRRITATION

Eye injury can be caused by many insults. These can be physical such as puncture by sharp objects. Eye injury can be caused by substances such as systemic drugs that can enter into the eye through the blood stream (e.g. Cyclosporine, vaccines, intravenous immunoglobulines, intravenous streptokinase). Various degrees of eye injury can also be caused by direct (topical) contact with substances or mixtures such as acids, alkalis, solvents or surfactants. These

materials may come into contact with 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, substances or mixtures which come directly into contact with the eye 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 substances and mixtures cause eye injury e.g. cell membrane lysis, saponification and coagulation (see Table R.7.2-5).

Table R.7.2-5 Categories of irritant substances and their typical mode of action in eye irritation.

Substance / mixtures	Mode of Action
Inert substances	May cause effect due to large size. Protrusions may cause direct puncture of the eye.
Acids	May react directly with cellular components e.g. eye proteins and cause coagulation, lysis or precipitation resulting in relatively localised injury.
Bases (Alkalis)	May actively disrupt the cell membrane lipids by alkaline action i.e. saponification. May penetrate to the deeper layers of the eye tissue. May react directly with cellular components and cause coagulation or lysis of the tissue.
Solvents	May cause membrane lysis by dissolving lipids in plasma membranes of epithelial and underlying cells resulting in loss of the cells affected and, as a result, tissue degradation, which might be transient, depending on the repair mechanisms (cell proliferation, tissue restoration). May also cause coagulation.
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 substance or 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 e.g. substances that react with cellular constituents via alkylation or oxidative attack on macromolecules. An example of these types of substances are e.g. peroxides, mustards and bleaches (Scott *et al.*, 2010).

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 mucus leading to an increase in discharge. Visual acuity can be impaired. Effects on the cornea may be more severe (e.g. destruction of the cornea, or persistent cornea opacity or discoloration of the cornea by a dye substance), or reversible where effects are limited to the epithelia. 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 substance. 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 adversive 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 TRACT CORROSION AND IRRITATION

Corrosion of the respiratory tract includes destruction of the mucosa followed by proliferation of epithelial cells. Remodeling of tissue may occur with chronic injury if repair mechanisms are unable to keep pace. Mild epithelial or endothelial injury without basement membrane damage, severe inflammation, or persistence of the inciting agent may be resolved by simple cellular regeneration. With more severe damage, a significant inflammation component may be elicited which may be followed by tissue destruction or fibrosis. In some cases, persistence of the inciting agent within the tissue may lead to the development of a granulomatous disease, as observed with inhalation exposure to crystalline silica or carbon nanotubes (Harkema *et al*., 2013).

Corrosive effects in the respiratory tract may be non-specific, e.g. induced by highly acidic or basic substances like sulphuric acid. However, acute necrosis and loss of olfactory epithelium may also be observed following inhalation or bloodborne exposure to toxicants that require metabolic activation by the P450 system, such as 3-methylfuran. Once the basement membrane is exposed, cytokines are released and inflammation takes place (Harkema *et al*., 2013).

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.

Cytotoxic effects in the respiratory tract are comparable to dermal and eye irritation. Those 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. A recent publication has proposed the term “tissue irritation” for this kind of effects (Brüning *et al.,* 2014).

Chronic irritation can lead to repeated episodes of cell proliferation in the affected tissues, and this may increase the risk of tumour development. The nature of effects depends on the substance and its primarily targeted region, the severity of effects depends on the concentration and duration of exposure. In general, repeated exposure studies in animals focus on observing (histo)pathological evidence for tissue damage. 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. Substance or substance-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 odour sensation, which is mediated via different nerve pathways (olfactory). However, there is evidence that odour 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 (Alarie, 1973)) 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 of the correlation between the results of the Alarie test and 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. Emphasis was given to develop a spectrum of objective measurements (see review by Arts *et al*., 2006). Compiling toxicological profiles for substances in the workplace demonstrate that sensory irritation often appears to be a very sensitive and relevant end point in human risk assessment. Accordingly, 40 % of the occupational exposure limit values (OELs) are based on the avoidance of sensory irritation (Dick and Ahlers 1998; Edling and Lundberg 2000; van Thriel et al., 2006). This end point is related to the interaction of volatile substances with neuronal sensors located in mucous membranes of the respiratory tract and the eyes. In many cases, data from controlled human studies are either not available or inadequate, so OELs are predominantly derived from animal data investigating local effects in the respiratory tract. These effects are usually measured as tissue irritation. Comparison of human data on sensory irritation with data from subacute and subchronic inhalation studies in animals led to the proposal of an default assessment factor of 3 for extrapolating animal data concerning local irritating effects to humans (Brüning et al., 2014).

