Guidance on the Application of the CLP Criteria

Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures

Third draft (to generate Version 4.0)
for RAC & Forum

XXX 201X

PART 3: HEALTH HAZARDS

NOTE regarding revisions to the Guidance due to Reg XXX/XXXX (4th ATP)

This Guidance includes revisions due to the 4th ATP. The Regulation XXX/XXXX will apply from 1 December 2014 for substances and 1 June 2015 for mixtures.

Changes in the legal text due to the 4th ATP, are highlighted in orange. Where the change is to a "P statement" in the Hazard Comminication legal boxes, the line is shaded in orange and a note is also added to the lines to say **4th ATP change**. All changes are preceded by a note highlighting the changes.

The complete sections 3.7.4.1 and 3.7.5.1 in Reproductive Toxicity are shaded in orange because they will be replaced. Both sections are preceded with a note highlighting the changes.

Once the 4th ATP is applied a Guidance corrigendum will be made to update the Guidance document and change the colour of relative legal text boxes from orange to green. No consultation will be made to do this.

3.1 ACUTE TOXICITY

3.1.1 Definitions and general considerations for acute toxicity

Annex I: 3.1.1.1. Acute toxicity means those adverse effects occurring following oral or dermal administration of a single dose of a substance or a mixture, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours.

Acute toxicity relates to effects occurring after a single or relatively brief exposure to a substance or mixture. The definition in CLP reflects the fact that the evidence for acute toxicity is usually obtained from animal testing. In particular, acute toxicity is usually characterised in terms of lethality and exposure times are based around those used in experimental protocols. However, classification for acute toxicity can also be based on human evidence which shows lethality following human exposure.

There are two hazard classes for acute toxicity – "Acute toxicity" and "STOT-SE (Specific Target Organ Toxicity – Single Exposure)". These are independent of each other and both may be assigned to a substance or a mixture if the respective criteria are met. However, care should be taken not to assign each class for the same effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such a case the most appropriate (the most severe hazard) class should be assigned.

Acute toxicity classification is generally assigned on the basis of evident lethality (e.g. an LD_{50}/LC_{50} value), or, where the potential to cause lethality can be concluded from evident toxicity (e.g. from the fixed dose procedure). STOT-SE should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality (see Chapter 3.8 of this Guidance).

For more details see Guidance on IR/CSA, Section R.7.4.1.1.

Annex I: 3.1.1.2. The hazard class Acute Toxicity is differentiated into:

- Acute oral toxicity;
- Acute dermal toxicity;

- Acute inhalation toxicity.

The classification must be considered for each route of exposure, using the appropriate approach as described in Sections 3.1.2.2 and 3.1.2.3 of this Guidance. If different hazard categories are assigned, the more severe hazard category will be used for the classification for acute toxicity, with the appropriate pictogram and signal word. For each relevant route of exposure, the hazard statement will correspond to the classification of this specific route.

3.1.2 Classification of substances for acute toxicity

3.1.2.1 Identification of hazard information

3.1.2.1.1 Identification of human data

Relevant information with respect to acute toxicity may be available from sources such as case reports, epidemiological studies, medical surveillance and reporting schemes and national poison centres. Human data to be considered for acute toxicity should report severe effects after single exposure or exposure of less than 24h, but data on severe effects after a few exposures over a few days can also be considered on a case by case basis.

For more details see Guidance on IR/CSA, Section R.7.4.3.2.

3.1.2.1.2 Identification of non-human data

Non-testing data:

Physicochemical data

Physico-chemical properties, such as pH, physical state, form, solubility, vapour pressure and particle size, can be important parameters in evaluating toxicity studies and in determining the most appropriate classification. This is especially valid with respect to inhalation where physical form and particle size can have a significant impact on toxicity (see Section 3.1.2.3.2 of this Guidance).

(Q)SAR models, expert systems and grouping methods

"Non-testing data can be provided by the following approaches: a) structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), collectively called (Q)SARs; b) expert systems incorporating (Q)SARs and/or expert rules; and c) grouping methods (read-across and categories. These approaches can be used to assess acute toxicity if they provide relevant and reliable (adequate) data for the chemical of interest. […] Compared with some endpoints, there are relatively few (Q)SAR models and expert systems capable of predicting acute toxicity." (Guidance on IR/CSA, Section R.7.4.3.1).

Testing data:

In vitro data

There are currently no *in vitro* tests that have been officially adopted by the EU or OECD for assessment of acute toxicity (Guidance on IR/CSA, Section R.7.4.3.1). Any available studies should be assessed by using expert judgement.

Animal data

A number of different types of studies have been used to investigate acute toxicity. Older standard studies were designed to determine lethality and estimate the LD_{50}/LC_{50}. In contrast, contemporary study protocols, such as the fixed dose procedure, use signs of evident

("~~evident~~") toxicity rather than lethality as indications of acute toxicity. These studies are generally conducted using preferred species, i.e. the rat for acute oral and inhalation toxicity studies, and in addition the rabbit for acute dermal toxicity studies.

The animal studies are listed in the Guidance on IR/CSA, Section R.7.4.3.1.

3.1.2.2 Classification criteria

Annex I: 3.1.2.1. Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 3.1.1. Acute toxicity values are expressed as (approximate) LD_{50} (oral, dermal) or LC_{50} (inhalation) values or as acute toxicity estimates (ATE). Explanatory notes are shown following Table 3.1.1.

Table 3.1.1

Acute toxicity hazard categories and acute toxicity estimates (ATE) defining the respective categories

Exposure Route	Category 1	Category 2	Category 3	Category 4
Oral (mg/kg bodyweight) See: Note (a) Note (b)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2000
Dermal (mg/kg bodyweight) See: Note (a) Note (b)	ATE ≤ 50	50 < ATE ≤ 200	200 < ATE ≤ 1000	1000 < ATE ≤ 2000
Gases (ppmV ([1])) see: Note (a) Note (b) Note (c)	ATE ≤ 100	100 < ATE ≤ 500	500 < ATE ≤ 2500	2500 < ATE ≤ 20000
Vapours (mg/l) see: Note (a) Note (b) Note (c) Note (d)	ATE ≤ 0.5	0.5 < ATE ≤ 2.0	2.0 < ATE ≤ 10.0	10.0 < ATE ≤ 20.0
Dusts and mists (mg/l) see: Note (a) Note (b) Note (c)	ATE ≤ 0.05	0.05 < ATE ≤ 0.5	0.5 < ATE ≤ 1.0	1.0 < ATE ≤ 5.0

([1]) Gas concentrations are expressed in parts per million per volume (ppmV).

Notes to Table 3.1.1:

(a) The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50}/LC_{50} where available.

(b) The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:

- the LD_{50}/LC_{50} where available,
- the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or
- the appropriate conversion value from Table 3.1.2 that relates to a classification category.

(c) Generic concentration limits for inhalation toxicity in the table are based on 4-hour testing exposures. Conversion of existing inhalation toxicity data which have been generated using a 1-hour exposure can be carried out by dividing by a factor of 2 for gases and vapours and 4 for dusts and mists.

(d) For some substances the test atmosphere will not just be a vapour but will consist of a mixture of liquid and vapour phases. For other substances the test atmosphere may consist of a vapour which is near the gaseous phase. In these latter cases, classification shall be based on ppmV as follows: Category 1 (100 ppmV), Category 2 (500 ppmV), Category 3 (2500 ppmV), Category 4 (20 000 ppmV).

The terms 'dust', 'mist' and 'vapour' are defined as follows:

- dust: solid particles of a substance or mixture suspended in a gas (usually air),

- mist: liquid droplets of a substance or mixture suspended in a gas (usually air),

- vapour: the gaseous form of a substance or mixture released from its liquid or solid state.

Dust is generally formed by mechanical processes. Mist is generally formed by condensation of supersaturated vapours or by physical shearing of liquids. Dusts and mists generally have sizes ranging from less than 1 to about 100 µm.

Guidance update to legal text "Annex I: 3.1.2.1" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures.

Annex I: 3.1.2.1. Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 3.1.1. Acute toxicity values are expressed as (approximate) LD_{50} (oral, dermal) or LC_{50} (inhalation) values or as acute toxicity estimates (ATE). Explanatory notes are shown following Table 3.1.1.

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Gases (ppmV ([1])) see: Note (a) Note (b) Note (c)	ATE ≤ 100	100 < ATE ≤ 500	500 < ATE ≤ 2500	2500 < ATE ≤ 20000
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Dusts and mists (mg/l) see: Note (a) Note (b) Note (c)	ATE ≤ 0.05	0.05 < ATE ≤ 0.5	0.5 < ATE ≤ 1.0	1.0 < ATE ≤ 5.0

([1]) Gas concentrations are expressed in parts per million per volume (ppmV).

Notes to Table 3.1.1:

(a) The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50}/LC_{50} where available.

(b) The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:

- the LD_{50}/LC_{50} where available,
- the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or
- the appropriate conversion value from Table 3.1.2 that relates to a classification category.

(c) The ranges of the acute toxicity estimates (ATE) for inhalation toxicity in the table are based on 4-hour testing exposures. Conversion of existing inhalation toxicity data which have been generated using a 1-hour exposure can be carried out by dividing by a factor of 2 for gases and vapours and 4 for dusts and mists.

(d) For some substances the test atmosphere will not just be a vapour but will consist of a mixture of liquid and vapour phases. For other substances the test atmosphere may consist of a vapour which is near the gaseous phase. In these latter cases, classification shall be based on ppmV as follows: Category 1 (100 ppmV), Category 2 (500 ppmV), Category 3 (2500 ppmV), Category 4 (20 000 ppmV).

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- dust: solid particles of a substance or mixture suspended in a gas (usually air),

- mist: liquid droplets of a substance or mixture suspended in a gas (usually air),

- vapour: the gaseous form of a substance or mixture released from its liquid or solid state.

Dust is generally formed by mechanical processes. Mist is generally formed by condensation of supersaturated vapours or by physical shearing of liquids. Dusts and mists generally have sizes ranging from less than 1 to about 100 µm.

Comment to CLP Annex I, Table 3.1.1, Note (c):

The classification criteria for acute inhalation toxicity relate to a 4-hour experimental exposure period. Where LC_{50} values have been obtained in studies using exposure durations shorter or longer than 4 hours these values may be adjusted to a 4-hour equivalent using Haber’s law ($C^n \cdot t=k$) for direct comparison with the criteria. The value of n, which is specific to individual substances, should be chosen using expert judgement. If an appropriate value of n is not available in the literature then it may sometimes be derived from the available

mortality data using probits (i.e. the inverse cumulative distribution functions associated with the standard normal distribution). Alternatively, some default values are recommended (Guidance on IR/CSA, Section R.7.4.4.1).

Particular care should be taken when using Haber's law to assess inhalation data on substances which are corrosive or locally active. In all cases, Haber's law should only be used in conjunction with expert judgement.

It is noted that the statements in the Guidance on IR/CSA, Section R.7.4.4.1, with respect to Haber's law are not consistent with those of CLP. However ,the CLP approach must be used for classification and labelling.

3.1.2.3 Evaluation of hazard information

3.1.2.3.1 Evaluation of human data

The evaluation of human data often becomes difficult due to various limitations frequently found with the types of studies and data highlighted in Section 3.1.2.1.1 of this Guidance. These include uncertainties relating to exposure assessment (i.e. unreliable information on the amount of substance the subjects were exposed to) and uncertain exposure to other substances. As such, human data needs careful expert evaluation to properly judge the reliability of the findings. It should be acknowledged that human data often do not provide sufficiently robust evidence on their own to support classification. They may, however, contribute to a weight of evidence assessment with other available information such as data from animal studies.

The classification for acute toxicity is based primarily on the dose/concentration that causes mortality (the Acute Toxicity Estimate, ATE), which is then related to the numerical values in the classification criteria according to CLP Annex I, Table 3.1.1 (see Section 3.1.2.2 of this Guidance) for substances or for use in the additivity formula in CLP Annex I, 3.1.3.6.1 and 3.1.3.6.2.3 for mixtures (see Section 3.1.3.3 of this Guidance). The ATE is usually obtained from animal studies but in principle suitable human data can also be used if available. Where human data are available, they should be used to estimate the ATE which can be used directly for classification as described above.

The minimum dose or concentration or range shown or expected to cause mortality after a single human exposure can be used to derive the human ATE directly, without any adjustments or uncertainty factors. See Example 1 (methanol) in Section 3.1.6.1.1 of this Guidance.

If there are no exact or quantitative lethal dose data the procedure described in CLP Annex I, 3.1.3.6.2.1(b) (see Section 3.1.3.3.4 of this Guidance) would have to be followed using Table 3.1.2 (see Section 0 of this Guidance) with an assessment of the available information on a semi-quantitative or qualitative basis.

Expert judgement is needed in a total weight of evidence approach taking relevance, reliability, and adequacy of the information into account. See Example 2 (N,N-dimethylaniline) in Section 3.1.6.1.2 of this Guidance.

3.1.2.3.2 Evaluation of non-human data

Annex I: 3.1.2.2. *Specific considerations for classification of substances as acutely toxic*

3.1.2.2.1. The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat, while the rat or rabbit are preferred for evaluation of acute dermal toxicity. When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD_{50} value from among valid, well-performed tests.

Evaluation of non- testing and *in vitro* data:

Results of (Q)SAR, grouping and read-across may be used instead of testing, and substances will be classified and labelled on this basis if the method fulfils the criteria described in Annex XI of REACH. See also the Guidance on IR/CSA, Section R.7.4.4.1.

ATE – establishing:

- Basis LD_{50}/LC_{50}: An available LD_{50}/LC_{50} is an ATE at first stage.

- Results from a range test: According to CLP Annex I, Table 3.1.2 results from range tests (i.e. doses/exposure concentrations that cause acute toxicity in the range of numeric criteria values) can be assigned to the four different categories of acute toxicity for each possible route of exposure (centre column). Further, CLP AnnexI, Table 3.1.2 allows allocating a single value, the converted acute toxicity point estimate (cATpE), to each experimentally obtained acute toxicity range estimate or classification category (right column), see Note (b) to Table 3.1.1. This cATpE can be used in the additivity formulae (CLP Annex I, 3.1.3.6.1 and 3.1.3.6.2.3) to calculate the acute toxicity of mixtures.

- In case of multiple LD_{50}/LC_{50} values or LD_{50}/LC_{50} values from several species:

Where several experimentally determined ATE values (i.e. LD_{50}, LC_{50} values or ATE derived from studies using signs of non-lethal toxicity) are available, expert judgement needs to be used to choose the most appropriate value for classification purposes. Each study needs to be assessed for its suitability in terms of study quality and reliability, and also for its relevance to the substance in question in terms of technical specification and physical form. Studies not considered suitable on reliability or other grounds should not be used for classification.

In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification. If there is information available to inform on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species. If there is a wide range of ATE values from the same species, it may be informative to consider the studies collectively, to understand possible reasons for the different results obtained. This would include consideration of factors such as the animal strains used, the experimental protocols, the purity of the substance and form or phase in which it was tested (e.g. the particle size distribution of any dusts or mists tested), as well as exposure mode and numerous technical factors in inhalation studies. This assessment may aid selection of the most appropriate study on which to base the classification.

If there are different LD_{50} values from tests using different vehicles (e.g. water vs. corn oil or neat substance vs. corn oil), generally the lowest valid value would be the basis for classification. It is not considered appropriate to combine or average the available ATE values. The studies may not be equivalent (in terms of experimental design such as protocol, purity of material tested, species of animal used, etc.) making such a collation or combination unsound.

If there is a study available with a post-observation period of less than the 14 days according to the OECD guidelines and effects are observed at the end of the study, the resulting LD_{50} might be misleading. A long persistency of effects may be indicative of cumulative toxicity,

sometimes coinciding with flat dose-response relationships, and sometimes with species differences. Such information should be included in the weight of evidence consideration.

Annex I: 3.1.2.3. *Specific considerations for classification of substances as acutely toxic by the inhalation route*

3.1.2.3.1. Units for inhalation toxicity are a function of the form of the inhaled material. Values for dusts and mists are expressed in mg/l. Values for gases are expressed in ppmV. Acknowledging the difficulties in testing vapours, some of which consist of mixtures of liquid and vapour phases, the table provides values in units of mg/l. However, for those vapours which are near the gaseous phase, classification shall be based on ppmV.

Conversions:

Differentiation between vapour and mist will be made on the basis of the saturated vapour concentration (SVC) for a volatile substance, which can be estimated as follows:

SVC [mg/l] = 0.0412 x MW x vapour pressure (vapour pressure in hPa at 20°C).

The conversion from mg/l to ppm assuming an ambient pressure of 1 atm = 101.3 kPa and 25°C is: ppm= 24,450 x mg/l x 1/MW.

An LC_{50} well below the SVC will be considered for classification according to the criteria for vapours; whereas an LC_{50} close to or above the SVC will be considered for classification according to the criteria for mists (see also Draft OECD GD 39).

Considerations with respect to physical forms or states or bioavailability:

Article 9(5) When evaluating the available information for the purposes of classification, the manufacturers, importers and downstream users shall consider the forms or physical states in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used.

For further details see Sections 1.2 and 1.3 of this Guidance.

Special considerations concerning aerosols (dusts and mists):

Annex I: 3.1.2.3.2. Of particular importance in classifying for inhalation toxicity is the use of well articulated values in the high toxicity categories for dusts and mists. Inhaled particles between 1 and 4 microns mean mass aerodynamic diameter (MMAD) will deposit in all regions of the rat respiratory tract. This particle size range corresponds to a maximum dose of about 2 mg/l. In order to achieve applicability of animal experiments to human exposure, dusts and mists would ideally be tested in this range in rats.

The test guidelines for acute inhalation toxicity with aerosols require rodents to be exposed to an aerosol containing primarily respirable particles (with a Mass Median Aerodynamic Diameter (MMAD) of 1 – 4 µm), so that particles can reach all regions of the respiratory tract. The use of such fine aerosols helps to avoid partial overloading of extra-thoracic airways in obligate nasal breathing species like rats. Results from studies in which substances with particle size with a MMAD > 4 µm have been tested can generally not be used for classification, but expert judgement is needed in cases where there are indications of high toxicity.

The use of highly respirable dusts and mists is ideal to fully investigate the potential inhalation hazard of the substance. However, it is acknowledged that these exposures may not necessarily reflect realistic conditions. For instance, solid materials are often micronised to a highly respirable form for testing, but in practice exposures will be to a dust of much lower

respirability. Similarly, pastes or highly viscous materials with low vapour pressure need strong measures to be taken to generate airborne particulates of sufficiently high respirability, whereas for other materials this may occur spontaneously. In such situations, specific problems may arise with respect to classification and labelling, as these substances are tested in a form (i.e. specific particle size distribution) that is different from all the forms in which these substances are placed on the market and in which they can reasonably be expected to be used.

A scientific concept has been developed as a basis for relating the conditions of acute inhalation tests to those occurring in real-life, in order to derive an adequate hazard classification. This concept is applicable only to substances or mixtures which are proven to cause acute toxicity through local effects and do not cause systemic toxicity (Pauluhn, 2008).

Corrosive substances

> **Annex I: 3.1.2.3.3.** In addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity was corrosivity, the substance or mixture shall also be labelled as 'corrosive to the respiratory tract' (see note 1 in 3.1.4.1). Corrosion of the respiratory tract is defined by destruction of the respiratory tract tissue after a single, limited period of exposure analogous to skin corrosion; this includes destruction of the mucosa. The corrosivity evaluation can be based on expert judgment using such evidence as: human and animal experience, existing (*in vitro*) data, pH values, information from similar substances or any other pertinent data.

It is presumed that corrosive substances (and mixtures) will cause toxicity by inhalation exposure. In cases where no acute inhalation test has been performed special consideration should be given to the need to communicate this potential hazard.

Corrosive substances (and mixtures) may be acutely toxic after inhalation to a varying degree and by different modes of action. Therefore, it is not possible to estimate the acute inhalation toxicity from the corrosivity data alone.

There are special provisions for hazard communication of acutely toxic substances by a corrosive effect, see Section 3.1.4.2 of this Guidance.

3.1.2.3.3 Weight of evidence

In cases where there is sufficient human evidence that meets the criteria given in Section 3.1.2.2 of this Guidance then this will normally lead to classification for acute toxicity, irrespective of other information available.

If there are human data indicating no classification but there are also non-human data indicating classification then the classification is based on the non-human data unless it is shown that the human data cover the exposure range of the non-human data or that the non-human data are not relevant for humans. If the human and non-human data both indicate no classification then classification is not required.

If there are no human data then the classification is based on the non-human data.

For the role and application of expert judgement and weight of evidence determination, see CLP Annex I, 1.1.1.

3.1.2.4 Decision on classification

The classification has to be performed with respect to all routes of exposure (oral, dermal, inhalation) on the basis of all adequate and reliable available information.

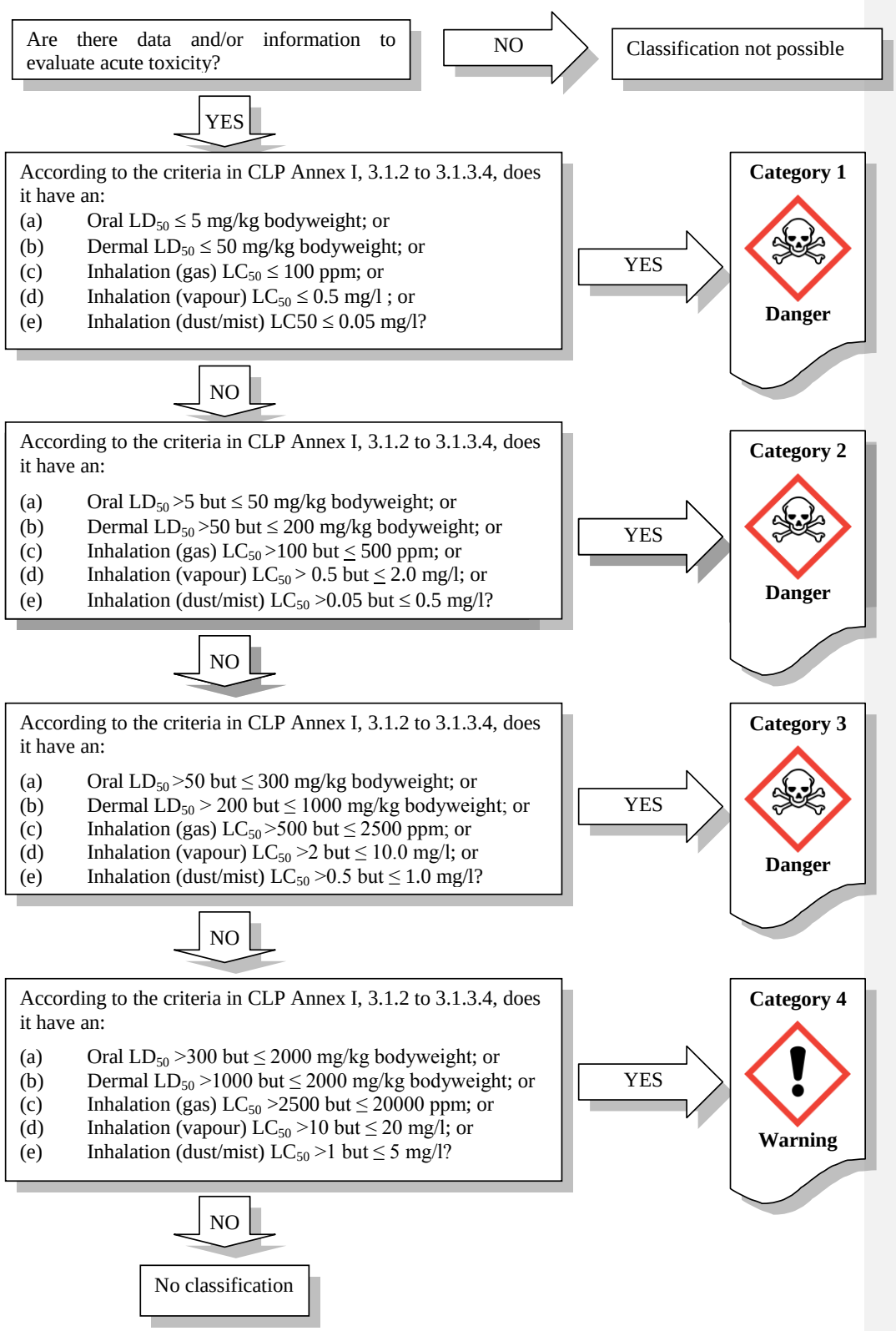
3.1.2.5 Setting of specific concentration limits

Specific concentration limits are not applicable for acute toxicity classification. Rather, the relative potency of substances is implicitly taken into account in the additivity formula (see Section 0 of this Guidance). For this reason specific concentration limits for acute toxicity will not appear in CLP Annex VI, Table 3.1 or in the classification and labelling inventory (CLP Article 42).

3.1.2.6 Decision logic

The decision logic below is provided as additional guidance. It is strongly recommended that the person responsible for classification is fully familiar with the criteria for acute toxicity classification before using the decision logic.

For a complete classification of a substance, the decision logic must be worked out for each route of exposure for which data and/or information is available. For example, if a certain substance is classified in Category 1 based on an oral $LD_{50} \leq 5$ mg/kg bodyweight (the answer was 'Yes' in box 2 for item (a)), it is still necessary to go back to box 2 in the decision logic and complete the classification for the dermal (b) and inhalation (c)-(e) route of exposure, when data is available for one or both of these routes of exposure. In case there are data for all three routes of exposure, the classification for acute toxicity of the substance will include the three differentiations of the hazard class, which might end up in three different categories. The route of exposure will then be specified in the corresponding hazard statement.


Are there data and/or information to evaluate acute toxicity?
NO
Classification not possible
YES
According to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4, does it have an:
(a) Oral LD50 ≤ 5 mg/kg bodyweight; or
(b) Dermal LD50 ≤ 50 mg/kg bodyweight; or
(c) Inhalation (gas) LC50 ≤ 100 ppm; or
(d) Inhalation (vapour) LC50 ≤ 0.5 mg/l ; or
(e) Inhalation (dust/mist) LC50 ≤ 0.05 mg/l?
YES
Category 1
Danger
NO
According to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4, does it have an:
(a) Oral LD50 >5 but ≤ 50 mg/kg bodyweight; or
(b) Dermal LD50 >50 but ≤ 200 mg/kg bodyweight; or
(c) Inhalation (gas) LC50 >100 but ≤ 500 ppm; or
(d) Inhalation (vapour) LC50 > 0.5 but ≤ 2.0 mg/l; or
(e) Inhalation (dust/mist) LC50 >0.05 but ≤ 0.5 mg/l?
YES
Category 2
Danger
NO
According to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4, does it have an:
(a) Oral LD50 >50 but ≤ 300 mg/kg bodyweight; or
(b) Dermal LD50 > 200 but ≤ 1000 mg/kg bodyweight; or
(c) Inhalation (gas) LC50 >500 but ≤ 2500 ppm; or
(d) Inhalation (vapour) LC50 >2 but ≤ 10.0 mg/l; or
(e) Inhalation (dust/mist) LC50 >0.5 but ≤ 1.0 mg/l?
YES
Category 3
Danger
NO
According to the criteria in CLP Annex I, 3.1.2 to 3.1.3.4, does it have an:
(a) Oral LD50 >300 but ≤ 2000 mg/kg bodyweight; or
(b) Dermal LD50 >1000 but ≤ 2000 mg/kg bodyweight; or
(c) Inhalation (gas) LC50 >2500 but ≤ 20000 ppm; or
(d) Inhalation (vapour) LC50 >10 but ≤ 20 mg/l; or
(e) Inhalation (dust/mist) LC50 >1 but ≤ 5 mg/l?
YES
Category 4
Warning
NO
No classification

3.1.3 Classification of mixtures for acute toxicity

3.1.3.1 General considerations for classification

Annex I: 3.1.3.1. The criteria for classification of substances for acute toxicity as outlined in section 3.1.2 are based on lethal dose data (tested or derived). For mixtures, it is necessary to obtain or derive information that allows the criteria to be applied to the mixture for the purpose of classification. The approach to classification for acute toxicity is tiered, and is dependent upon the amount of information available for the mixture itself and for its ingredients.

The procedure for classifying mixtures is a tiered i.e. a stepwise approach based on a hierarchy principle and depending on the type and amount of available data/information. If valid test data are available for the whole mixture they have precedence. If no such data exist, the so called bridging principles have to be applied if possible. If the bridging principles are not applicable an assessment on the basis of ingredient information will be applied (see Sections 0, 3.1.3.3.4 and 3.1.3.5 of this Guidance).

3.1.3.2 Identification of hazard information

Where toxicological information from human evidence and animal studies is available on a mixture, this should be used to derive the appropriate classification. Such information may be available from the mixture manufacturer. Where such information on the mixture itself is not available, information on similar tested mixtures and, the component substances in the mixture must be used, as described in Section 3.1.3.3 of this Guidance.

Alternatively, the hazard information on all individual components in the mixture could be identified as described in Section 3.1.2.2 of this Guidance.

3.1.3.3 Classification criteria

Annex I: 3.1.3.2. For acute toxicity each route of exposure shall be considered for the classification of mixtures, but only one route of exposure is needed as long as this route is followed (estimated or tested) for all components and there is no relevant evidence to suggest acute toxicity by multiple routes. When there is relevant evidence of toxicity by multiple routes of exposure, classification is to be conducted for all appropriate routes of exposure. All available information shall be considered. The pictogram and signal word used shall reflect the most severe hazard category and all relevant hazard statements shall be used.

The classification must be considered for each route of exposure, using the appropriate approach as described in Section 3.1.2.3 of this Guidance. If different hazard categories are assigned, the most severe hazard category will be used to select the appropriate pictogram and signal word on the label for acute toxicity. For each relevant route of exposure, the hazard statement will correspond to the classification of this specific route.

