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GUIDANCE

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.

Document history

Version	Changes	Date
[…]	[…]	[…]
Version 4.0	Full revision addressing the content of Section R.7.2 related to *Skin-, eye and respiratory tract irritation/corrosion*. The update includes the following: · Update of the information on new/revised EU test methods and OECD test guidelines for skin- and eye irritation/corrosion; · Update of the information on respiratory tract irritation/corrosion assessment; · Replacement of the terms "respiratory irritation" by "respiratory tract irritation/corrosion"; · Update of the information on non-testing methods, in particular in Appendices R.7.2-2 *QSARs and expert systems for skin irritation and corrosion* and R.7.2-3 *QSARs and expert systems for eye irritation and corrosion*; · Update of the recommended assessment and testing strategy for irritation/corrosion in Section R.7.2.6 and further sub-division of that section into the new Sections R.7.2.6.2 and R.7.2.6.3 for skin and eye, respectively; · Replacement of the terms "Integrated Testing Strategy (ITS)" by "assessment and and testing 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 irritation/corrosion* and 3.3 *Serious Eye damage/Eye irritation* of the *Guidance on the Application of the CLP Criteria* (version 4.0, November 2013).	XX 2014

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

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 or mixture of substances. These changes are considered local effects. A distinction in local effects can be made between those observed after single and those after repeated exposure. In this guidance document, the focus will be on local effects after single ocular or dermal exposure. However, wherever possible, use should also be made of existing repeated dose data as far as they may contain valuable information for the purpose of assessing and classifying effects after single ocular or dermal exposure.

Substances causing local effects after single exposure can be further distinguished as irritant or corrosive substances, depending on the severity, reversibility or irreversibility of the effects observed. *Corrosive substances* are those which may destroy living tissues with which they come into contact. *Irritant substances* are non-corrosive substances which, through immediate contact with the tissue under consideration may cause inflammation. These tissues are in the present context skin, eye (cornea and conjunctiva) and mucous epithelia such as the respiratory tract. Criteria for classification of irritant and corrosive substances are given in Annex 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 substances may have defatting properties (Ad-hoc Working group on Defatting substances, 1997). Chemicals that have a similar mechanism 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, eye and respiratory tract irritation*.

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

Definitions of skin-, eye and respiratory tract irritation/corrosion can be found in the CLP Regulation or in relevant EU/OECD test guidelines (TGs).

Skin irritation: Defined in Section 3.2.1.1 of Annex I to the CLP Regulation and in OECD TG 404/EU B.4 as *"[…] the production of reversible damage of the skin following the application of a test substance for up to 4 hours."*.

Dermal irritation after repeated exposure: Used for a substance which may cause skin dryness, flaking or cracking upon repeated exposure but which cannot be considered as skin irritant.

Skin corrosion: Defined in Section 3.2.1.1 of Annex I to the CLP Regulation and in OECD TG 404/EU B.4 as *"[…] the production of irreversible damage to skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. […]"*.

Eye irritation: Defined in 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 required 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*. *"[…] 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 required 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-, eye and/or respiratory tract irritation/corrosion can be further characterised by their classification under the CLP Regulation.

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).

a) For corrosion

- **Skin corrosives** are classifed in Category 1 with the Hazard statement H314 *"Causes severe skin burns and eye damage"*. Further subcategorisation is based on the results of animal testing (Draize skin corrosion test):
  - Subcategory 1A: Destruction of skin tissue occurs after exposure times up to 3 minutes and is observed within 1 hour after exposure,
  - subcategory 1B: Destruction of skin tissue occurs after exposure times between 3 minutes and 1 hour and is observed within 14 days after exposure,
  - subcategory 1C: Destruction of skin tissue occurs after exposure times between 1 hour and 4 hours and is observed within 14 days after exposure.

- **Substances or mixtures causing eye corrosion/serious eye damage/irreversible effects on the eye** are classifed in Category 1 with the Hazard statement H318 *"Causes serious eye damage".*

b) For irritation

- **Skin irritants** are classifed in Category 2 with the Hazard statement H315 *"Causes skin irritation".*

- **Substances or mixtures causing eye irritation/reversible effects on the eye** are classifed 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 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** are classifed in Specific Target Organ Toxicity after Single Exposure (STOT-SE) Category 3 with the Hazard statement H335 *"May cause respiratory irritation".*

In addition, for substances or mixtures classified for inhalation toxicity, and if data are available that indicate that the mechanism of toxicity is corrosivity, the supplemental statement EUH071 *"Corrosive to the respiratory tract"* must be used (see Section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I to the CLP Regulation).

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-, eye and respiratory tract irritation / corrosion

The general objectives are:

a. to establish whether information from physical/chemical data, from non-testing methods (grouping, QSARs and expert systems), from *in vitro* studies, from animal studies or human experience provide evidence that the substance is, or is likely to be, corrosive.

b. to establish whether information from physical/chemical data, from non-testing methods (grouping, QSARs and expert systems), from *in vitro* studies, from animal studies or human experience provide evidence of significant skin, eye or respiratory tract irritation/corrosion.

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

d. to gather, in the process of hazard identification, any quantitative data on dose-response relationships that might allow the derivation of DNELs essential for a complete risk assessment.

If a risk assessment is necessary, both the severity of the identified hazard (in so far at it can be judged from the test data) and the probability of the occurrence of an acute corrosive or irritant response in humans must be assessed based on the likelihood of any exposure to the substance and in relation to the route, pattern and extent of the expected exposure.

Please note that there are currently no standard tests and no OECD TGs available for acute respiratory tract irritation and there is no testing requirement for respiratory tract irritation under the REACH Regulation. Consequently respiratory tract irritation/corrosion is not included in the testing strategies suggested in this guidance.

Nevertheless, account should be taken of any existing and available data that provide evidence of the respiratory tract irritation/corrosion potential of a substance. 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. 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 irritation/corrosion potency of a substance. Information from acute and repeated dose inhalation toxicity studies may also be considered sufficient to show that the substance causes respiratory tract irritation/corrosion at a specific concentration level or range. The data need to be carefully evaluated with regard to the exposure conditions (sufficient documentation required). Possible confounding factors should be taken into account.

R.7.2.2 Information requirements on skin / eye irritation / corrosion

The information on irritation and corrosion 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 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. Furthermore, information on exposure, use and risk management measures should also be collected and evaluated.

If these data are inadequate for hazard and risk assessment, further testing should be carried out in accordance with the requirements of Annexes VII (^{3}1 tpa) and VIII (^{3}10 tpa) to the REACH Regulation.

Information requirements for quantities of ≥1 tpa (Annex VII to the REACH Regulation)

If new testing data are necessary, these must be derived from *in vitro* methods only. Annex VII does not foresee *in vivo* testing for irritancy or corrosivity.

The standard information requirements at this tonnage level for skin irritation/corrosion (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, 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).

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

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").

The *in vitro* methods that can be used for adaptation of the standard information requirements are detailed in Section R.7.2.4.1 of this Guidance, under "*In vitro* data".

Information requirements for quantities of ≥10 tpa (Annex VIII to the REACH Regulation)

For substances manufactured or imported in quantities of ≥10 tpa *in vivo* testing is required to meet the standard information requirements of Annex VIII (Column 1).

Column 2 of Annex VIII lists specific rules that allow deviating from the standard testing regime.

In summary, the specific rules for adapting the standard information required by Annex VIII are:

a. for skin irritation/corrosion:

- 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, 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).

b. For eye irritation/corrosion:

- 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 (i.e. as 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").

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

The use of *in vitro* methods in a *Weight-of-Evidence* approach (see Section 1.2 of Annex XI to the REACH Regulation) to comply with the information requirements at this tonnage is outlined in Section R.7.2.6.2 of this Guidance.

Importantly, the standard testing regime of Annexes VII and VIII can be adapted according to the general rules laid down in Annex XI, e.g. 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.7.2.4.1 of this Guidance for possible alternatives to animal testing). However, 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, 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 and its sources on irritation / corrosion

R.7.2.3.1 Non-human data on irritation / corrosion

Non-testing data on irritation / corrosion

Physico-chemical properties

Information of relevance to irritation/corrosion can be inferred from basic physico-chemical characteristics of a substance (extreme pH). Substances with *extreme* pH values will be inevitably skin corrosives or severe eye irritants:

IF pH ≤ 2 or pH ≥ 11.5, THEN predict to be corrosive to skin and severely irritating to eyes (See also Section R.7.2.4.1 of this Guidance).

Grouping, (Q)SARs and expert systems

Non-testing methods can be divided into three categories: 1) grouping approaches (read-across, SARs and categories), 2) QSARs, and 3) expert systems, generally incorporating multiple (Q)SARs, expert rules and data. These methods can be used for the assessment of skin and eye irritation and corrosion, if they provide relevant and reliable data for the substance of interest. Generally this means that the use of non-testing methods should be justified by means of detailed descriptions. In the case of QSARs and expert systems, registrants can refer to the QSAR Model Reporting Format (QMRF) and to the QSAR Prediction Reporting Format (QPRF). The QMRF is a harmonised template for summarising and reporting key information on (Q)SAR model validitie, 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 QPRF is a harmonised template for summarising and reporting substance-specific predictions generated by (Q)SAR models. 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 the case of skin irritation and corrosion, many of the models have a mechanistic basis, which provides additional information on the relevance of the model.

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

SARs and read-across are treated together in this Guidance 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 thought likely to cause the effect. They are generally considered to reflect some kind of chemical or biochemical reactivity that underlies the toxicological effect. The presence of a structural alert in a structure most often suggests the presence of effect. This evidence, in combination with the occurrence of structural analogues that exhibit corrosion (or irritation) potential can be used to predict a corrosive effect for the substance of interest and adapt further assessment, as indicated in the OECD Integrated Approach on Testing and Assessment (IATA) for skin irritation/corrosion (OECD, 2014). Negative data from structural analogues may also be used to make predictions in certain cases, provided that there are no other substructures or other properties of the substance that are thought likely to cause the effect.

The non-reactive chemicals, which lack alerts for reactivity, will normally not exhibit irritant or corrosive effects. However, irritant effects such as irritant contact dermatitis can occur in the case of exposure to organic solvents, which have defatting properties. Chemicals that have a similar mechanism need to be considered for 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 (R1-O-O-R2), based on the fact that peroxides are oxidising agents. These SARs are mentioned in the Classification and Labelling guide (EC, 2006). The validity of these models, however, is not given there. Rorije *et al*. (2007) showed that 75 and 60% of the hydroperoxides and peroxides are classified for corrosivity and irritancy, respectively.

A variety of SARs for predicting the presence of irritation or corrosion have been described by Hulzebos *et al*. (2001, 2003, 2005a), and others have been incorporated into the BfR (*Bundesinstitut für Risikobewertung*/German Federal Institute for Risk Assessment) rulebase, and the SICRET tool (Walker *et al*., 2005, see *Appendix R.7.2-2 QSARs and expert systems for skin irritation and corrosion*). The BfR alerts have more recently been incorporated into 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.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm).

Read-across has been used to a limited extent in the New Chemicals notification procedure for the classification of skin irritants (Hoffmann *et al*., 2005). As of May 2006, one substance had been classified as R38 ("*Irritating to skin*") under the former Council Directive 67/548/EEC on the classification, packaging and labelling of Dangerous Substances (DSD) by read-across from an analogue, and seven substances had been unclassified for R38 on the basis of read-across from analogues that had not been found to meet the classification criteria for skin irritation (Thomas Cole, ECB, personal communication).

- QSARs and expert systems on skin irritation and corrosion:

QSARs and expert systems for skin irritation and corrosion have been described in several reviews (Hulzebos *et al*., 2001, 2003, 2005a; 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 *QSARs and expert systems for skin irritation and corrosion*, 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 compounds, although in some cases more diverse and larger datasets were also examined. In general, it has been suggested that basic physico-chemical parameters such as acidity, basicity, hydrophobicity, and molecular size as well as electrophilic reactivity, are useful to predict the toxic potential of homologous chemicals. In contrast, models intended to predict the toxic potential of heterogeneous groups of chemicals emphasise the commonality of structural features.