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Appendix R.7.2-2 (Q)SARs and expert systems for skin corrosion and irritation

Content of Appendix R.7.2-2:

- Literature-based QSAR models
- Commercial models
- BfR rule-base
- OECD QSAR Toolbox

In principle, Annex XI to the REACH Regulation allows for an adaptation of the standard information requirements by using (Q)SARs, including the prediction of non-irritancy. However, for the endpoint skin corrosion/irritation, only very few of the currently available models are suitable for that purpose if used as stand-alone methods. Nevertheless, such models can still have their merit when used as supporting information or in *Weight-of-Evidence* approaches and for positive prediction of skin corrosion/irritation.

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 substances, 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, 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.

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 substances, 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 substances 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. 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, 1989, 1990), 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, 1995a). These studies did not address the question of how to use pKa where there are multiple functional groups in the substance of interest, and therefore multiple ionisation constants. Based on current knowledge, no clear recommendations can be made about how to use pKa information.

An overview on the available literature-based models for skin corrosion/irritation is given in the Table R.7.2-6.

Table R.7.2-6 Available literature-based models for skin corrosion/irritation.

Reference	*Content*
QSAR models	
Barratt (1996a) Quantitative structure-activity relationships (QSARs) for skin corrosivity of organic acids, bases and phenols: Principal components and neural network analysis of extended datasets.	This paper describes QSAR models relating skin corrosivity data of organic acids, bases and phenols to their log(octanol/water partition coefficient), molecular volume, melting point and pK(a).
Barratt (1996b) Quantitative structure-activity relationships for skin irritation and corrosivity of neutral and electrophilic organic chemicals.	This paper describes QSAR models derived by relating skin irritation and corrosivity data of neutral and electrophilic organic chemicals to their log(octanol/water partition coefficient) (logP), molecular volume, dipole moment and 1/molecular weight.
Barrat (1996c) The use of in vitro cytotoxicity measurements in (Q)SAR methods for the prediction of the skin corrosivity potential of acids.	This paper describes quantitative structure-activity relationships (QSAR) methods that relate the severity of skin corrosivity (designated by the EC risk phrases R34 and R35) of acids to parameters that model their skin permeability and cytotoxicity. Skin permeability was modelled by log(octanol/water partition coefficient), molecular volume and melting point, while the cytotoxicity of the acids was accounted for by their pKa values and the in vitro cytotoxicity of their sodium salts towards Swiss mouse embryo 3T3 cells.
Gerner *et al.* (2004) Quantitative structure-property relationships modeling of skin irritation.	This paper describes limit values for specific physico-chemical properties that are appropriate for identifying chemical substances that have no skin irritation or corrosion potential. These physicochemical properties include melting point, molecular weight, octanol-water partition coefficient, surface tension, vapour pressure, aqueous solubility and lipid solubility.
Golla *et al.* (2009) Quantitative structure-property	This paper describes a skin irritation QSPR model based on rabbit Draize test data for 186 compounds, which included chemicals from diverse molecular classes. The effectiveness of using a combination of