3.1.3.3.1 When data are available for the complete mixture

Annex I: 3.1.3.4.1. Where the mixture itself has been tested to determine its acute toxicity, it shall be classified according to the same criteria as those used for substances, presented in Table 3.1.1. […]

In general, where a mixture has been tested those data should be used to support classification according to the same criteria as used for substances. However, there should be some consideration of whether the test is appropriate. For instance, if the mixture contains a

substance for which the test species is not considered appropriate (for instance a mixture containing methanol tested in rats which are not sensitive to methanol toxicity), then the appropriateness of these data for classification should be considered using expert judgement.

With respect to the classification of mixtures in the form of dust or mist for acute inhalation toxicity, the particle size can affect the toxicity and the resulting classification should take this into account (see Section 3.1.2.3.2 of this Guidance).

3.1.3.3.2 When data are not available for the complete mixture: bridging principles

Annex I: 3.1.3.5.1. Where the mixture itself has not been tested to determine its acute toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging rules set out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture (see Section 1.6.3 of this Guidance).

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified based on its ingredients as in Sections 3.1.3.3.3, 3.1.3.3.4, 3.1.3.3.5 and 3.1.3.4 of this Guidance.

3.1.3.3.3 When data are available for all ingredients

Annex I: 3.1.3.3.

(c) If the converted acute toxicity point estimates for all components of a mixture are within the same category, then the mixture should be classified in that category.

(d) When only range data (or acute toxicity hazard category information) are available for components in a mixture, they may be converted to point estimates in accordance with Table 3.1.2 when calculating the classification of the new mixture using the formulas in sections 3.1.3.6.1 and 3.1.3.6.2.3.

Annex I: 3.1.3.6. *Classification of mixtures based on ingredients of the mixture (Additivity formula)*

3.1.3.6.1. Data available for all ingredients

In order to ensure that classification of the mixture is accurate, and that the calculation need only be performed once for all systems, sectors, and categories, the acute toxicity estimate (ATE) of ingredients shall be considered as follows:

(a) include ingredients with a known acute toxicity, which fall into any of the acute toxicity categories shown in Table 3.1.1;

(b) ignore ingredients that are presumed not acutely toxic (e.g., water, sugar);

(c) ignore components if the data available are from a limit dose test (at the upper threshold for Category 4 for the appropriate route of exposure as provided in Table 3.1.1) and do not show acute toxicity.

Components that fall within the scope of this section are considered to be components with a known acute toxicity estimate (ATE). See note (b) to Table 3.1.1 and section 3.1.3.3 for appropriate application of available data to the equation below, and section 3.1.3.6.2.3.

The ATE of the mixture is determined by calculation from the ATE values for all relevant ingredients according to the following formula below for Oral, Dermal or Inhalation Toxicity:

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

where:

C_i = concentration of ingredient i (% w/w or % v/v)

i = the individual ingredient from 1 to n

n = the number of ingredients

ATE_i = Acute Toxicity Estimate of ingredient i.

For mixtures containing substances tested for inhalation toxicity as vapours and others as dust, the additivity formula cannot be used because it is unclear when the numeric values for vapours or dusts must be used. Therefore for acute inhalation toxicity the additivity formula should be used separately for each relevant physical form (i.e. gas, vapour and/or dust/mist), using the appropriate categories in CLP Annex I, Table 3.1.1. In case of different outcomes, the most severe classification applies.

Annex I: *Table 3.1.2*
Conversion from experimentally obtained acute toxicity range values (or acute toxicity hazard categories) to acute toxicity point estimates for use in the formulas for the classification of mixtures

Exposure routes	Classification category or experimentally obtained acute toxicity range estimate	Converted acute toxicity point estimate (see Note 1)
Oral (mg/kg bodyweight)	0 < Category 1 ≤ 5	0.5
	5 < Category 2 ≤ 50	5
	50 < Category 3 ≤ 300	100
	300 < Category 4 ≤ 2000	500
Dermal (mg/kg bodyweight)	0 < Category 1 ≤ 50	5
	50 < Category 2 ≤ 200	50
	200 < Category 3 ≤ 1000	300
	1000 < Category 4 ≤ 2000	1100
Gases (ppmV)	0 < Category 1 ≤ 100	10
	100 < Category 2 ≤ 500	100
	500 < Category 3 ≤ 2500	700
	2500 < Category 4 ≤ 20000	4500
Vapours (mg/l)	0 < Category 1 ≤ 0,5	0,05
	0,5 < Category 2 ≤ 2	0.5
	2,0 < Category 3 ≤ 10,0	3
	10,0 < Category 4 ≤ 20,0	11

Dust/mist (mg/l)	0< Category 1 ≤ 0,05	0,005
	0,05 < Category 2 ≤ 0,5	0,05
	0,5 < Category 3 ≤ 1,0	0,5
	1,0 < Category 4 ≤ 5,0	1,5

Note 1:
These values are designed to be used in the calculation of the ATE for classification of a mixture based on its components and do not represent test results.

Some converted Acute Toxicity point Estimates (cATpEs) are equal to the upper limit of the next lower category, for example the cATpE of oral Category 2 (5 mg/kg bw) is equal to the upper limit of oral Category 1 (also 5 mg/kg bw).

This can lead to a problem when using the cATpE values for calculating the acute toxicity of mixtures. For instance, using the cATpEs for a mixture containing only substances classified in Category 2 actually results in a Category 1 classification for the mixture. Similarly, a mixture containing substances classified as Category 3 for dust/mist results in a Category 2 classification. Clearly these outcomes are incorrect and are an unintended side-effect of the approach. In such cases, CLP Annex I, 3.1.3.3.(c) should be applied.

Annex I: 3.1.3.3.(c) If the converted acute toxicity point estimates for all components of a mixture are within the same category, then the mixture should be classified in that category.

As a result, the mixtures in the examples highlighted above would be classified in Categories 2 and 3, respectively.

Annex I: 3.1.3.3.(b) where a classified mixture is used as an ingredient of another mixture, the actual or derived acute toxicity estimate (ATE) for that mixture may be used, when calculating the classification of the new mixture using the formulas in section 3.1.3.6.1 and paragraph 3.1.3.6.2.3.

It is important that the downstream user has sufficient information in order to enable him to perform a correct classification of mixtures.

3.1.3.3.4 When data are <u>not</u> available for all ingredients

Annex I: 3.1.3.6.2.1. Where an ATE is not available for an individual ingredient of the mixture, but available information such as that listed below can provide a derived conversion value such as those laid out in Table 3.1.2, the formula in paragraph 3.1.3.6.1 shall be applied.

This includes evaluation of:

(a) extrapolation between oral, dermal and inhalation acute toxicity estimates ([1]). Such an evaluation could require appropriate pharmacodynamic and pharmacokinetic data;

(b) evidence from human exposure that indicates toxic effects but does not provide lethal dose data;

(c) evidence from any other toxicity tests/assays available on the substance that indicates toxic acute effects but does not necessarily provide lethal dose data; or

(d) data from closely analogous substances using structure/activity relationships.

_____

([1]) When mixtures contain components that do not have acute toxicity data for each route of exposure, acute toxicity estimates may be extrapolated from the available data and applied to the appropriate routes (see section 3.1.3.2). However, specific legislation may require testing for a specific route. In those cases, classification shall be performed for that route based upon the legal

requirements.

Derivation of ATEs from available information:

When ingredients have a known acute toxicity (LC_{50} or LD_{50} values), this value has to be used in the additivity formula. However, for many substances, acute toxicity data will not be available for all exposure routes.

CLP allows for two ways of deriving acute toxicity conversion values. One option is to use the converted acute toxicity point estimates supplied in CLP Annex I, Table 3.1.2. The other option, expert judgement would recommend in substantiated cases the use of the directly derived ATE values.

a) Route-to-route extrapolation (CLP Annex I, 3.1.3.6.2.1.(a)):

Route-to-route extrapolation is defined as the prediction of the total amount of a substance administered by one route that would produce the same systemic toxic response as that obtained by a given amount of a substance administered by another route. Thus, route-to-route extrapolation is only applicable for the evaluation of systemic effects. It is not appropriate to assess direct local effects.

This extrapolation is possible if certain conditions are met, which substantiate the assumption that an internal dose causing a systemic effect at the target is related to an external dose/concentration; preferably the absorption can be quantified. Therefore information on the physico-chemical and biokinetic properties should be available and assessed in order to allow such a conclusion and performing an extrapolation across routes. In the absence of any information on absorption, 100% absorption has to be presumed as a worst case for the dermal and inhalation route. Extrapolating from the oral route to other routes, the assumption of absorption of 100% for the oral route is, however, not a worst case. Absorption of less than 100% by the oral route will lead to lower ATEs. Another important factor is the local and systemic metabolic pathways; in particular it must be ensured that no route-specific metabolism/degradation of substance occurs.

If extrapolating from oral data, the influence of first-pass metabolism in the stomach/intestines and the liver should be considered, especially if the substance is detoxified. Such first pass metabolism is unlikely to occur to any significant extent by the dermal or inhalation routes, and so this would lead to an underestimate of toxicity by these routes. Thus if based on kinetic or (Q)SAR data a specific first-pass effect is excluded, oral data may be used for extrapolation purposes.

For an extrapolation to the dermal route, information on the potential skin penetration may be derived from the chemical structure (polar vs. nonpolar structure elements, Log P_{ow}, molecular weight) if kinetic data are not available which would allow a quantitative comparison. When no such information is available 100% dermal absorption should be presumed. Further information and guidance on dermal absorption can be found on the OECD and EFSA websites OECD (http://www.oecd.org/chemicalsafety/testingofchemicals/48532204.pdf) and EFSA (http://www.efsa.europa.eu/en/efsajournal/doc/2665.pdf).

Similarly for an extrapolation to the inhalation route if there is no quantitative information on absorption then 100% absorption should be presumed. Inhalation volatility is an important factor which on one hand may increase the exposure, but on the other hand may reduce absorption due to higher exhalation rates. The solubility (in water and non-polar solvents) has to be considered, as well as particle size, which plays a particularly important role in inhalation toxicity.

Route-to-route extrapolation is not always appropriate. For example where there is a substantial difference in absorption between oral and inhalation uptake (e.g. poorly soluble particles, substances that decompose within the gastro intestinal-tract), or where the substance causes local effects, the toxicity by different routes may be significantly different, and route-to-route extrapolation may not be appropriate (ECETOC TR 86, 2003).

i: Extrapolation oral → inhalation:

If the mentioned conditions are met an extrapolation from oral data would be performed as follows:

Incorporated dose = concentration x respiratory volume x exposure time

1 mg/kg bw = 0.0052 mg/l/4h

using a respiratory volume for a 250 g rat of 0.20 l/min and 100 % absorption and postulating 100% deposition and absorption (Guidance on IR/CSA, Chapter R7C, Table R.7.12-10).

Valid information that the deposition and/or absorption rate for the extrapolated route is lower would allow a higher equivalent derived ATE (see Section 3.1.6.1.9 Example 9 of this Guidance).

ii: Extrapolation oral → dermal

If based on kinetic or SAR data a high penetration rate can be assumed and a specific first pass-effect is excluded, oral and dermal toxicity might be regarded as equivalent. This is rarely the case.

Solids themselves may have a very low absorption rate, but if diluted in an appropriate solvent there may be an appreciable absorption of the substance. Thus, depending on the kinetic and physico-chemical properties and kind of mixture, varying ATEs will result. For example, butyn-1,4-diol causes no mortality in rats when dermally applied as a solid at 5000 mg/kg bw, whereas when an aqueous solution of butyn-1,4-diol is administered, a dermal LD_{50} of 659 and 1240 mg/kg bw in male and female rats, respectively, and an oral LD_{50} of about 200 mg/kg bw in both sexes can be ~~measured~~ determined.

For more details on inter-route extrapolation see the Guidance on IR/CSA, Section R.7c.12.1.5. Examples 9 and 10 illustrate this approach.

b) Evidence from human exposure:

Human evidence can be used to derive an appropriate ATE to use in the additivity approach for mixtures (CLP Annex I, 3.1.3.6.1 and 3.1.3.6.2.3). Therefore it is necessary to extrapolate from adequate and reliable data and taking the potency (i.e. the magnitude of the lethal dose reported) of the effects in humans into account. Thus an equivalent ATE may be derived on the basis of valid human toxicity data (minimum dose/concentration) and used directly in the additivity formulae (see Section 3.1.6.1.1 Example 1 of this Guidance). The alternative to the derivation of an equivalent ATE is the allocation to a category. The category should be justified by semi-quantitative or qualitative data and a subsequent derivation of a converted ATE (cATpE) according to CLP Annex I, Table 3.1.2 and subsequently use in the formulae (see Section 3.1.6.1.2 Example 2 of this Guidance). See also Section 3.1.2.3.1 of this Guidance for more details.

c) Evidence from other toxicity tests:

Information from other types of studies can sometimes be useful in deriving an acute toxicity classification (see Section 3.1.2.2 of this Guidance). These studies will not usually provide an LD_{50}/ATE value that can be used directly for classification, but they may provide enough

information to allow an estimate of acute toxicity to be made, which would be sufficient to support a decision on classification.

Example:

Available information: In a range finding study with respect to repeated dose toxicity daily oral doses of 1000 mg/kg bw over 5 days prove to be neither lethal nor cause serious symptoms in rats at the end of the observation period of 14 days.

Conclusion: the LD_{50}=ATE is >2000 mg/kg bw since 2 doses following (within roughly) 24h are not lethal (see Section 3.1.2.2 of this Guidance). Thus this ingredient can be ignored in the additivity procedure.

d) Use of (Q)SAR:

LD_{50}/LC_{50} values predicted by a highly reliable model (see Section 3.1.2.3.2 of this Guidance) may be used according to Note (a) to CLP Annex I, Table 3.1.1 directly as LD_{50}/LC_{50}=ATE in the additivity formula CLP Annex I, 3.1.3.6.1. If the assessment using (Q)SARs gives a more general result a cATpE according to Table 3.1.2 may be derived. It has to be emphasised that these approaches generally require substantial technical information, and expert judgement, to reliably estimate acute toxicity.

Further guidance on how to apply this provision is given in Section 3.1.3.3.5 of this Guidance.

Annex I: 3.1.3.6.2.3. If the total concentration of the ingredient(s) with unknown acute toxicity is ≤ 10 % then the formula presented in section 3.1.3.6.1 shall be used. If the total concentration of the ingredient(s) with unknown toxicity is > 10 %, the formula presented in section 3.1.3.6.1 shall be corrected to adjust for the total percentage of the unknown ingredient(s) as follows:

$$\frac{100-\sum C_{umknown} \text{if } >10\%}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

Guidance update to legal text "Annex I: 3.1.3.6.2.3" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.1.3.6.2.3. If the total concentration of the relevant ingredient(s) with unknown acute toxicity is ≤ 10 % then the formula presented in section 3.1.3.6.1 shall be used. If the total concentration of the relevant ingredient(s) with unknown toxicity is > 10 %, the formula presented in section 3.1.3.6.1 shall be corrected to adjust for the total percentage of the unknown ingredient(s) as follows:

$$\frac{100-\sum C_{umknown} \text{if } >10\%}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

3.1.3.3.5 Ingredients that should be taken into account for the purpose of classification

Annex I: 3.1.3.3.(a) the 'relevant ingredients' of a mixture are those which are present in concentrations of 1 % (w/w for solids, liquids, dusts, mists and vapours and v/v for gases) or greater, unless there is a reason to suspect that an ingredient present at a concentration of less than 1 % is still relevant for classifying the mixture for acute toxicity (see Table 1.1).

When a mixture contains a "relevant" ingredient (i.e. constituting ≥ 1%; CLP Annex I, 3.1.3.3 (a)) for which there is no adequate acute toxicity data then the mixture must be

classified on the basis of the ingredients with known toxicity, with an additional statement on the label and in the SDS to indicate that the mixture consists of "x percent" of ingredients of unknown acute toxicity (CLP Annex I, 3.1.3.6.2.2). The determination of the classification depends on what proportion of the mixture such ingredients of unknown toxicity constitute. If these ingredients constitute ≤10% of the total mixture, the additivity formula in CLP Annex I, 3.1.3.6.1 must be used. However, in cases where these ingredients constitute over 10%, a modified additivity formula in CLP Annex I, 3.1.3.6.2.3 must be used, which adjusts for the presence of a significant proportion of ingredients of unknown toxicity, is used. This reflects the greater uncertainty as to the true toxicity of the mixture).

Annex I: *Excerpt of Table 1.1* **Generic cut-off values**	
Hazard class	Generic cut-off values to be taken into account
Acute Toxicity:	
- Category 1-3	0,1 %
- Category 4	1 %
Note: Generic cut-off values are in weight percentages except for gaseous mixtures for those hazard classes where the generic cut-off values may be best described in volume percentages.	

As indicated in CLP Annex I, Table 1.1, when components are present in low concentrations they do not need to be taken into account when determining the classification of the mixture, according to the approaches detailed in CLP Annex I, 3.1.3.6.1 and 3.1.3.6.2.3 (see Section 3.1.6.3.1 Example 11 of this Guidance). Accordingly, all components classified in Categories 1-3 at a concentration <0.1% and Category 4 <1% are not taken into account. Similarly unknown ingredients present at <1% are not taken into account.

3.1.3.4 Generic concentration limits for substances triggering classification of mixtures

Generic concentration limits as such are not applicable for acute toxicity classification; therefore specific concentration limits are also not applicable (see Section 3.1.2.5 of this Guidance). Nevertheless, according to CLP Annex VI, 1.2.1 the classification for entries with the reference * in the column specific concentration limits is of special concern; the * means that those entries have an SCL in CLP Annex VI, Table 3.2 originating from Annex I to DSD. Therefore when assessing a mixture according to the procedure set out in CLP Annex I, a thorough search for the data (animal, human experience or other information) which had been the basis for the respective SCL in Annex I of DSD is indicated as being necessary. The assessment must take all available information into account using a weight of evidence approach and expert judgement with special emphasis on possibly available human experience or information. These validated data will then be used in the additivity formula in CLP Annex I, 3.1.3.6.1 as ATEs or cATpEs (CLP Annex I, Table 3.1.2).

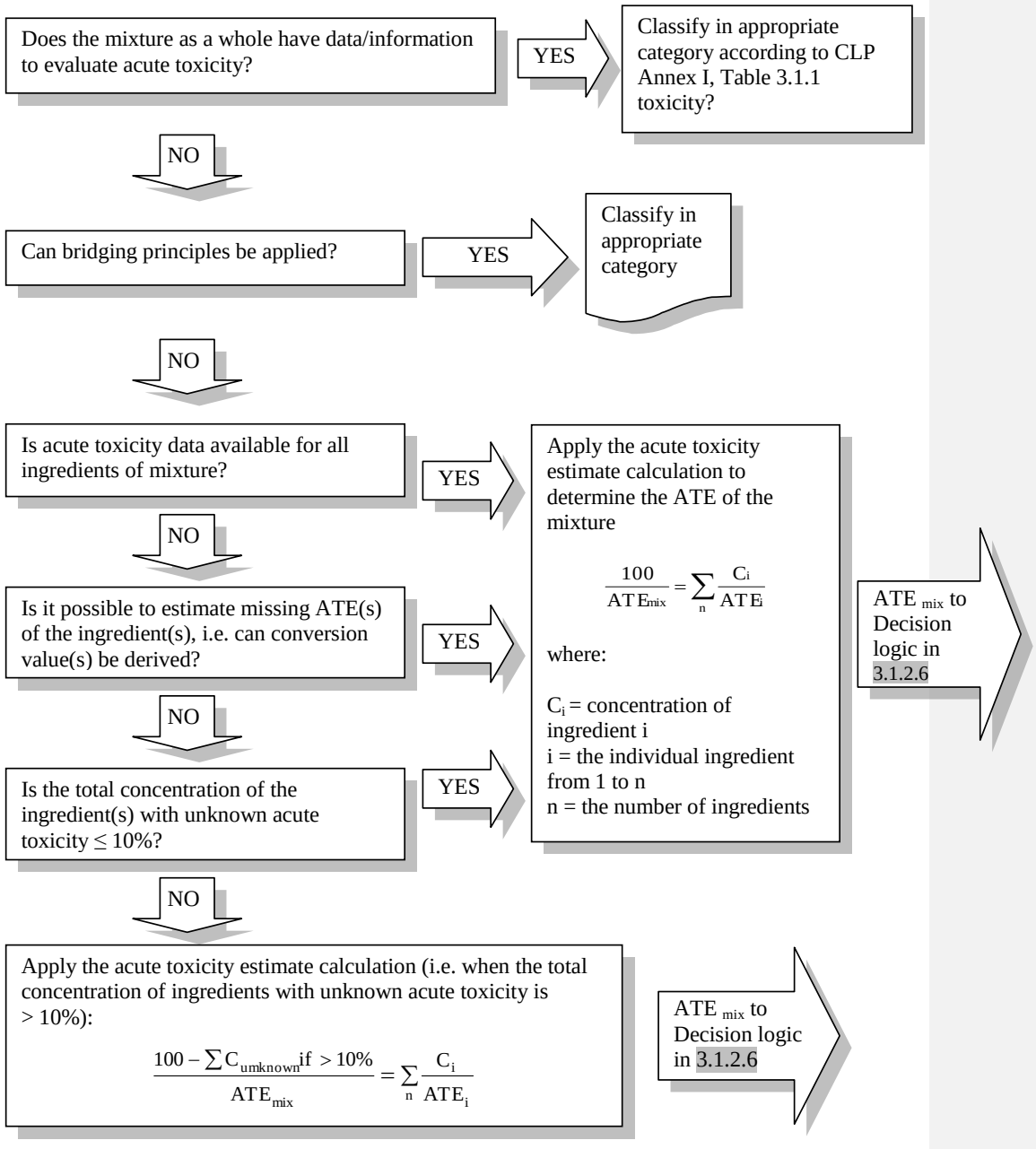
3.1.3.5 Decision on classification

The assessment on classification has to be performed with respect to all the relevant routes of exposure (oral, dermal, inhalation) on the basis of all adequate reliable data. If there is evidence of toxicity by multiple routes of exposure classification is warranted for all the routes of exposure, The label should include one pictogram and signal word reflecting the most severe hazard category. If for example, a mixture fulfils the criteria for oral toxicity Category 3 and for inhalation Category 2, then the mixture will be classified in Category 3

for oral toxicity and Category 2 for inhalation toxicity and assigned the corresponding hazard statements; it will be labelled withthe acute toxicity Category 2 pictogram (skull and corss bones) and the signal word “Danger” and both the hazard statements for inhalation Category 2 (H330) and oral Category 3 (H301) (see CLP Annex I Table 3.1.3 in next section 3.1.4.1 of this Guidance).

3.1.3.6 Decision logic

The decision logic is provided as additional guidance. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.



3.1.4 Hazard communication in form of labelling for acute toxicity

3.1.4.1 Pictograms, signal words, hazard statements and precautionary statements

Guidance update to legal text "Annex I: Table 3.1.3" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: *Table 3.1.3*

Acute toxicity label elements

Classification	Category 1	Category 2	Category 3	Category 4
GHS Pictograms				
Signal Word	Danger	Danger	Danger	Warning
Hazard Statement: – Oral	H300: Fatal if swallowed	H300: Fatal if swallowed	H301: Toxic if swallowed	H302: Harmful if swallowed
– Dermal	H310: Fatal in contact with skin	H310: Fatal in contact with skin	H311: Toxic in contact with skin	H312: Harmful in contact with skin
– Inhalation (see Note 1)	H330: Fatal if inhaled	H330: Fatal if inhaled	H331: Toxic if inhaled	H332: Harmful if inhaled
Precautionary Statement Prevention (oral)	P264 P270	P264 P270	P264 P270	P264 P270
Precautionary Statement Response (oral)	P301 + P310 P321 P330	P301 + P310 P321 P330	P301 + P310 P321 P330	P301 + P312 P330
Precautionary Statement Storage (oral)	P405	P405	P405	
Precautionary Statement Disposal (oral)	P501	P501	P501	P501
Precautionary Statement Prevention (dermal)	P262 P264 P270	P262 P264 P270	P280	P280

	P280	P280		
Precautionary Statement Response (dermal)	P302 + P350 P310 P322 P361 P363	P302 + P350 P310 P322 P361 P363	P302 + P352 P312 P322 P361 P363	P302 + P352 P312 P322 P363
Precautionary Statement Response (dermal) **4th ATP change**	P302 + P352 P310 P321 P361 + P364	P302 + P352 P310 P321 P361 + P364	P302 + P352 P312 P321 P361 + P364	P302 + P352 P312 P321 P362 +P364
Precautionary Statement Storage (dermal)	P405	P405	P405	
Precautionary Statement Disposal (dermal)	P501	P501	P501	P501
Precautionary Statement Prevention (inhalation)	P260 P271 P284	P260 P271 P284	P261 P271	P261 P271
Precautionary Statement Response (inhalation)	P304 + P340 P310 P320	P304 + P340 P310 P320	P304 + P340 P311 P321	P304 + P340 P312
Precautionary Statement Storage (inhalation)	P403 + P233 P405	P403 + P233 P405	P403 + P233 P405	
Precautionary Statement Disposal (inhalation)	P501	P501	P501	

Note 1

In addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity is corrosivity, the substance or mixture shall also be labelled as EUH071: 'corrosive to the respiratory tract' — see advice at 3.1.2.3.3. In addition to an appropriate acute toxicity pictogram, a corrosivity pictogram (used for skin and eye corrosivity) may be added together with the statement 'corrosive to the respiratory tract'.

Note 2

In the event that an ingredient without any useable information at all is used in a mixture at a concentration of 1 % or greater, the mixture shall be labelled with the additional statement that 'x percent of the mixture consists of ingredient(s) of unknown toxicity' — see advice at 3.1.3.6.2.2.

EUH071 can also be applied to inhaled corrosive substances not tested for acute inhalation toxicity according to CLP Annex II, Section 1.2.6

If a substance or a mixture fulfils the classification criteria with respect to different routes ~~hazard category~~ the pictogram and signal word will be based on the most severe one, however the hazard statements for each route must be included on the label. .

3.1.4.2 Additional labelling provisions

Annex I: 3.1.3.6.2.2. In the event that a component without any useable information for classification is used in a mixture at a concentration of 1 % or greater, it is concluded that the mixture cannot be attributed a definitive acute toxicity estimate. In this situation the mixture shall be classified based on the known components only, with the additional statement on the label and in the SDS that "x percent of the mixture consists of component(s) of unknown toxicity".

In addition to the statement required under CLP Annex I, 3.1.3.6.2.2, it would be appropriate to specify the relevant exposure route of toxicity concerned on a case-by-case basis: For example "'x percent of the mixture consists of component(s) of unknown acute oral toxicity. In the case of different values being available for the % of ingredients having unknown acute toxicity (as a result of different route of exposure), the % value to be included in the sentence on the label should be selected based on the route where the % of ingredients having unknown toxicity is the highest.".

Guidance update to legal text "Annex I: 3.1.3.6.2.2" and section 3.1.4.2 of the guidance in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures.

Annex I: 3.1.3.6.2.2. In the event that a component without any useable information for classification is used in a mixture at a concentration ≥ 1 %, it is concluded that the mixture cannot be attributed a definitive acute toxicity estimate. In this situation the mixture shall be classified based on the known components only, with the additional statement on the label and in the SDS that: "x percent of the mixture consists of component(s) of unknown acute toxicity", taking into account the provisions set out in section 3.1.4.2.

Annex I: 3.1.4.2

The acute toxicity hazard statements differentiate the hazard based on the route of exposure. Communication of acute toxicity classification should also reflect this differentiation. If a substance or mixture is classified for more than one route of exposure then all relevant classifications should be communicated on the safety data sheet as specified in Annex II to Regulation (EC) No 1907/2006 and the relevant hazard communication elements included on the label as prescribed in section 3.1.3.2. If the statement "x % of the mixture consists of ingredient(s) of unknown acute toxicity" is communicated, as prescribed in section 3.1.3.6.2.2, then, in the information provided in the safety data sheet, it can also be differentiated based on the route of exposure. For example, "x % of the mixture consists of ingredient(s) of unknown acute oral toxicity" and "x % of the mixture consists of ingredient(s) of unknown acute dermal toxicity.

In case section 3.1.3.6.2.2 applies and the statement "x % of the mixture consists of ingredient(s) of unknown acute toxicity" has to be communicated, the same statement can be differentiated on the base of the route of exposure in the safety data sheet in accordance with CLP Annex I 3.1.4.2. For example on the label and in SDS should appear "x % of the mixture consists of ingredient(s) of unknown acute toxicity"; in the SDS the route of exposure can also be specified: For example "x % of the mixture consists of ingredient(s) of unknown acute oral toxicity" and "x % of the mixture consists of ingredient(s) of unknown

acute dermal toxicity." In case of different values being available for the % of ingredients having unknown toxicity (as a result of different route of exposure), the % value to be included in the sentence on the label should be selected based on the route where the % of ingredients having unknown toxicity is the highest."

Corrosivity:

Annex I: 3.1.2.3.3.

In addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity was corrosivity, the substance or mixture shall also be labelled as 'corrosive to the respiratory tract' (see note 1 in 3.1.4.1). Corrosion of the respiratory tract is defined by destruction of the respiratory tract tissue after a single, limited period of exposure analogous to skin corrosion; this includes destruction of the mucosa. The corrosivity evaluation can be based on expert judgment using such evidence as: human and animal experience, existing (*in vitro*) data, pH values, information from similar substances or any other pertinent data.