A number of models are actually expert systems, which are computer programs that guide hazard assessment by predicting toxicity endpoints of certain chemical structures based on the available information. Expert systems can be based on an automated rule-induction system (e.g. TOPKAT, HazardExpert and MultiCASE), or on a knowledge-based system (e.g. DEREK for Windows, the BfR-DSS, and SICRET). More details on commercial expert systems are reported in Appendix R.7.2-2.

The freely available for download software OECD QSAR Toolbox contains two profilers relevant for skin irritation/corrosion, which encode inclusion rules (alerts) with a suggestion that exclusion for certain physico-chemical properties is also an option. The use in combination of profilers and data for analogue could allow the prediction of skin irritation/corrosion for new chemicals through read-across or category approach.

Not all of the models were developed with EU regulatory purposes in mind, so it is important to assess in each case whether the endpoint or effect being predicted corresponds with the regulatory endpoint of interest. In principle, such models could be redeveloped (re-parameterised) by using updated or alternative datasets, and used instead of the published models. The BfR model (also reported in Appendix R.7.2-2) has been developed to predict EU regulatory endpoints, and it has been evaluated (Rorije and Hulzebos, 2005; Gallegos Saliner *et al.*, 2007).

- Use of (Q)SAR models for skin corrosion:

In the case of classification models for skin corrosion, where it is not indicated in the supporting documentation whether the predicted classification should be Skin corrosive subcategory 1A, 1B or 1C, a 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 chemicals. For classification and labelling, the BfR rulebase 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).

- SARs and read-across for eye irritation and corrosion:

The occurrence of structural analogues that exhibit eye corrosion (or irritation) potential can also be used to predict the effect in the substance of interest and adapt further assessment. Negative data from structural analogues may also be used to make predictions in certain cases, provided that there are no other substructures or other properties of the substance that are thought likely to cause the effect. Read-across had been used in the New Chemicals notification procedure for the classification of eye irritants. An example is provided by the classification as R36 (*"Irritating to eyes"*) under the former DSD of Neodol HS, a branched alcohol ethoxy sulphate, by read-across from structurally related anionic surfactants. The adequacy of the read-across was justified in multiple ways:

i. by comparing the *in vitro* results of Neodol HS with that of SLS in the Cytosensor Microphysiometer Test. Since SLS is classified as R36 and used as the positive control in this assay, and since the test result showed that Neodol has a lower eye irritancy

than SLS, it was argued that Neodol HS should also be (conservatively) classified as R36;

ii. by referring to the Critical Micelle Concentration (CMC). Below this concentration, the surfactant is in the monomer form, which has irritant properties, whereas above the CMC, the surfactant forms micelles, which are less irritant. Thus, the higher the CMC, the greater the proportion of monomers present, and the more likely the surfactant is to be an irritant. Neodol HC was shown to have a lower CMC than similar surfactants classified as R36;

iii. by referring to the fact that alkyl ethoxy sulphates, such as Neodol HC, tend to be weaker eye irritants than alkyl sulphates and sulphonates, and that alkyl sulphates and sulphonates with similar chain lengths to Neodol HC were classified as R36.

This illustrates the use of *in vitro* data to support read-across by comparing the *in vitro* effect of the chemical of interest with that of a suitable benchmark chemical.

- QSARs and expert systems for eye irritation and corrosion:

An extensive review of the 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. These models also include literature-based QSAR models, and expert systems.

From the scientific literature, it appears that more emphasis has been placed on the QSAR modelling of ocular irritation compared with dermal irritation. Examples of models based on classical regression and classification techniques, together with more innovative approaches, are collected in Appendix R.7.2-3.

The most widely used commercial expert systems for assessing eye irritation are the same as those used for assessing skin irritation and corrosion. Details on automated rule-induction systems (e.g. TOPKAT and MultiCASE), and on knowledge-based systems (e.g. DEREK for Windows, and the BfR-DSS) are reported in Appendix R.7.2-3.

The freely available for download software OECD QSAR Toolbox contains two profilers relevant for eye irritation/corrosion, which encode inclusion rules (alerts) with a suggestion that exclusion for certain physico-chemical properties is also an option. The use in combination of profilers and data for analogues could allow the prediction of skin irritation/corrosion for new chemicals through a read-across or category approach.

Not all of the models were developed with EU regulatory purposes in mind, so it is important to assess in each case whether the endpoint or effect being predicted corresponds with the regulatory endpoint of interest. In the case of the more transparent, literature-based models, the examples could be more useful in terms of illustrating the feasibility of developing a model by using defined descriptors and by applying a defined statistical approach to a suitable dataset. If alternative or extended datasets are available, such models could be redeveloped (re-parameterised) and used instead of the published models. The BfR model for the prediction of eye irritation has been developed to predict EU regulatory endpoints, and it has been assessed (Tsakovska *et al*., 2005 and Tsakovska *et al*., 2007).

- Use of (Q)SAR models for eye irritation/corrosion:

In the case of classification models for eye irritation, the classification criteria used in 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.7.2-1 Overview of available (Q)SARs for skin and eye irritation/corrosion and the availability of QSAR model reporting formats (QMRFs), in which the application of the OECD principles for QSARs is illustrated. See Appendices R.7.2-2 and R.7.2-3 for more information on these models.

Category of model or source	Reference or name of the model	Type of model	Applicability domain	Draft QMRF* developed
Literature models	Barratt, 1995a	Statistical model	Acids, Bases , Phenols and pKa	no
	Berner *et al.*,1988, 1989, 1990	Mathematical model	pKa related acids	no
	Nangia *et al.*, 1996	Mathematical model	pKa related for bases	no
	Barratt, 1996b	Statistical model	Electrophiles	no
	Smith *et al.* 2000 a,b	Statistical model	Esters	no
	Barratt, 1996b	Statistical model	Neutral organics	no
	Gerner *et al.*, 2004, 2005; Walker *et al.*, 2004	Rule-based model	New Chemicals Database, organic chemicals	yes
	Golla *et al.*, 2009	Mathematical model	Organic chemicals from diverse classes	no
Computerised models	TOPKAT, commercial	Mathematical model using connectivity descriptors	Organic chemicals	yes
	DerekfW, commercial	Expert system using structural alerts	Organic chemicals and some metals	yes
	MultiCASE, commercial	Mathematical model using fragments	Organic chemicals	no
	Hazard expert, commercial	Organic chemicals using structural alerts	Organic chemicals	no
	BfR rulebase, free, available in-house at BfR	Rule-based model	New Chemicals Database, organic chemicals	yes
Review papers	Hulzebos *et al.*, 2001, 2003, 2005a	N.A.	N.A.	N.A.
	Patlewicz *et al.*, 2003	N.A.	N.A.	N.A.
	Gallegos Saliner *et al.*, 2006, 2008	N.A.	N.A.	N.A.

	Mombelli, 2008	N.A.	N.A.	N.A.

* QMRF: (Q)SAR model reporting format (available at http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/laboratories-research/predictive_toxicology/qsar_tools/QRF). For further details See 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).
Abbreviation: N.A. = not applicable.

Testing data for irritation / corrosion

The internationally accepted testing methods for skin and eye irritation/corrosion are described in OECD TGs (available at http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm#Test_Guidelines) and the Annex to the EU Test Methods (TM) Regulation (Council Regulation (EC) No 440/2008). Those regarding skin effects can be found in OECD TGs 404 (EU B.4), 430 (EU B.40), 431 (EU B.40bis), 435, 439 (EU B.46), and those for the endpoint eye in OECD TGs 405 (EU B.5), 437 (EU B.47), 438 (EU B.48), 460.

The testing strategies developed (see Section R.7.2.6 of this Guidance) emphasise the need to evaluate all available information (including physico-chemical properties) before attempting any *in vivo* testing. They both employ screening elements designed to avoid, as far as possible, *in vivo* testing of corrosive and severely irritating substances. In particular, it is recommended to perform *in vitro* tests for both skin and eye irritation/corrosion endoints in order to assess whether *in vivo* testing can be completely avoided. To date, there is no method for testing respiratory tract irritation or corrosion.

In vitro data

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

Accepted *in vitro* tests for skin and eye irritation/corrosion are listed in Table R.7.2-2 and described below.

- *In vitro* tests for skin corrosion:
  - Transcutaneous electrical resistance test (TER) (EU B.40/OECD TG 430),
  - Reconstructed Human Epidermis (RHE) test method, which includes more than one protocol (EU B.40 bis/OECD TG 431),
  - *In vitro* membrane barrier test method (OECD TG 435).
- *In vitro* tests for skin irritation:
  - Reconstructed human epidermis (RHE) tests, which include more than one protocol (EU B.46/OECD TG 439).

Irritant/corrosive substances are identified in the human skin assays by:

i. their ability to induce a decrease in cell viability (measured by the MTT test) below defined threshold levels.

ii. their ability to release inflammatory mediators (Interleukin 1α) when the cell viabiliy is above the defined threshold levels.

In vitro tests for eye irritation/corrosion:

Accepted *in vitro* tests to detect serious eye damage (Category 1 under CLP) are listed in Table R.7.2-2:

- Bovine corneal opacity and permeability (BCOP) test (EU B.47/OECD TG 437),
- Isolated chicken eye (ICE) test (EU B.48/OECD TG 438),
- Fluorescein Leakage Test Method (OECD TG 460).

In addition, negative results obtained with the BCOP and ICE test methods can be used for classification purposes under CLP, and can lead to no classification for eye hazard.

Currently, there are several *in vitro* methods under validation or already validated addressing serious damage to the eyes (Category 1 under CLP):

- Short-time exposure test (STE)
- Cytosensor® microphysiometer test method (CM)
- Isolated rabbit eye (IRE) test
- Hen's egg test – chorio-allantoic membrane (HET-CAM) test.
- EpiOcular™ Eye Irritation Test
- SkinEthic™ Human Corneal Model

Positive results in those *in vitro* tests can be considered and can lead to classification of the substances tested in Category 1 for eye damage and labelling with H318 *"Causes serious eye damage"* according to CLP.

The CM (http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFE8725DF1F9F5D) and STE (http://ntp.niehs.nih.gov/?objectid=2D70C7A2-CCDB-D782-06CB38302BD7D10E) test methods have been validated by ICCVAM and OECD draft TGs are available (see http://www.oecd.org/env/ehs/testing/section4healtheffects.htm). Concerning the IRE and HET-CAM test methods, ICCVAM validation assessment in 2007 concluded that these test methods were not sufficiently accurate for regulatory use and recommended additional studies (http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFE8725DF1F9F5D).

There are two human corneal epithelium models commercially available: EpiOcular™ (MatTek Inc., USA) and SkinEthic™ HCE (SkinEthic, France) which have undergone assessment in industry-organised trials for pre-validation and validation purposes. Formal validation of these two test methods is ongoing and coordinated by EURL ECVAM.

The above tests are either organotypic assays (BCOP, ICE, IRE and HET-CAM) or cytotoxicity and cell function based assays (CM, FL, EpiOcular™ and SkinEthic™). These test methods are mainly concerned with modelling the immediate effects of chemicals on the cornea**.** *In vivo* eye irritation endpoints which are not covered by the above-mentioned optimised protocols are the following:

i. persistence/reversibility of effects
ii. effects on conjunctivae or other eye tissue
iii. mechanical irritation produced by solid materials

Assessment and testing strategies combining the different tests according to their applicability domain and capacity to classify in the different ranges of eye irritation will still need to be developed, once the individual tests have been completely evaluated.