relationships modeling of skin irritation.	traditional, functional group and structural descriptors has been studied. The effects of molecular size, reactivity and skin penetration on skin irritation have been also analysed.
Hayashi *et al*. (1999) A quantitative structure-activity relationship study of the skin irritation potential of phenols.	This paper describes QSARs for skin irritation potential derived using twenty-four phenols, using the following descriptors: the absolute hardness calculated from HOMO and LUMO energy levels for reactivity, and log P for permeability. The selection of the descriptors was based on the hypothesis that skin irritation is induced by reaction of phenols with macromolecules present in epidermal and dermal levels of the skin.
Hulzebos *et al.* (2005) Use of structural alerts to develop rules for identifying chemical substances with skin irritation or skin corrosion potential.	This paper describes the identification and categorisation of structural alerts for acute skin lesions as irritation or corrosion or a combination of corrosion/irritation alerts.
Kodithala *et al.* (2002) Prediction of skin irritation from organic chemicals using membrane-interaction QSAR analysis.	This paper describes membrane-interaction QSAR analysis carried out for a training set of 22 hydroxy organic compounds for which the Draize skin irritation scores, PII, had been determined. Skin irritation potency is predicted to increase with (1) increasing effective concentration of the compound available for uptake into phospholipid-rich regions of a cellular membrane, (2) increasing binding of the compound to the phospholipid-rich regions of a cellular membrane, and (3) the chemical reactivity of the compound as reflected by the highest occupied molecular orbital (HOMO) and/or lowest unoccupied molecular orbital (LUMO) of the molecule.
Walker *et al.* (2004) (Q)SARs for Predicting Skin Irritation and Corrosion: Mechanisms, Transparency and Applicability of Predictions.	This paper describes previously-developed (Q)SARs for predicting skin irritation and corrosion, proposes mechanisms of skin irritation and corrosion, and discusses the transparency and applicability of predictions.
Walker *et al.* (2005) The Skin Irritation Corrosion Rules Estimation Tool (SICRET).	This paper describes the Skin Irritation Corrosion Rules Estimation Tool (SICRET) that was developed to allow others to estimate whether their chemicals are likely to cause skin irritation or skin corrosion. SICRET uses physicochemical property limits to identify chemicals with no skin corrosion or skin irritation potential.
Whittle (1996) Skin corrosivity potential of fatty acids: *In vitro* rat and human testing and (Q)SAR studies.	This paper investigates the corrosive potential of a series of fatty acids-propanoic acid (C3), butanoic acid (C4), hexanoic acid (C6), octanoic acid (C8), decanoic acid (C10) and dodecanoic acid (C12) according to in-vitro skin corrosivity test (IVSCT) using both rat skin and human skin. The results are discussed in the context of a QSAR for the corrosivity of organic acids, with the putative mechanism that corrosivity is a function of the ability of the chemical to permeate the skin together with its cytotoxicity, expressed in this case as acidity (pK(a)).
Worth and Cronin (2001) The use of pH measurements to predict the potential of chemicals to cause acute dermal	This paper presents a the development of classification models based on pH data for predicting the potential of chemicals to cause skin corrosion, skin irritation and eye irritation. The possible application of these models in the context of tiered testing strategies is discussed.

and ocular toxicity.	
Reviews and evaluation of existing models	
Gallegos Saliner *et al.* (2006) Review of Literature-Based Models for Skin and Eye Irritation and Corrosion.	This report reviews the state-of-the-art of in silico and in vitro methods for assessing dermal and ocular irritation and corrosion. In this review, emphasis is placed on literature-based QSAR models for skin and eye irritation and corrosion as well as computer-based expert systems.
Gallegos Saliner *et al.* (2008) Review of (Q)SAR Models for Skin and Eye Irritation and Corrosion.	This paper reviews the state-of-the-art of in silico methods for assessing dermal and ocular irritation and corrosion. It is based on an in-depth review performed by the European Chemicals Bureau of the European Commission: Joint Research Centre . The most widely used in silico approaches are classified into methods to assess (1) skin irritation, (2) skin corrosion and (3) eye irritation. In this review, emphasis is placed on literature-based (Q)SAR models.
Gallegos Saliner *et al.* (2007) Evaluation of SARs for the prediction of skin irritation/corrosion potential: structural inclusion rules in the BfR decision support system.	This work evaluates the structural inclusion rules implemented in the Decision Support System for skin irritation and corrosion developed at the German Bundesinstitut für Risikobewertung (BfR) for predicting the absence of skin irritation and/or corrosion. The following assessments were performed: (a) a confirmation of the structural rules by rederiving them from the original training set (1358 substances), and (b) an external validation by using a test set of 200 chemicals not used in the derivation of the rules.
Mombelli (2008) An evaluation of the predictive ability of the QSAR software packages, DEREK, HAZARDEXPERT and TOPKAT, to describe chemically-induced skin irritation.	This paper reports the performance of the skin irritation module of three commercially-available software packages: DEREK, HAZARDEXPERT and TOPKAT. Their performances were tested on the basis of data published in the literature, for 116 chemicals.
Rorije and Hulzebos (2005) Evaluation of (Q)SARs for the prediction of Skin Irritation/Corrosion Potential. Physicochemical exclusion rules.	This work evaluates the physical-chemical rule-base incorporated in the Decision Support System for skin irritation and corrosion developed at the German Bundesinstitut für Risikobewertung (BfR) for predicting the absence of skin irritation and/or corrosion. This evaluation includes 1) the compliance of the rule-base with the OECD principles on (Q)SARs, 2) the derivation of the (Q)SAR rules, 3) the external validation of these rules, including an assessment of the suitability of the dataset used for validation.