In addition to the application of the classification for acute inhalation toxicity, the substance or mixture must also be labelled as EUH071 where data are available which indicate that the mode of toxic action was corrosivity (see Note 1 to Table 3.1.3). Such information can be derived from data which warrant classification as corrosive according to the hazard skin corrosion/irritation (see Chapter 3.2 of this Guidance). In this case the substance or mixture has to be classified and labelled for skin corrosion with the pictogram for corrosivity, GHS05, hazard statement H314 and also labelling with EUH071 (for criteria, see CLP Annex II) is required. (see Chapter 3.2.4.2 of this Guidance).

Annex II: 1.2.6. EUH071 — 'Corrosive to the respiratory tract'

For substances and mixtures in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I.

For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled.

Corrosive substances and mixtures may be acutely toxic after inhalation to a varying degree, although this is only occasionally proved by testing. In case no acute inhalation study is available for a corrosive substance or mixture, and such substance or mixture may be inhaled, a hazard of respiratory tract corrosion may exist. As a consequence, substances and mixtures have to be supplementary labelled with EUH071, also taking into consideration the saturated vapour concentration, as appropriate, (see also chapter 3.8.2.5 of this Guidance. It is strongly recommended to apply the precautionary statement P260: Do not breathe dust/fume/gas/mist/vapours/spray.

Toxic by eye contact:

Annex II: 1.2.5 EUH070 — 'Toxic by eye contact'

For substances or mixtures where an eye irritation test has resulted in overt signs of systemic toxicity or mortality among the animals tested, which is likely to be attributed to absorption of the substance or mixture through the mucous membranes of the eye. The statement shall also be applied if there is evidence in humans for systemic toxicity after eye contact.

The statement shall also be applied where a substance or a mixture contains another substance labelled for this effect, if the concentration of this substance is equal to, or greater than 0,1 %, unless otherwise specified in part 3 of Annex VI.

In cases where a substance or mixture has shown clear signs of severe systemic toxicity or mortality in an eye irritation study a supplemental labelling phrase EUH070 "Toxic by eye contact" is required. This additional labelling, based on relevant data, is independent of any classification in an acute toxicity category.

Liberation of toxic gases

Annex II: 1.2.1. EUH029 — 'Contact with water liberates toxic gas'

For substances and mixtures which in contact with water or damp air, evolve gases classified for acute toxicity in category 1, 2 or 3 in potentially dangerous amounts, such as aluminium phosphide, phosphorus pentasulphide.

Annex II: 1.2.1 EUH031 — 'Contact with acids liberates toxic gas'

For substances and mixtures which react with acids to evolve gases classified for acute toxicity in category 3 in dangerous amounts, such as sodium hypochlorite, barium polysulphide.

Annex II: 1.2.3. EUH032 — 'Contact with acids liberates very toxic gas'

For substances and mixtures which react with acids to evolve gases classified for acute toxicity in category 1 or 2 in dangerous amounts, such as salts of hydrogen cyanide, sodium azide.

3.1.5 Re-classification of substances and mixtures classified for acute toxicity according to DSD and DPD

3.1.5.1 Is direct "translation" of classification and labelling possible?

The CLP allows a minimum classification of substances and mixtures classified according to DSD and DPD, by use of a translation table in CLP Annex VII (Table 1.1) into the corresponding classification under CLP. For more details see Chapter 1.7 of this Guidance for the application of Annex VII.

3.1.5.2 Re-evaluation of data

If there is new information which might be relevant with respect to classification a re-evaluation has to be performed. Classified gases should be re-evaluated because the guidance values changed from general guidance values in mg/l for dusts and mists, vapours and gases to a specific guidance value for gases in ppm (see section 3.1.2.3.2 of this guidance: Conversions). Often the values for classification are higher according to CLP compared to DSD which may require a re-evaluation on a case by case basis.

3.1.6 Examples of classification for acute toxicity

Remark: The classification proposals for the examples refer only to Acute Toxicity.

3.1.6.1 Examples of substances fulfilling the criteria for classification

3.1.6.1.1 Example 1: Methanol

Application	Use of adequate and reliable human data allowing derivation of an equivalent ATE according to CLP Annex I, Table 3.1.1. Animal data not appropriate.		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data:** Oral LD_{50} rat ≥ 5000 mg/kg bw	Classification not possible	The rat is known to be insensitive to the toxicity of methanol and is thus not considered to be a good model for human effects (different effect/mode of action)
	Human experience: Methanol is known to cause lethal intoxications in humans (mostly via ingestion) in relatively low doses: "…minimal lethal dose in the absence of medical treatment is between 300 and 1000 mg/kg bw" (IPCS, Environmental Health Criteria 196, Methanol, WHO, 1997)	Category 3	The minimum lethal dose reported of 300 mg/kg bw is used as equivalent ATE; according to CLP Annex I, Table 3.1.1 the resulting classification is Category 3
Remarks	Test data in rats from mixtures containing methanol should not be used directly in additivity formula.		

3.1.6.1.2 Example 2: N,N-Dimethylaniline

Application	Use of qualitative human data and of SAR information with extrapolation to an ATE (CLP Annex I, 3.1.3.6.2.1(b) and Table 3.1.2). Animal data are not appropriate.		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data:** Acute dermal toxicity: LD_{50} values > 1690 mg/kg bw rabbit.	Category 4	
	Human experience: Broad human experience, reported in many case reports, demonstrating death from MetHB following relatively low oral/dermal/inhalation exposure to aromatic amines such as N,N-dimethylaniline. For N,N-Dimethyl - aniline itself no exact human	Category 3 (oral, dermal, inhalation)	The extensive and consistent human experience is considered to be sufficiently robust by expert judgement to be used for classification into Category 3. The rabbit LD_{50} suggests lower sensitivity to MetHB formation than humans which is consistent with what is known from other rabbit tests with

	toxicity values are available.		substances known to induce MetHB in humans. The rabbit data are therefore not considered to be adequate for acute toxicity classification. Therefore the human data on this and structurally related substances are used to give a converted Acute Toxicity point Estimate (cATpE) according to CLP Annex I, Table 3.1.2 for Category 3; e.g. cATpE dermal = 300 mg/kg bw, which is then falling in a higher category than the rabbit data.
Remarks			

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2 **3.1.6.1.3 Example 3**

Application	No exact LD_{50} value available. Expert judgement needed.		
	Test Data	**Classification**	**Rationale**
Available information	Corrosive volatile liquid (not classified for skin corrosion). **Animal data:** In a GLP-compliant acute oral toxicity study in rats, the following results were observed: At a test dose of 200 mg/kg bw: no mortality, only transient symptoms and no necropsy findings. At a test dose of 500 mg/kg: 100% mortality, symptoms: poor general state; necropsy findings: hyperemia in stomach (due to local irritation /corrosivity), no other organs affected	Category 4	Since at a dose of 200 mg/kg bw no mortality and only slight transient symptoms without necropsy findings were observed, and at 500 mg/kg bw the high amount/concentration of the corrosive substance caused serious effect only at the site of action and mortality, based on expert judgement it can be assumed that the likely LD_{50} is > 300 mg/kg bw. Therefore, the Acute Toxicity Estimate (ATE) value for classification purpose is between 300 and 500 mg/kg bw, corresponding to Category 4 classification for acute toxicity.
Remarks	Labelling (in addition to the labelling provisions for Acute tox Cat. 4): Corrosive pictogram (pictogram is not mandatory, it may be added) (see Annex I: Note 1 of Table 3.1.3) Additional Hazard statement: EUH071 Corrosive to the respiratory tract		

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4 **3.1.6.1.4 Example 4**

Application	Use of non-standard-guideline test data.

	Test Data	Classification	Rationale
Available information	**Animal data:** A study to evaluate the acute dermal (percutaneous) toxicity was performed in rabbits. The following test data results were reported: - At the dose level of 50 mg/kg bw: no mortality was observed - At 200 mg/kg bw: 100% mortality Therefore, LD_{50} was estimated to be between 50 mg/kg bw and 200 mg/kg bw	Category 2	Rationale for classification: Since the dermal LD_{50} is above 50 mg/kg bw and less than 200 mg/kg bw, Category 2 classification is warranted (see CLP Annex I, Table 3.1.2)
Remarks			

3.1.6.1.5 Example 5

Application	Use of CLP Annex I, Table 3.1.1 and experimentally obtained LC_{50} value		
	Test Data	**Classification**	**Rationale**
Available information	A gas **Animal data**: A GLP-compliant test for acute inhalation toxicity (gaseous form) was performed in accordance with OECD TG 403 in rats. The following LC_{50} was calculated: LC_{50}: 4500 ppm/4h	Category 4	Rationale for classification: LC_{50} = 4500 ppm is considered an Acute Toxicity Estimate (ATE) for classification purposes; according to the classification criteria for acute inhalation toxicity for gases (CLP Annex I, Table 3.1.1), this value corresponds to Category 4. Therefore Category 4 Acute Inhalation Toxicity classification is warranted.
Remarks			

3.1.6.1.6 Example 6

Application	Time extrapolation; Note (c) in CLP Annex I, Table 3.1.1; Haber's law		
	Test Data	**Classification**	**Rationale**
Available information	Solid substance **Animal data**: The acute inhalation toxicity was studied in rats in a GLP-compliant study performed in principle according to OECD	Category 3	The classification criteria for acute inhalation toxicity in CLP Annex I, Table 3.1.1 refer to a 4h exposure time; therefore to classify a substance, existing inhalation toxicity data generated from 1-

	TG 403 in rats, but with respect for transport only with 1-h exposure. The LC_{50} (1-h) of 3 mg/l was calculated.		hour exposure should be converted accordingly: LC_{50} values with 1h have to be converted by dividing by 4 (Haber's rule/law, dusts and mists) LC_{50} (4-h) = (LC_{50} (1-h) **:** 4) = (3 mg/l **:** 4) = 0.75 mg/l, thus Category 3 classification is warranted according to CLP Annex I, Table 3.1.1.
Remarks			

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2 **3.1.6.1.7 Example 7: 2,3-Dichloropropene**

Application	Discrimination from STOT-SE		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data**: - Oral LD_{50}, rat 250-320 mg/kg bw (assumption: results from different tests; lowest LD_{50} is valid) - Inhalation LC_{50} rat 2.3 mg/l/4h (vapour) Observations: extensive liver and kidney damage following oral and inhalation exposure to lethal doses (insufficient information)	Category 3 oral and Category 3 inhalation	Classification according to criteria for acute inhalation and oral toxicity in CLP Annex I, Table 3.1.1.
Remarks	The substance is classified for acute toxicity and not for STOT-SE, since the observed organ toxicity is clearly the cause of the lethality.		

3

4 **3.1.6.1.8 Example 8**

Application	Route-to-route extrapolation: oral to inhalation (Section 3.1.3.3.4 of this Guidance). Expert judgement.		
	Test Data	**Extrapolated inhalation ATE/CATpE**	**Rationale**
Available information	**Animal data:** LD_{50} oral rat: 250 mg/kg bw (Category 3) 100 % oral absorption assumed a) No specific kinetic information b) Robust kinetic information	0.5 mg/l/4h (cATpE) 2.6 mg/l/4h (ATE)	a) Using the extrapolation formula 1 mg/kg bw = 0.0052 mg/l/4h: 250 x 0.0052 mg/l/4h = 1.3 mg/l/4h → Category 2

	allows the conclusion that only 50% is absorbed due to an exhalation rate of 50 %.		according to CLP Annex I, Table 3.1.2 b)Based on the 50% inhalation absorption rate the equivalent ATE would be 2.6 (2 x 1.3) → Category 3 according to CLP Annex I, Table 3.1.2
Remarks	Robust kinetic and other information would allow the use of directly derived ATEs in the additivity formulae by expert judgement		

3.1.6.1.9 Example 9

Application	Route-to-route extrapolation: oral to dermal (Section 3.1.3.3.4 of this Guidance). Expert judgement		
	Test Data	**Extrapolated dermal ATE/cATpE**	**Rationale**
Available information	**Animal data:** LD_{50} rat oral: 270 mg/kg bw; 100 % oral absorption assumed a) Assumed dermal absorption rate: 100% b) Dermal absorption rate based on robust kinetic/SAR information: 25%	300 mg/kg bw LD_{50} dermal 1080 mg/kg bw	a) Based on the assumption of 100% dermal absorption the converted dermal ATE will be derived by using Table 3.1.2 for Category 3 → 300 mg/kg bw as cATpE. b) Since dermal absorption is only 25%, the dermal ATE has to be accordingly increased → 4x270 mg/kg bw = 1080 mg/kg bw. This is regarded as an equivalent ATE which can be directly used in the additivity formulae.
Remarks	Robust kinetic and other information would allow the use of directly derived ATEs in the additivity formulae by expert judgement		

3.1.6.2 Examples of substances not fulfilling the criteria for classification

3.1.6.2.1 Example 10

Application	Available data are of different quality. Expert judgement. WoE		
	Test Data	**Classification**	**Rationale**
Available information	A liquid **Animal data**: Three studies for acute inhalation toxicity (vapour) in	No classification	With 3 different available values a validity check proved that the study with LC_{50} = 19 mg/l is not fully valid in contrast to the two others;

	rats are described. Two studies were performed in accordance with test guideline 403 and were GLP-compliant. One study has deficiencies with respect to study methodology and description of study performance and documentation of the test results; no GLP-compliance. The LC_{50} were as follows: – LC_{50}: 19 mg/l/4h (no GLP) – LC_{50}: 23 mg/l/4h (TG 403, GLP) – LC_{50}: 28 mg/l/4h (TG 403, GLP)		thus in a weight of evidence approach it is concluded that the LC_{50} = ATE > 20 mg/l/4h. The criteria for Category 4 are not fulfilled.
Remarks			

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2 **3.1.6.3 Examples of mixtures fulfilling the criteria for classification**

3 **3.1.6.3.1 Example 11**

Application	Application of the "Relevant ingredient" (CLP Annex I, 3.1.3.3 (a)) and "Generic cut-off values to be taken into account" concepts (CLP Annex I, Table 1.1) for mixtures with data gaps using the equation in CLP Annex I, 3.1.3.6.2.3. For dermal and inhalation routes, there is no acute toxicity data available for ingredients 2 and 4. For ingredients 1, 3 and 5 the data indicates no classification for acute toxicity.		
	Test Data	**Classification (ingredient)**	**Rationale**
Available information	**Animal data (oral rat):**		
Ingredient 1 (4%)	LD_{50}: 125 mg/kg bw	Oral Category 3	Apply the equation in CLP Annex I, 3.1.3.6.2.3: $\frac{100-(\sum C_{unknown} if\ >10\%)}{ATE_{mix}} = \sum_n \frac{C_i}{ATE_i}$ $\frac{100-92}{ATE_{mix}} = \frac{4}{125} + \frac{3}{1500} + \frac{0.2}{10} =$ $= 0.032 + 0.002 + 0.02 = 0.054$ ATEmix = 148 mg/kg bw → Category 3
Ingredient 2 (92%)	No data available	-	
Ingredient 3 (3%)	LD_{50}: 1500 mg/kg bw	Oral Category 4	
Ingredient 4 (0.9%)	No data available	-	
Ingredient 5 (0.2%)	LD_{50}: 10 mg/kg bw	Oral Category 2	
Remarks	Rationale for classification of the mixture in Category 3: 1. Classification via application of substance criteria is not possible since acute		

	toxicity test data was not available for the complete mixture (CLP Annex I, 3.1.3.4). 2. Classification via the application of bridging principles is not possible since data on a similar mixture was not available (CLP Annex I, 3.1.3.5.1). 3. Classification based on ingredient data for the mixture can be considered (CLP Annex I, 3.1.3.6). 4. Applying the "relevant ingredients" concept from CLP Annex I, 3.1.3.3 (a) means that Ingredient 4 is excluded from the ATE_{mix} calculation since its concentration is < 1%. The same reasoning cannot apply to Ingredient 5, though its concentration is below the "relevant ingredients" threshold of 1% but it is higher than the cut-off value of 0.1% for a Category 2 ingredient in CLP Annex I, Table 1.1. 5. The total concentration of ingredients with unknown acute toxicity (i.e., Ingredient 2) is 92%; therefore, the ATE_{mix} equation in CLP Annex I, 3.1.3.6.2.3 must be used. This corrected calculation adjusts for the total percentage of the ingredient with unknown acute toxicity. 6. Ingredients 1, 3 and 5 are included in the ATE_{mix} calculation because they have data that fall within a CLP acute toxicity category, CLP Annex I, 3.1.3.6.1 (a). 7. Applying the guidance in Note (b) to CLP Annex I, Table 3.1.1 results in using the actual LD_{50} data for Ingredients 1, 3 & 5 in the ATE_{mix} calculation since data is available. **Additional Labelling**: "92% of the mixture consists of components of unknown acute toxicity." (see section 3.1.4.2 of this guidance)

3.1.6.3.2 Example 12 a

Application	Different phases in inhalation exposure. Extrapolation		
	Test Data	**Classification**	**Rationale**
Available information	Use /exposure as **aerosol** (mist) Animal data (rat): LC_{50} (mg/l/4h)		
Ingredient 1 solid (6%)		Category 4	Conv. ATE (mg/l/4h) = 1.5 mg/1/4h
Ingredient 2 solid (11%)	0.6	Category 3	ATE = LC_{50}
Ingredient 3 solid (10%)	6 (dust)	-	Neglected, since not classified in any acute category.
Ingredient 4 liquid (40 %)	11 (vapour)	Category 4	Conv. ATE (mg/l/4h) = 1.5 mg/1/4h, assuming identical category for vapour and mist by expert judgement
Ingredient 5 (33%)		-	Water; neglected

Remarks	**Classification:** Category 4 No test data available for the whole mixture. Bridging principles not applicable since no test data on similar mixtures available. Classification therefore based on ingredients. Use additivity formula in Annex I, 3.1.3.6.1, as information is available for all ingredients. $100/ATE_{mix} = 6/1.5+11/0.6+0+40/1.5+0 = 49$ → ATE_{mix} = 2.04 mg/l/4h →Category 4

Conclusion: The mixture Example 12a) has to be classified formally in Category 4 with respect to inhalation toxicity. It is notable that this classification is only derived from the calculation for the aerosol phase, not for the vapour phase.

3.1.6.4 Examples of mixtures not fulfilling the criteria for classification

3.1.6.4.1 Example 12 b

Application	Different phases in inhalation exposure. Extrapolation		
	Test Data	**Classification**	**Rationale**
Available information	Use / exposure as vapour Animal data (rat): LC_{50} (mg/l/4h)		
Ingredient 1 solid (6%)		Category 4	A solid with no sublimation, therefore not present in the vapour phase; neglected.
Ingredient 2 solid (11%)	0.6 (dust)	Category 3	As Ingredient 1
Ingredient 3 solid (10%)	6 (dust)	-	Neglected, since not classified in any acute category.
Ingredient 4 liquid (40%)	11 (vapour)	Category 4	ATE = LC_{50}
Ingredient 5 (33%)		-	Water; not relevant
Remarks	**Classification:** NC Inhalation is appropriate route since one hazardous ingredient with appreciable vapour pressure. No test data on the whole mixture. Bridging principles not applicable since no test data on similar mixtures available. Classification is therefore based on ingredients. Use additivity formula in CLP Annex I, 3.1.3.6.1 as information is available for all ingredients.		

	There is no contributions from ingredients 1 and 2 in the formula since the diluted solid ingredients do not sublime, and thus are not present in the vapour phase; ingredient 3 is in addition not classified in any acute toxicity category. Ingredient 5 does not show acute toxicity. 100/ATEmix = 0+0+0+40/11+0 = 3.64→ATEmix = 27.5 27.5 mg/l/4h is above the upper generic concentration limit for vapour → NC

3.1.7 References

OECD (2009) Series on testing and assessment number 39: Guidance document on acute inhalation toxicity testing ENV/JM/MONO(2009)28 (21 July 2009).

ECETOC (2003) TR 86: European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, Technical report N°86.

Pauluhn, J. (2008) Inhalation toxicology: methodological and regulatory challenges. Exp Toxicol Pathol. **60**(2-3):111-24.

3.2 SKIN CORROSION/IRRITATION

3.2.1 Definitions for classification for skin corrosion/irritation

Annex I: 3.2.1.1. Skin Corrosion means the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 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. Histopathology shall be considered to evaluate questionable lesions.

Skin Irritation means the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

3.2.2 Classification of substances for skin corrosion/irritation

3.2.2.1 Identification of hazard information

3.2.2.1.1 Identification of human data

CLP Article 7(3) specifies that testing on humans is not allowed for the purposes of CLP; however it does acknowledge that existing data obtained from other sources can be used for classification purposes.

Human data may be retrieved from a number of sources, e.g. epidemiological studies, clinical studies, well-documented case reports, poison information units and accident databases or occupational experience.

In this context the quality and relevance of existing human data for hazard assessment should be critically reviewed. There may be a significant level of uncertainty in human data due to poor reporting and lack of specific information on exposure. Diagnosis confirmed by expert physicians may be missing. Confounding factors may not have been accounted for. Small group sizes may flaw the statistical strength of evidence. Many other factors may compromise the validity of human data. In clinical studies the selection of individuals for the

test and the control groups must be carefully considered. A critical review of the value of human studies is provided in the Guidance on IR/CSA Section R.4.3.3 and more specific considerations for skin corrosion/irritation are given in the Guidance on IR/CSA Section R.7.2.4.2.

Data indicates that human skin is, in most cases, less sensitive than rabbits (ECETOC, 2002).

3.2.2.1.2 Identification of non human data

Non human data include physico-chemical properties, results from (Q)SARs and expert systems, and results from *in vitro* and *in vivo* tests. Available skin corrosion/irritation information on substances may include existing data generated by the test methods in the Test Methods Regulation (Commission Regulation (EC) No 440/2008) or by methods based on internationally recognised scientific principles.

Several of the following non-testing methods and *in vitro* methods have been validated against the DSD criteria but not against CLP criteria for classification. As the criteria differ slightly between DSD and CLP, it should be checked whether the method is sufficiently validated for classification according to CLP.

3.2.2.1.2.1 Consideration of physico-chemical properties

Substances with oxidising properties can give rise to highly exothermic reactions in contact with other substances and human tissue. High temperatures thus generated may damage/destroy biological materials. This applies, for example, to organic peroxides, which can be assumed to be skin irritants, unless evidence suggests otherwise (Guidance on IR/CSA Section R.7.2.3.1).

For a hydro peroxide classification as Skin Corrosive Category 1B should be considered, whereas Skin Irritation Category 2 should be considered for peroxides. Appropriate evidence must be provided in order to consider non-classification of substances with oxidising properties.

3.2.2.1.2.2 Testing-methods: pH and acid/alkaline reserve

Annex I: 3.2.2.2. Likewise, pH extremes like ≤ 2 and ≥ 11,5 may indicate the potential to cause skin effects, especially when buffering capacity is known, although the correlation is not perfect. Generally, such substances are expected to produce significant effects on the skin. If consideration of alkali/acid reserve suggests the substance may not be corrosive despite the low or high pH value, then further testing shall be carried out to confirm this, preferably by use of an appropriate validated in vitro test.

The acid/alkaline reserve is a measure of the buffering capacity of chemicals. For details of the methodology, see Young *et al*, 1988, and Young and How, 1994. The higher the buffer capacity the higher in general the potential for corrosivity.

3.2.2.1.2.3 Non-testing methods: (Q)SARs and expert systems

Non-testing methods such as (Q)SARs and expert systems may be considered on a case-by-case basis. (Q)SAR systems that also account for skin effects are for example TOPKAT, TerraQSAR, and the BfR-DSS. These systems go beyond the structural similarity considerations encompassing also other parameters such as topology, geometry and surface properties. For full guidance consult the Guidance on IR/CSA Sections R.6 and R.7.2.3.1.

The BfR-DSS has been recommended in the Guidance on IR/CSA Section R.7.2.4 since there is no other model that sufficiently describes the absence of effects. The BfR rules to predict

skin irritation and corrosion have been integrated in the internet tool "toxtree", http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree.

Conclusion on no classification can be made if the (Q)SAR or expert system has been shown to adequately predict the absence of the classified effect (Guidance on IR/CSA Figure R.7.2-2, footnote f).

Since a formal adoption procedure for those non-testing methods is not foreseen and no formal validation process is in place, appropriate documentation is very important. In order to achieve acceptance under REACH the documentation must conform the so-called QSAR Model Reporting Format (QMRF). For more details consult the Guidance on IR/CSA Section R.6.1.

3.2.2.1.2.4 Testing methods: *in vitro* methods

Table R.7.2-2 in the Guidance on IR/CSA lists the status of validation and regulatory acceptance for *in vitro* test methods for skin corrosion and skin irritation. The information given below is current at the time of publication, however further information on newly adopted OECD Test Guidelines can be found on the OECD website (http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdguidelinesforthetestingofchemicals.htm).

In vitro methods for skin corrosion

In recent years, the OECD has accepted new guidelines for *in vitro* skin corrosion tests as alternatives for the standard *in vivo* rabbit skin test (OECD TG 404). Accepted *in vitro* tests for skin corrosivity are found in the Test Methods (TM) Regulation and in OECD Test Guidelines (TG):

- The transcutaneous electrical resistance (TER; using rat skin) test (TM B.40; OECD TG 430)
- Human skin model (HSM) tests (TM B.40 bis; OECD TG 431)
- The *in vitro* membrane barrier test method (OECD TG 435)

Positive *in vitro* results do not generally require further testing and can be used for classification. Negative *in vitro* corrosivity responses must be subject to further evaluation.

Whereas the TER test and the human skin models at present only allow a classification into Skin Corrosion Category 1A, the membrane barrier test allows for the differentiation into the three Categories 1A, 1B and 1C. The applicability domain of the three tests outlined here (TER-, HSM- and membrane barrier test) with regard to the alkalinity and acidity of the tested substance should be carefully considered to decide which data are most appropriate for the actual substance.

The TER and the HSM assays have been validated for the classification of skin corrosion. The results of this validation are well founded, because the CLP criteria for skin corrosion are identical with the ones referred to in the past validation study.

The membrane barrier method has been endorsed as a scientifically validated test for a limited range of substances – mainly acids, bases and their derivatives (ECVAM/ESAC, 2000).

In vitro methods for skin irritation

Three *in vitro* skin irritation test methods based on reconstructed human epidermis (RHE) technology have been recently accepted by the OECD in the OECD TG 439 (TM B.46). They

serve to reliably distinguish non-irritants from irritant substances using one single irritant category. The three assays are the EpiSkin™, the modified EpiDerm™ and the SkinEthic™ RHE test method. The EpiSkin™ and EpiDerm™ assays have undergone formal ECVAM validation from 2003 – 2007 (Spielmann *et al*, 2007). In 2007 the EpiSkin™ assay was considered valid by ESAC as a full replacement test (ECVAM/ESAC, 2007). Originally validated for use in a testing strategy for the identification of positives only (ECVAM/ESAC, 2007), the EpiDerm™ test methods protocol was subsequently modified. In November 2008, also the modified EpiDerm™ and the SkinEthic™ RHE assay were found reliable and relevant test methods capable of distinguishing non-irritants from irritants and may therefore fully replace the traditional skin irritation test (ECVAM/ESAC, 2008). It should be noted that conclusions on the applicability domain of the three methods rest mainly on the optimisation and validation data set. All three methods are valid for the classification of substances for skin irritancy according to CLP criteria (ECVAM/ESAC, 2009).

The Skin Integrity Function Test (SIFT) is also listed in the Guidance on IR/CSA, Table R.7.2-2. This test has only undergone prevalidation so far and the applicability domain is limited to surfactants. Positive data from SIFT may be used in a weight of evidence approach to consider classification for irritation, while negative data are not conclusive for a non-classification.

Information on the current developments of *in vitro* tests and methodology can be found on the ECVAM website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam).

Other suitable in vitro methods

Positive data from other suitable *in vitro* methods may be used in a weight of evidence approach to determine classification as irritant, while negative data are not conclusive for a non-classification. In this context ‘suitable’ means sufficiently well developed according to internationally agreed development criteria (see REACH Annex XI, section 1.4).

3.2.2.1.2.5 Testing methods: *In vivo* data

The *in vivo* test in rabbits according to TM B.4 (OECD TG 404) is the standard in vivo test for the hazard assessment under REACH . However it should be noted that according to REACH (Annexes VII to X) *in vivo* testing of corrosive substances at concentration/dose levels causing corrosivity should be avoided.

Until 1987 the OECD standard protocol used occlusive patching for the application of the test substance, which resulted in more rigorous test conditions compared to the semi-occlusive patching used today. Especially in borderline cases of classification the method of application should be accounted for in the evaluation of effects.

Studies performed according to the USA Federal Hazardous Substances Act (US-FHSA), may be used for classification purposes although they deviate in their study protocol from the OECD TG 404. They do not include a 48-hour observation time and involve a 24-hour test material exposure followed by observations at 24 hour and 72 hours. Moreover, the test material is patched both on abraded and on intact skin of six rabbits. Studies usually are terminated after 72 hours. In case of no or minimal responses persisting until the 72 hours time points it is feasible to use such data for classification by calculating the mean values for erythema and oedema on the basis of only the 24 and 72 hours time points. Calculation of mean scores should normally be restricted to the results obtained from intact skin. In case of pronounced responses at the 72 hours time point an expert judgement is needed as to whether the data is appropriate for classification.

Data on skin effects on animals may be available from tests that were conducted for other primary purposes than the investigation of skin corrosion / irritation. Such information may be gained from acute or repeated dose dermal toxicity studies on rabbits or rats (TM B.3, OECD TG 402; TM B.9, OECD TG 410), guinea pig skin sensitisation studies (TM B.6, OECD guideline 406) and from irritation studies in hairless mice.