Table R.7.2-2 Validation status, regulatory acceptance, relevant guidelines for skin and eye irritation / corrosion

Area of concern	Test	Validation status, regulatory acceptance, use, limitations	OECD test guideline	EU Test Methods Regulation	EURL ECVAM DB-ALM protocol Nr.
Skin corrosion					
	TER	Validated	TG 430	B.40	115
	EpiDerm ™	Validated	TG 431	B.40 bis	119
	EpiSkin ™	Validated	TG 431	B.40 bis	118
	SkinEthic ™	Validated	TG 431	B.40 bis	-
	epiCS®	Validated	TG 431	B.40 bis	-
	Corrositex	Validated	TG 435	Not yet	116
Skin irritation					
	EpiDerm ™	Validated	TG 439	B.46	138
	EpiSkin ™	Validated	TG 439	B.46	131
	SkinEthic ™	Validated	TG 439	B.46	135
	SIFT	Only prevalidation so far. Applicability domain limited.	N.A.	N.A.	-
Eye irritation					
	BCOP	Validated	OECD 437	B.47	98, 124
	ICE	Validated	OECD 438	B.48	80
	FL	Validated	OECD 460	N.A.	71
	CM	Validation completed	N.A.	N.A.	130
	STE	Validation completed	N.A.	N.A.	
	HET-CAM	Pending, but regulatory acceptance for severe irritants*,***	N.A.	N.A.	47, 96
	RBC	Pending. Used by industry for screening purposes.***	N.A.	N.A.	37, 99
	NRR	Pending. Used by industry for screening purposes.	N.A.	N.A.	54

	SMP	Pending. Used by industry for screening purposes.	N.A.	N.A.	97, 102
	EpiOcular ™	Pending. Used by industry for screening purposes.	N.A.	N.A.	-
	SkinEthic ™	Pending. Used by industry for screening purposes.	N.A.	N.A.	-
	IRE	Pending, but regulatory acceptance for severe irritants *,***	N.A.	N.A.	85

* see Section 13.5.2 of the *Manual of Decisions for Implementation of the 6th and 7th Amendments to Directive 67/548/EEC on Dangerous Substances (*EC, 2006).

** see ICCVAM's report (ICCVAM, 2010).

*** see ECVAM Scientific Advisory Committee (ESAC)'s report (ESAC, 2009a).

Abbreviations: BCOP = Bovine Corneal Opacity and Permeability; CM = Cytosensor Microphysiometer; FL = Fluorescein Leakage; HET-CAM = Hen's Egg Test on Chorioallantoic Membrane; ICE = Isolated Chicken Eye; IRE = Isolated Rabbit Eye; N.A. = not applicable; NRR = Neutral Red Release; RBC = Red Blood Cell Haemolysis Test; SIFT = Skin Integrity Function Test in Mouse; SMP = Silicon Microphysiometer; STE = Short-Time Exposure; TER = Transcutaneous Electrical Resistance.

Animal data

- Skin and eye irritation/corrosion:

Annex I to the CLP Regulation defines both skin and eye irritation as local toxic effects, and, as such, an assessment of irritation/corrosion is normally part of the acute testing phase of a toxicity programme and it is an early requirement of all regulatory programmes. As a consequence, testing for irritation/corrosion has, historically, used animal models and a variety of test methodologies depending upon, for example, the laboratory undertaking the test, the era and intended application.

Current approaches for irritation/corrosion testing *in vivo* are covered by the following test guidelines:

- Acute Dermal Irritation/Corrosion (EU B.4/OECD TG 404),
- Acute Eye Irritation/Corrosion (EU B.5/OECD TG 405).

The guidelines for skin and eye irritation/corrosion testing require a tiered approach, using one animal (the rabbit is the preferred species) initially, which in the absence of severe effects is followed by a further two animals (a total of three animals).

Both 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 for both eye and skin, *irritants* (Category 2 Eye irritants and Category 2 Skin irritants, respectively) cause significant inflammation of the eye (conjunctiva redness/oedema, cornea and/or iris) and/or skin (erythema and/oedema) but these effects are transient, i.e. the affected sites are repaired within the observation period of the test. A *severe eye irritant* causes considerable damage to the cornea and/or iris and is classified in Category 1 for Serious Eye Damage. The criteria for classification in Category 1 for Serious Eye Damage

include persistence of effects (any score), irreversible staining of the eye and/or criteria for the degree of severity. Guidance on how industry interprets eye irritation data in the light of EU classification and labelling is summarised in a publication by ECETOC (1997).

A corrosive substance causes full thickness destruction of the skin tissue and is classified as 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 substances, 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.

In addition to the EU and OECD TGs mentioned above, further animal data may be available from:

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

See Section R.7.2.6 of this Guidance for comments on how to use information from these tests in an assessment and testing strategy for skin and eye irritation/corrosion.

Data on chemosensory effects obtained in the Alarie test for respiratory tract irritation (Alarie, 1973; Arts *et al*., 2006) may be useful as supportive evidence for human eye irritation after exposure to airborne chemicals (e.g. vapours).

- Respiratory tract irritation/corrosion:

There are currently no EU or OECD adopted test guidelines that deal specifically with respiratory tract irritation or corrosion. Studies that could inform on the respiratory tract irritation/corrosion potential of the chemical concerned are single or repeated inhalation exposure studies (information on (histo-)pathological changes) or, in case of sensory irritation, the Alarie assay (information on sensory irritation) (Alarie, 2000; ASTM, 2004).

Single inhalation exposure studies may provide information on nasal irritation such as rhinitis, whereas histopathological examination of respiratory tract tissues of animals repeatedly exposed by inhalation (28-day and 90-day inhalation studies) may provide information on inflammatory/cytotoxic effects such as hyperemia, edema, inflammation or mucosal thickening. Data from bronchoalveolar lavage may give additional information on the inflammatory response.

The evaluation of respiratory tract corrosion can be based on expert judgment using evidence such as: human and animal experience, existing (*in vitro*) data, pH values, information from similar substances or any other pertinent data.

R.7.2.3.2 Human data for irritation / corrosion

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

Existing data can be obtained from case reports, poison information centres, medical clinics, and occupational experience or from epidemiological studies. Their quality and relevance for hazard assessment should be critically reviewed. However, in general, human data can be

used to determine a corrosive or irritating potential of a substance. Good quality and relevant human data have precedence over other data. However, lack of positive findings in humans does not necessarily overrule good quality animal data that are positive.

Specifically with regard to respiratory 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.7.2.4 Evaluation of information on irritation/corrosion

R.7.2.4.1 Non-human data on irritation/corrosion

Non-testing data on irritation/corrosion (skin and eye)

In 2014, the OECD approved an IATA for skin irritation/corrosion. 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, 2014).

Physico-chemical properties

According to the current EU and OECD guidelines, substances should not be tested on animals for irritation/corrosion if they can be predicted to be corrosive from their physico-chemical properties. In particular, substances exhibiting strong acidity (pH ≤2.0) or alkalinity (pH ≥11.5) in solution are predicted to be corrosive, and should not be tested. However, no conclusion can be made regarding corrosivity when the pH has an intermediate value (when 2.0< pH <11.5).

- Physico-chemical properties for **skin** corrosion/irritation:

Chemicals that have other pH values will need to be considered further for their potential for skin and eye irritation/corrosion.

The following decision rule can be used in a tiered testing strategy:

IF pH ≤ 2 or pH ≥ 11.5 THEN assume the chemical to be corrosive (Category 1A).

This model is included in OECD IATA for skin irritation and corrosion (OECD, 2014). 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).

However, where extreme pH is the only basis of classification as corrosive, it may also be important to take into consideration the acid/alkaline reserve, a measure of the buffering capacity of a chemical substance (Young *et al*., 1988; Botham *et al*., 1998; Young and How, 1994), as mentioned in the OECD TG 404. However, the buffering capacity should not be used alone to exonerate from classification as corrosive. Indeed, when the Acid/Alkaline reserve

suggests that the substance might be non-corrosive, further *in vitro* testing should be considered.

- Physico-chemical properties for **eye** irritation/corrosion:

A chemical known or predicted to be corrosive to the skin is automatically considered to cause Serious Eye Damage (Category 1). However, no conclusion can be made regarding eye irritation/corrosion potential when the pH has an intermediate value (when 2< pH <11.5). Thus, the following decision rule may be used in a tiered testing strategy:

IF pH ≤2 or pH ≥11.5 THEN consider the chemical for classification as a severe eye irritant.

To predict the eye irritation/corrosion potential of non-corrosive chemicals, the distribution of pH values for irritants and non-irritants in a data set of 165 chemicals has been analysed (Worth, 2000). The irritants spanned a wide range of pH values from 0 to about 12, whereas the non-irritants spanned a much narrower range from about 3 to 9. Using the cut off values generated by classification tree analysis, the following model was formulated:

IF pH <3.2 or if pH >8.6, then consider the chemical for eye irritation classification; otherwise make no prediction.

According to the way the model was developed, and referring to the former EU Risk Phrases for irritation, *irritant* can either be R41 (Category 1 under CLP) or R36 (Category 2 under CLP). Further information and/or reasoning is needed to conclude on classification. The more severe classification (R41 or Category 1 under CLP) should be assumed if no further information is available.

This model had a sensitivity of 53% (and therefore a false negative rate of 47%), a specificity of 97% (and therefore a false positive rate of 3%), and a concordance of 76%. A QSAR Model Reporting Format (QMRF) has been developed (see Section R.6.1 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; and JRC QSAR Model Database accessible at http://qsardb.jrc.ec.europa.eu/qmrf/index.jsp).

Based on these statistics, this model is not recommended for the stand-alone discrimination between eye irritants and non-irritants. However, it could be used in the context of a tiered testing strategy to identify eye irritants (due to its very low false positive rate) but not non-irritants (due to its relatively high false negative rate).

Grouping, (Q)SARs and expert systems

Guidance has been developed by the 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 guidance on the use of read-across/category approaches is given in Section R.6.2.

First the model should be descripted in accordance with OECD principles on (Q)SARs (OECD, 2004c), and documented by means of a QMRF. Interpretation of the model is additionally needed. For example a model based on the logarithm of the octanol/water partition coefficient (K_{ow}) might indicate how the log K_{ow} should be derived, measured, calculated, with which program, whether ionised chemicals can be used as well. For more complicated parameters e.g. the quantum descriptors HOMO (Highest Occupied Molecular Orbital energy) and LUMO (Lowest Unoccupied Molecular Orbital energy) this is even more crucial as the calculation outcome depend on the configuration state of the molecule. The performance parameters for the model (i.e. correlation coefficient, sensitivity/specificity, etc.) have to be reported. When

the predictivity of a model is assessed, it should be assessed whether the test set is within the applicability domain of the model. The guidance given by the authors/builders of the model should be a starting point.

The second step is to evaluate the prediction of a specific chemical. The OECD principles on (Q)SARs can again be used. In this regard, the fit of the target chemical within the applicability domain has to be evaluated extensively (e.g. is the target chemical similar to the molecules in the training set, are the most similar analogues in the training set well predicted, does information exist on the predictivity). The outcome of the prediction should be assessed and documented in the form of a QPRF.

The third and last step of the evaluation explicitly needs to meet regulatory requirements. In this last evaluation the (Q)SAR prediction is weighed against the possible mechanism of skin irritation and corrosion. It has to be compared with the effects that can be observed in the *in vivo* test, to see whether all skin irritation/corrosion pathways are covered. In this last step, the hazard of defatting properties has to be assessed as well. (Q)SAR models have to be evaluated in considering the possible mechanism and how this would relate to CLP classification.

The mechanism of irritation and corrosion involves toxicodynamic and toxicokinetic parameters. Models that predict irritation and corrosion based on toxicodynamic properties only (e.g. acidity or basicity, electrophilicity, other reactivity, surfactant activity, solving membranes) have to be additionally evaluated for their taking into consideration of toxicokinetic parameters. These parameters can be physico-chemical parameters or others and indicate the potential to cross the skin (stratum corneum) and be active in the living tissue underneath the stratum corneum. Conversely models that predict (the absence of) irritation and corrosion solely from e.g. physico-chemical properties that illustrate the toxicokinetic behaviour of a chemical, have to be evaluated for their taking into consideration of its activity (toxicodynamics).

For example, the BfR physico-chemical rulebase predicts the absence of skin and eye irritation. Evaluations of the BfR rulebases for the prediction of no skin irritation/corrosion (Rorije and Hulzebos, 2005; Gallegos Saliner *et al.*, 2007) and for the prediction of no eye irritation (Tsakovska *et al.*, 2005) have been carried out independently. However, when the absence of irritation cannot be excluded, further information on the structure of the chemical is needed to predict presence of irritation/corrosion.