Further details on these models can be found in Chapter 3 of the JRC report “Alternative methods for regulatory toxicology - a state-of-the-art review” (Worth *et al.*, 2014).

COMMERCIAL MODELS

There is a number of software tools that provide access to QSARs for skin corrosion/ irritation.

TOPKAT, which is commercialised by Accelrys (http://accelrys.com/solutions/scientific-need/predictive-toxicology.html), 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). The algorithm of TOPKAT is not very 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.

There is a rule-base for irritation in **Derek Nexus** (Sanderson and Earnshaw, 1991; Combes and Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.lhasalimited.org/products/derek-nexus.htm). 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 Derek Nexus rule-base has 25 structural alerts for the prediction of skin corrosion/irritation. There are some combined alerts for the respiratory tract irritation and irritation of the gastrointestinal tract but these are not specific to skin corrosion or irritation. If Derek Nexus does not make a prediction of corrosion or irritation, it cannot be concluded that there is no effect – it could mean that none of known alerts was found to be present in the substance of interest or it was outside the applicability domain of that specific alert. The Derek Nexus 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 substances. The example substances are supposed to support the mechanistic reasoning. The Derek Nexus model can be used for positive identification of skin irritation. The confidence levels have to be taken into account for the purpose of classification. The Derek Nexus model cannot be used to predict non-corrosion/irritation as the model only contains alerts that detect the presence of corrosion/irritation.

HazardExpert is a rule-based software tool developed and commericalised by CompuDrug Chemistry Ltd. (http://www.compudrug.com/hazardexpertpro) for predicting the toxicity of organic substances in humans and in animals (Smithing and 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/case-ultra-models#skin_eye_tox_bundle), 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.

ACD/Labs Percepta Predictors (http://www.acdlabs.com/products/percepta/predictors.php), developed by ACD/Labs, includes a module for skin and eye irritation. It estimates the

potential of a compound to cause eye or skin irritation in a standard rabbit Draize test. The predictions are reported as qualitative irritation categories (not irritating, slightly irritating, irritating, highly irritating, and corrosive). Probabilistic models are supplemented by an expert system that identifies Structural Alerts relevant to the irritation properties of compounds. Overall, 21 structural alerts were formulated for rabbit eye irritation, and 17 alerts – for the rabbit skin irritation case. The categorisation of effect needs to be compared to the CLP cut-offs if application under REACH is intended.

PaDEL-DDPredictor includes several models for skin and eye irritation and corrosion (http://padel.nus.edu.sg/software/padelddpredictor/). The models have been built on a training set of 1707 compounds using one and two dimensional descriptors. The final predictions rely on consensus models based on majority voting from base models predictions. The applicability domain is defined on the range of descriptors for compounds in the training set.

QSAR PREDICTION REPOSITORY

The Danish EPA (http://qsar.food.dtu.dk/) 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 former official list of EU-classified substances (Annex I of Directive 67/548/EEC, now replaced by Annex VI to the CLP Regulation). 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 corrosion/irritation potential in combination with structural inclusion rules (SARs) to predict the presence of such potential (Gerner *et al.*, 2004; Hulzebos *et al.*, 2005; 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 sub-structural molecular features. The physico-chemical rules are assumed to implicitly take into account bioavailability (skin penetration) whereas the structural rules take reactivity into account. The physico-chemical and structural rule-bases are designed to predict the former EU risk phrases for skin irritation (R38) and skin corrosion (R34 and R35).