3.2.2.2 Classification criteria

Annex I: 3.2.2.6. *Corrosion*

3.2.2.6.1. On the basis of the results of animal testing a substance is classified as corrosive, as shown in Table 3.2.1. A corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. Corrosive reactions are typified by ulcers, bleeding, bloody scabs and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia and scars. Histopathology shall be considered to discern questionable lesions.

3.2.2.6.2. Three subcategories are provided within the corrosive category: subcategory 1A – where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B – where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C – where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

3.2.2.6.3. The use of human data is discussed in paragraphs 3.2.2.1 and 3.2.2.4 and also in paragraphs 1.1.1.3, 1.1.1.4 and 1.1.1.5.

Table 3.2.1

Skin Corrosive category and subcategories

		Corrosive in ≥ 1 of 3 animals[*]	
	Corrosive subcategory	Exposure	Observation
Category 1: Corrosive	1A	≤ 3 minutes	≤ 1 hour
	1B	> 3 minutes - ≤ 1 hour	≤ 14 days
	1C	> 1 hour - ≤ 4 hours	≤ 14 days

3.2.2.7. *Irritation*

3.2.2.7.1. Using the results of animal testing a single irritant category (Category 2) is presented in Table 3.2.2. The use of human data is discussed in paragraphs 3.2.2.1 and 3.2.2.4 and also in paragraphs 1.1.1.3, 1.1.1.4 and 1.1.1.5. The major criterion for the irritant category is that at least 2 of 3 tested animals have a mean score of ≥ 2,3 - ≤ 4,0.

Table 3.2.2

Skin irritation category

Category	Criteria
Category 2: Irritant	(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or (3) In some cases where there is pronounced variability of response among

	animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

3.2.2.8. *Comments on responses obtained in skin irritation tests in animals*

3.2.2.8.1. Animal irritant responses within a test can be quite variable, as they are with corrosion. The major criterion for classification of a substance as irritant to skin, as shown in paragraph 3.2.2.7.1, is the mean value of the scores for either erythema/eschar or oedema calculated in at least 2 of 3 tested animals. A separate irritant criterion accommodates cases when there is a significant irritant response but less than the mean score criterion for a positive test. For example, a test material might be designated as an irritant if at least 1 of 3 tested animals shows a very elevated mean score throughout the study, including lesions persisting at the end of an observation period of normally 14 days. Other responses could also fulfil this criterion. However, it should be ascertained that the responses are the result of chemical exposure.

3.2.2.8.2. Reversibility of skin lesions is another consideration in evaluating irritant responses. When inflammation persists to the end of the observation period in 2 or more test animals, taking into consideration alopecia (limited area), hyperkeratosis, hyperplasia and scaling, then a material shall be considered to be an irritant.

* *Note*: In Table 3.2.1 it should read "Corrosive in ≥ 1 of 3 animals". There is a misprint in the BG, CS, ET, EL, EN, LV, PT, and RO versions of CLP published in the Official Journal 31.12.2008.

3.2.2.3 Evaluation of hazard information

Annex I: 3.2.2.4.

[…]

Although information might be gained from the evaluation of single parameters within a tier (see paragraph 3.2.2.5), e.g. caustic alkalis with extreme pH shall be considered as skin corrosives, there is merit in considering the totality of existing information and making an overall weight of evidence determination. This is especially true when there is information available on some but not all parameters. Generally, primary emphasis shall be placed upon existing human experience and data, followed by animal experience and testing data, followed by other sources of information, but case-by-case determinations are necessary.

3.2.2.5. A tiered approach to the evaluation of initial information shall be considered, where applicable, recognising that all elements may not be relevant in certain cases.

3.2.2.3.1 Evaluation of human data

The usefulness of human data for classification purposes will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. Further guidance on evaluation of human data for skin corrosion/irritation can be found in the Guidance on IR/CSA Section R.7.2.4.2.

The criteria in CLP Annex I, Table 3.2.2 are not applicable to human data.

3.2.2.3.2 Evaluation of non human data

3.2.2.3.2.1 *In vitro* data

In evaluation of data from *in vitro* tests the applicability domain has to be taken into account. For instance, the *in vitro* membrane barrier test method is mainly applicable for acids and bases and is not applicable for solutions with pH values between 4.5 and 8. Normally, recommendations for classification according to GHS criteria based on the results of an *in vitro* test are mentioned in the corresponding OECD test guideline.

3.2.2.3.2.2 *In vivo* data

Tests in albino rabbits (OECD TG 404)

Evaluation criteria for local effects on the skin are *severity* of the damage and *reversibility*.

For the *severity* of damage the responses are evaluated according to the Draize score ranking from "0" (no response") up to "4" (severe response"). Evaluation takes place separately for erythema and oedema.

Reversibility of skin lesions is the other decisive factor in evaluating responses in the animal test. The criteria are fulfilled if, for

- *corrosion*
  - the full thickness of the skin is destroyed resulting in ulcers, bleeding, bloody scabs discoloration, complete areas of alopecia and scars. In questionable cases a pathologist should be consulted. One animal showing this response at the end of the observation period is sufficient for the classification as corrosive.
- *irritation*
  - a limited degree of alopecia, hyperkeratosis, hyperplasia and scaling occurs. Two animals showing this response are sufficient for the classification as irritant.
  - very elevated mean scores throughout the study are revealed, including lesions persisting at the end of an observation period of normally 14 days. One animal showing this response throughout and at the end of the observation period is sufficient for the classification as irritant (In cases of suspected corrosives, existing test data may only be available for one animal due to testing restrictions, see Example 2.).

With regard to severity the main criterion for classification of a substance as irritant to skin, is the mean score per animal for either erythema/eschar or oedema. During the observation period following the removal of the patch each animal is scored on erythema and oedema. For each of the three test animals the average scores for three consecutive days (usually 24, 48 and 72 hours) are calculated separately for oedema and erythema. If 2/3 animals exceed the cut-off-values defined in the CLP, the classification has to be done accordingly.

With regard to reversibility the test report must prove that these effects are transient i.e. the affected sites are repaired within the observation period of the test (see Example 1).

Non-classification as corrosive can be only justified, if the test was performed with at least three animals and the test results were negative for all three animals.

Tests that have been conducted with more than three animals

Current guidelines foresee a sequential testing of rabbits until a response is confirmed. Typically, up to 3 rabbits may be used. The basis for a positive response is the individual rabbit value averaged over days 1, 2, and 3. The mean score for each individual animal is used as a criterion for classification. The Skin Irritant Category 2 is used if at least 2 animals show a mean score of 2.3 or above. Other test methods, however, have been using up to 6 rabbits. This is also the case for the studies performed according to the US-FSHA.

For existing test data with more than three animals, specific guidance adopted at UN level in June 2011 (http://www.unece.org/fileadmin/DAM/trans/doc/2011/dgac10c4/ST-SG-AC10-C4-42e.pdf) needs to be applied (http://www.unece.org/fileadmin/DAM/trans/doc/2011/dgac10c4/ST-SG-AC10-C4-2011-e.pdf).

The average score is determined per animal. (see Example 3).

*In case of **6** rabbits the following applies:*

(a) Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least one animal after exposure up to 4 hours.

(b) Classification as skin irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema;

*In case of **5** rabbits the following applies:*

(a) Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least 1 animal after exposure up to 4 hours.

(b) Classification as skin irritation – Category 2 if at least 3 out of 5 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema;

*In case of **4** rabbits the following applies:*

(a) Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least one animal after exposure up to 4 hours.

(b) Classification as skin irritation – Category 2 if at least 3 out of 4 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema;

Other dermal tests in animals

Relevant data may also be available from animal studies that were conducted for other primary purposes than the investigation of skin corrosion/irritation. However, due to the different protocols and the interspecies differences in sensitivity, the use of such data in general needs to be evaluated on a case-by-case basis. These are considered significant if the effects seen are comparable to those described above. For further guidance on how to evaluate data from studies on dermal toxicity or skin sensitisation, see the Guidance on IR/CSA Figure R.7.2-2 footnotes d) and e), respectively.

3.2.2.3.3 Weight of evidence

Where the criteria cannot be applied directly to available identified information, a weight of evidence determination using expert judgement shall be applied in accordance with CLP Article 9(3).

A weight of evidence determination means that all available and scientifically justified information bearing on the determination of hazard is considered together, such as physico-chemical parameters (e.g., pH, reserve alkalinity/acidity), information from the application of the category approach (grouping, read-across), (Q)SAR results, the results of suitable *in vitro* tests, relevant animal data, skin irritation information/data on other similar mixtures, human experience such as occupational data and data from accident databases, epidemiological and clinical studies and well-documented case reports and observations. The quality and consistency of the data shall be given appropriate weight. Both positive and negative results shall be assembled together in a single weight of evidence determination.

Evaluation must be performed on a case-by-case basis and with expert judgement. However, normally positive results that are adequate for classification should not be overruled by negative findings.

> **Annex I: 1.1.1.4.** For the purpose of classification for health hazards (Part 3) established hazardous effects seen in appropriate animal studies or from human experience that are consistent with the criteria for classification shall normally justify classification. Where evidence is available from both humans and animals and there is a conflict between the findings, the quality and reliability of the evidence from both sources shall be evaluated in order to resolve the question of classification. Generally, adequate, reliable and representative data on humans (including epidemiological studies, scientifically valid case studies as specified in this Annex or statistically backed experience) shall have precedence over other data. However, even well-designed and conducted epidemiological studies may lack a sufficient number of subjects to detect relatively rare but still significant effects, to assess potentially confounding factors. Therefore, positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness, quality and statistical power of both the human and animal data.

For further guidance, if both human and animal data are available, see the Guidance on IR/CSA Section R.7.2.3.2.

3.2.2.4 Decision on classification[1]

Where the substance is classified as a skin corrosive but the data used for classification does not allow differentiation between the skin corrosion subcategories 1A/1B/1C, then the substance should be assigned skin corrosive Category 1.

3.2.2.5 Setting of specific concentration limits

> **Article 10(1)** Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous.
>
> Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.

It is more difficult to prove the absence of a hazardous property; the legal text states that:

> **Article 10(1)**
>
> In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.

A specific concentration limit (SCL) set in accordance with the above mentioned provisions shall take precedence over the generic concentration limit (GCL) set out in Tables 3.2.3 and 3.2.4 of Annex I to CLP (Article 10(6)). Furthermore, an SCL is substance-specific and

[1] Please note that using the general hazard Category 1 for Skin Corrosives in relation to the use of subcategories is currently under discussion at GHS level and is not yet implemented into the CLP Regulation. It is therefore agreed not to revise the Guidance at this point in time.

should be applicable to all mixtures containing the substance, instead of any GCL that otherwise would apply to a mixture containing the substance.

What type of information may be the basis for setting a specific concentration limit?

Existing human data may in certain cases (especially if dose-response information is available) indicate that the threshold for the irritation hazard in humans for a substance in a mixture, would be higher or lower than the GCL. A careful evaluation of the usefulness and the validity of such human data, as well as their representativeness and predictive value (IR/CSA, sections R.4.3.3. and R.7.2.4.2), should be performed. As pointed out in 1.1.1.4 (Annex I to CLP), positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of robustness, quality and a degree of statistical certainty of both the human and animal data.

The aim of the standard test method for "Acute Dermal Irritation/Corrosion" TM B.4/OECD TG 404[2] is to **identify** potential skin corrosion or irritation. The test material is generally administered undiluted, thus, no dose-response relationship can be obtained from an individual test.

However, if there are adequate, reliable, relevant and conclusive existing data from other already performed animal studies with a sufficient number of animals tested to ensure a high degree of certainty, and with information on dose-response relationships, such data may be considered for setting a lower or, in exceptional cases, a higher SCL on a case-by-case basis.

It should be noted that generating data specifically for the purpose of setting SCLs is not a requirement according to the CLP Regulation. Article 8(1) CLP specifies that new tests may only be performed (in order to determine the hazard of a substance or mixture) if all other means of generating information has been exhausted and Article 7(1) specifies that where new tests are carried out, tests on animals must be undertaken only when no other alternatives, which provide adequate reliability and quality of data, are possible. The GCLs must be applied for the classification of a mixture on the basis of its ingredient substances classified for skin irritation and corrosivity, if there are no already existing specific data justifying an SCL which is lower or, in exceptional cases, higher than the GCL (see Article 10(1) CLP). Therefore, information will **always** be available, for mixtures containing substances already classified for skin corrosion/irritation, making it possible to identify the hazard for the mixture by using the GCLs (Article 9(4) CLP). The possibilities to use *in vitro* test methods are being explored as a basis for setting SCLs, but an accepted common approach is not yet available. Thus, at the present point in time, it is not possible to provide guidance for the use of *in vitro* methods for the purpose of setting SCLs. However, this does not exclude that a method to set SCLs based on *in vitro* tests could be developed in the future, as they provide a promising option for SCL setting.

An SCL should apply to any mixture containing the substance instead of the GCL (that otherwise would apply to the mixture containing the substance). Thus, if the SCL is based on data derived from tests with dilutions of the substance in a specific solvent, it has to be considered that the derived concentration should be applicable to all mixtures for which the SCL should apply.

[2] TO NOTE: In OECD TG 404 *test substance* refers to the test material, test article or test item. The term *substance* may be used differently from the REACH/CLP definition.

Annex VI Part 3 (Table 3/1 and 3.2) to CLP includes examples of substances for which a higher or lower SCL was set under Directive 67/548/EEC (old DSD system) and which were transferred to CLP.

3.2.2.6 Decision logic for classification of substances[3]

The decision logic, which is based on the Guidance on IR/CSA Figure R.7.2-2 is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification, studies the criteria for classification, as well as the guidance above, before and during use of the decision logic.

Step		
1a	Is the substance an organic hydro peroxide or an organic peroxide? **YES** ➔ **NO** ⬇	Consider to classify as – corrosive (Skin Corr. 1B) if the substance is a hydro peroxide, or – irritating (Skin Irrit. 2) if the substance is a peroxide. OR Provide evidence for the contrary and proceed to step 1b
1b	Is the pH of the substance ≤ 2 or ≥ 11.5? **YES** ➔ **NO** ⬇	Consider to classify as corrosive. – Where classification is based upon consideration of pH alone (i.e. buffering capacity is not known), Skin Corr. 1 should be applied. – Where consideration of alkali/acid reserve suggests that the substance is not corrosive, this has to be confirmed (preferably by use of an appropriate *in vitro* test). Proceed to step 1c
1c	Are there other physical or chemical properties that indicate that the substance is irritating / corrosive? **YES** ➔ **NO** ⬇	Use this information for weight of evidence (WoE) determination (step 7). Proceed to step 2
2	Are there adequate existing human data which provide evidence that the substance is corrosive or irritant? **YES** ➔ **NO** ⬇	Classify accordingly.
3	Are there data from existing studies *on*	Classify accordingly (either Skin Corr.

[3] Please note that using the general hazard Category 1 for Skin Corrosives in relation to the use of subcategories is currently under discussion at GHS level and is not yet implemented into the CLP Regulation. It is therefore agreed not to revise the Guidance at this point in time.

	irritation and corrosion in laboratory animals, which provide sound conclusive evidence that the substance is a corrosive, irritant or non-irritant? **YES** ➔ **NO** 🡻	1A/1B/1C or Skin Irrit. 2 or no classification).
4a	Has the substance proven to be a corrosive, irritant or non-irritant in a suitable acute dermal toxicity test? **YES** ➔ **NO** 🡻	If test conditions are consistent with OECD TG 404, classify accordingly (Skin Corr. 1A/1B/1C or Skin Irrit. 2 or no classification) If test conditions are not consistent with OECD TG 404, use this information in the WoE determination (step 7) and proceed to step 4b
4b	Has the substance proven to be a corrosive or an irritant in sensitisation studies or after repeated exposure? **YES** ➔ NO 🡻	Classification cannot be considered directly. Use this information for WoE determination (step 7). Proceed to step 5a
5a	Are there structurally related substances (suitable "read-across" or grouping), which are classified as corrosive (Skin Cat. 1) on the skin, or do suitable (Q)SAR methods indicate corrosive potential of the substance? **YES** ➔ **NO** 🡻	Consider to classify as Skin Corr. 1. Proceed to step 5b
5b	Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritant on the skin (Skin Cat. 2), or do suitable (Q)SAR methods indicate the presence of irritating potential of the substance? **YES** ➔ **NO** 🡻	Consider to classify as Skin Irrit. 2. Proceed to step 6a
6a	Has the substance demonstrated corrosive properties in an OECD adopted *in vitro* test? **YES** ➔ **NO** 🡻	Classify as corrosive. If discrimination between Skin Corr. 1A/1B/1C is not possible, Skin Corr. 1 must be chosen.
6b	Are there acceptable data from a validated *in vitro* test (adopted by OECD or not), which provide evidence that the substance is an	Consider to classify accordingly (Skin Irrit. 2 or no classification). Proceed to step 6c

	irritant or non-irritant? **YES** ➔ **NO** 🡻	
6c	Are there data from a suitable *in vitro* test, which provide sound conclusive evidence that the substance is an irritant? **YES** ➔ **NO** 🡻	Consider to classify as Skin Irrit. 2 Proceed to step 7
7	Taking all existing and relevant data (steps 1-6) into account, is there sufficient information to make a decision on classification? **YES** ➔ **NO** 🡻	Classify accordingly (Skin Corr. 1 or Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification)
Unable to classify substance for skin corrosion/irritation		Decision to undertake generation of new test data should be made in compliance with REACH and Article 8 of CLP. It is recommended that the Guidance on IR/CSA R.7.2.6 should also be considered.

3.2.3 Classification of mixtures for skin corrosion/irritation

3.2.3.1 Identification of hazard information

The procedure for classifying mixtures is a tiered, i.e. a stepwise, approach based on a hierarchy principle and depending on the type and amount of available data/information starting from evaluating existing human data on the mixture, followed by a thorough examination of the existing *in vivo* data, physico-chemical properties, and finally *in vitro* data available on the mixture. For mixtures that have been on the market for a long time, human data and experience may exist that may provide useful information on the skin irritation potential of the respective mixtures. Although human data from accident or poison centre databases can provide evidence for classification, absence of incidents is not itself evidence for no classification, as exposures may be unknown or uncertain. See Section 3.2.2.1.1 of this Guidance for further information on the identification of human data.

If valid test data are available for the whole mixture they have precedence. If no such data exist, the so called bridging principles have to be applied if possible. If the bridging principles are not applicable an assessment on the basis of data for the components of the mixture will be applied.

Where it is decided to base the classification of a mixture upon consideration of pH alone, Skin corrosion Category 1 should be applied.

3.2.3.2 Classification criteria for mixtures[4]

[4] Please note that using the general hazard Category 1 for Skin Corrosives in relation to the use of subcategories is currently under discussion at GHS level and is not yet implemented into the CLP Regulation. It is therefore agreed not to revise the Guidance at this point in time.

3.2.3.2.1 When data are available for the complete mixture

Annex I: 3.2.3.1.1. The mixture will be classified using the criteria for substances, and taking into account the testing and evaluation strategies to develop data for these hazard classes.

3.2.3.1.2. Unlike other hazard classes, there are alternative tests available for skin corrosivity of certain types of substances and mixtures that can give an accurate result for classification purposes, as well as being simple and relatively inexpensive to perform. When considering testing of the mixture, classifiers are encouraged to use a tiered weight of evidence strategy as included in the criteria for classification of substances for skin corrosion and irritation (paragraph 3.2.2.5), to help ensure an accurate classification as well as avoid unnecessary animal testing. A mixture is considered corrosive to skin (Skin Category 1) if it has a pH of 2 or less or a pH of 11.5 or greater. If consideration of alkali/acid reserve suggests the substance or mixture may not be corrosive despite the low or high pH value, then further testing shall be carried out to confirm this, preferably by use of an appropriate validated in vitro test.

There are a range of available *in vitro* test systems that have been validated for their suitability in assessing skin corrosion/irritation potential of substances. Some but not all test systems have been validated for mixtures and not all available *in vitro* test systems work equally well for all types of mixtures. Prior to testing a mixture in a specific *in vitro* assay for classification purposes, it has to be ensured that the respective test has been previously shown to be suitable for the prediction of skin corrosion/irritation properties for the type of mixture to be evaluated.

3.2.3.2.1.1 Mixtures with extreme pH

As a general rule, mixtures with a pH of ≤ 2 or ≥ 11.5 should be considered as corrosive. However, assessment of the buffering capacity of the mixture indicated by its acid or alkali reserve should be considered.

Low values of acid or alkaline reserve indicate a low buffer capacity. Mixtures showing a low buffer capacity are less or even not corrosive or irritant. The relation is quantitatively expressed by:.- pH + 1/12 alkaline reserve>= 14.5 or pH - 1/12 acid reserve<= -0.5. If the sums are >= 14.5 or <= -0.5 the chemical has to be considered as corrosive (see Decision logic 3.2.3.4, step 1a).

If the additional consideration of the acid/alkaline reserve according to Young *et al.* (1987, 1994) suggests that classification for corrosion or even irritation may not be warranted, then further *in vitro* testing to confirm final (or no) classification shall be carried out. The consideration of acid/alkali reserve should not be used alone to exonerate mixtures from classification.

Where the mixture has an extreme pH value but the only corrosive/irritant ingredient present in the mixture is an acid or base with an assigned SCL (either in CLP Annex VI or set by supplier), then the mixture should be classified according to the SCL. In this instance, pH of the mixture should not be considered a second time since it would have already been taken into account when deriving the SCL for the substance.

If this is not the case, then the steps to be taken into consideration when classifying a mixture with pH ≤ 2 or ≥ 11.5 are described in the following decision logic:

Mixture without *in vivo* data on skin corrosion or relevant data from similar tested mixtures, **pH is ≤ 2 or ≥ 11.5**	
Does the acid alkaline reserve indicate that the mixture may not be corrosive? **NO ➔** **YES** ↓	Classify as corrosive, Skin Corr. Cat. 1.
Is the mixture tested in an OECD adopted *in vitro* test for skin corrosion? **NO ➔** **YES** ↓	Classify as corrosive, Skin Corr. Cat. 1 .
Does the mixture demonstrate corrosive properties in an OECD adopted *in vitro* test? **YES ➔** **NO** ↓	Classify as corrosive. If discrimination between Skin Corr. 1A/1B/1C is not possible, Skin Corr. 1 must be chosen.
Apply methods in CLP Annex I, sections 3.2.3.3.2 (Table 3.2.3)/3.2.3.3.4 (Table 3.2.4) ➔ (Validated *in vitro* skin irritation test methods are available, and these should be used to generate data to classify the mixture instead of using the summation method.)	Classify accordingly.

The mixture must be classified as Skin corrosion Category 1 should the supplier decide not to carry out the required confirmatory testing.

It is also important to note that the pH-acid/alkali reserve to change classification from corrosive to irritant, or from irritant to not classified, assumes that the potential corrosivity or irritancy is due to the effect of the ionic entities. When this is not the case, especially when the mixture contains non-ionic (non-ionisable) substances themselves classified as corrosive or irritant, then the pH-reserve method cannot be a basis for modifying the classification but should be considered in a weight of evidence analysis. If a mixture with corrosive constituents also contains surfactants (e.g. tensids or detergent substances), it can be assumed that corrosivity might be amplified (Kartono & Maibach 2006). Even if only one corrosive substance with an assigned SCL is present in such a mixture, the possible synergistic effect has to be taken into account when classifying the mixture.

3.2.3.2.2 When data are not available for the complete mixture: bridging principles

Annex I: 3.2.3.2.1. Where the mixture itself has not been tested to determine its skin irritation/corrosion hazards, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging rules set out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture. (see Section 1.6.3 of this Guidance)

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified based on its ingredients as described in Sections 3.2.3.2.3 and 3.2.3.3 of this Guidance.

3.2.3.2.3 When data are available for all ingredients or only for some ingredients

3.2.3.2.3.1 Ingredients that should be taken into account for the purpose of classification

Annex I: 3.2.3.3.1. […] Assumption: the 'relevant ingredients' of a mixture are those which are present in concentrations of 1% (w/w for solids, liquids, dusts, mists and vapours and v/v for gases) or greater, unless there is a presumption (e.g., in the case of corrosive ingredients) that an ingredient present at a concentration of less than 1% can still be relevant for classifying the mixture for skin irritation/corrosion.

3.2.3.2.3.2 The additivity approach is applicable

Annex I: 3.2.3.3.2. In general, the approach to classification of mixtures as irritant or corrosive to skin when data are available on the components, but not on the mixture as a whole, is based on the theory of additivity, such that each corrosive or irritant component contributes to the overall irritant or corrosive properties of the mixture in proportion to its potency and concentration. A weighting factor of 10 is used for corrosive components when they are present at a concentration below the generic concentration limit for classification with Category 1, but are at a concentration that will contribute to the classification of the mixture as an irritant. The mixture is classified as corrosive or irritant when the sum of the concentrations of such components exceeds a concentration limit.

3.2.3.3.3. Table 3.2.3 provides the generic concentration limits to be used to determine if the mixture is considered to be an irritant or a corrosive to the skin.

When the supplier is unable to derive the classification using either data on the mixture itself or bridging principles, he must determine the skin corrosion/irritation properties of the mixture using data on the individual ingredients. Although the general approach is the additivity principle which has been used succesfully in the DPD for long periods, the supplier must ascertain whether the additivity approach is applicable, the first step in the process being to identify all the ingredients in the mixture (i.e. their name, chemical type, concentration level, hazard classification and any SCLs) and the pH of the mixture. In addition to for example surfactant interaction, neutralisation of acids/bases could also occur in a mixture, which also makes it important to consider effects of the entire mixture (i.e. pH and the acid/alkaline reserve) rather than considering contributions of individual ingredients. Additivity may not apply where the mixture contains substances mentioned in CLP Annex I, 3.2.3.3.4, see Section 3.2.3.2.3.3 of this Guidance.

Application of SCLs when applying the additivity approach

The generic concentration limits (GCLs) are specified in CLP Annex I, Table 3.2.3. However, according to CLP Article 10(5) SCLs take precedence over GCLs. Thus, if a given substance has an SCL, then this limit has to be taken into account when applying the summation (additivity) method for skin corrosion/irritation (see Examples 5 and 6).

In cases where additivity applies for skin corrosion/irritation to a mixture with two or more substances some of which may have SCLs assigned, then the following formula should be used:

The mixture is classified for skin corrosion/irritation if the

Sum of (ConcA / clA) + (ConcB / clB) + ….+ (ConcZ / clZ) is ≥ 1

Where ConcA = the concentration of substance A in the mixture;

clA = the concentration limit (either specific or generic) for substance A;

ConcB = the concentration of substance B in the mixture;

clB = the concentration limit (either specific or generic) for substance B; etc.

This approach is similar to that used in the DPD where a substance SCL replaces the default limits in the conventional method equations.

3.2.3.2.3.3 The additivity approach is not applicable

Guidance update to legal text "Annex I: 3.2.3.3.4.1" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.2.3.3.4.1. Particular care must be taken when classifying certain types of mixtures containing substances such as acids and bases, inorganic salts, aldehydes, phenols, and surfactants. The approach explained in paragraphs 3.2.3.3.1 and 3.2.3.3.2 may not be applicable given that many of such substances are corrosive or irritant at concentrations < 1%.

3.2.3.3.4.2. For mixtures containing strong acids or bases the pH shall be used as a classification criterion (see paragraph 3.2.3.1.2) since pH is a better indicator of corrosion than the concentration limits of Table 3.2.3.

3.2.3.3.4.3. A mixture containing ingredients that are corrosive or irritant to the skin and that cannot be classified on the basis of the additivity approach (Table 3.2.3), due to chemical characteristics that make this approach unworkable, shall be classified as Skin Corrosive Category 1A, 1B or 1C if it contains ≥ 1% of an ingredient classified in Category 1A, 1B or 1C respectively or as Category 2 when it contains ≥ 3% of an irritant ingredient. Classification of mixtures with ingredients for which the approach in Table 3.2.3 does not apply is summarised in Table 3.2.4.

3.2.3.3.5. On occasion, reliable data may show that the skin corrosion/irritation hazard of an ingredient will not be evident when present at a level at or above the generic concentration limits mentioned in Tables 3.2.3 and 3.2.4. In these cases the mixture shall be classified according to that data (see also Articles 10 and 11). On other occasions, when it is expected that the skin corrosion/irritation hazard of an ingredient is not evident when present at a level at or above the generic concentration limits mentioned in Tables 3.2.3 and 3.2.4, in section 3.2.3.3.6. In testing of the mixture shall be considered. In those cases the tiered weight of evidence strategy shall be applied, as described in paragraph 3.2.2.5.

3.2.3.3.6. If there are data showing that (an) ingredient(s) is/are corrosive or irritant at a concentration of < 1 % (corrosive) or < 3 % (irritant), the mixture shall be classified accordingly.

3.2.3.3 Generic concentration limits for substances triggering classification of mixtures

3.2.3.3.1 When the additivity approach is applicable

Annex I: *Table 3.2.3* **Generic concentration limits of ingredients classified for skin corrosive/irritant hazard (Category 1 or 2)that trigger classification of the mixture as corrosive/irritant to skin**		
Sum of ingredients classified as:	Concentration triggering classification of a mixture as:	
	Skin Corrosive	Skin Irritant
	Category 1 (see note below)	Category 2
Skin corrosive Categories 1A, 1B, 1C	≥ 5%	≥ 1% but < 5%
Skin irritant Category 2		≥ 10%
(10 x Skin corrosive Category 1A, 1B, 1C) + Skin irritant Category 2		≥ 10%
Note The sum of all ingredients of a mixture classified as Skin Corrosive Category 1A, 1B or 1C respectively, shall each be ≥ 5% respectively in order to classify the mixture as either Skin Corrosive Category 1A, 1B or 1C. If the sum of the Skin Corrosive Category 1A ingredients is < 5% but the sum of Category 1A+1B ingredients is ≥ 5%, the mixture shall be classified as Skin corrosive Category 1B. Similarly, if the sum of Skin corrosive Category 1A+1B ingredients is < 5% but the sum of Category 1A+1B+1C ingredients is ≥ 5% the mixture shall be classified as Skin Corrosive Category 1C.		