Evaluations of the predictive performance of the physico-chemical (Rorije and Hulzebos, 2005) and structural (Gallegos Saliner *et al.*, 2007) rules showed a high negative predictivity (99% for non-corrosives and 97% for non-irritancy for skin; 87% for eye) of the physico-chemical exclusion rules, and a high positive predictivity of the structural inclusion rules (95% for corrosives and 68% for irritants for skin, >80% depending on the hazard class). These evaluations were performed before the BfR rulebases were implemented in Toxtree and the OECD Toolbox, so it would be worthwhile to revisit the analysis, using modern tools and more recently published data. Both rules would need to be fulfilled in order to predict that a substance is non-irritant, i.e. a substance should fulfil the exclusion rules, and not have any alert for the inclusion rules. A limitation of this system is that the BfR exclusion rules depend on physico-chemical properties of the substance, such as surface tension or lipid solubility, for which there is no reliable calculation method either in the OECD Toolbox or in Toxtree. Hence, to use this system with some confidence, it would be necessary to have the measured values of the relevant physico-chemical properties, unlike for the above-mentioned commercial tools which can be applied using chemical structure as the sole input. Alerts from the inclusion rules might potentially be used on their own for a positive prediction, since this would represent a conservative approach. There is no other model available which sufficiently describes the absence of effects. Neutral organics are expected not to be irritants, however their defatting potential should be discussed. The definition of a neutral organic is a chemical which does not

have potential reaction centres, even after skin metabolism. Absence of reactivity needs to be described in sufficient detail or be substantiated with other information.

Presence of effects has been well established using the pH cut-off values for high acidity and basicity and can be applied. Structural alerts for the presence of effects can be used, however further characterisation of the effects needs to be described in sufficient detail or be substantiated with other information. For instance, the BfR structural rulebases for the prediction of skin irritation/corrosion (Rorije *et al*., 2007 and Gallegos Saliner *et al*. 2007) and for the prediction of eye irritation (Tsakovska *et al*., 2007) have been evaluated.

Testing data on irritation / corrosion (skin and eye)

In vitro data

There are OECD and EU adopted test guidelines (see Section R.7.2.3), under which substances can be classified as irritants (currently for skin irritation only) or corrosives, or not classified.

Annex VII to the REACH Regulation requires information from *in vitro* tests for skin and 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.7.2.6 of this document.

As a consequence of the general rules in Annex XI, data from the following types of tests may be accepted as described below:

- 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 and non-irritant chemicals 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:

  - While the validation database contained pure chemicals and mixtures of pure chemicals, the experience with complex formulations is still limited. A report containing data on mixtures (e.g. agrochemicals) is available but difficult to interpret as the composition of the mixtures is not known (Kolle *et al*., 2013).
  - They discriminate skin irritants (Category 2) from chemicals not classified for skin irritation (No Category) under CLP. However they are not designed to classify chemicals into the optional UN GHS Category 3 (mild irritants).
  - 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 chemical 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 chemical 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.
  - They do not allow testing of gases and aerosols.

- For skin corrosion:

- **Transcutaneous electrical resistance test** (TER) (EU B.40/OECD TG 430)
- **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)

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 other two tests have some limitations with regard to subcategorisation.
- Concerning the RHE test method, while the validation database contained pure chemicals and mixtures of pure chemicals, the experience with complex formulations is still limited. However, some data indicated at least a high specificity for the classification of corrosive vs. non-corrosive complex formulations (Kolle *et al*., 2013)
- 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 chemical detection system (4.5 < pH < 8.5) may not be tested.

· For eye irritation/corrosion:

- **Bovine Corneal Opacity and Permeability test method (BCOP)** (EU B.47/OECD TG 437): The specific scope and limitations are:
  - This test is recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified in Eye Damage Category 1 under CLP, without further testing, and also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage.
  - If, as a result of testing, the substance is not classified as Eye Damage Category 1 under CLP, or not identified as not requiring classification, more testing is required.
  - This test may result in false positive predictions for alcohols and ketones and false negative predictions for solids. Also this test method is does not allow the identification of chemicals classified as Category 1 Eye Damage under CLP based on persistency of the effects and hence may provide false negative outcomes.
  - This test does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.
  - This test does not allow testing of gases and aerosols.

- **Isolated Chicken Eye test (ICE)** (EU B.48/OECD TG 438): The specific scope and limitations are:
  - This test is recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified in Eye Damage Category 1 under CLP, without further

testing, and also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage.

- If, as a result of testing, the substance is not classified as Eye Damage Category 1 under CLP or not identified as not requiring classification, more testing is required.

- This test may result in false positive predictions for alcohols and false negative predictions for solids and surfactants. Also this test method is does not allow the identification of chemicals classified as Category 1 Eye Damage under CLP based on persistency of the effects and hence may provide false negative outcomes.

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- This test does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.

- This test does not allow testing of gases and aerosols.

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

- This test is recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified in Eye Damage Category 1 under CLP, without further testing.

- This test is not recommended for the identification of chemicals which should be classified as mild/moderate irritants or of chemicals which should not be classified for ocular irritation (substances and mixtures).

- This test is only applicable to water soluble chemicals (substances and mixtures) 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 chemicals.

- If, as a result of testing, the substance is not classified as Eye Damage Category 1 under CLP, more testing is required.

Positive outcome from four *in vitro* assays (i.e. the IRE, HET-CAM, CM, STE) are accepted in the EU to classify a substance as Eye Damage Category 1 under CLP under Annex VII and Annex VIII to the REACH Regulation using the adaptations of the standard testing regime specified in Annex XI. They have undergone a formal retrospective evaluation, and their scientific validity has been the subject of a statement by ESAC (2007, 2009a) and reports from ICCVAM (available at http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFE8725DF1F9F5D and http://ntp.niehs.nih.gov/?objectid=2D70C7A2-CCDB-D782-06CB38302BD7D10E).

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

· Quality Aspects:

For the case of such quality assessments as 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

Section R.5.2.1.2 of Chapter R.5 of the *Guidance on IR&CSA* for aspects that need to be taken into account in such a *Weight of Evidence*.

Animal data

Well-reported studies, particularly if conducted in accordance with principles of GLP, can be used to identify substances which would be considered to be, or not to be, corrosive or irritant to the skin or eye. There may be a number of skin or eye irritation/corrosion 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 and/or eye irritation/corrosion 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 and eye.

Data from studies other than skin or eye irritation/corrosion studies (e.g. other toxicological studies on the substance in which local responses of skin, eye mucous membranes and/or respiratory system have been reported) may provide useful information though they may not be well reported in relation to, for example, the basic requirements for information on skin and eye irritation. However, information from studies in animals on mucous membrane and/or respiratory system irritation can be very useful for risk assessment provided the irritation is clearly substance-induced, and particularly if it can be related to exposure levels.

- Quality Aspects:

Data from **existing** irritation studies in animals must be taken into account before further testing is considered. A quality assessment of any such reports should be done using, for example, the system developed by Klimisch *et al*. (1997), as described in Section R.4.2 of Chapter R.4 of the *Guidance on IR&CSA*, and a judgement will need to be made as to whether any further testing is required. Some examples to note are:

i. Was the animal species 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 3 animals but 6 were frequently used in the past.

iii. How many dose levels were used? If dilutions were included, what solvent was used (as this may have influenced absorption)? Which dose volume was used?

iv. For skin testing, which exposure period was used? Single or repeated exposure?

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

vii. For eye irritation, was initial pain noted after instillation of the test substance? Was the substance washed out from the eye? Was fluorescent staining used?

viii. For eye irritation, 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 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.

- Specific considerations for eye irritation/corrosion:

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 is currently under review of EURL ECVAM for the detergent and cleaning preparations 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. 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 chemicals (Griffith *et al*., 1980) and detergent and cleaning preparations (Freeberg *et al*., 1984; Freeberg *et al*. 1986a,b; Cormier *et al*., 1995; Roggeband *et al*., 2000), and has shown to be a very good predictor of the effects on man. It still overpredicts, but much less than the classical Draize test of OECD TG 405.

A retrospective validation study of the refinement/reduction LVET method for the use domain of household detergents and cleaning products as well as their main ingredient classes took place between 2006 and 2009.

After peer review, the LVET was not recommended for prospective use, i.e. to generate new data but it was recommended that existing LVET data of the limited use domain mentioned above may be used for purposes of classification and labeling decisions. Moreover, it was recommended 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 (ESAC, 2009b).

In summary, available data from the LVET on substances and preparations should be considered and must be carefully evaluated. For the classification of substances however it must be taken into account that the test up to now has a limited applicability domain (detergent and cleaning products). Consequently, positive LVET data (be it Category 2 or Category 1) are a trigger for the appropriate classification for eye irritancy, but negative data

from LVET as a *stand alone method* (in the absence of any other information) are not conclusive for *no classification*.

· Specific considerations for respiratory tract irritation and corrosion:

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

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

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.

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 a 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*).

- **Animal data**:Data from inhalation studies (acute, repeated exposure):
  1. Clinical symptoms of dyspnoea or breathing difficulties,
  2. Histomorphology of the respiratory tract,
  3. Lavage examination (nasal, bronchoalveolar).
- Alarie assay.

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 Adverse Effect Concentration (NOAEC) and/or a Low Adverse Effect Concentration (LOAEC) 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. Also the Alarie test might be used as an additional element in a *Weight-of-Evidence* evaluation.

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”* might 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 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 (preparations) in sub-corrosive concentrations, it needs to be ensured that an appropriate classification for potential respiratory tract irritation is applied.

R.7.2.4.2 Human data for irritation / corrosion

Well-documented *existing* human data of different sources can often provide very useful information on skin, eye or respiratory tract irritation/corrosion, sometimes for a range of exposure levels. Often the only useful information available on irritation is obtained from human experience (occupational settings). The usefulness of all human data on irritation will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. Experience has shown that it is difficult to obtain useful data on substance-induced eye irritation, but data may be available on human ocular responses to certain types of preparations (e.g. Freeberg *et al.*, 1986a).

The quality and relevance of existing human data for hazard assessment should be critically reviewed. For example, in occupational studies with mixed exposure it is important that the substance causing the irritation or corrosion has been accurately identified. There may also be a significant level of uncertainty in human data due to poor reporting and lack of specific information on exposure.

Examples of how existing human data can be used in hazard classification for irritancy are provided in a recent ECETOC monograph (ECETOC, 2002).

Human data for skin and eye irritation/corrosion

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 (see Section 3.2 of Annex I to the CLP Regulation), i.e. corrosivity is an irreversible damage. With this characterisation it should be possible to discern corrosive properties in humans. However, to distinguish between corrosives from subcategory 1A, subcategory 1B and subcategory 1C (3 minutes', 1 hour' and 4 hours' exposure in rabbits, respectively) may not be so obvious in practice. A clear case for classification in subcategory 1A would be an accidental splash which gave rise to necrosis of the skin. In cases where it is obvious that a prolonged exposure is needed (not to be mixed with delayed effects) before necrosis occurs, classification in subcategory 1B/1C seems more reasonable. If the distinction between subcategories 1A, 1B and 1C is not clearly apparent then the general Category 1 (without subcategorisation) should be used. Discrimination between corrosives and skin irritants in rabbits is made on the effects caused after 4 hours' exposure. Irritants to the skin cause a significant inflammation which is reversible (for further information, see Section 3.2 of the *Guidance on the application of the CLP criteria*).

Severe eye irritants (Category 1 under CLP) give more severe corneal opacity and iritis than eye irritants (Category 2). Category 1 compounds induce considerable tissue damage which can result in serious physical decay of vision. The effects normally do not reverse within 21 days (relates to animals) (see Section 3.3 of Annex I to the CLP Regulation). In contrast, the effects of Category 2 compounds are reversible within 21 days. In humans, a sight control by a physician would reveal a decay of vision. If it is not transient but persistent it implies classification in Category 1. If the discrimination between Category 1 and Category 2 is not obvious, then Category 1 should be chosen (for further information, see Section 3.3 of the *Guidance on the application of the CLP criteria*).

Human data for respiratory tract irritation

Consideration should be given to real-life human observational experience, if this is properly collected and documented (Arts *et al.*, 2006), e.g. data from well-designed workplace surveys, worker health monitoring programmes. For substances with an array of industrial uses and with abundant human evidence, the symptoms of respiratory 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.

R.7.2.4.3 Exposure considerations for irritation/corrosion

Exposure-based waiving from testing is not applicable to the endpoints of skin and eye irritation/corrosion. 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 irritation / corrosion

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 according to internationally-accepted test methods will usually give very good information on the skin or eye irritancy of a substance in the test species, and, in general, it is assumed that substances which are irritant in EU or OECD TG-compliant studies in animals will be skin and/or eye irritants in humans, and those which are not irritant in EU or OECD TG-compliant studies will not be irritant in humans. Good data, often clearly related to exposure levels, can be obtained on respiratory and mucous membrane irritation, from well-designed and well-reported inhalation studies in animals. However, inconsistent results from a number of similar studies increases the uncertainty in deriving data from animal studies.