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 substance does not need to be considered for classification

Some of the exclusion rules can be applied to all structures within the domain, whereas others are only referring to a subset containing certain elements.

The structural inclusion rules take the following general form:

IF (substructure) A THEN predict the occurrence of toxic effect B

Example: IF *Chlorosilane alert is present* THEN the substance needs to be considered for "corrosive" classification

The performance of the BfR physico-chemical rule-base for predicting the absence of skin effects has been assessed by the RIVM (Rorije and Hulzebos, 2005), whereas the structural rule-base for predicting the occurrence of skin effects has been assessed by the ECB (Gallegos Saliner *et al.*, 2007). The endpoint is the former EU (DSD) classification, the algorithms and domain of applicability are transparent. However, the exact chemical structures of the training set are not disclosed to users of the model, due to the data originating from the confidential notification procedure at the time of the development of the system. Though the rules are empirically derived, a mechanism of action can be deduced. Thus, in principle, the resulting predictions can be used as the basis for classification by comparison with CLP criteria. It should be determined, on a case-by-case basis, whether the predictions for a given substance provide a sufficient basis for classification, or whether additional information is needed in a *Weight-of-Evidence* approach.

OECD QSAR TOOLBOX

The freely downloadable OECD QSAR Toolbox software (http://www.qsartoolbox.org/) covers the skin corrosion/irritation endpoint with one experimental database and two profilers.

In more detail, the database of experimental data (called “Skin irritation” in the software) refers to the endpoint primary irritation index and collects the data available in:

1. The RIVM Skin Irritation database, which contains Primary Skin Irritation Indices from skin irritation tests from the following sources: ECVAM Workshop 6 on Corrosivity (Barratt (1995b); Botham *et al.* (1995)), and ECETOC Technical Report No.66 on Skin Irritation and Corrosion Reference Chemicals Data Bank (ECETOC, 1995).

2. Experimental results for Primary Skin Irritation Indices from LJMU. Additional experimental results gathered from OECD SIDS Dossiers published between 1992 and 2009 were added in 2010.

The OECD QSAR Toolbox allows for the identification of analogues based on mechanistic and endpoint specific profilers, and for the prediction of skin irritation/corrosion through the use of profilers (BfR rule-base), readacross, trend analysis and QSAR models. Information about inclusion and exclusion rules, details on the performance of the exclusion rules, and applicable chemical class-specific rules for the results of the Skin irritation/corrosion profiler can be found by searching the context menu in the the OECD QSAR Toolbox software.

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Appendix R.7.2-3 QSARs and expert systems for serious eye damage and eye irritation

Content of Appendix R.7.2-3:

- Literature-based QSAR models
- Commercial models
- BfR decision support system
- OECD QSAR Toolbox

In principle, Annex XI to the REACH Regulation allows for an adaptation of the standard information requirements by using (Q)SARs, including the prediction of non-irritancy. However, for the endpoint serious eye damage/eye irritation, only very few of the currently available models are suitable for that purpose if used as stand-alone methods. Nevertheless, such models can still have their merit when used as supporting information or in *Weight-of-Evidence* approaches and for positive prediction of serious eye damage/eye irritation.

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) and 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, 1993; Abraham *et al.*, 1998). The mechanistic 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 and Cronin, 2000), so that irritants can be transparently identified as those substances 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).

Different methods were applied to a dataset of 119 organic liquids classified as I (irritant) or NI (non-irritant) according to former 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). (Worth and Cronin, 2003).

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, but it does so with a high degree of confidence. Conversely, the CT does not identify many of the non-irritants, but it has a low false negative rate. Thus, the combined use of the BLR and CT models could be useful for distinguishing between eye irritants and non-irritants.

An overview on the available literature-based models for serious eye damage/eye irritation is provided in Table R.7.2-7.

Table R.7.2-7 Available literature-based models for serious eye damage / eye irritation.