3.2.3.3.2 When the additivity approach is not applicable

Annex I: *Table 3.2.4* **Generic concentration limits of ingredients of a mixture for which the additivity approach does not apply, that trigger classification of the mixture as corrosive/irritant to skin**		
Ingredient:	Concentration:	Mixture classified as: Skin
Acid with pH ≤ 2	≥ 1%	Category 1
Base with pH ≥ 11,5	≥ 1%	Category 1
Other corrosive (Categories 1A, 1B, 1C) ingredients for which additivity does not apply	≥ 1%	Category 1
Other irritant (Category 2) ingredients for which additivity does not apply, including acids and bases	≥ 3%	Category 2

3.2.3.4 Decision logic for classification of mixtures[5]

The decision logic, which is based on the Guidance on IR/CSA Figure R.7.2-2, is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification, study the criteria for classification, as well as the guidance above, before and during use of the decision logic.

1. When data are available for the complete mixture		
1a	Is the pH of the mixture ≤ 2 or ≥ 11.5? **YES** ➔ **NO** 🡻	Consider to classify as corrosive. − Where classification is based upon consideration of pH alone (i.e. buffering capacity is not known), Skin Corr. 1 should be applied. − Where consideration of alkali/acid reserve suggests that the substance is not corrosive, this has to be confirmed (preferably by use of an appropriate *in vitro* test). Proceed to step 1b.
1b	Are there other physical or chemical properties that indicate that the mixture is corrosive/irritating? **YES** ➔ **NO** 🡻	Use this information for WoE analysis (step 6). Proceed to step 2
2	Is there adequate existing human experience which provides evidence that the mixture is corrosive or irritant? **YES** ➔ **NO** 🡻	Classify accordingly (Skin Corr. 1 or Skin Irrit. 2).
3	Are there data from existing studies *on irritation and corrosion* in laboratory animals, which provide sound conclusive evidence that the mixture is corrosive, irritant or non-irritant? **YES** ➔ **NO** 🡻	Classify accordingly (Skin Corr. 1, or Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification).
4a	Has the mixture proven to be a corrosive, irritant or non-irritant in a suitable acute dermal toxicity test? **YES** ➔ **NO** 🡻	− If test conditions are consistent with OECD TG 404, classify accordingly (Skin Corr. 1A/1B/1C or Skin Irrit. 2 or no classification). − If test conditions are not consistent with OECD TG 404, use this

[5] Please note that using the general hazard Category 1 for Skin Corrosives in relation to the use of subcategories is currently under discussion at GHS level and is not yet implemented into the CLP Regulation. It is therefore agreed not to revise the Guidance at this point in time.

		information in the WoE determination (step 6) and proceed to step 4b
4b	Has the mixture proven to be a corrosive or an irritant in sensitisation studies or after repeated exposure? **YES** ➔ **NO** 🡻	Classification cannot be considered directly. Use this information for WoE determination (step 6). Proceed to step 5a
5a	Has the mixture demonstrated corrosive properties in an OECD adopted *in vitro* test? **YES** ➔ **NO** 🡻	Classify as corrosive. If discrimination between Skin Corr. 1A/1B/1C is not possible, Skin Corr. 1 must be chosen.
5b	Are there acceptable data from a validated *in vitro* test (adopted by OECD or not), which provide evidence that the mixture is an irritant or non-irritant? **YES** ➔ **NO** 🡻	Consider to classify accordingly (Skin Irrit. 2 or no classification). Proceed to step 5c
5c	Are there data from a suitable *in vitro* test, which provide sound conclusive evidence that the mixture is an irritant? **YES** ➔ **NO** 🡻	Consider to classify as Skin Irrit. 2. Proceed to step 6
6	Taking all existing and relevant data (steps 1-5) into account including potential synergistic/antagonistic effects and bioavailability, is there sufficient information to make a decision on classification? **YES** ➔ **NO** 🡻	Classify accordingly (Skin Corr. 1, or Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification**)**
2. When data are not available for the complete mixture: bridging principles		
7a	Are existing sufficient skin corrosion/irritation data available on similar tested mixtures and on the individual ingredients? **NO** ➔ **YES** 🡻	Proceed to step 8
7b	Can bridging principles be applied? **YES** ➔ **NO** 🡻	Classify in appropriate category (Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification)
3. When data are available for all components or only for some components of the mixture		
8a	Is pH of the mixture ≤ 2 or ≥ 11.5? **YES** ➔	Follow decision logic in Section

	NO ↓	3.2.3.2.1.1 of this Guidance and classify accordingly.
8b	Is there any indication that the additivity principle does not apply? **YES** ➔ **NO** ↓	CLP Annex I, section. 3.2.3.3.4 and Table 3.2.4 may apply. Take into account relevant ingredients (CLP Annex I, 3.2.3.3.1). and SCLs as appropriate. Classify in appropriate category (Skin Corr. 1A/1B/1C or Skin Irrit. 2 or no classification)
	CLP Annex I, section 3.2.3.3.2 and Table 3.2.3 applies. Take into account relevant ingredients (CLP Annex I, 3.2.3.3.1. and SCLs as appropriate. Classify in appropriate category (Skin Corr. 1A/1B/1C or Skin Irrit. 2 or no classification)	Where the mixture is classified as corrosive but the data used for classification does not allow differentiation between the skin corrosion subcategories 1A/1B/1C, then the mixture should be assigned Skin corrosion Category 1.

3.2.4 Hazard communication in form of labelling for skin corrosion/irritation

3.2.4.1 Pictograms, signal words, hazard statements and precautionary statements

Guidance update to legal text "Annex I: Table 3.2.5" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.2.4.1. Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.2.5.

Table 3.2.5
Label elements for skin corrosion/irritation

Classification	Category 1A / 1B / 1C	Category 2
GHS Pictograms		
Signal Word	Danger	Warning
Hazard Statement	H314: Causes severe skin burns and eye damage	H315: Causes skin irritation
Precautionary Statement Prevention	P260 P264 P280	P264 P280
Precautionary Statement Response	P301 + P330 + P331 P303 + P361 + P353 P363 P304 + P340 P310 P321 P305 + P351 + P338	P302 + P352 P321 P332 + P313 P362

Precautionary Statement Response **4th ATP change**	P301 + P330 + P331 P303 + P361 + P353 P363 P304 + P340 P310 P321 P305 + P351 + P338	P302 + P352 P321 P332 + P313 P362 + P364
Precautionary Statement Storage	P405	
Precautionary Statement Disposal	P501	

3.2.4.2 Additional labelling provisions

Annex II: 1.2.6. *EUH071 — Corrosive to the respiratory tract*
For substances and mixtures in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I.

For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled.

Corrosive substances (and mixtures) may be acutely toxic after inhalation to a varying degree, which is only occasionally proved by testing. In case no acute inhalation study is available for a corrosive substance (or mixture) and such substance (or mixture) may be inhaled, a hazard of respiratory tract corrosion may exist. As a consequence, substances and mixtures have to be supplementary labelled with EUH071, also taking the saturated vapour concentration into consideration, as appropriate, (see also chapter 3.8.2.5 of this Guidance. Moreover, in such a case it is strongly recommended to apply the precautionary statement P260: "Do not breathe dust/fume/gas/mist/vapours/spray."

Annex II: 1.2.4. *EUH066 — Repeated exposure may cause skin dryness or cracking*
For substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the criteria for skin irritancy in section 3.2 of Annex I, based on either:
— practical observations; or
— relevant evidence concerning their predicted effects on the skin.

3.2.5 Re-classification of substances and mixtures classified for skin corrosion/irritation according to DSD and DPD

3.2.5.1 Is direct "translation" of classification and labelling possible?

A direct translation as indicated in the translation table in Annex VII to CLP is generally possible. Translation from classification according to DSD or DPD to the classification according to CLP is as follows:

- C; R35 is translated into Skin Corr. 1A; H314. This automatic translation can be applied when the classification in DSD was based on *in vivo/in vitro* data, which are the same as the criteria used in CLP.
- C; R34 is translated into Skin Corr. 1B; H314 with the following note:

Annex VII: *Table 1.1*

Note 2
It is recommended to classify in Category 1B even if it also could be possible that 1C could be applicable for certain cases. Going back to original data, may not result in a possibility to distinguish between Category 1B or 1C, since the exposure period has normally been up to 4 hours according to Regulation (EC) No 440/2008. However, for the future, when data are derived from tests following a sequential approach as foreseen in the Regulation (EC) No 440/2008, Category 1C should be considered.

- Xi; R38 is translated into Skin Irrit. 2; H315. The criteria in CLP and DSD are almost identical

It should be noted that where mixtures containing substances with risk phrase R34 have been classified on the basis of the hazards of individual ingredients, the use of the translation table may lead to an under-classification of the mixture. This is because the general concentration limits, to be applied for mixtures, are lowered under CLP compared to DPD. For mixtures containing substances with this classification the use of the translation table may therefore not be appropriate and re-classification done by using the existing data would be more correct. For more details see Section 1.7 of this Guidance.

3.2.5.2 Re-evaluation of data

If there is new information which might be relevant with respect to classification a re-evaluation has to be performed.

3.2.6 Examples of classification for skin corrosion/irritation

3.2.6.1 Examples of substances fulfilling the criteria for classification

3.2.6.1.1 Example 1: Standard test according to OECD TG 404 with three animals

In a guideline test according to OECD TG 404 the test substance was applied for three minutes and 1 hour. No scars or other irreversible effects were found. The scoring results obtained after a 4-hour application time are listed in the following table:

Animal Nr.	Degree of erythema after …[observation time]						Degree of oedema after …[observation time]						**<u>∅ 24/48/72 h ≥2.3 ?</u>**	
	1h	24h	48 h	72 h	7d	14d	1h	24h	48 h	72 h	7d	14d	Erythe-ma	Oede-ma
1	3	3	3	2	0		1	2	2	2	0		Yes	No
		<u>∅ 24/48/72 h = 2.7</u>						**<u>∅ 24/48/72 h = 2.0</u>**					=>"positive Responder"	
2	3	3	3	3	0		1	2	2	1	0		Yes	No
		<u>∅ 24/48/72 h = 3</u>						**<u>∅ 24/48/72 h = 1.7</u>**					=>"positive Responder"	
3	1	1	1	0	0		1	1	1	1	0		No	No

		<u>∅ 24/48/72 h = 0.66</u>				**<u>∅ 24/48/72 h = 1</u>**				

Classification: Skin Irritant Category 2

Rationale: The classification is made on the basis of 2/3 "positive responder" exceeding 2.3 mean score for erythema.

3.2.6.1.2 Example 2: Test carried out with one animal with a test substance which is suspected as corrosive

Due to the unprecedented structure the biological effects of the substance cannot be anticipated. Therefore, the test according to OECD TG 404 was started with one animal only in line with testing restrictions. Exposure times were 3 min and 1h. The following scores/effects were observed:

Exposure time	Degree of erythema after ……[observation time]					Degree of oedema after ……[observation time]					Visible necrosis, irreversible skin damage
	1h	24h	48h	72h	...	1h	24h	48h	72h	...	After 14d
3 min	0	0	0	0		0	0	0	0		No
1h	0	1	2	3		0	2	2	3		Yes

Classification: Skin Corrosion Category 1B

Rationale: The classification is based on the destruction of the tissue after 1 hour of exposure.

3.2.6.1.3 Example 3: Test carried out with more than three animals

A substance was tested on acute skin irritation / corrosion according to OECD TG 404. Contact time was 4 hours. No effects were seen after a contact time of 3 min and one hour. The following scores were obtained after a contact time of 4 hours:

	Observation time													
	1h	24h	48h	72h	7d	14d	1h	24h	48h	72h	7d	14d	Pos responder	
Animal Nr	Erythema						Oedema						Erythe-ma	Oed-ema
1	3	3	2	2	1	0	2	3	2	2	1	0	Yes	Yes
2	3	2	2	2	1	0	2	2	2	2	1	0	No	No
3	2	2	1	1	1	0	2	2	2	2	1	0	No	No
4	2	2	1	1	1	0	2	2	2	2	1	0	No	No

Evaluation is made based on the average score per animal.

Only 1/4 of the animals reached the cut-off value of 2.3, i.e. only animal No 1 is a positive responder. No classification is warranted with regard to skin irritation.

3.2.6.2 Examples of mixtures fulfilling the criteria for classification

Where the mixture is made up of ingredients with no assigned SCLs, the appropriate summation(s) and generic concentration limits from CLP Annex I, Table 3.2.3 should be used.

3.2.6.2.1 Example 4 Mixture without extreme pH, with ingredients with no assigned SCLs,

Ingredient	Skin corrosion / irritation classification	Concentration (% w/w)	SCL
Surfactant A	Skin Cat 2	1.8	Not assigned
Substance B	Not classified	0.5	
Substance C	Skin Cat 2	5.4	Not assigned
Substance D	Not classified	4	
Acid	Skin Cat 1A	2	Not assigned
Water	Not classified	86.3	

pH of the mixture is 9.0 - 10.0, thus extreme pH provisions do not apply. The mixture contains a surfactant and an acid but neither are corrosive/irritant below 1% (as identified by the absence of SCLs in either CLP Annex VI or the Classification and Labelling Inventory). Additivity is considered to apply.
Substance B, substance D and water can be disregarded as they are not classified for skin corrosion/irritation.

The mixture contains 2% acid, the only ingredient classified as Skin Corr. Cat 1. As this is below the 5% GCL, the mixture is not classified Skin Corr. Cat. 1 but is classified Skin Irrit. Cat. 2 (≥ 1% but < 5%).

3.2.6.2.2 Example 5 Mixture without extreme pH, with ingredients with SCLs,

Ingredient	Skin corrosion / irritation classification	Concentration (% w/w)	SCL
Surfactant A	Skin Cat 2	3.8	Not assigned
Substance B	Not classified	0.5	
Base E	Skin Cat 1B	5.4	C ≥ 10 %: Skin Cat 1B 5 % ≤ C < 10 %: Skin Cat 2
Substance D	Not classified	4	
Substance F	Skin Cat 1B	2	Not assigned
Water	Not classified	84.3	

pH of the mixture is 10.5 – 11.0, thus extreme pH provisions do not apply. The mixture contains a surfactant and a base but none are corrosive/irritant below 1% (as identified by absence of specific concentration limits in either CLP Annex VI or the Classification and Labelling Inventory). Additivity is considered to apply.

Substance B, substance D and water can be disregarded as they are not classified for skin corrosion/irritation.

SCLs are neither assigned to substance F nor surfactant A, thus GCLs apply for these ingredients. SCLs are assigned to Base E (see section 3.2.3.2.3.2 under *Application of SCLs when applying the additivity approach*).

Skin Cat 1:

(% substance F/GCL) + (% base E/SCL) = (2/5) + (5.4/10) = 0.94 ⇨ < 1, thus the mixture is not classified as Skin Corr. Cat 1

Skin Cat 2:

(% substance F/GCL) + (% base E/SCL) + (% surfactant A/GCL) = (2/1) + (5.4/5) + (3.8/10) = 3.46 which is > 1, thus the mixture is classified Skin Irrit. Cat. 2

3.2.6.3 Examples of mixtures not fulfilling the criteria for classification

3.2.6.3.1 Example 6 Example 6: Mixture without extreme pH, with ingredients with SCLs.

Ingredient	Skin corrosion / irritation classification	Concentration (% w/w)	SCL
Surfactant C	Skin Cat 2	0.4	Not assigned
Surfactant G	Skin Cat 2	3.0	Not assigned
Surfactant A	Skin Cat 2	0.7	Not assigned
Substance H	Skin Cat 1A	3.0	C ≥ 70 %: Skin Cat 1A 50 % ≤ C < 70 %: Skin Cat 1B 35 % ≤ C < 50 %: Skin Cat 2
Substance D	Not classified	2	
Water	Not classified	90.9	

pH of the mixture is: 2.5 – 3.0, thus extreme pH provisions do not apply. The mixture contains three surfactants but none are corrosive/irritant below 1% (as identified by the absence of specific concentration limits in either CLP Annex VI or the Classification and Labelling Inventory) Additivity is considered to apply.

Substance D and water can be disregarded as they are not classified for skin corrosion/irritation. Also surfactant C and surfactant A can be disregarded as both are present below 1%.

No SCL is assigned to surfactant G, thus GCL apply for this ingredient.

Skin Cat 1:

The mixture contains 3% substance H, the only ingredient classified as Skin Corr. Cat. 1. As this is below the 50% SCL for substance H, the mixture is not classified as Skin Corr. Cat. 1.

Skin Cat 2:

(% substance H/SCL) + (% surfactant G/GCL) = (3/35) + (3/10) = 0.39 which is < 1, thus the mixture is not classified Skin Irrit. Cat. 2.

3.2.7 References

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Young J.R., How M.J. (1994): Product classification as corrosive or irritant by measuring pH and acid / alkali reserve. In Alternative Methods in Toxicology vol. 10 - *In Vitro* Skin Toxicology: Irritation, Phototoxicity, Sensitization, eds. A.Rougier, A.M. Goldberg and H.I Maibach, Mary Ann Liebert, Inc. 23-27.

3.3 SERIOUS EYE DAMAGE/EYE IRRITATION

It should be noted that if a substance or mixture is classified as Skin corrosive Category 1 then serious damage to eyes is implicit and the substance or mixture is classified for serious eye damage but there is no need to label as such.

3.3.1 Definitions for classification for serious eye damage/eye irritation

Annex I: 3.3.1.1. Serious eye damage means 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.

Eye irritation means the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

3.3.2 Classification of substances for serious eye damage/eye irritation

3.3.2.1 Identification of hazard information

3.3.2.1.1 Identification of human data

Existing data on eye effects in humans may include well-documented epidemiological studies, clinical studies, case reports, and data from poison information units and accident databases or occupational experience. Their quality and relevance for hazard assessment should be thoroughly reviewed. A critical review of the value of human studies is provided in the Guidance on IR/CSA Section R.4.3.3 and more specific considerations for eye damage/irritation are given in the Guidance on IR/CSA Section R.7.2.4.2.

3.3.2.1.2 Identification of non human data

Available serious eye damage/eye irritation information on substances may include existing data generated by the test methods in the Test Methods Regulation or by methods based on internationally recognised scientific principles.

Several of the following non-testing and *in vitro* methods have been validated against the DSD criteria but not against the CLP criteria for classification. Therefore it should be checked whether the method is sufficiently validated for classification according to CLP.

3.3.2.1.2.1 Consideration of physico-chemical properties

Substances with oxidising properties can give rise to highly exothermic reactions in contact with other substances and human tissue. High temperatures thus generated, or direct oxidative impact, may damage/destroy biological materials. This applies, for example, to organic peroxides, which can be assumed to be eye irritants, unless evidence suggests otherwise (Guidance on IR/CSA Section R.7.2.3.1).

For a hydro peroxide classification as Eye Damage Category 1 should be considered, whereas Eye Irritation Category 2 should be considered for peroxides. Appropriate evidence must be provided in order to consider non-classification of substances with oxidising properties.

3.3.2.1.2.2 Non-testing methods: (Q)SARs and expert systems

Non-testing methods such as (Q)SARs and expert systems may be considered on a case-by-case basis. (Q)SARs are in general not very specific for eye irritancy. In many cases rules are used in a similar manner to those used for skin irritation and corrosion. (Q)SAR systems that also account for eye effects are for example TOPKAT, Derek for Windows, and SICRET. For full guidance, consult the Guidance on IR/CSA Section R.6 ("QSAR and grouping of chemicals"), in which also the many shortcomings of the existing systems are discussed.

Since a formal adoption procedure for those non-testing methods is not foreseen and no formal validation process is in place, appropriate documentation is crucial. In order to achieve acceptance under REACH, the documentation must conform to the so-called QSAR Model Reporting Format (QMRF). For more details consult the Guidance on IR/CSA Section R.6.1.

3.3.2.1.2.3 Testing-methods: pH and the acid/alkaline reserve

Annex I: 3.3.2.3. […] Likewise, pH extremes like ≤ 2 and ≥ 11,5 may produce serious eye damage, especially when associated with significant buffering capacity. Such substances are expected to produce significant effects on the eyes. Possible skin corrosion has to be evaluated prior to consideration of serious eye damage/eye irritation in order to avoid testing for local effects on eyes with skin corrosive substances. […]

Substances can be predicted to be corrosive, if the pH is ≤ 2 or ≥ 11.5. Where extreme pH is the only basis for classification as serious eye damage, it is important to take into consideration the acid/alkaline reserve, a measure of the buffering capacity (Young *et al*, 1988, and Young and How, 1994). However, lack of buffering capacity should not be used

alone to exonerate from classification as corrosive (see also section 3.2.3.2.1.1 of this Guidance).

If pH is < 3.2 but > 2, or > 8.6 but < 11.5, then consider the substance for serious eye damage/eye irritation (Guidance on IR/CSA Section R.7.2.4.1). Further information and/or reasoning is needed to conclude whether the substance is causing severe eye damage or eye irritation. 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 for non-irritants (due to its relatively high false negative rate).

3.3.2.1.2.4 Testing methods: *in vitro* methods

The OECD has at present adopted three *in vitro* tests for the identification of substances inducing serious eye damage, i.e. the Isolated Chicken Eye (ICE) test (OECD TG 438; TM B.48), the Bovine Corneal Opacity and Permeability (BCOP) test (OECD TG 437; TM B.47) and the the Fluorescein Leakage (FL) (OECD TG 460). Both tests are recommended for use as part of a tiered-testing strategy for regulatory classification and labelling (e.g. Top-Down Approach). A substance can be considered as causing serious eye damage (Category 1) based on positive results in the ICE test, the BCOP test, the Isolated Rabbit Eye (IRE) test or the Hen's Egg Test on Chorio-allantoic Membrane (HET-CAM) test[6]. Negative *in vitro* corrosivity responses in these tests must be followed by further testing (Guidance on IR/CSA Section R.7.2.4.1).

In addition an *in vitro* test method has been validated by ECVAM and is under consideration for the development of an OECD TG: the Cytosensor Microphysiometer (CM) test. This can be used for the identification of category 1-substances within a Top-Down Approach. The CM test can also be used within a Bottom-Up Approach to identify non-irritants for some types of substances (ECVAM/ESAC, 2009a).

There are no *in vitro* tests with regulatory acceptance for eye irritation at present. However, two human corneal epithelium models, EpiOcular™ and SkinEthic™, have been submitted to ECVAM for validation.

Further information on newly adopted OECD Test Guidelines can be found on the OECD website:
(http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdguidelinesforthetestingofchemicals.htm).

Information on the current developments of *in vitro* tests and methodology can be found on the ECVAM website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam).

3.3.2.1.2.5 Testing methods: *In vivo* data

Testing for eye irritation would not be carried out on substances known or predicted to be corrosive to skin. Such substances are automatically considered to be severely damaging to the eye and are classified but not labelled for serious eye damage in addition to skin corrosion.

The *in vivo* test in rabbits according to OECD TG 405 (B.5 in the Test Methods Regulation) is the standard in vivo test for the hazard assessment under REACH.

[6] ICCVAM published a report on the HET-CAM in 2010 http://iccvam.niehs.nih.gov/docs/ocutox_docs/InVitro-2010/Body.pdf

The Low Volume Eye Test (LVET; Griffith *et al* 1980) is a modification of the standard OECD TG 405 test method, the differences being:

- the test material is placed directly on the cornea instead of introducing it in the conjunctival sac inside the lower lid;
- a reduction in the volume of test material applied (0.01 ml (or corresponding weight for solids) compared with the standard 0.1 ml).

Data from the LVET should be considered but must be carefully evaluated. The applicability domain up to now is limited to detergent and cleaning products. It is stated that positive data are a trigger for appropriate classification, but that negative data are not conclusive for a non-classification (Guidance on IR/CSA R.7.2.4.1). However, they should be considered in a weight of evidence determination.

In 2009, the ESAC gave its conclusions on the use of the LVET data for classification and labelling (ECVAM/ESAC, 2009b).

3.3.2.2 Classification criteria

Annex I: 3.3.2.6. *Irreversible effects on the eye/serious damage to eyes (Category 1)*

3.3.2.6.1. Substances that have the potential to seriously damage the eyes are classified in Category 1 (irreversible effects on the eye). Substances are classified in this hazard category on the basis of the results of animal testing, in accordance with the criteria listed in Table 3.3.1. These observations include animals with grade 4 cornea lesions and other severe reactions (e.g., destruction of cornea) observed at any time during the test, as well as persistent corneal opacity, discoloration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight. In this context, persistent lesions are considered those which are not fully reversible within an observation period of normally 21 days. Substances are also classified in Category 1 if they fulfil the criteria of corneal opacity ≥ 3 or iritis > 1,5 detected in a Draize eye test with rabbits, recognising that such severe lesions usually do not reverse within a 21 days observation period.

Table 3.3.1
Category for irreversible eye effects

Category	Criteria
Irreversible effects on the eye (Category 1)	If, when applied to the eye of an animal, a substance produces: − at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or − at least in 2 of 3 tested animals, a positive response of: − corneal opacity ≥ 3 and/or − iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

Annex I: 3.3.2.7. *Reversible effects on the eye (Category 2)*

3.3.2.7.1. Substances that have the potential to induce reversible eye irritation are classified in Category 2 (irritating to eyes).

Table 3.3 2
Category for reversible eye effects

Category	Criteria

Irritating to eyes (Category 2)	if, when applied to the eye of an animal, a substance produces:
	− at least in 2 of 3 tested animals, a positive response of:
	  − corneal opacity ≥ 1 and/or
	  − iritis ≥ 1, and/or
	  − conjunctival redness ≥ 2 and/or
	  − conjunctival oedema (chemosis) ≥ 2
	− calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

3.3.2.7.2. For those substances where there is pronounced variability among animal responses, this information shall be taken into account in determining the classification	

The classification criteria apply to the results of the OECD TG 405 and to the results of the LVET. Negative data from the LVET are not conclusive for non-classification, but should be considered in a weight of evidence determination.

3.3.2.3 Evaluation of hazard information

Annex I: 3.3.2.5. A tiered approach to the evaluation of initial information shall be considered where applicable, while recognising that all elements may not be relevant in certain cases.

3.3.2.4. […] Although information may be gained from the evaluation of single parameters within a tier (e.g. caustic alkalis with extreme pH shall be considered as local corrosives), the totality of existing information shall be considered in making an overall weight of evidence determination, particularly when there is information available on some but not all parameters. Generally, primary emphasis shall be placed upon expert judgement, considering human experience with the substance, followed by the outcome of skin irritation testing and of well-validated alternative methods. […]

3.3.2.3.1 Evaluation of human data

Quality data on substance-induced eye irritation in humans are likely to be rare. Where human data are available, the usefulness of such data for classification purposes will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. The quality and relevance of such data for hazard assessment should be critically reviewed.

If a substance is diagnostically confirmed by a physician to be the cause for decay in vision with the effects not being transient but persistent this should lead to the most serious eye classification, i.e. Eye Damage Category 1.

Further information on the evaluation of human data for eye irritation can be found in the Guidance on IR/CSA Section R7.2.4.2.

3.3.2.3.2 Evaluation of non-human data

The results of the non-testing methods fulfilling the criteria of REACH Annex XI paragraphs 1.3 and 1.5 should be used instead of testing or as part of the weight of evidence approach.

3.3.2.3.2.1 *In vitro* data

Currently only positive results in the, IRE and HET-CAM *in vitro* assays can be used for classification of a substance as causing serious eye damage. Negative results are not conclusive for a non-classification.

There are currently no validated *in vitro* eye irritation test methods available. However, two human corneal epithelium models (EpiOcular^{TM} and SkinEthic^{TM}) are undergoing formal validation by ECVAM.

3.3.2.3.2.2 *In vivo* data

Tests in albino rabbits (OECD TG 405)

Evaluation criteria for local effects on the eye are *severity* of the damage and *reversibility*.

For the *severity* of damage the degree of inflammation is assessed. Responses are graded according to the grading of ocular lesions in OECD TG 405.

Evaluation takes place separately for cornea, iris and conjunctiva (erythema and swelling). If the scoring meets the criteria in CLP Annex I, Tables 3.3.1 and 3.3.2, the substances are classified as Category 1 for serious eye damage or Category 2 for eye irritation, respectively.

Reversibility of eye lesions is the other decisive factor in evaluating responses in the animal test. If the effects are not transient within the observation time of 21 days but cause persistent damage, they are considered irreversible and the test substance needs to be classified into Category 1. In the case of studies with a shorter observation period with irreversible effects, classification based on expert judgement should be considered.

With regard to reversibility the test report must prove that these effects are transient, i.e. the affected sites are repaired within the observation period of the test (see Example 1). Evaluation of reversibility or irreversibility of the observed effects does not need to exceed 21 days after instillation for the purpose of classification.

According to OECD TG 405, in cases of suspected serious eye damage, the test is started with one animal only. If effects in this animal are irreversible until the end of the observation period, sufficient information is available to classify the substance for serious eye damage. For a decision on no classification for serious eye damage and/or irritation or for a decision on classification as irritant, two additional animals have to be tested.

For each of the three test animals the average scores for three consecutive days (usually 24, 48 and 72 hours) are calculated separately for the cornea, iris and conjunctiva (erythema and swelling). If the mean scores for 2 out of 3 animals exceed the values in CLP Annex I, Tables 3.3.1 and 3.3.2, classification has to be assigned accordingly.