Data obtained from *in vitro* studies may include many dose levels and replicates: when such a study has a well-defined mechanistic basis and indicates that a substance is expected to be irritating, this may suffice for defined hazard identification purposes.

R.7.2.5 Conclusions for irritation / corrosion

R.7.2.5.1 Concluding on suitability for Classification and Labelling

In order to conclude on 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.7.2.5.2 Concluding on suitability for Chemical Safety Assessment

A dose-response assessment is difficult to make for skin and eye irritation and corrosion simply because up to the present time most data have been produced with undiluted chemicals in accordance with test guidelines and traditional practice (which continues today). From a risk characterisation perspective it is therefore advisable to use the outcome of the classification procedure, i.e. a substance that is classified is assumed to be sufficiently characterised. However, a complete risk assessment requires both hazard and dose-response data. Consequently, if the latter 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 toxicity studies as well as from human experience.

For respiratory tract irritation or corrosion, 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.

Guidance on the possibilities for derivation of DNELs for skin and eye irritation/corrosion and respiratory tract 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 following testing strategies can be adopted (see Section R.7.2.6 of this Guidance).

R.7.2.6 Assessment and testing strategy for irritation / corrosion

As pointed out above, the OECD has approved an IATA for skin irritation/corrosion (OECD, 2014). The IATA includes 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 vivo* or *in vitro* testing of the substance is necessary. While the OECD IATA provides slightly more detailed guidance than the Assessment and testing strategy below, there is no conceptual difference between these two.

For eye irritation/corrosion, currently no OECD IATA is available.

R.7.2.6.1 Objective / General principles

For substances with no or very few data, the following sequential test strategy is recommended for developing adequate and scientifically sound data for assessment/evaluation and classification of the corrosive and irritating properties of substances. For existing substances with insufficient data, this strategy can also be used to decide which additional data, beside those already available, are needed.

The objective of the testing strategies is to propose a stepwise approach to hazard identification with regard to skin and eye irritation/corrosion. A principle of the strategy is that the results of one study are evaluated before another study is initiated. The strategy seeks to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

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

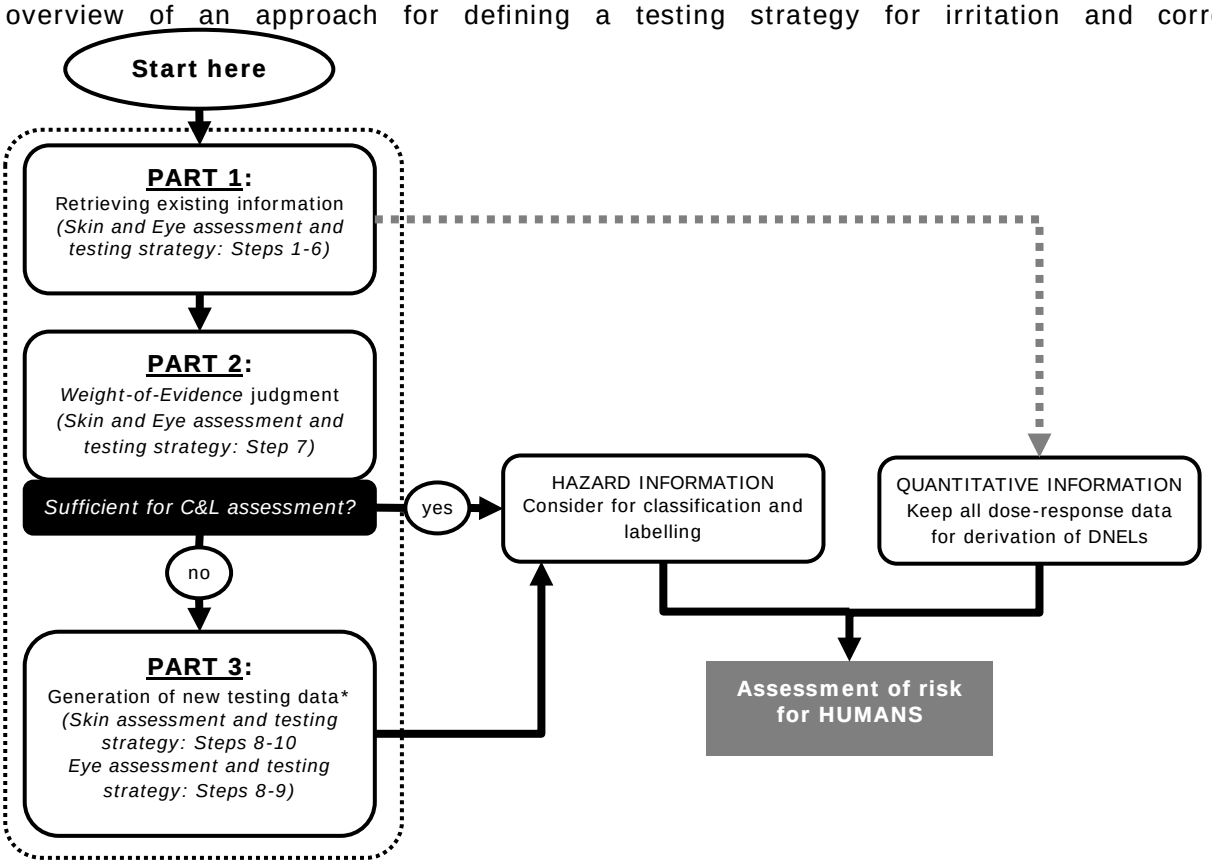
Risk assessment of the irritating/corrosive potential of a substance is normally made in a qualitative way provided that the substance has been classified as being irritant or corrosive to the skin or to the eyes. Existing test guidelines do not contain dose-response assessment, so that a quantitative 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 information strategy be followed until step 6 (Figure R.7.2-1, **Error! Reference source not found.**Figure R.7.2-2 and Figure R.7.2-3) in all cases and thereafter the *Weight-of-Evidence* analysis be performed. Clearly, not all steps will necessarily be accompanied by data but it is important that all potential data sources are explored prior to

starting the *Weight-of-Evidence* analysis. Note that before the *Weight-of-Evidence* analysis in step 7 no new *in vitro* or *in vivo* tests should be conducted: instead, the assessment should be solely based on existing data. Furthermore, prior to performing any new *in vivo* test, the use of *in vitro* methods should be fully exploited (see Article 25 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 and eye irritation/corrosion, 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**Error! Reference source not found.**) gives an overview of an approach for defining a testing strategy for irritation and corrosion.



*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.7.2-1 Overview of the assessment and testing strategy for irritation/corrosion

R.7.2.6.2 Assessment and testing strategy for skin irritation/corrosion

Stepwise approach

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

In the information retrieval part, existing and available information from the literature and databases is gathered and considered in a stepwise process. At the end of this part all the information collected is analysed using a *Weight-of-Evidence* approach (step 7). It is therefore necessary to run through all preceding steps before arriving at step 7. This means that in cases of "yes, consider to classify…", the registrant should nevertheless proceed to the next step. However, the assessment and testing strategy may be exited in the sole exception if the substance is spontaneously flammable at room temperature in contact with air or water (box 1a). In this case testing is not required.

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

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

Step	Information	Conclusion
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable in contact with air (pyrophoric) or water at room temperature? → ↓ **NO** ↓	**YES**: No testing required. No need to proceed.
1b	Is the substance an organic hydroperoxide or an organic peroxide? → ↓ **NO** ↓	**YES**: Consider classifying as: ■ corrosive (Skin Corrosive Cat. 1B) if the substance is a hydroperoxide, or ■ irritating (Skin Irritant Cat. 2) if the substance is a peroxide. **OR** Provide evidence for the contrary. Proceed to next step.
1c	Is the pH of the substance lower than 2 or higher than 11.5? [a] → ↓ **NO** ↓	**YES**: Consider classifying as corrosive. Where classification is based upon consideration of pH alone (see step 7), Skin Corrosive Cat. 1A should be applied. Proceed to next step.
1d	Are there other physical or chemical properties that indicate that the substance is irritating/corrosive? → ↓ **NO** ↓	**YES**: Use this information for *Weight-of-Evidence* analysis (step 7). Proceed to next step.
Existing human data		
2	Are there adequate existing human data[b] which provide evidence that the substance is an irritant or corrosive? → ↓ **NO** ↓	**YES**: Consider classifying accordingly. Proceed to next step.
Existing animal data from irritation/corrosivity studies		
3	Are there data from existing studies *on irritation and corrosion* in laboratory animals, which provide sound conclusive evidence that the substance is a corrosive, irritant or non-irritant? →	**YES**: Consider classifying accordingly (either Skin Corrosive Cat. 1A/1B/1C or Skin Irritant Cat. 2 or no classification).

	↓ **NO** ↓	Proceed to next step.
Existing data from general toxicity studies via the dermal route and from sensitisation studies		
4a	Is the substance acutely toxic (LD_{50} £ 400 mg/kg bw) or very toxic (LD_{50} £ 50 mg/kg bw) via the dermal route? [c] → ↓ **NO** ↓	**YES**: The substance will be classified for its acute dermal toxicity. Proceed to next step.
4b	Has the substance proven to be a corrosive, irritant or non-irritant in a suitable acute dermal toxicity test? [d] → ↓ **NO** ↓	**YES**: If test conditions are consistent with OECD TG 404, consider classifying accordingly (Skin Corrosive Cat. 1A/1B/1C or Skin Irritant Cat. 2 or no classification). Proceed to next step.
4c	Has the substance proven to be a corrosive or an irritant in sensitisation studies or after repeated exposure? [e] → ↓ **NO** ↓	**YES**: This information cannot be used for considering a concrete classification conclusion but must be used exclusively within the integrated *Weight-of-Evidence* judgment. Proceed to next step.
Existing (Q)SAR data and read-across		
5a	Are there structurally related substances (suitable "read-across" or grouping), which are classified as corrosive to the skin (Skin Corrosive Cat. 1), or do suitable (Q)SAR methods indicate corrosion potential of the substance? [f] → ↓ **NO** ↓	**YES**: Consider classifying as Skin Corrosive Cat. 1. Proceed to next step.
5b	Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritant to the skin (Skin Irritant Cat. 2), or do suitable (Q)SAR methods indicate irritating potential of the substance? [f] → ↓ **NO** ↓	**YES**: Consider classifying as Skin Irritant Cat. 2. Proceed to next step.
Existing in vitro data		
6a	Has the substance demonstrated corrosive properties in an EU/OECD adopted *in vitro* test? →	**YES**: Consider classifying as corrosive. If

	↓ **NO** [g] ↓	discrimination between Skin Corrosive Cat. 1A, 1B and 1C is not possible, Cat. 1 must be chosen. Proceed to next step.
6b	Are there acceptable data from a validated *in vitro* test (adopted by EU/OECD or not), which provide evidence that the substance is an irritant or non-irritant? → ↓ **NO** ↓	**YES**: Consider classifying accordingly (Skin Irritant Cat. 2 or no classification). Proceed to next step.
6c	Are there data from a non-validated *in vitro* test, which provide sound conclusive evidence that the substance is an irritant[h]? → ↓ **NO** ↓	**YES**: Consider to classify as Skin Irritant Cat. 2, Proceed to next step.
Weight-of-Evidence analysis		
7	Taking all existing and relevant data (steps 1-6) into account, is there sufficient information to make a decision on whether classification/labelling is necessary, and – if so – how to classify and label? → ↓ **NO** ↓	**YES**: Classify accordingly (Skin Corrosive Cat. 1A/1B/1C or Skin Irritant Cat. 2 or no classification).
New in vitro/ex vivo tests for corrosivity (Annex VII to the REACH Regulation)		
8	Does the substance demonstrate corrosive properties in an EU/OECD adopted *in vitro* or *ex vivo* tests for skin corrosion? → ↓ **NO** [g] ↓	**YES**: Classify as Skin Corrosive Cat. 1A, 1B or 1C. If discrimination between Cat. 1A, 1B and 1C is not possible, Cat. 1 must be chosen.
New in vitro/ex vivo tests for irritation (Annex VII to the REACH Regulation)		
9a	Does the substance demonstrate irritating or non-irritating properties in validated *in vitro* tests (adopted by EU/OECD or not) for skin irritation? → ↓ **NO** ↓	**YES**: Classify accordingly.
9b	Does the substance demonstrate irritating properties in a non-validated *in vitro* test for skin irritation[h]? → ↓ **NO**	**YES**: Classify accordingly.