Reference	*Content*
QSAR models	
Abraham *et al.* (2003) Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis.	Draize rabbit eye test scores, as modified maximum average score (MMAS), for 68 pure bulk liquids were adjusted by the liquid-saturated vapor pressure P. These 68 adjusted scores, as log (MMAS/P), were shown to be completely equivalent to eye irritation thresholds (EIT), expressed as log (1/EIT), for 23 compounds in humans. Thus, for the first time the Draize eye test in rabbits for pure bulk liquids is shown to be perfectly compatible with eye irritation thresholds in humans.
Barratt (1995) The role of structure-activity relationships and expert systems in alternative strategies for the determination of skin sensitisation, skin corrosivity and eye irritation.	This paper describes the derivation of a set of structural alerts for skin sensitisation, which have been incorporated into the expert system DEREK, and of Quantitative structure-activity relationships (QSARs) derived for predicting the skin corrosivity (for organic acids and bases) and for the eye irritation potential (for neutral organic chemicals).
Gerner *et al.* (2005) Assessment of the Eye Irritating Properties of Chemicals by Applying Alternatives to the Draize Rabbit Eye Test: The Use of QSARs and *In Vitro* Tests for the Classification of Eye Irritation.	This paper evaluates and discusses the nature of eye lesions and their importance for classification and labelling of possible hazards to human eyes, with a view to promoting the development of specific in vitro assays which are able to discriminate between eye damage, moderate eye irritation, and minor irritation effects which are completely reversible within a few days. Structural alerts for the prediction of eye irritation/corrosion hazards to be classified and labelled according to international classification criteria, are presented, which should be validated in accordance with internationally agreed (OECD) principles for (Q)SAR system validation. Physicochemical limit values for prediction of the absence of any eye irritation potential relevant for human health

	can make available a definition of the applicability domains of alternative methods developed for the replacement of the Draize eye irritation test.
Solimeo *et al.* (2012) Predicting Chemical Ocular Toxicity Using a Combinatorial QSAR Approach.	This paper describes QSAR models for a set of small molecules with animal ocular toxicity data compiled by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods.
Reviews and evaluation of existing models	
Gallegos Saliner *et al.* (2006) Review of Literature-Based Models for Skin and Eye Irritation and Corrosion.	This report reviews the state-of-the-art of in silico and in vitro methods for assessing dermal and ocular irritation and corrosion. In this review, emphasis is placed on literature-based QSAR models for skin and eye irritation and corrosion as well as computer-based expert systems.
Gallegos Saliner *et al.* (2008) Review of (Q)SAR Models for Skin and Eye Irritation and Corrosion.	This paper reviews the state-of-the-art of in silico methods for assessing dermal and ocular irritation and corrosion. It is based on an in-depth review performed by the European Chemicals Bureau of the European Commission: Joint Research Centre . The most widely used in silico approaches are classified into methods to assess (1) skin irritation, (2) skin corrosion and (3) eye irritation. In this review, emphasis is placed on literature-based (Q)SAR models.
Tsakovska *et al.* (2005) Evaluation of (Q)SARs for the prediction of Eye Irritation/Corrosion Potential - physicochemical exclusion rules.	In this study, an evaluation was performed of the physicochemical BfR-DSS rule-base (comprising 31 physicochemical exclusion rules) for predicting the absence of eye irritation/corrosion. According to the results of this study: a) the physicochemical exclusion rules for eye irritation/corrosion comply well with the OECD validation principles; b) predictions of no adverse effect (NOT R34/R35/R36/R41) can be made for 20 out of the 199 chemicals in the test set; c) 3 of the 45 irritants/corrosives are falsely predicted as non-irritant or non corrosive; d) the probability of a negative prediction being correct (Negative Predictive Value) is 0.87; and e) approximately 10% of Draize rabbit eye tests could be avoided by relying on the predictions of no adverse effect.
Tsakovska *et al.* (2007) Evaluation of SARs for the prediction of eye irritation/corrosion potential - structural inclusion rules in the BfR decision support system.	This work summarises the results of a study carried out by the ECB to assess the performance of the BfR structural rule-base. The assessment included: (a) evaluation of the structural alerts by using the training set of 1341 substances with experimental data for eye irritation and corrosion; and (b) external validation by using an independent test set of 199 chemicals. The test set of 199 substances contained 154 (77%) non-labeled substances and 45 (23%) labeled as eye irritants/corrosives, subdivided as follows: (i) 10 R36 substances (5%); (ii) 28 R41 substances (14%); and (iii) 7 substances (4%) labeled R34 or R35.