Tests that have been conducted with more than three animals

Older test methods, however, have been using up to six rabbits. The current UN Criteria (http://www.unece.org/fileadmin/DAM/trans/doc/2011/dgac10c4/ST-SG-AC10-C4-2011-2e.pdf) adopted in June 2011 (http://www.unece.org/fileadmin/DAM/trans/doc/2011/dgac10c4/ST-SG-AC10-C4-42e.pdf) should be applied (see Example 2):

*In case of **6** rabbits the following applies:*

(a) Classification as serious eye damage – Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or(ii) at least 4 out of 6 rabbits show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis

(b) Classification as eye irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of:

(i) ≥ 1 for corneal opacity and/or

(ii) ≥ 1 for iritis and/or

(iii) ≥ 2 conjunctival erythema (redness) and/or

(iv) ≥ 2 conjunctival oedema (swelling) (chemosis)

and which fully reverses within an observation period of normally 21 days.

*In case of **5** rabbits the following applies:*

(a) Classification as serious eye damage – Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(ii) at least 3 out of 5 rabbits show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis.

(b) Classification as eye irritation – Category 2 if at least 3 out of 5 rabbits show a mean score per animal of:

(i) ≥ 1 for corneal opacity and/or

(ii) ≥ 1 for iritis and/or

(iii) ≥ 2 conjunctival erythema (redness) and/or

(iv) ≥ 2 conjunctival oedema (swelling) (chemosis)

and which fully reverses within an observation period of normally 21 days.

*In case of **4** rabbits the following applies:*

(a) Classification as serious eye damage – Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(ii) at least 3 out of 4 rabbits show a mean score per animal of

≥ 3 for corneal opacity and/or

> 1.5 for iritis

(b) Classification as eye irritation – Category 2 if at least 3 out of 4 rabbits show a mean score per animal of:

(i) ≥ 1 for corneal opacity and/or

(ii) ≥ 1 for iritis and/or

(iii) ≥ 2 conjunctival erythema (redness) and/or

(iv) ≥ 2 conjunctival oedema (swelling) (chemosis)

and which fully reverses within an observation period of normally 21 days.

In this case the irritant categories 1 and 2 are used if 4 of 6 rabbits show a mean score per animal as outlined in the criteria. Likewise, if the test was performed with 4 or 5 animals, for

at least 3 individuals the mean score per animal must exceed the values laid down in the classification criteria. A single animal showing irreversible or otherwise serious effects consistent with corrosion will necessitate classification as serious eye damage Category 1 irrespective of the number of animals used in the test.

Other animal tests

The LVET uses the same scoring system as for results from the OECD TG 405, but data from the test is not conclusive for a non-classification. However, they can be included in a weight of evidence determination.

Note that in case there are test data that originate from non-OECD tests and scoring has not been performed according to the Draize system, the values in CLP Annex I, Tables 3.3.1 and 3.3.2 are no longer applicable for classification purposes. However these data from non-OECD tests should be considered in a weight of evidence determination.

3.3.2.3.3 Weight of evidence

Where the criteria cannot be applied directly to available identified information, a weight of evidence determination using expert judgement shall be applied in accordance with CLP Article 9(3).

A weight of evidence determination means that all available and scientifically justified information bearing on the determination of hazard is considered together, such as human experience (including occupational data and data from accident databases, epidemiological and clinical studies, and well-documented case reports and observations), relevant animal data, skin irritation information/data, physico-chemical parameters (e.g. pH, reserve alkalinity/acidity), the results of suitable *in vitro* tests, information from the application of the category approach (grouping, read-across), QSAR results. The quality and consistency of the data shall be given appropriate weight. Both positive and negative results shall be assembled together in a single weight of evidence determination. Evaluation must be performed on a case-by-case basis and with expert judgement. However, normally positive results that are adequate for classification should not be overruled by negative findings.

> **Annex I: 1.1.1.4**. For the purpose of classification for health hazards (Part 3) established hazardous effects seen in appropriate animal studies or from human experience that are consistent with the criteria for classification shall normally justify classification. Where evidence is available from both humans and animals and there is a conflict between the findings, the quality and reliability of the evidence from both sources shall be evaluated in order to resolve the question of classification. Generally, adequate, reliable and representative data on humans (including epidemiological studies, scientifically valid case studies as specified in this Annex or statistically backed experience) shall have precedence over other data. However, even well-designed and conducted epidemiological studies may lack a sufficient number of subjects to detect relatively rare but still significant effects, to assess potentially confounding factors. Therefore, positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness, quality and statistical power of both the human animal data.

For further guidance, if both human and animal data are available, see the Guidance on IR/CSA Section R.7.2.3.2.

3.3.2.4 Decision on classification

A skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H 314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required but the

hazard statement H318 "Causes serious eye damage" is not indicated on the label because of redundancy (CLP Article 27).

3.3.2.5 Setting of specific concentration limits

Article 10(1) Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous.

Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.

It is more difficult to prove the absence of a hazardous property, the legal text states that:

Article 10(1)

In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.

A specific concentration limit (SCL) set in accordance with the above mentioned provisions shall take precedence over the generic concentration limit (GCL) set out in Tables 3.2.3 and 3.2.4 of Annex I to CLP (Article 10(6)). Furthermore, an SCL is substance-specific and should be applicable to all mixtures containing the substance, instead of any GCL that otherwise would apply to a mixture containing the substance.

What type of information may be the basis for setting a specific concentration limit?

Existing human data may in certain cases (especially if dose-response information is available) indicate that the threshold for the irritation hazard in humans for a substance in a mixture, would be higher or lower than the GCL. A careful evaluation of the usefulness and the validity of such human data as well as their representativeness and predictive value (IR/CSA, sections R.4.3.3. and R.7.2.4.2) should be performed. As pointed out in Section 1.1.1.4 of Annex I, CLP, positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of robustness, quality and a degree of statistical certainty of both the human and animal data.

The aim of the standard test method for "Acute Eye Irritation/Corrosion" TM B.5/OECD TG 405[7] is to **identify** potential serious eye damage or eye irritation. The test material is generally administered undiluted. Thus, no dose-response relationship can be obtained from an individual test.

However, if there are adequate, reliable, relevant and conclusive existing data from other already performed animal studies with a sufficient number of animals tested to ensure a high degree of certainty, and with information of dose-response relationships, such data may be considered for setting a lower or, in exceptional cases, a higher SCL on a case-by-case basis.

[7] TO NOTE: In OECD TG 404 the term test substance refers to the test material, test article or test item. The term substance may be used differently from the REACH/CLP definition.

It should be noted that generating data specifically for the purpose of setting SCLs is not a requirement according to the CLP Regulation. Article 8(1) CLP specifies that new tests may only be performed (in order to determine the hazard of a substance or mixture) if all other means of generating information has been exhausted and Article 7(1) specifies that where new tests are carried out, test on animals shall be undertaken only when no other alternatives, which provide adequate reliability of data, are possible. The GCLs must be applied for the classification of a mixture on the basis of its ingredient substances classified as causing serious eye damage or as an eye irritant, if there are no already existing specific data justifying an SCL which is lower or, in exceptional cases, higher than the GCL (see Article 10(1) CLP). Therefore, information will **always** be available, for mixtures containing substances already classified for serious eye damage/eye irritation, making it possible to identify the hazard for the mixture by using the GCLs (Article 9(4) CLP). The possibilities to use *in vitro* test methods as a basis for setting SCLs have not yet been explored and therefore, at the present point in time, it is not possible to provide guidance for the use of *in vitro* methods for the purpose of setting SCLs. However, this does not exclude that a method to set SCLs based on *in vitro* tests could be developed in the future, and these tests may provide a promising option for SCL setting.

An SCL should apply to any mixture containing the substance instead of the GCL (that otherwise would apply to the mixture containing the substance). Thus, if the SCL is based on data derived from tests with dilutions of the substance in a specific solvent, it has to be considered that the derived concentration, should be applicable to all mixtures for which the SCL should apply.

Annex VI Part 3 (Table 3.2) to CLP Regulation includes examples of substances for which a higher or lower SCL was set under Directive 67/548/EEC (old Dangerous Substances Directive (DSD) system).

3.3.2.6 Decision logic

The decision logic which is based on the Guidance on IR/CSA Figure R.7.2-3 is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

Step		
0	Is the substance classified as a skin corrosive? **YES** ➔ **NO** 🡻	When classified as Skin Corr. 1, the risk of severe damage to eyes is considered implicit. The substance is classified for serious eye damage but not labelled for serious eye damage.
1a	Is the substance an organic hydro peroxide or an organic peroxide? **YES** ➔ **NO** 🡻	− Consider to classify as serious eye damage (Eye Dam. 1) if the substance is a hydro peroxide, or − eye irritating (Eye Irrit. 2) if the substance is a peroxide. OR Provide evidence for the contrary and proceed to step 1b
1b	Is the pH of the substance ≤ 2 or ≥ 11.5?	− Where classification is based upon consideration of pH alone (i.e. buffering

	YES ➔ **NO** **⬇**	capacity not known), Eye Dam. 1 should be applied.. – Where consideration of the alkali/alkaline reserve suggests that the substance is not corrosive, this has to be confirmed (preferably by use of an appropriate *in vitro* test). Proceed to step 1c
1c	Are there other physico-chemical properties that indicate that the substance has the potential to cause serious eye damage or is irritating to the eye? **YES ➔** **NO** **⬇**	Use this information for weight of evidence (WoE) determination (step 6). Proceed to step 2
2	Is there adequate existing human experience which provides evidence that the substance has the potential to cause serious eye damage or is irritating to the eye? **YES ➔** **NO** **⬇**	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2).
3	Are there data from existing studies *on eye irritation* in laboratory animals, which provide sound conclusive evidence that the substance has the potential to cause serious eye damage, is an eye irritant or non-irritant? **YES ➔** **NO** **⬇**	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification).
4	Are there structurally related substances (suitable "read-across" or grouping), which are classified as serious eye damage or eye irritant, or do valid QSAR methods indicate the presence/absence of serious eye damage/eye irritation potential of the substance? **YES ➔** **NO** **⬇**	Consider to classify accordingly (Eye Dam. 1 or Eye Irrit. 2). If discrimination between Eye Dam. 1 and Eye Irrit. 2 is not possible, Eye Dam. 1 must be chosen. Proceed to step 5a
5a	Are there data from a validated *in vitro* test (adopted by OECD or not), which provide evidence that the substance is an eye irritant or non-irritant? **YES ➔** **NO** **⬇**	Consider to classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification). If discrimination between Eye Dam. 1 and Eye Irrit. 2 is not possible, Eye Cat. 1 must be chosen. Proceed to step 5b
5b	Are there acceptable data from a suitable *in vitro* test, which provide evidence that the	Consider to classify as Eye Dam. 1. Proceed to step 6

	substance is a severe eye irritant? **YES** ➔ **NO** 🡻	
6	Taking all existing and relevant data into account, is there sufficient information to make a decision on classification? **YES** ➔ **NO** 🡻	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification).
	Unable to classify substance for serious eye damage/eye irritation	Decision to undertake generation of new test data should be made in compliance with REACH and Article 8 of the CLP. It is recommended that ECHA Guidance R.7.2.6 should also be considered.

3.3.3 Classification of mixtures for serious eye damage/eye irritation

3.3.3.1 Identification of hazard information

The procedure for classifying mixtures is a tiered i.e. a stepwise approach based on a hierarchy principle and depending on the type and amount of available data/information starting from evaluating existing human data on the mixture, followed by a thorough examination of the existing *in vivo* data, physico-chemical properties, and finally *in vitro* data available on the mixture. If valid test data are available for the whole mixture they have precedence. If no such data exist, the so called bridging principles have to be applied if possible. If the bridging principles are not applicable an assessment on the basis of data for the components of the mixture will be applied.

Where it is decided to base the classification of a mixture upon consideration of pH alone, Eye Damage Category 1 should be applied. In this case no further retrieval of information on the mixture itself is needed.

3.3.3.1.1 Identification of existing human data

For mixtures that have been on the market for a long time, some human data and experience may exist that could provide useful information on the eye irritation potential of the respective mixtures. However, lack of data on effects in humans may be due to, for example, poor reporting or adequate preventive measures. Therefore, lack of data cannot be taken as evidence of the mixture being non-hazardous. See Section 3.3.2.1.1 of this Guidance for further information on the identification of human data.

3.3.3.2 Classification criteria for mixtures

3.3.3.2.1 When data are available for the complete mixture

Annex I: 3.3.3.1.1. The mixture will be classified using the criteria for substances, and taking into account the testing and evaluation strategies used to develop data for these hazard classes.

3.3.3.1.2. Unlike other hazard classes, there are alternative tests available for skin corrosivity of certain types of mixtures that give an accurate result for classification purposes, as well as being simple and relatively inexpensive to perform. When considering testing of the mixture classifiers are encouraged to use a tiered weight of evidence strategy as included in the criteria for

classification of substances for skin corrosion and serious eye damage and eye irritation to help ensure an accurate classification, as well as avoid unnecessary animal testing. A mixture is considered to cause serious eye damage (Category 1) if it has a pH ≤ 2,0 or ≥ 11,5. If consideration of alkali/acid reserve suggests the mixture may not have the potential to cause serious eye damage despite the low or high pH value, then further testing needs to be carried out to confirm this, preferably by use of an appropriate validated in vitro test.

Where the criteria cannot be applied directly to available identified information, a weight of evidence determination using expert judgement shall be applied in accordance with CLP Article 9(3). A weight of evidence determination means that all available and scientifically justified information bearing on the determination of hazard is considered together, such as physico-chemical parameters, the results of suitable *in vitro* tests, relevant animal data, and human experience. The quality and consistency of the data shall be given appropriate weight. Both positive and negative results shall be assembled together in a single weight of evidence determination.

The integration of all information to come to a final hazard assessment based on weight of evidence in general requires in-depth toxicological expertise.

There are a number of available *in vitro* test systems that are currently being validated for their suitability in assessing serious eye damage/eye irritation potential of substances and mixtures. When validated *in vitro* eye irritation test methods are available in the future the results from such tests can be used for classification. Then these results can also be used to classify the mixture. However, not all available in vitro test systems work equally well for all types of mixtures. Prior to testing a mixture in a specific *in vitro* assay for classification purposes, it has to be assured that the respective test has been previously shown to be suitable for the prediction of serious eye damage/eye irritation properties for the type of mixture to be evaluated.

3.3.3.2.1.1 Mixtures with extreme pH

Where the mixture has an extreme pH value but the only corrosive/irritant ingredient present in the mixture is an acid or base with an assigned SCL (either CLP Annex VI or set by supplier), then the mixture should be classified accordingly. In this instance, pH of the mixture should not be considered a second time since it would have already been taken into account when deriving the SCL for the substance.

If this is not the case, then the steps to be taken into consideration when classifying a mixture with pH ≤ 2 or ≥ 11.5 are described in the following decision logic:

Mixture not classified as Skin Corr. 1 and without *in vivo* data on serious eye damage/eye irritation or relevant data from similar tested mixtures. **pH is ≤ 2 or ≥ 11.5**	
Does the acid/alkaline reserve indicate that the mixture may not be corrosive? **NO ➔** **YES** **↓**	Classify as serious eye damaging, Eye Dam. 1.
Is the mixture tested for serious eye damaging properties in an accepted *in vitro* test? **NO ➔** **YES** **↓**	Classify as serious eye damaging, Eye Dam. 1.
Does the mixture demonstrate serious eye damaging	Classify as serious eye damaging, Eye

properties in an accepted *in vitro* test? **YES ➔** **NO** **↓**	Dam. 1.
Apply methods in CLP Annex I, 3.3.3.3.2 (Table 3.3.3) / 3.3.3.3.4 (Table 3.3.4) ➔ (When validated *in vitro* eye irritation test methods are available, these may be used to generate data to classify the mixture instead of using the summation method.)	Classify accordingly.

If consideration of extreme pH and acid/alkaline reserve indicates the mixture may not have the potential to cause serious eye damage, then the supplier should carry out further testing to confirm this (CLP Annex I, Section 3.3.3.2.1). The mixture must be classified as Serious eye damage Category 1 if the supplier decides not to carry out the required confirmatory testing.

If further testing confirms that the mixture should not be classified for serious eye damage effects, then the supplier should assess the mixture for eye irritation either using *in vitro* eye irritation test methods when available or the summation method.

It must be noted that the pH-acid/alkali reserve method assumes that the potential corrosivity or irritancy is due to the effect of the ionic entities. When this is not the case, especially when the mixture contains non-ionic (non-ionisable) substances themselves classified as corrosive or irritant, then the pH-reserve method cannot be a basis for modifying the classification.

Where the mixture has an extreme pH value and contains some other corrosive/irritant ingredients (some of which may have SCLs assigned) in addition to an acid or base with or without an assigned SCL, then the mixture shall follow the procedure described in the decision logic.

3.3.3.2.2 When data are not available for the complete mixture: bridging principles

Annex I: 3.3.3.2.1. Where the mixture itself has not been tested to determine its skin corrosivity or potential to cause serious eye damage or irritation, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging rules set out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture (see Section 1.6.3 of this Guidance).

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified based on its ingredients as described in Section 3.3.3.2.3 and 3.3.3.3 of this Guidance.

3.3.3.2.3 When data are available for all ingredients or only for some ingredients of the mixture

3.3.3.2.3.1 Ingredients that should be taken into account for the purpose of classification

Annex I: 3.3.3.3.1. […] Assumption: The 'relevant ingredients' of a mixture are those which are present in concentrations of 1% (w/w for solids, liquids, dusts, mists and vapours and v/v for gases) or greater, unless there is a presumption (e.g. in the case of corrosive ingredients) that an ingredient present at a concentration of less than 1% is still relevant for classifying the mixture for eye irritation/serious eye damage.

3.3.3.2.3.2 The additivity approach is applicable

Annex I: 3.3.3.3.2. In general, the approach to classification of mixtures as eye irritant or seriously damaging to the eye when data are available on the components, but not on the mixture as a whole, is based on the theory of additivity, such that each corrosive or irritant component contributes to the overall irritant or corrosive properties of the mixture in proportion to its potency and concentration. A weighting factor of 10 is used for corrosive components when they are present at a concentration below the generic concentration limit for classification in Category 1, but are at a concentration that will contribute to the classification of the mixture as an irritant. The mixture is classified as seriously damaging to the eye or eye irritant when the sum of the concentrations of such components exceeds a concentration limit.

3.3.3.3.3. Table 3.3.3 provides the generic concentration limits to be used to determine if the mixture shall be classified as irritant or as seriously damaging to the eye.

When the supplier is unable to derive the classification using either data on the mixture itself or bridging principles, he must determine the serious eye damage/eye irritation properties of his mixture using data on the individual ingredients. Although the general approach is the additivity principle which has been used succesfully in the DPD for long periods, the supplier must ascertain whether the additivity approach is applicable, the first step in the process being to identify all the ingredients in the mixture (i.e. their name, chemical type, concentration level, hazard classification and any SCLs) and the pH of the mixture. In addition, for example surfactant interaction or neutralisation of acids/bases could occur in a mixture, which makes it important to consider not only the contribution of individual ingredients but also the effects of the entire mixture.

Additivity may not apply where the mixture contains substances mentioned in CLP Annex I, 3.3.3.3.4.1 which may be corrosive/irritant at concentrations below 1%, see Section 3.3.3.2.3.3 of this Guidance.

Application of SCLs when applying the additivity approach

The generic concentration limits are specified in Table 3.3.3. However, CLP Article 10(5) indicates that specific concentration limits (SCLs) take precedence over generic concentration limits. Thus, if a given substance has an SCL, then this specific concentration limit has to be taken into account when applying the summation (additivity) method for serious eye damage/eye irritation (see Examples 4 and 5).

In cases where additivity applies for serious eye damage/eye irritation to a mixture with two or more substances some of which may have SCLs assigned, then the following formula should be used:

The mixture is classified for serious eye damage/eye irritation if the

$$\text{Sum of } (\text{ConcA} / \text{clA}) + (\text{ConcB} / \text{clB}) + \ldots + (\text{ConcZ} / \text{clZ}) \text{ is } \geq 1$$

Where ConcA = the concentration of substance A in the mixture;

clA = the concentration limit (either specific or generic) of substance A;

ConcB = the concentration of substance B in the mixture;

clB = the concentration limit (either specific or generic) of substance B; etc.

This approach is similar to that used in the DPD where a substance SCL can replace the default limits in the conventional method equations.

3.3.3.2.3.3 The additivity approach is not applicable

Guidance update to legal text "Annex I: 3.3.3.3.5" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I; 3.3.3.3.4.1. Particular care must be taken when classifying certain types of mixtures containing substances such as acids and bases, inorganic salts, aldehydes, phenols, and surfactants. The approach explained in paragraphs 3.3.3.3.1 and 3.3.3.3.2 might not work given that many of such substances are corrosive or irritant at concentrations < 1 %.

3.3.3.3.4.2. For mixtures containing strong acids or bases the pH shall be used as classification criteria (see paragraph 3.3.2.3) since pH will be a better indicator of serious eye damage than the generic concentration limits of Table 3.3.3.

3.3.3.3.4.3. A mixture containing corrosive or irritant ingredients that cannot be classified based on the additivity approach (Table 3.3.3), due to chemical characteristics that make this approach unworkable, shall be classified as Category 1 for effects on the eye if it contains ≥ 1 % of a corrosive ingredient and as Category 2 when it contains ≥ 3 % of an irritant ingredient. Classification of mixtures with ingredients for which the approach in Table 3.3.3 does not apply is summarised in Table 3.3.4.

3.3.3.3.5. On occasion, reliable data may show that the reversible/irreversible eye effects of an ingredient will not be evident when present at a level at or above the generic concentration limits mentioned in Tables 3.3.3 and 3.3.4. In these cases the mixture shall be classified according to those data. On other occasions, when it is expected that the skin corrosion/irritation hazards or the reversible/irreversible eye effects of an ingredient will not be evident when present at a level at or above the generic concentration limits mentioned in Tables 3.3.3 and 3.3.4, and in section 3.3.3.3.6. In testing of the mixture shall be considered. In those cases, the tiered weight of evidence strategy shall be applied.

3.3.3.3.6. If there are data showing that (an) ingredient(s) may be corrosive or irritant at a concentration of < 1 % (corrosive) or < 3 % (irritant), the mixture shall be classified accordingly.

3.3.3.3 Generic concentration limits for substances triggering classification of mixtures

3.3.3.3.1 When the additivity approach is applicable

Annex I: *Table 3.3.3*
Generic concentration limits of ingredients of a mixture classified as Skin corrosive Category 1 and/or eye Category 1 or 2 for effects on the eye that trigger classification of the mixture for effects on the eye (Category 1 or 2)

Sum of ingredients classified as:	Concentration triggering classification of a mixture as:	
	Irreversible Eye Effects	Reversible Eye Effects
	Category 1	Category 2
Eye effects Category 1 or Skin corrosive Category 1A, 1B, 1C	≥ 3 %	≥ 1 % but < 3 %
Eye Effects Category 2		≥ 10 %
(10 x Eye Effects Category 1) + Eye effects Category 2		≥ 10 %
Skin Corrosive Category 1A, 1B, 1C + Eye effects Category 1	≥ 3 %	≥ 1 % but < 3 %

10 x (Skin corrosive Category 1A, 1B, 1C + Eye Effects Category 1) + Eye Effects Category 2		≥ 10 %

3.3.3.3.2 When the additivity approach is not applicable

Annex I: *Table 3.3.4* **Generic concentration limits of ingredients of a mixture for which the additivity approach does not apply, that trigger classification of the mixture as hazardous to the eye**		
Ingredient	Concentration	Mixture classified as: Eye
Acid with pH ≤ 2	≥ 1%	Category 1
Base with pH ≥ 11,5	≥ 1%	Category 1
Other corrosive (Categories 1) ingredients for which additivity does not apply	≥ 1%	Category 1
Other irritant (Category 2) ingredients for which additivity does not apply, including acids and bases	≥ 3%	Category 2

There are ongoing discussions at UN level whether 'Other irritant (Category 2) ingredients' in CLP Annex I, Table 3.3.4 (last row) include skin and eye irritants or only eye irritants.

3.3.3.4 Decision logic

The decision logic which is based on the Guidance on IR/CSA Figure R.7.2-3 is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.

1. When data are available for the complete mixture		
0	Is the mixture classified as a skin corrosive? **YES** ➔ **NO** 🡻	When assigned Skin Corr. 1, the risk of severe damage to eyes is considered implicit. The mixture is classified for serious eye damage but not additionally labelled, as labelling for Skin Cor 1 already covers serious eye damage.
1a	Is the pH of the mixture ≤ 2 or ≥ 11.5? **YES** ➔ **NO** 🡻	– Where classification is based upon consideration of pH alone (i.e. buffering capacity not known), Eye Dam. 1 should be applied. – Where consideration of the acid/alkaline reserve suggests that the substance is not corrosive, this has to be confirmed (preferably by use of an appropriate *in vitro* test). Proceed to step 1b.

1b	Are there other physical or chemical properties that indicate that the mixture has the potential to cause serious eye damage or is irritating to the eye? **YES** ➔ **NO** 🡻	Use this information for weight of evidence (WoE) determination (step 6). Proceed to step 2.
2	Are there adequate existing human experience data which provide evidence that the mixture has the potential to cause serious eye damage or is irritating to the eye? **YES** ➔ **NO** 🡻	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2).
3	Are there data from existing studies *on eye irritation* in laboratory animals, which provide sound conclusive evidence that the mixture has the potential to cause serious eye damage, is an eye irritant or non-irritant? **YES** ➔ **NO** 🡻	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification).
4a	Are there data from a validated *in vitro* or *ex vivo* test (adopted by OECD or not), which provide evidence that the mixture is an eye irritant or non-irritant? **YES** ➔ **NO** 🡻	Consider to classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification). If discrimination between Eye Dam. 1 and Eye Irrit. 2 is not possible, Eye Dam. 1 must be chosen. Proceed to step 4b
4b	Are there acceptable data from a suitable *in vitro* test, which provide evidence that the mixture is an irritant to the eye? **YES** ➔ **NO** 🡻	Consider to classify accordingly (Eye Dam. 1 or Eye Irrit. 2). If discrimination between Eye Dam. 1 and Eye Irrit. 2 is not possible, Eye Dam. 1 must be chosen. Proceed to step 5
5	Taking all existing and relevant data (steps 1-4) into account including potential synergistic/antagonistic effects and bioavailability, is there sufficient information to make a decision on classification? **YES** ➔ **NO** 🡻	Classify accordingly (Eye Dam. 1 or Eye Irrit. 2 or no classification)
2. When data are not available for the complete mixture: bridging principles		
6a	Are existing eye irritation data available on similar tested mixtures and on the individual ingredients? **NO** ➔ **YES**	Proceed to step 7a

	↓	
6b	Can bridging principles be applied? **YES** → **NO** ↓	Classify in appropriate category (Eye Dam. 1 or Eye Irrit. 2 or no classification)
3. When data are available for all components or only for some components of the mixture		
7a	Is pH of the mixture ≤ 2 or ≥ 11.5? **YES** → **NO** ↓	Follow decision logic in Section 3.3.3.2.1.1 of this Guidance and classify accordingly.
7b	Is there any indication that the additivity principle does not apply? **YES** → **NO** ↓	CLP Annex I, 3.3.3.3.4 and Table 3.3.4 may apply. Take relevant ingredients (CLP Annex I, 3.2.3.3.1) and SCLs into account, as appropriate. Classify in appropriate category (Eye Dam. 1 or Eye Irrit. 2 or no classification)
	CLP Annex I, 3.3.3.3.2 and Table 3.3.3 apply. Take relevant ingredients (CLP Annex I, 3.2.3.3.1) and SCLs into account, as appropriate. Classify in appropriate category (Eye Dam. 1 or Eye Irrit. 2 or no classification).	

3.3.4 Hazard communication in form of labelling for serious eye damage/eye irritation

3.3.4.1 Pictograms, signal words, hazard statements and precautionary statements

Annex I; 3.3.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.3.5.

Table3.3.5
Label elements for serious eye damage/eye irritation

Classification	Category 1	Category 2
GHS Pictograms		
Signal Word	Danger	Warning
Hazard Statement	H318: Causes serious eye damage	H319: Causes serious eye irritation
Precautionary Statement Prevention	P280	P264 P280
Precautionary Statement	P305 + P351 + P338	P305 + P351 + P338

Response	P310	P337 + P313
Precautionary Statement Storage		
Precautionary Statement Disposal		

A skin corrosive mixture is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion, H314: Causes severe skin burns and eye damage. Thus, in this case a mixture has to be classified for both classifications (Skin Corr. 1 and Eye Dam. 1) but the hazard statement H318 "Causes serious eye damage" is not indicated on the label because of redundancy (CLP Article 27)

3.3.5 Re-classification of substances and mixtures classified for serious eye damage/eye irritation according to DSD and DPD

3.3.5.1 Is direct "translation" of classification and labelling possible?

A direct translation as indicated in the translation table in Annex VII to CLP is generally possible. However, an evaluation and classification must be carried out in accordance with CLP Articles 9 – 13 when data for the mixture are available. It should be noted that if classified as a skin corrosive under DSD then this will translate to skin corrosive and serious eye damage under CLP although this is not listed in Annex VII. Translation from classification according to DSD to the classification according to CLP is as follows:

- Xi; R41 is translated into Eye Dam. 1; H318. The criteria in DSD are completely covered by the criteria in CLP.
- Xi; R36 is translated into Eye Irrit. 2; H319. The criteria in DSD are completely covered by the criteria in CLP.