	↓	
New in vivo test for irritation (Annex VIII to the REACH Regulation) [i]		
10	Does the substance demonstrate irritancy in an EU/OECD adopted *in vivo* test? → ↓ **NO** ↓ No classification	**YES**: Classify accordingly.

Notes to the information scheme on skin irritation / corrosion:

[a)] Note that if the buffering capacity suggests that the substance may not be corrosive, further data are needed to confirm this.

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

[c)] If the substance is acutely toxic (LD_{50} £ 400 mg/kg bw) or very toxic (LD_{50} £ 50 mg/kg bw) via the dermal route further testing for irritation/corrosion would result in severe suffering or death of the animal. Thus, further testing is not required and sufficient labelling (warning) is provided by the Hazard statement H311 *"Toxic in contact with skin"* or H310 *"Fatal in contact with skin"* and the GHS Pictogram GHS06 with the signal word "Danger". Please note, that although the derogation regarding acute toxicity (LD_{50} £ 400 mg/kg bw) is not a specific rule for adaptation from column 1 in REACH Annexes, it is considered here to be scientific common sense.

[d)] Has the substance proven to be either an irritant or a corrosive in an acute dermal toxicity test carried out with rabbits with the undiluted test substance (liquids) or with a suitable suspension (solids)? In case of signs of skin corrosion,classify as Skin Corrosive Category 1A,1B or 1C. 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 irritation/corrosion 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, 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 sensitiation studies, the skin of guinea pigs is less sensitive than the skin of rats which is less sensitive than the skin of rabbits. Only in case of evidence of skin corrosivity in the 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 irritating 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 chemicals. 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).

[g] No classification for corrosivity if a negative result can be supported by a *Weight-of-Evidence* determination using other existing information, e.g. pH, SAR, human and/or animal data (according to OECD TG 430 and 431/EU B.40 and B.40 bis). If not corrosive, the irritating potential needs to be determined, proceed.

[h] Conclusion on no classification can only be made if it has been concluded in the evaluation process that the test allows the identification of non-irritants and the data are used in a *Weight-of-Evidence* approach following Section 1.2 of Annex XI to the REACH Regulation.

[i] *In vivo* testing can be avoided in case the substance falls under the scope of the specific *in vitro* tests performed, and there are no chemical-specific limitations to use those tests, and the Registrant formulates a *Weight-of-Evidence* adaptation according to Section 1.2 of Annex XI to the REACH Regulation.

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

For skin irritation and corrosion it is currently accepted that no single test method can fully replace the *in vivo* test for skin irritation. However, the *in vitro* methods specified in Section R.7.2.2 may be used for partial replacement within a tiered testing strategy or as stand-alone replacement tests depending on the outcome of the study.

There are some 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 Annex VII to the REACH Regulation and include:

1. Assessment of all available human and animal data (e.g. animal data from acute dermal toxicity, repeated dose toxicity and skin sensitisation studies, if available)

2. Acid and alkaline reserve. It should be noted that other substance properties e.g. pH or others could indicate that the substance is irritating or corrosive. Consideration of the use of (Q)SARs or looking into information on similar substances may provide useful information on skin irritation and corrosion potential (see Figure R.7.2-2).

   If a conclusion on the classification cannot be made at this step, *in vitro* studies for irritation/corrosion potential should be conducted.

3. An *in vitro* study for skin corrosion should be performed. In case of a positive result, the substance can be classified as corrosive (Category 1 under CLP). Since the study may only provide information on whether the substance is corrosive or non-corrosive, if a negative result is obtained (i.e. non-corrosive), further *in vitro* study(ies) to assess the skin irritation potential is/are needed.

4. An *in vitro* study for skin irritation should be performed in order to verify that the substance would be classified as skin irritant (Category 2 under CLP) or that the substance does not require classification.

After these steps, no *in vivo* test is necessary (for any tonnage level) in the case where:

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

b) the Registrant formulates a *Weight-of-Evidence* adaptation according to Section 1.2 of Annex XI to the REACH Regulation.

If an *in vivo* study for skin irritation is a standard information requirement (e.g. for substances registered at or above 10 tonnes per annum) and the steps above have been followed, the Registrant may choose to adapt the standard information requirement for the *in vivo* study by using Annex XI adaptation possibilities e.g. *Weight of Evidence* (see Section 1.2 of Annex XI). 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 OECD or EU TGs for skin irritation/corrosion purposes will provide results that will have regulatory acceptance under REACH.

R.7.2.6.3 Assessment and testing strategy for eye irritation / corrosion

Stepwise approach

The assessment and testing strategy for eye irritation/corrosion (see Figure R.7.2-3) is completely analogous in structure to that for skin corrosion/irritation. The testing strategy consists of an information retrieval part (steps 0a to 6) and a part on the generation of new information by testing (steps 8 to 9). These two parts are separated by a *Weight-of-Evidence* analysis and expert judgement (step 7).

In the information retrieval part, existing and available information from the literature and databases is gathered and considered in a stepwise process. At the end of this part all the information collected is analysed using a *Weight-of-Evidence* approach (step 7). It is therefore necessary to run through all preceding steps before arriving at step 7. This means that in cases of “yes, consider classifying…”, one should nevertheless “Proceed to next step”. An exception is a “yes” in one or all of the following boxes: 0a, 1a or 1c: if the substance is classified as a skin corrosive, or its pH is ≤ 2.0 or ≥ 11.5 (taking the buffer capacity into due consideration), the process of information retrieval can stop at this point, since the substance’s eye irritation potential is implicit in this classification or from this pH information. If the substance is spontaneously flammable at room temperature in contact with air (pyrophoric) or water, testing is not required.

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

Figure R.7.2-3 Assessment and testing strategy for evaluating the eye irritation/corrosion potential of substances.

Step	Information	Conclusion
Conclusion of the information strategy on skin irritation/corrosion		
0a	Is the substance classified as a skin corrosive? → ↓ **NO** ↓	**YES**: When assigned Skin Corrosive Cat. 1, 1A, 1B or 1C, the risk of severe damage to eyes is considered implicit (Eye Damage Cat. 1). No need to proceed.
Existing data on physico-chemical properties		
1a	Is the substance spontaneously flammable in contact with air (pyrophoric) or water at room temperature? → ↓ **NO** ↓	**YES**: No testing required. No need to proceed.
1b	Is the substance an organic hydroperoxide or an organic peroxide? → ↓ **NO** ↓	**YES**: Consider classifying for: ■ corrosivity (hydroperoxide) using Skin Corrosive Cat. 1B, thus implicitly also for severe ocular irritancy (Eye Damage Cat. 1), or ■ irritation (peroxide) using Eye Irritant Cat. 2. Proceed to next step.
1c	Is the pH of the substance lower than 2 or higher than 11.5? [a] → ↓ **NO** ↓	**YES**: When assigned Skin Corrosive Cat. 1A, the risk of severe damage to eyes is considered implicit (Eye Damage Cat. 1). No need to proceed.
1d	Are there other physical or chemical properties that indicate that the substance is irritating to the eye [b]? → ↓ **NO** ↓	**YES**: Use this information for *Weight-of-Evidence* analysis (step 7). Proceed to next step.
Existing human data		
2	Are there adequate existing human data [c] which provide evidence that the substance is irritating to the eye? → ↓ **NO**	**YES**: Consider classifying (Eye Damage Cat. 1 or Eye Irritant Cat. 2), or use for *Weight-of-Evidence* analysis (step 7). Proceed to next step.

	↓	
Existing animal data from eye irritation studies		
3	Are there data from existing studies *on eye irritation* in laboratory animals, which provide sound conclusive evidence that the substance is an eye (severe) irritant or non-irritant? → ↓ **NO** ↓	**YES**: Consider classifying accordingly (Eye Damage Cat. 1 or Eye Irritant Cat. 2 or no classification). Proceed to next step.
Existing data on acute dermal toxicity		
4	Is the substance acutely toxic (LD_{50} £ 400 mg/kg bw) or very toxic (LD_{50} £ 50 mg/kg bw) via the dermal route? [d] → ↓ **NO** ↓	**YES**: The substance will be classified for its acute dermal toxicity. Proceed to next step.
Existing (Q)SAR data and read-across		
5	Are there structurally related substances (suitable "read-across" or grouping), which are classified as damaging or irritating to the eye, or do valid (Q)SAR methods indicate eye irritation/corrosion by the substance? [e]→ ↓ **NO** ↓	**YES**: Consider classifying accordingly (Eye Damage Cat. 1 or Eye Irritant Cat. 2). If discrimination between Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Eye Damage Cat. 1 must be chosen. Proceed to next step.
Existing in vitro data		
6a	Are there data from a validated *in vitro* test (adopted by EU/OECD or not), which provide evidence that the substance is a (severe) eye irritant or non-irritant? → ↓ **NO** ↓	**YES**: Consider classifying accordingly (Eye Damage Cat. 1 or Eye Irritant Cat. 2 or no classification). If discrimination between Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Eye Damage Cat. 1 must be chosen. Proceed to next step.
6b	Are there acceptable data from a non-validated *in vitro* test, which provide evidence that the substance is a severe irritant to the eye [f]? → ↓ **NO** ↓	**YES**: Consider classifying as Eye Damage Cat. 1. Proceed to next step.
Weight of evidence analysis		

7	Taking all existing and relevant data (steps 1 – 6) into account, is there sufficient information to make a decision on whether classification/labelling is necessary, and – if so – how to classify and label? → ↓ **NO** ↓	**YES**: Classify accordingly (Eye Dam.age Cat. 1 or Eye Irritant Cat. 2 or no classification).
New in vitro/ex vivo tests for eye irritation/corrosion (Annex VII to the REACH Regulation)		
8a	Does the substance demonstrate (severe) irritating or non-irritating properties in validated *in vitro* or *ex vivo* tests (adopted by EU/OECD or not) for eye irritation? → ↓ **NO** ↓	**YES**: Classify accordingly (Eye Damage Cat. 1 or Eye Irritant Cat. 2or no classification). If discrimination between Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Eye Damage Cat. 1 must be chosen.
8b	Does the substance demonstrate severe irritating properties in acceptable (non-)validated *in vitro* or *ex vivo* tests for eye irritation but without regulatory acceptance (at present only IRE, CM, STE, HET-CAM, SkinEthic™ or EpiOcular™) [f]? → ↓ **NO** ↓	**YES**: Classify as Eye Damage Cat. 1.
***New in vivo test for eye irritation (Annex VIII to the REACH Regulation)*[g]**		
9	Does the substance demonstrate irritancy in an OECD adopted *in vivo* test? → ↓ **NO** ↓ No classification	**YES**: Classify accordingly.

Notes to the information scheme eye irritation:

[a] Note that if the buffering capacity suggests the substance be non-corrosive, further data are needed to confirm this.

[b] If pH < 3.2 or pH > 8.6, the substance is very likely to be an eye irritant.

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

[d] If the substance is acutely toxic (LD_{50} £ 400 mg/kg bw) or very toxic (LD_{50} £ 50 mg/kg bw) via the dermal route further testing for eye irritation would result in severe suffering or death of the animal. Thus, further testing is not required and sufficient labelling (warning) is provided by the Hazard statement H311 *"Toxic in contact with skin"* or H310 *"Fatal in contact with skin"* and the GHS Pictogram GHS06 with the signal word *"Danger"*.

[e] Conclusion on no classification can be made if the model has been shown to adequately predict the absence of the classified effect and if it fulfils the requirements of Annex XI 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 chemicals. 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).

[f] Conclusion on no classification can only be made if it has been concluded in the evaluation process that the test allows the identification of non-irritants and the data are used in a *Weight-of-Evidence* approach following Section 1.2 of Annex XI to the REACH Regulation.

[g] *In vivo* test(s) can be avoided in case the substance falls under the scope of the specific *in vitro* tests performed, and there are no chemical-specific limitations to using those tests, and the Registrant formulates a *Weight-of-Evidence* adaptation according to Section 1.2 of Annex XI to the REACH Regulation.