Further details on these models can be found in Chapter 4 of the JRC report “Alternative methods for regulatory toxicology - a state-of-the-art review” (Worth *et al.*, 2014).

COMMERCIAL MODELS

There is a number of software tools that provide access to QSARs for serious eye damage/eye irritation.

The **TOPKAT** software (http://accelrys.com/solutions/scientific-need/predictive-toxicology.html) includes models for eye irritation based on structural fragments. These models were originally developed by Enslein *et al.* (1988). The TOPKAT algorithm is not very 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.

There is a rulebase for irritation in **Derek Nexus** (Sanderson and Earnshaw, 1991; Combes and Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.lhasalimited.org/products/derek-nexus.htm). See for a general outline the skin irritation section on (Q)SARs. The Derek Nexus rule-base has five alerts that are specific to eye irritation, plus one for eye lachrymation. If Derek Nexus does not make a prediction of irritation 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 substance of interest or it was outside the applicability domain of that specific alert. The Derek Nexus 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 substances, which is not sufficient to consider them internally validated. The example substances underline the mechanistic reasoning. The Derek Nexus model can be used for positive identification of skin irritation. The confidence levels have to be translated to consider them for classification. The Derek Nexus model cannot be used to predict for non-serious eye damage/eye irritation as the model only contains alerts that detect the presence of serious eye damage/eye irritation.

The fragment-based **MultiCASE** approach (http://www.multicase.com/case-ultra-models#skin_eye_tox_bundle) 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. The prediction should be underlined with mechanistic reasoning using other models or expert judgment.

ACD/Labs Percepta Predictors (http://www.acdlabs.com/products/percepta/predictors.php), developed by ACD/Labs, includes a module for skin and eye irritation. It estimates the potential of a compound to cause eye or skin irritation in a standard rabbit Draize test. The predictions are reported as qualitative irritation categories (not irritating, slightly irritating, irritating, highly irritating, and corrosive). Probabilistic models are supplemented by an expert system that identifies Structural Alerts relevant in the irritational properties of compounds. Overall, 21 structural alerts were formulated for rabbit eye irritation, and 17 alerts for the rabbit skin irritation case.

PaDEL-DDPredictor includes several models for skin and eye irritation and corrosion (http://padel.nus.edu.sg/software/padelddpredictor/). The models have been built on a

training set of 1707 compounds using one and two dimensional descriptors. The final predictions rely on consensus models based on majority voting from base models predictions. The applicability domain is defined on the range of descriptors for compounds in the training set.

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 serious eye damage/eye irritation 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 corrosion and irritation section above. The physico-chemical and structural rule-bases are designed to predict the former EU risk phrases for eye irritation (R36) and severe eye irritation/corrosion (R41). Independent assessments by the ECB support the performance of the physico-chemical rule-base 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).

OECD QSAR TOOLBOX

The freely downloadable OECD QSAR Toolbox software (http://www.qsartoolbox.org/) covers the serious eye damage/eye irritation endpoint with one experimental database and two profilers.

In more detail, the database of experimental data (called “Eye irritation ECETOC” in the software) refers to the endpoint Modified Maximum Average Score (MMAS) and collects experimental results on rabbit eye irritation described in,ECETOC Technical Report No.48 on Eye Irritation Reference Chemicals Data Bank (ECETOC, 1992).

The OECD QSAR Toolbox allows for the identification of analogues based on mechanistic and endpoint specific profilers, and for the prediction of skin irritation/corrosion through the use of read across, trend analysis and QSAR models. Information about inclusion and exclusion rules, details on the performance of the exclusion rules, and applicable chemical class-specific rules for the results of the Eye irritation/corrosion profiler can be found by searching the context menu in the the OECD QSAR Toolbox software.

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