It should be noted that CLP eye irritation Category 2 will include more substances which are currently not classified under the DSD, but with values of cornea opacity >1 and <2 or values of conjunctival redness >2 and <2.5, will be classified as eye irritants under CLP.

It should be noted that where mixtures containing substances with risk phrase R41 have been classified on basis of the hazards of individual ingredients, the use of the translation table may lead to an under-classification of the mixture. This is because the general concentration limits, to be applied for mixtures, are lowered under CLP compared to DPD. For mixtures containing substances with this classification the use of the translation table may therefore not be appropriate and re-classification done by using the existing data would be more correct. For more details see Section 1.7 of this Guidance.

3.3.5.2 Re-evaluation of data

If there is new information which might be relevant with respect to classification a re-evaluation has to be performed.

3.3.6 Examples of classification for serious eye damage/eye irritation

3.3.6.1 Examples of substances fulfilling the criteria for classification

3.3.6.1.1 Example 1: Standard test according to OECD TG 405 with three animals

In a study according to OECD 405 the test substance was applied on the eyes of three rabbits. The scoring results obtained are listed in the following table:

Cornea:

Animal No.	Evaluation after …					**Positive responder?** Ø Score …	
	1 hr	24 hrs	48 hrs	72 hrs	21 days	≥ 1	≥ 3
1	0	2	2	2	0		
		Ø 24/48/72 h animal 1 is 2				Yes	No
2	2	2	2	2	0		
		Ø 24/48/72 h animal 2 is 2				Yes	No
3	2	2	1	1	0		
		Ø 24/48/72 h animal 3 is 1.3				Yes	No

Effects are reversible

Iris:

Animal No.	Evaluation after …					**Positive responder?** Ø Score …	
	1 hr	24 hrs	48 hrs	72 hrs	21 days	≥ 1	> 1.5
1	0	1	1	1	0		
		Ø 24/48/72 h animal 1 is 1				Yes	No
2	1	1	1	1	0		
		Ø 24/48/72 h animal 2 is 1				Yes	No
3	1	1	1	1	0		
		Ø 24/48/72 h animal 3 is 1				Yes	No

Effects are reversible

Conjunctiva – Erythema:

Animal No.	Evaluation after …					**Positive responder?** Ø Score …	
	1 hr	24 hrs	48 hrs	72 hrs	21 days	≥ 2	
1	2	2	2	2	0		
		Ø 24/48/72 h animal 1 is 2				Yes	
2	1	1	1	1	0		
		Ø 24/48/72 h animal 2 is 1				No	
3	1	1	1	1	0		
		Ø 24/48/72 h animal 3 is 1				No	

Effects are reversible

Conjunctiva – Swelling:

Animal No.	Evaluation after …					**Positive responder?** ∅ Score …	
	1 hr	24 hrs	48 hrs	72 hrs	21 days	≥ 2	
1	0	3	3	3	0		
		∅ 24/48/72 h animal 1 is 3				Yes	
2	2	2	2	1	0		
		∅ 24/48/72 h animal 2 is 1.7				No	
3	2	3	2	2	0		
		∅ 24/48/72 h animal 3 is 2.3				Yes	

Effects are reversible

Classification according to CLP: Eye irritant Category 2

Rationale: Cornea and Conjunctiva ”positive responder” ≥ 2: 2/3 animals

Iris ”positive responder” ≥ 1: 3/3 animals

3.3.6.1.2 Example 2: Test carried out with more than 3 rabbits

Cornea:

Animal No.	Evaluation after …							**Positive responder?** ∅ Score …	
	1h	24h	48h	72h	7d	14d	21d	≥ 3	≥ 1
1	1	2	3	3	1	1	0		
		∅ 24/48/72h = 2.7						no	yes
2	1	2	2	3	1	1	0		
		∅ 24/48/72h = 2.3						no	yes
3	1	2	3	3	2	1	0		
		∅ 24/48/72h = 2.7						no	yes
4	1	2	4	4	2	1	0		
		∅ 24/48/72h = 3.3						yes	yes

Effects are reversible

Iris:

Animal No.	Evaluation after …							**Positive responder?** ∅ Score …	
	1h	24h	48h	72h	7d	14d	21d	> 1.5	≥ 1
1	0	0	0	0	0	0	0		
		∅ 24/48/72h = 0						no	no
2	0	0	0	0	0	0	0		
		∅ 24/48/72h = 0						no	no
3	0	1	1	1	1	0	0		
		∅ 24/48/72h = 1						no	yes
4	0	0	0	0	0	0	0		
		∅ 24/48/72h = 0						no	no

Effects are reversible

Conjunctiva – Erythema:

Animal No.	Evaluation after …							**Positive responder?** ∅ Score …	
	1h	24h	48h	72h	7d	14d	21d	≥ 2	
1	2	2	2	1	1	1	0		
		∅ 24/48/72h = 1.7						no	
2	2	2	2	1	1	0	0		
		∅ 24/48/72h = 1.7						no	
3	2	2	2	1	1	1	1		
		∅ 24/48/72h = 1.7						no	
4	2	2	2	1	0	0	0		
		∅ 24/48/72h = 1.7						no	

Effects are NON-reversible

Conjunctiva – Swelling:

Animal No.	Evaluation after …							**Positive responder?** ∅ Score …	
	1h	24h	48h	72h	7d	14d	21d	≥ 2	
1	2	2	2	1	1	1	0		
		∅ 24/48/72h = 1.7						no	
2	2	2	1	1	1	0	0		

		∅ 24/48/72h = 1.3					no	
3	2	2	2	1	1	1	1		
		∅ 24/48/72h = 1.7					no	
4	2	2	2	1	1	1	1		
		∅ 24/48/72h = 1.7					no	

Effects are NON-reversible

Classification according to CLP: Serious eye damage Category 1

Rationale: Conjunctiva with irreversible effects

3.3.6.2 Examples of mixtures fulfilling the criteria for classification

3.3.6.2.1 Example 3: Application of the additivity approach for mixtures containing ingredients without SCLs

Where the mixture is made up of ingredients with no assigned SCLs, then the appropriate summation(s) from CLP Annex I, Table 3.3.3 should be used.

Ingredient	Skin / eye classification	Concentration (% w/w)	SCL
Surfactant A	Eye Cat 1	1.8	Not assigned
Substance B	Eye Cat 2	0.5	Not assigned
Substance C	Eye Cat 1	5.4	Not assigned
Substance D	Not classified	4.0	
Acid E	Skin Cat 1A	2.0	Not assigned
Water	Not classified	86.3	

pH of the mixture is 9.0 – 10.0, thus extreme pH provisions do not apply. The mixture contains a surfactant and an acid but neither are corrosive/irritant below 1% (as identified by the absence of specific concentration limits in either CLP Annex VI or the Classification and Labelling Inventory). Additivity is considered to apply.
Substance D and water can be disregarded as they are not classified for serious eye damage/eye irritation. Substance B can also be disregarded as present below 1%.

Mixture contains 7.2% Eye Cat 1 ingredients as well as 2% acid E so the summation {Skin corrosion Cat 1A, 1B, 1C + Eye Cat 1} applies and is > 3%, thus mixture is classified Eye Cat 1.

3.3.6.2.2 Example 4: Application of the additivity approach for mixtures containing ingredients which may have SCLs

Ingredient	Skin / eye classification	Concentration (% w/w)	SCL
Surfactant A	Eye Cat 1	2.0	Not assigned
Substance B	Eye Cat 2	0.5	Not assigned
Substance C	Skin Cat 1B	5.4	C ≥ 10 %: Skin Cat 1B 5 % ≤ C < 10 %: Eye Cat 2

Substance D	Not classified	4.0	
Substance E	Skin Cat 1B	2.0	Not assigned
Water	Not classified	86.1	

pH of the mixture is 10.5 – 11.0, thus extreme pH provisions do not apply. The mixture contains a surfactant, an acid and a base but none are corrosive/irritant below 1% (as identified by the absence of specific concentration limits in either CLP Annex VI or the Classification and Labelling Inventory). Additivity is considered to apply.

Substance D and water can be disregarded as they are not classified for serious eye damage/eye irritation. Substance B can also be disregarded as present below 1%.

SCLs are not assigned to substance E or surfactant A, thus generic concentration limits (GCL) apply for these ingredients

Eye Cat 1

(% surfactant A / GCL) + (% Substance C / SCL) + (% Substance E / GCL) = (2/3) + (5.4/10) + (2/3) = 1.9 ⇨ > 1 thus mixture is classified Eye Cat 1

3.3.6.2.3 Example 5: Application of the additivity approach for mixtures containing ingredients which may have SCLs

Ingredient	Serious eye damage/ eye irritation classification	Concentration (% w/w)	SCL
Surfactant B	Eye Cat 1	0.7	Not assigned
Substance C	Eye Cat 2	74.9	Not assigned
Substance D	Eye Cat 1	8.5	C ≥ 25 %: Eye Cat 1 10 % ≤ C < 25 %: Eye Cat 2
Substance E	Not classified	15.9	

pH of the mixture is 10.0 – 10.5 (10% solution), thus extreme pH provisions do not apply. The mixture contains a surfactant which is not corrosive/irritant below 1% (as identified by the absence of specific concentration limits in either CLP Annex VI or the Classification and Labelling Inventory). Additivity is considered to apply.

Substance E can be disregarded as it is not classified for serious eye damage/eye irritation. Surfactant B can also be disregarded as present below 1%.

SCLs are not assigned to substance C, thus GCL apply for this ingredient

Eye Cat 1

Mixture contains 8.5% substance D, the only 'relevant' ingredient classified as Eye Cat 1. As this is below the 25% SCL for substance D, the mixture is not classified Eye Cat 1

Eye Cat 2

(%substance D/ SCL) + (%substance C / GCL) = (8.5/10) + (74.9/10) which is > 1 thus mixture is classified Eye Cat 2

3.3.7 References

ECVAM/ESAC (2009a) Statement on the scientific validity of cytotoxicity/cell-function based in vitro assays for eye irritation testing. Online: http://ecvam.jrc.it/

ECVAM/ESAC (2009b) Statement on the use of existing low volume eye test (LVET) data for weight of evidence decisions on classification and labelling of cleaning products and their main ingredients. Online: http://ecvam.jrc.it/

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3.4 RESPIRATORY OR SKIN SENSITISATION

3.4.1 Definitions and general considerations for respiratory or skin sensitisation

Annex I: 3.4.1.1. Respiratory sensitiser means a substance that will lead to hypersensitivity of the airways following inhalation of the substance.

3.4.1.2. Skin sensitiser means a substance that will lead to an allergic response following skin contact.

In terms of prevention it might be important to note that respiratory sensitisation may be induced not only by inhalation but also by skin contact.

Annex I: 3.4.1.3. For the purpose of section 3.4, sensitisation includes two phases: the first phase is induction of specialised immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitised individual to an allergen.

3.4.1.4. For respiratory sensitisation, the pattern of induction followed by elicitation phases is shared in common with skin sensitisation. For skin sensitisation, an induction phase is required in which the immune system learns to react; clinical symptoms can then arise when subsequent exposure is sufficient to elicit a visible skin reaction (elicitation phase). As a consequence, predictive tests usually follow this pattern in which there is an induction phase, the response to which is measured by a standardised elicitation phase, typically involving a patch test. The local lymph node assay is the exception, directly measuring the induction response. Evidence of skin sensitisation in humans normally is assessed by a diagnostic patch test.

3.4.1.5. Usually, for both skin and respiratory sensitisation, lower levels are necessary for elicitation than are required for induction. Provisions for alerting sensitised individuals to the presence of a particular sensitiser in a mixture can be found in Annex II, section 2.8.

3.4.1.6. The hazard class Respiratory or Skin Sensitisation is differentiated into:

- Respiratory Sensitisation and;
- Skin Sensitisation.

3.4.2 Classification of substances for respiratory sensitisation

3.4.2.1 Identification of hazard information

There are no formally recognised and validated animal tests for respiratory sensitisation. However there may be data from human observations indicating respiratory sensitisation in exposed populations.

3.4.2.1.1 Identification of human data

Relevant information with respect to respiratory sensitisation may be available from case reports, epidemiological studies, medical surveillance, reporting schemes. For more details see the Guidance on IR/CSA, Section R.7.3.5.

3.4.2.1.2 Identification of non human data

~~At present~~ No formally validated methods ~~non-testing systems e~~xist to predict respiratory sensitising potential. ~~In addition,~~ There are some animal studies that are indicative of the potential of a substance to cause sensitisation by inhalation in humans and these data may provide supportive evidence and should be used in a weight of evidence assessment. For further information see the Guidance on IR/CSA, Section R.7.3.5.1

3.4.2.2 Classification criteria for substances

Annex I: 3.4.2.1. *Respiratory sensitisers*

3.4.2.1.1. Hazard categories

3.4.2.1.1.1. Respiratory sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation.

3.4.2.1.1.2. Where data are sufficient a refined evaluation according to 3.4.2.1.1.3 shall allow the allocation of respiratory sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other respiratory sensitisers.

3.4.2.1.1.3. Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory sensitisers. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.1 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

3.4.2.1.1.4. Substances shall be classified as respiratory sensitisers in accordance with the criteria in Table 3.4.1:

Table 3.4.1

Hazard category and sub-categories for respiratory sensitisers

Category	Criteria
Category 1	Substances shall be classified as respiratory sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:

	(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and /or (b) if there are positive results from an appropriate animal test.
Sub-category 1A:	Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests ([1]). Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests ([1]). Severity of reaction may also be considered.
([1]) At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.	

There is currently no clear way of establishing sub-categories for respiratory sensitisation, however if compelling evidence was available such as observations in the workplace, it may be possible to determine a sub-category.

Classification into sub-categories is only allowed if data are sufficient. Therefore care should be taken when classifying substances into category 1B when category 1A cannot be excluded. In such cases classification into category 1 should be considered.High frequency and low to moderate frequency cannot be defined as specific concentrations or percentages for human study data because when considering human evidence, it is necessary to take into account the size of the exposed population and the extent and conditions of exposure, including frequency. It is necessary, therefore, to reach a view on a case-by-case basis.

3.4.2.3 Evaluation of hazard information

3.4.2.3.1 Human data on respiratory sensitisation

Substances shall be classified as respiratory sensitisers if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity. This is further described in the CLP Annex I, 3.4.2.1.2.

Annex I: 3.4.2.1.2 Human evidence

3.4.2.1.2.1. Evidence that a substance can lead to specific hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.

3.4.2.1.2.2. When considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases:

(a) the size of the population exposed;

(b) the extent of exposure.

[…]

3.4.2.1.2.3. The evidence referred to above could be:

(a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include:

(i) in vivo immunological test (e.g. skin prick test)

(ii) in vitro immunological test (e.g. serological analysis);

(iii) studies that indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects;

(iv) a chemical structure related to substances known to cause respiratory hypersensitivity;

(b) data from one or more positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction.

3.4.2.1.2.4. Clinical history shall include both medical and occupational history to determine a relationship between exposure to a specific substance and development of respiratory hypersensitivity. Relevant information includes aggravating factors both in the home and workplace, the onset and progress of the disease, family history and medical history of the patient in question. The medical history shall also include a note of other allergic or airway disorders from childhood, and smoking history.

3.4.2.1.2.5. The results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own. It is however recognised that in practice many of the examinations listed above will have already been carried out.

3.4.2.3.2 Non human data on respiratory sensitisation

Annex I: 3.4.2.1.3. Animal studies

3.4.2.1.3.1. Data from appropriate animal studies (*) which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans (**) may include:

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice;

(b) specific pulmonary responses in guinea pigs.

(*) At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.

(**) The mechanisms by which substances induce symptoms of asthma are not yet fully known. For preventative measures, these substances are considered respiratory sensitisers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyper reactivity, they should not be considered respiratory sensitisers.

No formally recognised and validated animal tests currently exist for respiratory sensitisation. However data from some animal studies may be indicative of the potential of a substance to cause sensitisation by inhalation in humans (CLP Annex I, 3.4.2.1.3) and may provide supportive evidence in case human evidence is available.

3.4.2.4 Decision on classification

According to CLP Annex I, Section 3.4.2.1.1.4 substances fulfilling the criteria for respiratory sensitisation will be classified as such in Category 1 (and in Sub-category 1A or 1B when sufficient data are available),

3.4.2.5 Setting of specific concentration limits

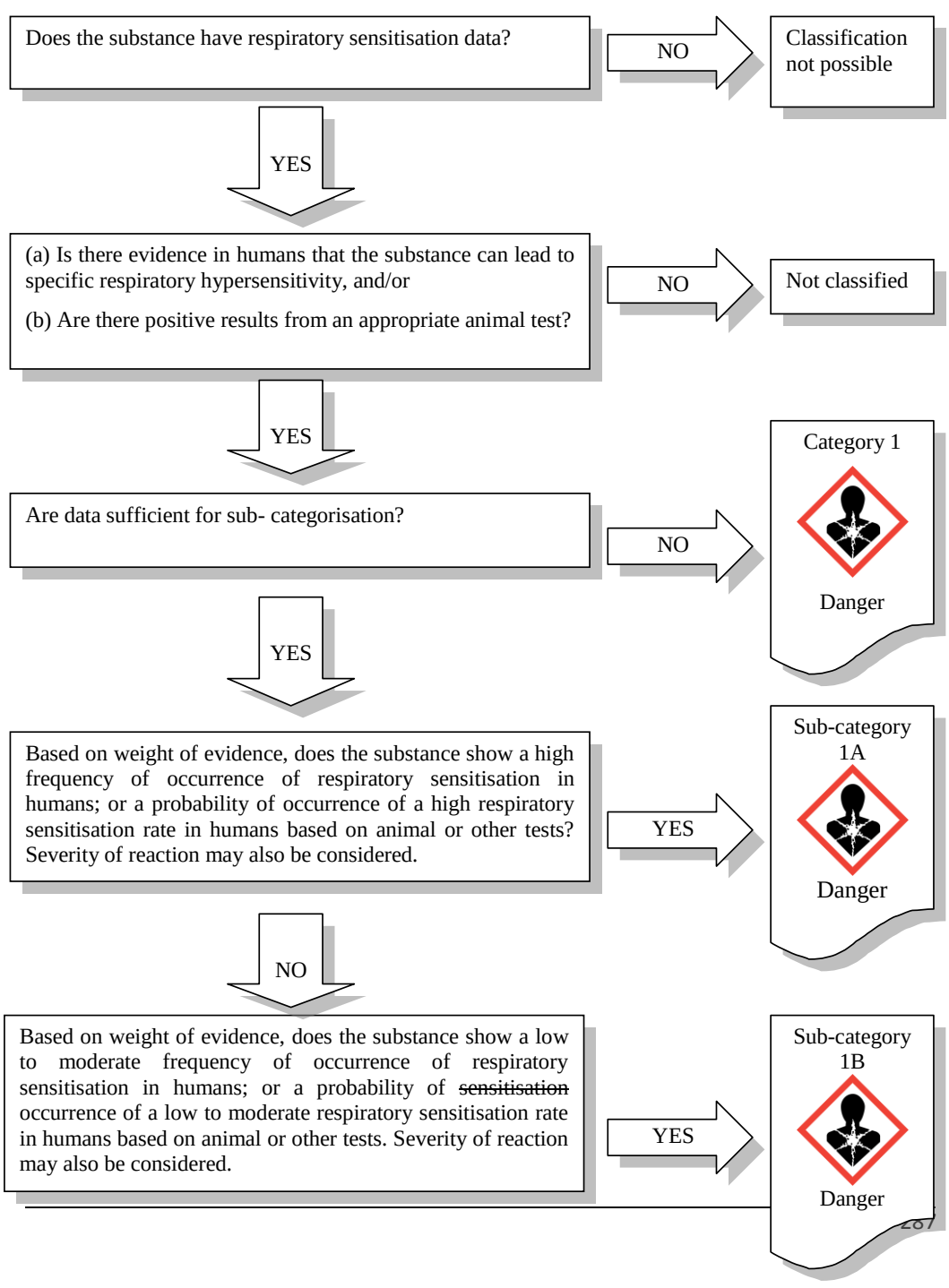
Respiratory sensitisers cannot be identified reliably on the basis of animal tests as yet, since no recognised validated test exists to determine sensitising potential and potency by inhalation. Therefore specific concentration limits (SCLs) cannot be set on the basis of

animal data alone. Moreover, there is no concept available to set SCLs on the basis of human data for respiratory sensitisers.

3.4.2.6 Decision logic for classification of substances

It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

Decision logic for respiratory sensitisation



3.4.3 Classification of substances for skin sensitisation

3.4.3.1 Identification of hazard information

With respect to identification of relevant information for skin sensitisation see the Guidance on IR/CSA, Section R.7.3.3.

3.4.3.1.1 Identification of human data

Relevant information with respect to skin sensitisation may be available from case reports, epidemiological studies, medical surveillance and reporting schemes based on human patch testing. For more details see the Guidance on IR/CSA, Section R.7.3.3.2.

3.4.3.1.2 Identification of non human data

At present no formally validated non-testing systems exist to predict skin sensitising potential. However data such as structural alert data or data to show that the chemical structure of a molecule is similar to that of known sensitisers (e.g. QSARs or expert systems) may form part of the weight of evidence for classification (see also Guidance on IR/CSA, Section R.7.3.3).

The subject of *in vitro* testing for skin sensitisation has also been dealt with in the Guidance on IR/CSA, Section R.7.3.3. At present no validated *in vitro* methods exist to identify the sensitising potential of a chemical.

Information on the current developments of *in vitro* tests and methodology can be found on the ECVAM website (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam).

There are three standard animal test methods used to evaluate skin sensitisation for substances: the mouse local lymph node assay (LLNA), the guinea pig maximisation test (GPMT) and the Buehler assay. They are further described in the Guidance on IR/CSA, Section R.7.3.3, and in the context of classification in Section 3.4.3.3.2 of this Guidance.

3.4.3.2 Classification criteria for substances

Annex I: 3.4.2.2. *Skin Sensitisers*

3.4.2.2.1. Hazard categories

3.4.2.2.1.1. Skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation.

3.4.2.2.1.2. Where data are sufficient a refined evaluation according to section 3.4.2.2.1.3 allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers.

3.4.2.2.1.3. Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitisers as described in section 3.4.2.2.2. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in sections 3.4.2.2.2.1 and 3.4.2.2.3.2 for sub-category 1A and in sections 3.4.2.2.2.2 and 3.4.2.2.3.3 for sub-category 1B.

3.4.2.2.1.4. Substances shall be classified as skin sensitisers in accordance with the criteria in Table 3.4.2:

Table 3.4.2

Hazard category and sub-categories for skin sensitisers

Category	Criteria
Category 1	Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test (see specific criteria in paragraph 3.4.2.2.4.1).
Sub-category 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

Classification into sub-categories is only allowed if data are sufficient. Therefore care should be taken when classifying substances into category 1B when category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent (in line with some test protocols where a maximised dose should be used).

When considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis. Human data should be incorporated with animal data to decide the sub-categorisation.

For a newly identified skin sensitiser, which might also be a substance newly introduced onto the market, or a substance not included in the baseline diagnostic patch test series, the high severity of responses might be used as an indication that classification as Category 1A is appropriate. For example, where the substance has caused:

- Hospitalisation due to acute skin reaction
- Chronic dermatitis (lasting > 6 months)
- Generalised (systemic/whole body) dermatitis

It should be noted that the severity/strength of diagnostic patch test reactions normally cannot be used for this purpose.

3.4.3.3 Evaluation of hazard information

3.4.3.3.1 Human data on skin sensitisationHuman evidence for classification of a substance can be based on positive data from patch testing, epidemiological studies showing allergic contact dermatitis caused by the substance, positive data from experimental studies in

man and/or well documented episodes of allergic contact dermatitis, using a weight of evidence approach (see Section 3.4.3.3.3 of this Guidance for details).

Criteria for sub-categorisation are listed in CLP Annex I, 3.4.2.2.2.1 and 3.4.2.2.2.2:

Annex I: 3.4.2.2.2.1. Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

3.4.2.2.2.2. Human evidence for sub-category 1B can include:

(a) positive responses at > 500 μg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

HRIPT: Human Repeat Insult Patch Test; HMT: Human Maximisation Test

CLP Article 7 (3) states "Tests on humans shall not be performed for the purposes of this Regulation". However, data obtained from other sources, such as clinical studies, can be used for the purposes of this Regulation." Thus human induction studies such as HRIPT or HMT must not be performed, although historical data may be used as weight of evidence for the sub-categorisation. To provide further guidance on the types of human data that may be considered as data from other sources, please refer to the following table:**Table 3.4.3.3.1.** – Types of Human Studies

Type	**Subjects**	**Endpoint studied**	**Comments**
Human Repeated Insult Patch Test (HRIPT) & Human Maximization Test (HMT)	Healthy volunteers	Induction of sensitisation	This is not a clinical study and is only of historical relevance. New studies for this regulation are not permitted.
Diagnostic patch test from individual clinics or collated clinic data	Eczema patients attending dermatology clinics	Elicitation (as an indicator of previous sensitisation)	Primary source of clinical information on the occurrence of skin sensitisation
Dose response study (eg patch test serial dilution; repeated open application test)	Sensitised individuals (usually from diagnostic patch tests)	Elicitation	Not yet a standardised protocol, but provides an indication of the degree of sensitivity and of safe limits of exposure
Epidemiology study	Eczema patients, selected occupational	Elicitation	Large general population studies are scarce; focused studies in

	groups,other selected groups, or general population		selected populations are more common and provide insights on frequency of sensitisation compared to exposure

The purpose of the material that follows is the provision of guidance concerning the evaluation of human data, particularly with respect to balancing considerations of exposure against the clinical evidence regarding the frequency of skin sensitisation. The concept of "guidance" should be applied generally to all of the numeric criteria – they represent indicators derived from expert opinion and are not to be taken as proven absolute values. Application of this guidance should permit sub-categorisation where the human data on exposure and sensitisation is clear. However, in the majority of cases the human data will be insufficient, therefore the most likely outcome of an evaluation of human data is that sub-categorisation is not possible.

Table 3.4.3.3.2.: Relatively high or low frequency of occurrence of skin sensitisation*

Human diagnostic patch test data	**High frequency**	**Low/moderate frequency**
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies: 1: all or randomly selected workers 2: selected workers with known exposure or dermatitis	 ≥ 0.4 % ≥ 1.0 %	 < 0.4 % < 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

* Note that only one or two types of information may be sufficient for sub-categorisation.

The figure of 0.2% for the general population is intended to reflect that the frequency of contact allergy in dermatitis patients is approximately 5 (range 2-10) times higher than in the general population (Mirshahpanah and Maibach, 2007).

The figure of 1% for consecutive (i.e. unselected) dermatitis patients is based on the generally agreed consideration that a contact allergy frequency of ≥ 1% in such patients is of high concern.

The figure of 0.4% for unselected workers in a workplace is derived from the use in REACH of a 2 times higher assessment factor for the general population than for workers.

It is important to note that the data from the testing of unselected, consecutive dermatitis patients is more standardised than testing which is undertaken on a specific patient group (e.g those with facial eczema) or worker group (e.g. individuals with a particular type of

exposure). Such clinical studies may be conducted on patients selected according to a particular type of eczema or based on their likelihood of occupational exposure and often involves patch testing with materials beyond those normally used i.e. "the standard series" (Andersen et al, 2011). It is important to consider also that there may be variations in positive patch test frequency related to age, gender or region.

Table 3.4.3.3.3: Relatively high or low exposure *

Exposure data	**Relatively low exposure (weighting)**	**Relatively high exposure (weighting)**
Concentration / dose	< 1.0% (score 0) < 500µg/cm^2	≥ 1.0% (score 2) ≥ 500µg/cm^2
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	<100 exposures (score 0)	≥100 exposures (score 2)

* To achieve the exposure index (see text below) a response in each row is necessary.

The scores in Table 2 represent weightings whose purpose is to enable an exposure index to be derived which best reflects our understanding of the relative importance of dose versus frequency of exposure. An additive exposure index of 1-4 equates to low exposure, whereas 5-6 reflects high exposure.

Careful consideration has to be given regarding the release (migration) of a sensitising substance from a solid object, and not the concentration. Ideally, skin exposure is best expressed in dose per unit area, but it is recognised that this data is often not available, hence concentration may be used as a surrogate indicator of exposure.

Table 3.4.3.3.4: Sub-categorisation decision table

	Relatively low frequency of occurrence of skin sensitisation	**Relatively high frequency of occurrence of skin sensitisation**
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

3.4.3.3.2 Non human data on skin sensitisation

Annex I: 3.4.2.2.3.2. Animal test results for sub-category 1A can include data with values indicated in Table 3.4.3

Table 3.4.3

Animal test results for sub-category 1A

Assay	Criteria

Local lymph node assay	EC3 value ≤ 2 %
Guinea pig maximisation test	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
Buehler assay	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose

3.4.2.2.3.3. Animal test results for sub-category 1B can include data with values indicated in Table 3.4.4 below:

Table 3.4.4

Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2 %
Guinea pig maximisation test	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B.

> **Annex I: 3.4.2.2.1.1:** Skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation.