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

It is generally accepted that no single *in vitro* eye irritation/corrosion test will be able to fully replace the OECD TG 405 for acute eye irritation/corrosion test *in vivo* (also known as the Draize eye test) across the full range of irritation for different chemical classes. Combinations of several alternative test methods may be able to replace use of the Draize eye test. Testing strategies such as the top-down or bottom-up approaches provide a means of incorporating existing information, QSAR predictions, and *in vitro* test results.

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:

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 eye irritant);

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 irritating to eyes or corrosive to the skin and classified as eye irritant. Consideration of information obtained from the use of (Q)SARs or from similar substances may be useful in predicting eye irritation potential (see Figure R.7.2-3).

After following steps 1 and 2, if a conclusion on the classification cannot be made, the next steps are:

3. Assessment of existing data on physico-chemical properties. No further testing is required if the substance is spontaneously flammable in air at room temperature. If not, the next step is an *in vitro* study for eye irritation/corrosion.

4. An *in vitro/ex vivo* study for eye irritation/corrosion should be performed, and the outcome can be:

    a) In the case of a positive and definitive result from the BCOP, ICE or FL test, the substance can be classified as inducing “serious eye damage” (Eye Damage Category 1 under CLP), and no further test *in vivo* is necessary.

    b) In addition, BCOP and ICE tests can also provide information on whether the substance does not require any classification for eye irritation/corrosion. If no classification is needed, no further testing *in vivo* is necessary.

c) For Annex VIII information requirements, if neither of these conclusions can be made, a further test conducted *in vivo* to assess the eye irritation potential 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 chemical-specific limitations to using those tests.

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 *Weight-of-Evidence* considerations based on the adaptation possibilities of Section 1.2 of Annex XI to the REACH Regulation and without testing on animals. 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.

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 is suitable for the substance in order to obtain adequate information from the *in vitro* studies. For most substances, the use of adopted OECD or EU TGs for eye irritation purposes will provide results that will have regulatory acceptance under REACH.

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

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

Content of Appendix R.7.2-1:

- Mechanisms of skin corrosion and irritation
- Mechanisms of eye irritation
- Mechanisms of respiratory tract irritation and corrosion

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 chemicals, the mechanisms leading to skin irritation are normally described by a two-stage process where a chemical first has to penetrate the *stratum corneum* and then trigger a biological response in deeper epidermal or dermal layers.

For strong inorganic acids and bases, no *stratum corneum* penetration is needed because they erode the *stratum corneum*. According to the Technical Guidance Document (TGD) supporting Commission Directive 93/67/EEC on risk assessment for new notified and existing substances (EC, 2003), the percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts is slow, since these chemicals are binding to macromolecules in 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 chemicals.

2. Mechanisms of skin corrosion:

   - Erosion of the *stratum corneum* by most inorganic acids and bases and by strong organic acids with pH ≤2.0 and bases with pH ≥11.5, and

   - Binding to skin components in the *stratum corneum* by cationic surfactants and percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts.

3. Mechanisms that may lead to both skin irritation and corrosion:

   - Penetration of the *stratum corneum* by anionic or non-surfactant organic chemicals with sufficient hydrophobic and hydrophilic properties, and

   - Elicitation of 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 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 chemicals 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 chemicals or chemical 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, chemicals or chemical mixtures which come into contact with the eye directly may cause local effects on the frontal tissues and substructures of the eye, e.g. cornea, conjunctiva, iris, lachrymal system and eye lids. There are several modes of action by which topical chemicals and chemical mixtures cause eye injury (see Table R.7.2-3).

Table R.7.2-3 Categories of irritant chemicals and their typical mode of action in eye irritation.

Chemical/chemical mixtures	Mode of Action
Inert chemicals	May cause effect due to large size. Protrusions may cause direct puncture of the eye.
Acids	May react directly with eye proteins and cause coagulation or precipitation resulting in relatively localised injury.
Bases (Alkalis)	May actively dissolve cell membranes. May penetrate to the deeper layers of the eye tissue.
Solvents	May dissolve lipids in plasma membranes of epithelial and underlying cells resulting in loss of the cells affected and, as a result, tissue degradation, which might be transient, depending on the repair mechanisms (cell proliferation, tissue restoration)..
Lachrymators	May stimulate the sensory nerve endings in the corneal epithelium causing an increase in tearing.

The degree of eye injury is usually dependent on the characteristics (chemical category/class) and concentration of the chemical or chemical mixture. Acids and alkalis usually cause immediate irritation to the eyes. Other substances may cause eye injuries that start as mild but progress to be more severe at a later period.

Upon exposure of the ocular surface to eye irritants, inflammation of the conjunctiva can be induced. This includes dilation of the blood vessels causing redness, increased effusion of water causing swelling (oedema/chemosis) and an increase in the secretion of mucus leading to an increase in discharge. Visual acuity can be impaired. Irritants may also produce an increase in tear production and changes to the tear film integrity such as increased wetness. Iritis may result from direct irritation or become a secondary reaction to the corneal injury. Once the iris is inflamed, infiltration of fluids can follow which affects the ability to adjust the size of the pupil and decreases the reaction to light leading to decreased visual acuity. Due to the richness of nerves in the iris, irritation also causes subjective symptoms such as itching, burning and stinging.

Eye injury can be reversible or irreversible depending on the degree of damage and degree of repair. Damage to the corneal epithelium alone can repair quickly, often with no permanent eye damage. The cornea may still repair fairly well if the damage goes beyond the basement membrane into the superficial part of the stroma but the repair process may take days or even weeks to occur. Once the damage extends significantly into the stroma, corneal ulceration can occur due to the subsequent series of inflammatory processes. If damage extends to and beyond the endothelium, corneal perforation may occur which is irreversible and may cause permanent loss of vision. Eye injury can cause different degrees of functional loss e.g. increase of tear production, opacification of the cornea, oedema and so decrease visual acuity.

The body has its own defence mechanisms, e.g. sensing the pain, stinging and burning, and the eyelids will blink to avoid full exposure to the chemical. Increased tear production and blinking of the eyes with the help of the drainage apparatus help to dilute or clear the causative agent. Such defence mechanisms are highly developed in man with rapid blinking and profuse tear production resulting from exposure of the eye to a foreign material that is irritating. It is well reported in the literature that species differences occur in the rate of blinking and tear production mechanism that can influence how effectively foreign materials are removed from the eye.

MECHANISMS OF RESPIRATORY TRACT IRRITATION AND CORROSION

The term "respiratory tract irritation" is often used to indicate either or both of two different toxicological effects. These are i) cytotoxic effects in the affected tissue, and ii) sensory irritation. The first type of irritation is comparable to dermal and eye irritation.

Cytotoxic irritant effects are characterised by inflammation (increased blood flow (hyperemia), local infiltration with white blood cells, swelling, oedema) and there may also be haemorrhage, and eventual necrosis and other pathological changes. The effects are in principle reversible.

Chronic irritation can lead to repeated episodes of cell proliferation in the affected tissues, and this may increase the risk of tumour development. The nature of effects depends on the chemical compound and its primarily targeted region, the severity of effects depends on the concentration and duration of exposure. In general, repeated exposure studies in animals 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. Compound or compound-group specific target sites of sensory irritation generating different responses can be identified: a) nasal (and eye) irritation, i.e. interaction with the trigeminal nerve, b) pharyngeal irritation, i.e. interaction with the glossopharyngeal nerve, and c) larynx and lower respiratory tract, i.e. interaction with the vagus nerve.

Sensory irritation leads to unpleasant sensations such as pain, burning, pungency, and tingling. The severity depends on the airborne concentration of the irritant rather than on the duration of exposure. Sensory irritation is a receptor-mediated effect, and usually occurs almost immediately upon exposure to the inhaled irritant. It leads to reflex involuntary responses such as sneezing, lacrimation, rhinorrhea, coughing, vasodilatation of blood vessels in the nasal passages, and changes in the rate and depth of respiration. In humans, protective behavioural responses such as covering the nose and mouth can also occur. Sensory irritation is distinct from 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) 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. During recent years, emphasis was given to develop a spectrum of objective measurements (see review by Arts *et al*., 2006).

There is a view in the occupational health literature that sensory irritation may be a more sensitive effect than overt tissue-damaging irritation (which is a non-receptor mediated unspecific mode inducing cell death at the site of contact). Sensory irritation-related effects are fully reversible given that its biological function is to serve as a warning against inhaled substances that could damage the airways, and that it triggers physiological reflexes that limit inhalation volumes and protect the airways. However, there is a lack of documented evidence to indicate that this is a generic position that would necessarily apply to all inhaled irritants. It should be noted that no clear relationship between the RD_{50} value and the onset of histologically observable lesions in animals has been observed.

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).

Appendix R.7.2-2 QSARs and expert systems for skin irritation and corrosion

Content of Appendix 7.2-2:

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

LITERATURE-BASED QSAR MODELS

In the open scientific literature, (Q)SARs have been based on continuous (e.g. Primary Irritation Indices) or categorical (e.g. EU classifications) measures of skin irritation.

For defined classes of chemicals, categorical QSARs have been reported for discriminating between corrosives and non-corrosives (Barratt, 1996a, 1996b), and between skin irritants and non-irritants (Smith *et al.*, 2000a, 2000b). These studies did not actually provide a transparent algorithm for classifying chemicals, so they are of limited value for regulatory use. However, they illustrate the feasibility of developing such models, so it should be possible for a QSAR specialist to redevelop the models in such a way that an algorithm is clearly defined.

A linear discriminant model for distinguishing between irritant and non-irritant liquid esters in human volunteers was reported by Smith *et al*. (2000a). As mentioned above the exact algorithm is not clear. In addition the primary irritation index for human irritation may need translation when these scores are considered for classification. However, the results could be informative for future model development for esters, since they indicate that irritant esters can be distinguished from non-irritants on the basis of a limited number of physico-chemical parameters.

For defined classes of chemicals, continuous QSARs for predicting the Primary Irritation Index (PII) have also been published (Barratt, 1996b; Hayashi *et al.*, 1999; Kodithala *et al.*, 2002). For example, the application of stepwise regression analysis to a set of 52 neutral and electrophilic organic chemicals produced the following model:

$$PII = 1.047 \log P - 0.244\ MV + 0.888\ DM + 0.353$$

$$N=52,\ r^2 = 0.422,\ r_{cv}^2 = 0.201,\ s=1.376,\ F=11.70$$

This equation indicates that the PII has a positive dependence on log P (logarithm of the octanol-water partition coefficient) and DM (dipole moment), and a negative dependence on MV (molecular volume). This model has a low goodness-of-fit (r^2) and a poor predictivity (as reflected by r_{cv}^2), so is not recommended for regulatory use. Nevertheless, the model does reveal three potentially useful descriptors for the development of new models for PII prediction. More research is needed into the development of models for predicting PII and it should be considered whether the information generated could be used in the setting of DNELs.

Some limited evidence indicates that the reactive effects of acids and bases can be predicted by using the acid/base dissociation constant (pKa), which can itself be predicted by using commercially available software products, such as the SPARC program. Evidence for the

usefulness of pKa as a predictor of skin irritation for acids has been provided by Berner *et al.* (1988, 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 chemical of interest, and therefore multiple ionization 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 is provided in the table below:

Reference	
Barratt (1996a)	
Barratt (1996b)	
Gallegos Saliner *et al.* (2006)	
Gallegos Saliner *et al.* (2008)	
Gerner *et al.* (2004)	
Golla *et al.* (2009)	
Hayashi M *et al*. (1999)	
Hulzebos *et al.* (2005b)	
Kodithala *et al.* (2002)	
Mombelli (2008)	
Rorije and Hulzebos (2005)	
Gallegos Saliner *et al.(*2007)	
Walker *et al.* (2004)	
Walker *et al.* (2005)	
Worth and Cronin (2001)	

COMMERCIAL MODELS

TOPKAT, which is commercialised by Accelrys (http://accelrys.com), incorporates models to discriminate severe irritants from non-severe irritants, as well as mild/moderate irritants from non-irritants. These models are based on work by Enslein *et al*. (1987), but due to a lack of documentation, it is not clear whether the current version of the software encodes the models that were originally published. A QMRF for the TOPKAT skin irritation model is provided as an appendix. The algorithm of the TOPKAT is not transparent. The model predicts a probability of a weak/mild/moderate and severe irritation. It states that probabilities <0.3 and >0.7 give

sufficient certainty of the prediction. The model gives the sensitivity and specificity values of the specific classes such as acyclic etc., which are mostly around or above 90%. It also shows similar structures from the TOPKAT perspective including the experimental result. The TOPKAT predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. The models indicate whether the prediction is in the applicability domain of the model. Due to the limitations of the model (lack of transparency for the algorithm, no external validation, no mechanistic reasoning), it cannot be used as stand-alone method. The TOPKAT prediction should be supported with mechanistic reasoning, using other models or expert judgment.

There is a rulebase for irritation in **Derek for Windows** (Sanderson and Earnshaw, 1991; Combes and Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.chem.leeds.ac.uk). To predict toxicity, the program checks whether any alerts within the query structure match previously characterised toxicophores (substructure with potential toxic effect) in the knowledge base. The reasoning engine then assesses the likelihood of a structure being toxic, and a message indicating the nature of the toxicological hazard is provided together with relevant literature references. There are nine levels of confidence: certain, probable, plausible, equivocal, doubted, improbable, impossible, open, contradicted. The DerekfW8.0 rulebase has 25 structural alerts for the prediction of skin irritancy/corrosion; four alerts are specific to eye irritancy, and some combined for the respiratory tract irritation and gastrointestinal tract, but none is specific to skin irritancy or corrosivity. If DerekfW does not make a prediction of irritancy or corrosivity, it cannot be concluded that there is no effect – it could mean that none of known alerts was found to be present in the chemical of interest or it was outside the applicability domain of that specific alert. The DerekfW model is transparent in its algorithm, when the model is fired showing the structural alert and its limitations. The alert is supported with literature references and sometimes with example chemicals, although this is not sufficient to consider them validated. The example chemicals support the mechanistic reasoning. The DerekfW model can be used for positive identification of skin irritation. The confidence levels have to be translated to consider them for classification. Due to the limitations (lack of validation) it cannot be used as stand-alone method, though the mechanistic reasoning provides supporting information. The DerekfW model cannot be used to predict non-irritation/corrosion as the model only contains alerts that detect the presence of irritation/corrosion.

HazardExpert is a rule-based software tool developed and commericalised by CompuDrug Chemistry Ltd. (http://www.compudrug.com) for predicting the toxicity of organic compounds in humans and in animals (Smithing 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), is an automated rule induction tool that automatically identifies molecular fragments likely to be relevant to the activity of molecules (Klopman, 1992; Klopman *et al.*, 1993). It also provides an indication of the importance of these fragments in relation to the potency of the molecules containing them. MultiCASE can be used to predict various human health endpoints, including eye irritation (Klopman *et al.*, 1993; Rosenkranz *et al.*, 1998). However, it is not clear how to relate the MultiCASE scoring system to Draize scores or regulatory classifications. In principle, the MultiCASE model can be used for positive and negative indications of skin irritation. The structural alert is provided as well as information on its internal validation. The MultiCASE model also indicates whether it is in the applicability domain of the model. The MultiCASE predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. Due to limitations (lack of external validation and mechanistic reasoning) the model cannot be used as a stand-

alone method. The prediction should be supported with mechanistic reasoning using other models or expert judgment.

The Danish EPA (http://eng.mst.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 irritation/corrosion potential in combination with structural inclusion rules (SARs) to predict the presence of such potential (Gerner *et al.*, 2004; Walker *et al.*, 2004). The exclusion rules are based on physico-chemical properties such as molecular weight, aqueous solubility, and log K_{ow}, whereas the inclusion rules are based on substructural molecular features. The physico-chemical rules implicitly take into account bioavailability (skin penetration) whereas the structural rules take reactivity into account. The physico-chemical and structural rulebases are designed to predict the former EU risk phrases for skin irritation (R38) and skin corrosion (R34 and R35). Further details are given in QSAR Model Reporting Format for the BfR skin and eye irritation rulebases (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/laboratories-research/predictive_toxicology/qsar_tools/QRF).

The exclusion rules have the following general form:

IF (physico-chemical property) A THEN predict the absence of toxic effect B

Example: IF Log K_{ow} < -3.1 THEN the chemical does not need to be considered for classification

The structural inclusion rules take the following general form:

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

Example: IF *Chlorosilane* THEN the chemical needs to be considered for “corrosive” classification

The performance of the BfR physico-chemical rulebase for predicting the absence of skin effects has been validated by the RIVM (Rorije and Hulzebos, 2005), whereas the structural rulebase for predicting the occurrence of skin effects has been validated by the ECB (Gallegos Saliner *et al.*, 2007). The endpoint is former EU classification, the algorithms and domain of applicability are transparent, the rules and alerts are independently validated by ECB and RIVM (Gallegos Saliner *et al.*, 2007, Rorije and Hulzebos, 2005). Though the rules are empirically derived, a mechanism of action can be deduced. For chemicals in the applicability domain of the rulebase, the rules may be used on their own to predict the presence or absence of hazard. Thus, the resulting predictions can be used as the basis for classification. It should be determined, on a case-by-case basis, whether the predictions for a given chemical provide a sufficient basis for classification, or whether additional information is needed in a *Weight-of-Evidence approach.*

OECD QSAR TOOLBOX

The freely available for download OECD QSAR Toolbox (http://www.qsartoolbox.org/) covers the skin irritation/corrosion 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.

SICRET

The so-called “Skin Irritation Corrosion Rules Estimation Tool” (SICRET), has been developed by Walker *et al.* (2005) to estimate whether chemicals are likely to cause skin irritation or skin corrosion SICRET is not actually a computer-based tool but a tiered approach based on the use of physico-chemical property limits, structural alerts and *in vitro* tests to classify chemicals that cause skin irritation or skin corrosion. The physico-chemical rules and alerts include those in the BfR rulebases as well as some additional rules and alerts published by Hulzebos *et al.* (2001, 2003, 2005a).

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

Content of Appendix 7.2-3:

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

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 chemicals located within the boundaries of the ellipse. The statistical significance of these “embedded clusters’ can be verified by cluster significance analysis (CSA), as illustrated for an eye irritation dataset by Cronin (1996).

Applying the methods of ECM and CSA, the following model, applicable to undiluted organic liquids, was developed by Worth and Cronin (2000):

Classify an undiluted, organic liquid as an eye irritant if:

$$(\log P\text{-}1.07)^2 / 2.06^2 + (dV1 + 0.98)^2 / 0.99^2 £ 1$$

This model was based on 73 diverse organic chemicals, using two descriptors: LogP (which accounts for diffusion) and a size-independent molecular connectivity index (dV1, which accounts for the degree of branching and cyclicity). The sensitivity, specificity and concordance of the model were 73%, 78% and 75%, respectively, whereas the positive and negative predictivities were 77% and 74%, respectively. The model is an explicit algorithm with a defined applicability domain and predicts former EU classifications directly.

The 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). The cut-off values below which a chemical should be predicted to be irritating to the eye were 121, 77, and 137 g/mol, in the LDA, BLR, and CT classification models, respectively (Table R.7.2-4) (Worth and Cronin, 2003).

Table R.7.2-4 Classification results of the different models of eye irritancy

CM (p<0.01)	Cut-off value	Sensitivity	Specificity	Accuracy
Linear Discriminant Analysis (LDA)	if MW ≤ 121 g/mol, then predict I; otherwise, predict NI	73	62	65
Binary Logistic Regression (BLR)	if MW ≤ 77 g/mol, then predict I; otherwise, predict NI	27	93	76
Classification Tree (CT)	if MW ≤ 137 g/mol, then predict I; otherwise, predict NI	97	49	61

All of these models are simple to apply and are associated with a transparent algorithm. The statistics illustrate the inevitable trade-offs that result from the selection of different cut-off values. Thus, the BLR model does not identify many irritants (only 27%), but it does so with a high degree of confidence (i.e. low false positive rate of 7%). Conversely, the CT does not identify many of the non-irritants (49%), but it has a low false negative rate of 3%). Thus, the combined use of the BLR and CT models could be useful for distinguishing between eye irritants and non-irritants.

An overview on the available literature-based model is provided in the table below:

Reference	
Solimeo *et al.* (2012)	
Gallegos Saliner *et al.* (2006)	
Gallegos Saliner *et al.* (2008)	
Tsakovska *et al.* (2005)	
Tsakovska *et al.* (2007)	
Abraham *et al.* (2003)	

COMMERCIAL MODELS

The **TOPKAT** software includes models for eye irritation based on structural fragments. These models were originally developed by Enslein *et al.* (1988), but the algorithms are not well defined in the TOPKAT documentation The TOPKAT algorithm is not transparent. The model predicts a probability of a weak/mild/moderate and severe irritation. It states that probabilities <0.3 and >0.7 give sufficient certainty of the prediction. The model gives the sensitivity and specificity values of the specific classes such as acyclic, which are mostly around or above 90%. It also shows similar structures from the TOPKAT perspective including the experimental result. The TOPKAT predictions weak/mild/moderate and severe irritation need to be translated to consider them for classification. The models indicate whether the prediction is in the applicability domain of the model. Due to the limitations of the model (lack of transparency for the algorithm, no external validation, no mechanistic reasoning), it cannot be used as stand alone method. The TOPKAT prediction should be underlined with a mechanistic reasoning, using other models or expert judgment.

There is a rulebase for irritation in **Derek for Windows** (Sanderson and Earnshaw, 1991; Combes and Rodford, 2004), which is developed and regularly updated by LHASA Ltd (http://www.chem.leeds.ac.uk). See for a general outline the skin irritation section on (Q)SARs. The DerekfW8.0 rulebase has four four alerts are specific to eye irritancy. If DerekfW does not make a prediction of irritancy or corrosivity, it cannot be concluded that there is no effect – it could mean that none of known alerts was found to be present in the chemical of interest or it was outside the applicability domain of that specific alert. The DerekfW model is transparent in its algorithm, when the model is fired showing the structural alert and its limitations. The alert is underlined with literature references and sometimes with example chemicals, which is not sufficient to consider them internally validated. The example chemicals underline the mechanistic reasoning. The DerekfW model can be used for positive identification of skin irritation. The confidence levels have to be translated to consider them for classification. Due to the limitations (lack of internal and external validation) it cannot be used as stand alone method, though the mechanistic reasoning possibly provides sufficient information. The DerekfW model cannot be used to predict for non-irritation/corrosion as the model only contains alerts that detect the presence of irritation/corrosion.

The fragment-based **MultiCASE** approach has been used to model eye irritation (Klopman *et al.*, 1993; Enslein *et al.*, 1988; Rosenkranz *et al.*, 1998; Klopman, 1998). The publications on these models do not define the algorithms. In principle, the MultiCASE model can be used for positive and negative indication for eye irritation. The structural alert is provided as well as the internal validation. The MultiCASE model also indicates whether it is in the applicability domain of the model. The MultiCASE predictions of weak/mild/moderate and severe irritation need to be translated to consider them for classification. Due to limitations (lack of external validation and mechanistic reasoning) the model cannot be used as a stand alone method. The prediction should be underlined with mechanistic reasoning using other models or expert judgment.

BFR DECISION SUPPORT SYSTEM

The decision support system (DSS) developed by the German Federal Institute for Risk Assessment (BfR) uses physico-chemical exclusion rules to predict the absence of eye irritation/corrosion potential in combination with structural inclusion rules (SARs) to predict the presence of such potential (Gerner *et al.*, 2005). These rules are used analogously to those described in the skin irritation and corrosion section above. The physico-chemical and structural rulebases are designed to predict the former EU risk phrases for eye irritation (R36) and severe eye irritation/corrosion (R41). Independent validation exercises by the ECB support the performance of the physico-chemical

rulebase for predicting the absence of eye effects (Tsakovska *et al.*, 2005), as well as the performance of the structural rulebase for predicting the occurrence of eye effects (Tsakovska *et al*., 2007).

OECD QSAR TOOLBOX

The freely available for download OECD QSAR Toolbox (http://www.qsartoolbox.org/) covers the eye irritation/corrosion 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).

R.7.2.7 Useful links

JRC QSAR Model Database: http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsar-inventory

EURL ECVAM page: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam

EURL ECVAM database service on alternative methods to animal experimentation (DB-ALM): http://ecvam-dbalm.jrc.ec.europa.eu/

OECD QSAR Toolbox: http://www.qsartoolbox.org/

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