Classification into sub-categories is only allowed if data are sufficient (CLP Annex I 3.4.2.2.1.1). Therefore care should be taken when classifying substances into Category 1B when Category 1A cannot be excluded. This is particularly important if only data are available from the guinea pig tests or from the rLLNA showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent or in the absence of adequate dose-response information. Unless there is sufficient evidence to place such substances in sub category 1A or 1B, classification in category 1 should be the default position. In other words, although the criteria in the table 3.4.4 for classification to subcategory 1B are fulfilled, the classification for subcategory 1A may not be excluded and therefore the substance should be classified as a Category 1 skin sensitiser (see also examples 6 & 7)

Since it is possible to refine the evaluation of skin sensitisers on the basis of the potency of the sensitising effect, this guidance advises how to evaluate the potency on the basis of the recommended test methods. High potency is determined according to the results from the animal studies as given in CLP Annex I, Table 3.4.3 and low to moderate potency is determined according to the results from the animal studies as given in CLP Annex I, Table 3.4.4 The potency considerations may be used as a basis for setting specific concentration

limits (see Section 3.4.3.5 of this gGuidance). The three currently recognised and officially accepted animal test methods for skin sensitisation defined by OECD Test Guidelines are the Mouse Local Lymph Node Assay (LLNA) OECD TG 429 and its variations OECD TG 442A and 442B, Guinea Pig Maximisation Test by Magnusson & Kligman (GPMT) and the Buehler assay in the guinea pig OECD TG 406. The mouse and guinea pig methods differ fundamentally with respect to the endpoints used; whereas the mouse LLNA measures the responses provoked during the induction of sensitisation, the two guinea pig tests measure challenge induced elicitation reactions in previously sensitised animals. For new testing of substances the LLNA is now the method of first choice. In the exceptional circumstance that the LLNA is not appropriate, one of the alternative tests may be used (Buehler or GPMT), but justification shall be provided (see the Guidance on IR/CSA, Section R.7.3.2.1).

Test results from the LLNA, GPMT and the Buehler assay can be used directly for classification. They may also be used for potency evaluation.

A sensitising potential of a substance is identified if a significant effect has been obtained in an acceptable *in vivo* test. A significant skin sensitising effect in each of the three recognised animal tests is defined as follows:

Table 3.4.3.3.5: *Definition of significant skin sensitising effect*

Test	Result
Mouse local lymph node assay (LLNA) (OECD TG 429)	Stimulation Index ≥ 3
LLNA: DA (OECD TG 442A),	Stimulation Index ≥ 1.8
LLNA: BrdU-ELISA (OECD TG 442B)	Stimulation Index ≥ 1.6
Guinea pig maximisation test (GPMT) (OECD 406)	Redness (Score ≥ 1) in ≥ 30% of the test animals
Buehler assay (OECD 406)	Redness (Score ≥ 1) in ≥ 15% of the test animals

A substance may be classified as a skin sensitiser on the basis of a positive test result in one of the above described animal tests. A positive result obtained by another test method not officially recognised may also justify classification as a skin sensitiser, but can normally not overrule a negative result obtained in one of the three recognised, animal tests described above. A new animal study should not be conducted in an attempt to negate a clearly positive response in a test method not officially recognised particularly where there is other supporting evidence that the substance is a skin sensitiser.

3.4.3.3.2.1 Mouse Local Lymph Node Assay

The LLNA is used both for determination of skin sensitising potential (hazard identification) and for determination of relative skin sensitisation potency (hazard characterisation). In both instances the metric is cellular proliferation induced in draining lymph nodes following topical exposure to a chemical, lymph node cell proliferation being causally and quantitatively correlated with the acquisition of skin sensitisation (Basketter *et al.* 2002a, 2002b). A correlation has been demonstrated between the concentration of chemical required for the acquisition of skin sensitisation in humans according to historical predictive data and skin sensitisation potency as measured in the mouse LLNA (Schneider and Akkan 2004, Basketter *et al.* 2005b). Potency is measured as a function of derived EC3-values. The EC3-value is the amount of test chemical (% concentration, molar value or dose per unit area) required to elicit a stimulation index of 3 in the standard LLNA (Kimber *et al.* 2003). An

inverse relationship exists between EC3-value and potency meaning that extremely potent sensitisers have extremely low EC3-values. The relevance of potency derives from an appreciation that skin sensitisers vary by up to four or five orders of magnitude with respect to the minimum concentration required inducing skin sensitisation. Potency is graded on the basis of these minimum concentrations each grade reflecting a concentration range of approximately one order of magnitude. However, it should be noted that if the dose interval for LLNA is too low so that all the stimulation indexes are below 3, it is not possible to know whether the higher doses would have generated a stimulation index above 3. Also, if only high doses would be used in an LLNA test, the EC3 value may be associated with great uncertainty since the extrapolation is needed to low doses when the shape of the dose-response curve is not known. It is also known that the choice of vehicle may also provide a variable EC3 value, which may significantly influence the skin sensitising potency and make it difficult to categorise/subcategorise the substance.

Potency may be considered when setting a specific concentration limit for a substance in mixtures (see Section 3.4.3.5 of this Guidance).

Different variants of the LLNA exist, namely the reduced LLNA (rLLNA) described as an option in the OECD TG 429, the LLNA: DA (OECD TG 442A), and the LLNA: BrdU-ELISA (OECD TG 442B). The rLLNA uses fewer animals than the classical LLNA and should only be used in those circumstances where dose-response information are not required (e.g. to confirm a negative prediction of skin sensitising potential) and thus should not be used for sub-categorisation of skin-sensitisers. The two last variants avoid the use of DNA radiolabelling agent and provide quantitative data suitable for dose-response assessment. However, the guidance for determining the positive response is different from that of the traditional LLNA(OECD TG 429). Full details are given in the corresponding OECD Test Guidelines. There is no guidance for sub-categorisation.

3.4.3.3.2.2 Guinea Pig Maximisation Test (GPMT, OECD TG 406)

This test has been used for over 40 years, ~~although a less sensitive test,~~ to detect the sensitising potential of chemicals through a test system maximizing the sensitivity by both intradermal and epidermal induction and use of an adjuvant (Freund's Complete Adjuvant). The intradermal induction is made by injection. Consequently the test is not suited for substances which cannot be made up into a liquid formulation.

The GPMT was originally designed to maximise the ability to identify a sensitisation hazard, rather than to determine skin sensitisation potency. Yet, when only a GPMT test result is available, potency categorisation is possible on the basis of the concentration of test material used for intradermal induction and the percentage of guinea pigs sensitised. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the GPMT.

It should be noted that the guinea pig tests should be conducted at highest induction dose causing mild (Buehler Assay) or mild-to-moderate (GPMT) skin irritation. As a consequence, it is unlikely that substances (except strong irritants) would be tested at low concentration given in table 3.4.4 triggering classification as a skin sensitiser in sub category 1A.

Potency may be considered when setting a specific concentration limit for a substance in mixtures (see Section 3.4.3.5 of this Guidance).

3.4.3.3.2.3 Buehler assay (OECD TG 406)

This test has been in use for the last 40 years, although still a sensitive, test to detect skin sensitisers using epidermal occluded exposure. The skin barrier of the test species (guinea pig) is kept intact in this assay. Potency can be categorised using the results of the Buehler assay on the basis of the number of animals sensitised and the concentration of the test material used for the epidermal induction. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the Buehler assay.

Potency may be considered when setting a specific concentration limit for a substance in mixtures (see Section 3.4.3.5 of this Guidance).

It should be noted that the guinea pig tests should be conducted at highest induction dose causing mild (Buehler Assay) or mild-to-moderate (GPMT) skin irritation. As a consequence, it is unlikely that substances (except strong irritants) would be tested at low concentration given in table 3.4.4 triggering classification as a skin sensitiser in sub category 1A.

3.4.3.3.2.4 Non-compliant skin sensitisation tests

In vivo test methods which do not comply with recognised guidelines are strongly discouraged for the identification of skin sensitisers or assessment of skin sensitising potency. The results of such tests have to be well-validated with scientific justification and evaluated carefully, but may provide supportive evidence. If doubts exist about the validity and the interpretation of the results, the evaluation needs to be taken by using a weight-of-evidence approach as described below (see Section 3.4.3.3.3 of this Guidance).

3.4.3.3.2.5 Animal test methods conducted for purposes other than sensitisation

Occasionally signs of skin sensitisation occur in repeated dose tests. These tests are often dermal toxicity tests on rats. Clearly, if signs of erythema/oedema occur in animals after repeated application, the possibility of skin sensitisation should be considered, and ideally assessed in an appropriate study.

3.4.3.3.3 Weight of evidence

Annex I: 3.4.2.2.4. Specific considerations

3.4.2.2.4.1. For classification of a substance, evidence shall include any or all of the following using a weight of evidence approach:

(a) positive data from patch testing, normally obtained in more than one dermatology clinic;

(b) epidemiological studies showing allergic contact dermatitis caused by the substance. Situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small;

(c) positive data from appropriate animal studies

(d) positive data from experimental studies in man (see section 1.3.2.4.7);

(e) well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic;

(f) severity of reaction may also be considered.

3.4.2.2.4.2. Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather aspart of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on skin sensitisation are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken. Negative human data should not normally be used to negate positive results from animal studies. For both animal and human data, consideration should be given to the impact of vehicle.

3.4.2.2.4.3. If none of the abovementioned conditions are met, the substance need not be classified as a skin sensitiser. However, a combination of two or more indicators of skin sensitisation as listed below may alter the decision. This shall be considered on a case-by-case basis.

(a) Isolated episodes of allergic contact dermatitis;

(b) epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in section 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant;

(d) positive data from non-standard methods;

(e) positive results from close structural analogues.

3.4.2.2.4.4. Immunological contact urticaria

Substances meeting the criteria for classification as respiratory sensitisers may in addition cause immunological contact urticaria. Consideration should be given to classifying these substances also as skin sensitisers. Substances which cause immunological contact urticaria without meeting the criteria for respiratory sensitisers should also be considered for classification as skin sensitisers.

There is no recognised animal model available to identify substances which cause immunological contact urticaria. Therefore, classification will normally be based on human evidence which will be similar to that for skin sensitisation.

Positive effects seen in either humans or animals for skin sensitisation will normally justify classification. Evidence from animal studies on skin sensitisation is usually more reliable than evidence from human exposure, although adequate reliable and respresentative human data are usually more relevant. In cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to decide on the classification on a case-by-case basis. Negative human data should not normally negate positive findings in animal studies (CLP Annex I, 3.4.2.2.4.2).

Since the data used in hazard or risk assessment should be relevant, reliable and sufficient for the regulatory purpose, it is necessary to base the assessment on the totality of available information, i.e. to apply Weight of Evidence (WoE) considerations.

The WoE assessment can be based on the total of experimental data, as well as post-market surveys and/or occupational experience data. In the case of mixtures, extrapolation from similar mixtures or from data available on the components may often provide reliable means

of assessment. Estimated data might be used to supplement and increase confidence in the available experimental data, whereas in some others, such data might be used instead of experimental data.

WoE assessment can be divided into two stages:

a) Assessment of each single test result and, if needed, of other data. It may be helpful to apply criteria for reliability as defined by Klimisch *et al* (1997). These criteria include details on the recognition of the test method, reporting detail, method relevance, test parameters, etc.
b) Comparison of the weighed single test results.

Good quality data on the substance itself have more weight than such data extrapolated from similar substances.

3.4.3.4 Decision on classification

According to CLP Annex I, 3.4.2.2.1.4 substances fulfilling the criteria for skin sensitisation will be classified as such in Category 1 (or in Sub-category 1A or 1B when sufficient data are available). In addition substances classified in Categories 1, 1A, or 1B for skin sensitisation can be allocated specific concentration limits as described in Section 3.4.3.5 of this Guidance.

3.4.3.5 Setting of specific concentration limits

SCLs for skin sensitisation can be set based on the results from animal testing as reported below. SCLs are set on the basis of testing of the substance and never on the basis of testing of a mixture containing the sensitising substance (see CLP Annex I, 3.4.3.1.1). Setting of SCL is based on potency; potency is already considered for subcategorisation defining generic concentration limits. SCL generally applies for the most potent skin sensitisers classified in 1A.

The following schemes can be used for determination of potency categories for sensitisers. The potency categories given in the 3 tables below are described in Basketter *et al*. (2005a).

For the LLNA(OECD TG 429):

Table 3.4.3.5.1: *Skin Sensitisation Potency in the Mouse Local Lymph Node Assay*

EC3-value (% w/v)	Potency	Predicted sub-category (*)
≤ 0.2	Extreme	1A
> 0.2 - ≤ 2	Strong	1A
> 2	Moderate	1B

(*) based on Annex I Section 3.4.2.2.3.2. and Section 3.4.2.2.3.3.

For the Guinea Pig Maximisation Test (OECD TG 406)

Table 3.4.3.5.2: *Potency on basis of the Guinea Pig Maximisation Test*

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted sub-category (*)
≤ 0.1	≥ 60	Extreme	1A
≤ 0.1	≥30 - <60	Strong	1A
>0.1 - ≤ 1.0	≥60	Strong	1A

>0.1 - ≤ 1.0 (**)	≥30 - <60 (**)	Moderate	1B
> 1.0 (**)	≥ 30 (**)	Moderate	1B

(*) based on CLP Annex I Section 3.4.2.2.3.2. and Section 3.4.2.2.3.3.

(**) If the concentration used for intradermal induction or the incidence of sensitised guinea pigs is very high, care should be taken to exclude the possibility of the substance being a Cat 1A (a strong or an extreme) sensitiser.

For the Buehler Assay, (OECD TG 406)

Table 3.4.3.5.3: *Potency on basis of the Buehler assay*

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted sub-category (*)
≤ 0.2	≥ 60	Extreme	1A
≤ 0.2	≥15 - <60	Strong	1A
>0.2 - ≤ 20	≥ 60	Strong	1A
>0.2 - ≤ 20 (**)	≥15 - <60 (**)	Moderate	1B
> 20 (**)	≥ 15 (**)	Moderate	1B

(*) based on CLP Annex I Section 3.4.2.2.3.2. and Section 3.4.2.2.3.3.
(**) If the concentration used for intradermal induction or the incidence of sensitised guinea pigs is very high, care should be taken to exclude the possibility of the substance being a Cat 1A (a strong or an extreme) sensitiser.

The generic concentration limits (GCLs) for the classification of sensitisers in mixtures are given in CLP Annex I, Table 3.4.5 (see Section 3.4.4.3.1 of this Guidance). In some cases, the GCL may not be sufficiently protective and an SCL shall be set in accordance with CLP Article 10, which will better reflect the hazard of mixtures containing that skin sensitiser.

SCLs shall be set when there is adequate and reliable scientific information available showing that the specific hazard is evident below the GCL for classification. As such the recommended SCL should normally be as given in Table 3.4.3.5.4. However, supported by reliable data the SCL could have some other value below the GCL. Reliable data could be human data from e.g. work place studies where the exposure is defined.

It is more difficult to prove the absence of sensitising properties at certain concentration levels. Therefore an SCL above the GCL may only be set in exceptional circumstances, if scientific information is adequate, reliable and conclusive for that particular skin sensitiser. However there is currently no guidance on how to set an SCL above the GCL.

The concentration limits for skin sensitisers categorised according to their sensitisation potency in the Table 3.4.3.5.4 are based on the recommendations from an EU expert group on skin sensitisation (Basketter *et al.*, 2005a).

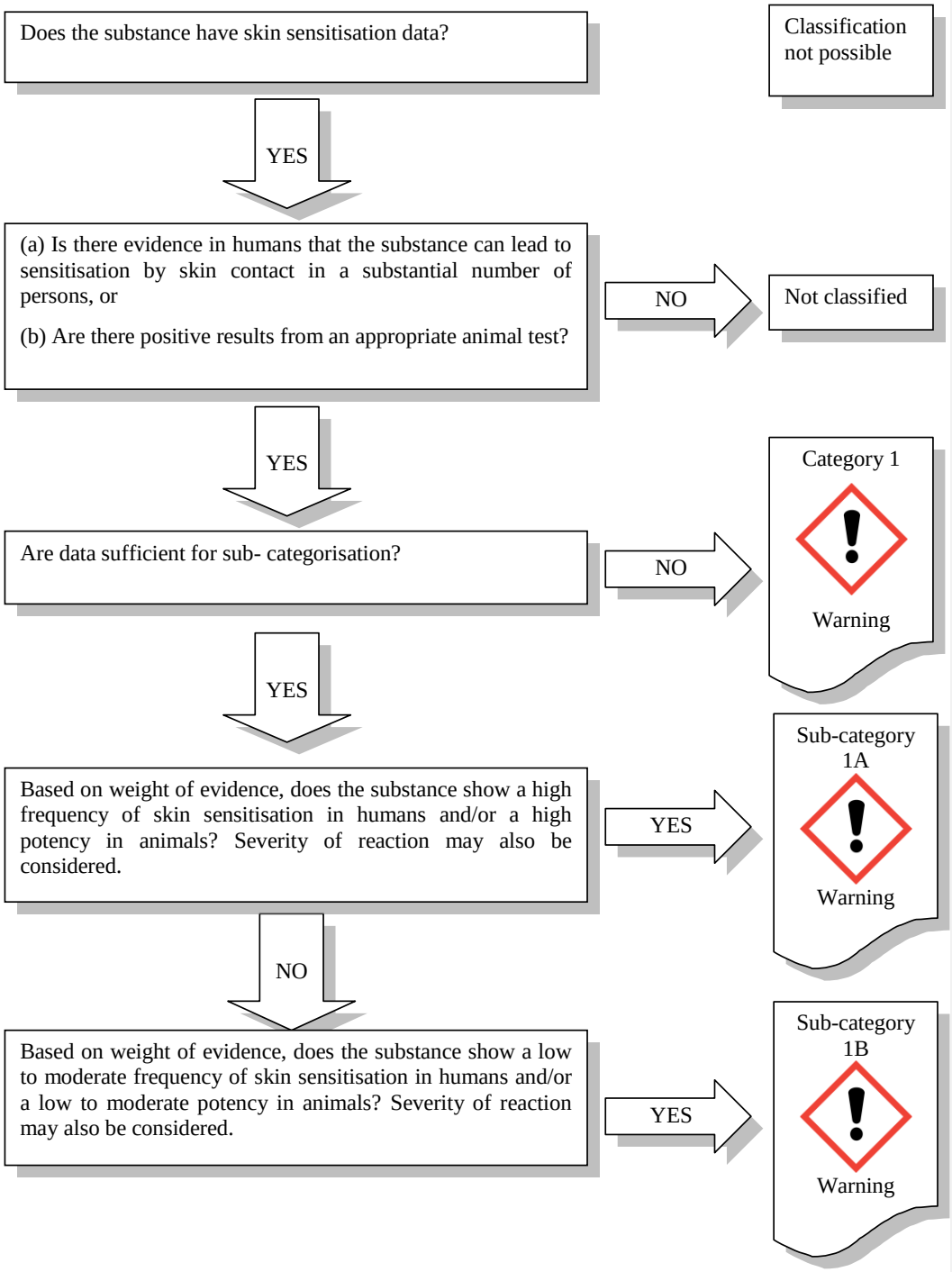
Table 3.4.3.5.4: Skin sensitising potency for substances and recommendations on concentration limits

Potency	Concentration Limit (% w/v)
Extreme	0.001 (SCL)
Strong	0.1 (GCL)
Moderate	1 (GCL)

3.4.3.6 Decision logic for classification of substances

It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

Decision logic for skin sensitisation



3.4.4 Classification of mixtures for respiratory or skin sensitisation

3.4.4.1 Identification of hazard information for respiratory sensitisation

The same principles apply as for substances (see Section 3.4.2 of this Guidance).

3.4.4.2 Identification of hazard information for skin sensitisation

For identification of the sensitisation potential of a mixture the following information may be available:

(a) test results on one or more, preferably all of its potentially sensitising components; or
(b) test results on the mixture itself; or
(c) test results of a similar mixture.

Test methods are outlined in Section 3.4.3.3.2 of this Guidance. However, these animal tests have been developed to identify sensitising substances and not mixtures. Therefore the results obtained on mixtures need to be evaluated with care. For a mixture the cut-off in the mouse LLNA should be seen as a threshold for identification of a sensitiser rather than as a threshold for sensitisation. A conclusion on the absence of sensitising potential of a mixture based on the negative outcome in a test must be taken with great caution.

On the other hand test data on a mixture takes into account effects of possible interactions of its components. For instance, it is known that the presence of a vehicle may significantly influence the skin sensitising potency, by influencing the penetration of the sensitising component(s) through the skin, (Basketter *et al.* 2001, Dearman *et al*. 1996, Heylings *et al*. 1996) or through other mechanisms involved in the acquisition of sensitisation (Cumberbatch *et al*. 1993; Dearman *et al*. 1996).

Repeated exposure to mixtures, that are non-sensitising under standard LLNA exposure conditions, might induce skin sensitisation, if the sensitising component in the mixture has sufficient accumulation potential in the skin to reach the minimum concentration for a positive effect (De Jong et al. 2007). Uncertainty also exists about the effect of such a mixture after exposure on a larger skin area. Therefore additional information is important, if the outcome of sensitisation tests on mixtures contrasts with the classification based on the content of sensitising component(s). For example, the validity of a well conducted LLNA on a mixture with a negative outcome can scientifically be confirmed by spiking the test mixture with another sensitiser (positive control) at different concentrations, or by showing a dose response relationship. Such LLNA tests could have been designed to provide such information without use of extra animals. Additional animal testing for the purpose of classification and labelling shall be undertaken only where no other alternatives, which provide adequate reliability and quality of data, are possible (CLP Article 7(1)).

3.4.4.3 Classification criteria for mixtures

When mixtures are classified as sensitizing based on the presence of a sensitizing substance at a concentration at or above the generic or specific concentration limit, no sub-categorisation is required.

3.4.4.3.1 When data are available for all ingredients or only for some ingredients

Annex I: 3.4.3.3.1. The mixture shall be classified as a respiratory or skin sensitiser when at least one ingredient has been classified as a respiratory or skin sensitiser and is present at or above the appropriate generic concentration limit as shown in Table 3.4.5 below for solid/liquid and gas respectively.

Table 3.4.5
Generic concentration limits of components of a mixture classified as either respiratory sensitisers or skin sensitisers that trigger classification of the mixture

Component classified as:	Concentration triggering classification of a mixture as:		
	Respiratory sensitiser Category 1		Skin sensitiser Category 1
	Solid/Liquid	Gas	All physical states
Respiratory sensitiser Category 1	≥ 1,0 %	≥ 0,2 %	
Respiratory sensitiser Sub-category 1A	≥ 0,1 %	≥ 0,1 %	
Respiratory sensitiser Sub-category 1B	≥ 1,0 %	≥ 0,2 %	
Skin sensitiser Category 1			≥ 1,0 %
Skin sensitiser Sub-category 1A			≥ 0,1%
Skin sensitiser Sub-category 1B			≥ 1,0 %

All sensitising components of a mixture at or above their generic or specific concentration limit should be taken into consideration for the purpose of classification. Specific concentration limits (see Section 3.4.3.5 of this Guidance) will always take precedence over the generic concentration limits.

The additivity concept is not applicable for respiratory or skin sensitisation, i.e. if one single classified substance is present in the mixture above the generic or specific concentration limit, the mixture must be classified for that hazard. If the mixture contains two substances each below the generic or specific concentration limits, the mixture will not be classified, as far as no SCL has been set.

Guidance update to legal text "Annex I: 3.4.3.3.2" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.4.3.3.2. Some substances that are classified as sensitisers may elicit a response, when present in a mixture in quantities below the concentrations established in Table 3.4.5, in individuals who are already sensitised to the substance or mixture (see Note 1 to Table 3.4.6).

Table 3.4.6
Concentration limits for elicitation of components of a mixture

Component classified as:	Concentration limits for elicitation		
	Respiratory sensitiser Category 1		Skin sensitiser Category 1
	Solid/Liquid	Gas	All physical states
Respiratory sensitiser Category 1	≥ 0,1 % (Note 1)	≥ 0,1 % (Note 1)	
Respiratory sensitiser Sub-category 1A	≥ 0,01 % (Note 1)	≥ 0,01 % (Note 1)	
Respiratory sensitiser Sub-category 1B	≥ 0,1 % (Note 1)	≥ 0,1 % (Note 1)	
Skin sensitiser Category 1			≥ 0,1 % (Note 1)
Skin sensitiser Sub-category 1A			≥ 0,01 % (Note 1)
Skin sensitiser Sub-category 1B			≥ 0,1 % (Note 1)
Note 1: This concentration limit for elicitation is used for the application of the special labelling requirements section 2.8 of Annex II to protect already sensitised individuals. A SDS is required for the mixture containing a component at or above this concentration. For sensitising substances with specific concentration limit lower than 0,1 %, the concentration limit for elicitation should be set at one tenth of the specific concentration limit.			

Further details on the additional labelling provisions to protect already sensitised individuals are provided in Section 3.4.5.2 of this Guidance.

3.4.4.3.2 When data are available for the complete mixture

Annex I: 3.4.3.1.1. When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture can be classified by weight-of-evidence evaluation of these data. Care shall be exercised in evaluating data on mixtures, that the dose used does not render the results inconclusive.

In case classification of a mixture is based on test results for the mixture as a whole, this data must be shown to be conclusive. Especially it should be taken into account that in case of skin sensitisation current test methods are based on application of maximised dose, which only can be obtained using a substance by itself and not diluted in a mixture.

It is recognised that mixtures not showing sensitisation in a test, may still contain a low concentration of sensitising component.

For specific guidance on the test methods and evaluation of the results see Section 3.4.4.2 of this Guidance and CLP Annex I, 3.4.3.1.1.

3.4.4.3.3 When data are not available for the complete mixture: Bridging Principles

Annex I: 3.4.3.2.1. Where the mixture itself has not been tested to determine its sensitising properties, but there are sufficient data on the individual ingredients and similar tested mixtures to

adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging rules out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture.

The same limitations apply for the use of existing test results of similar tested mixures generated with current test methods as those described for any mixture in sections 3.4.4.2 and 3.4.4.3.2. Care must be exercised in evaluating data on mixtures, that the dose used does not render the results inconclusive.

Note that the following bridging principles are not applicable to this hazard class:

- concentration of highly hazardous mixtures
- interpolation within one hazard category

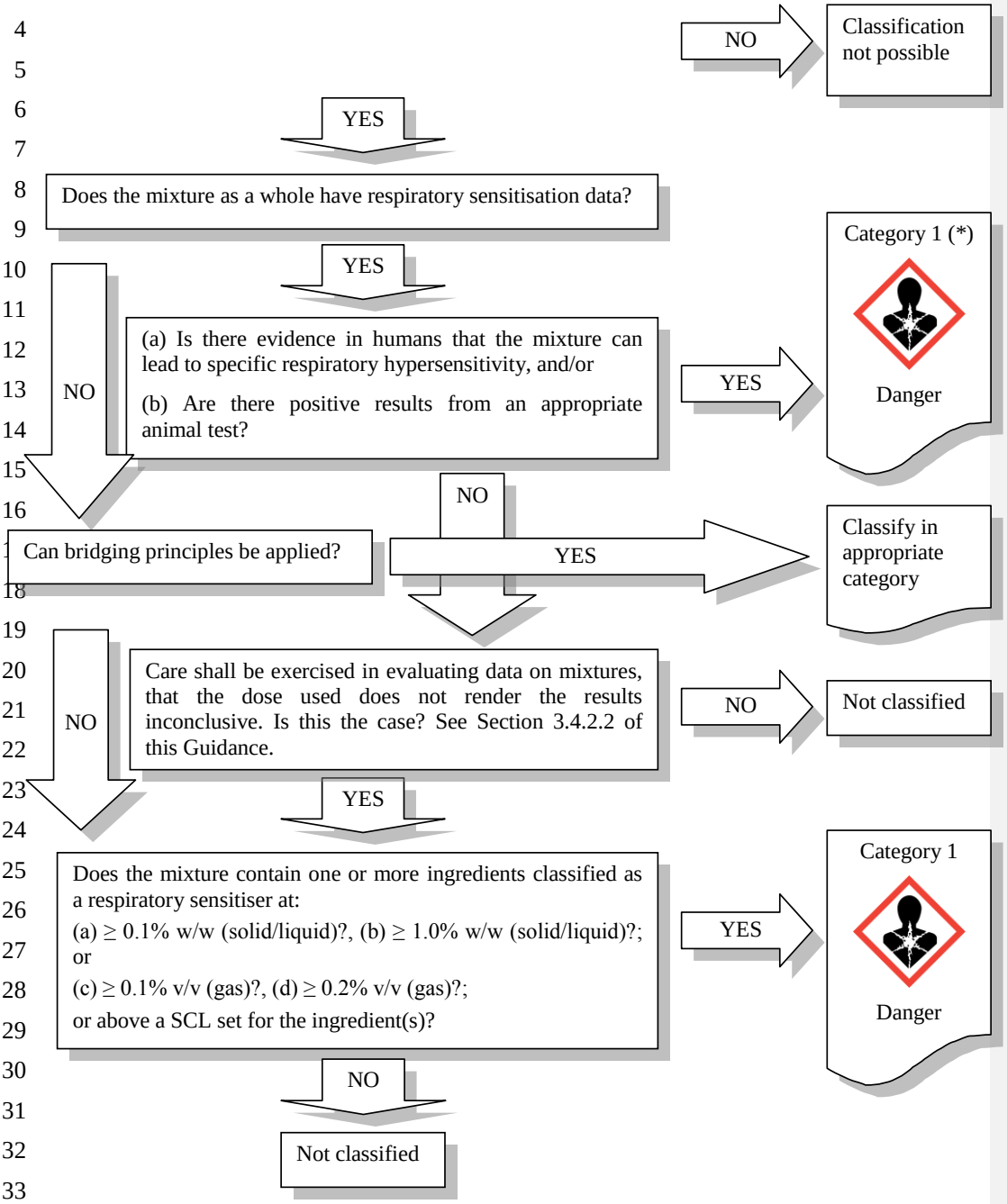
(see CLP Annex 1, 1.1.3.3 and 1.1.3.4).

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified using the method described in section 3.4.4.3.1 of this Guidance. .

3.4.4.4 Decision logic for classification of mixtures

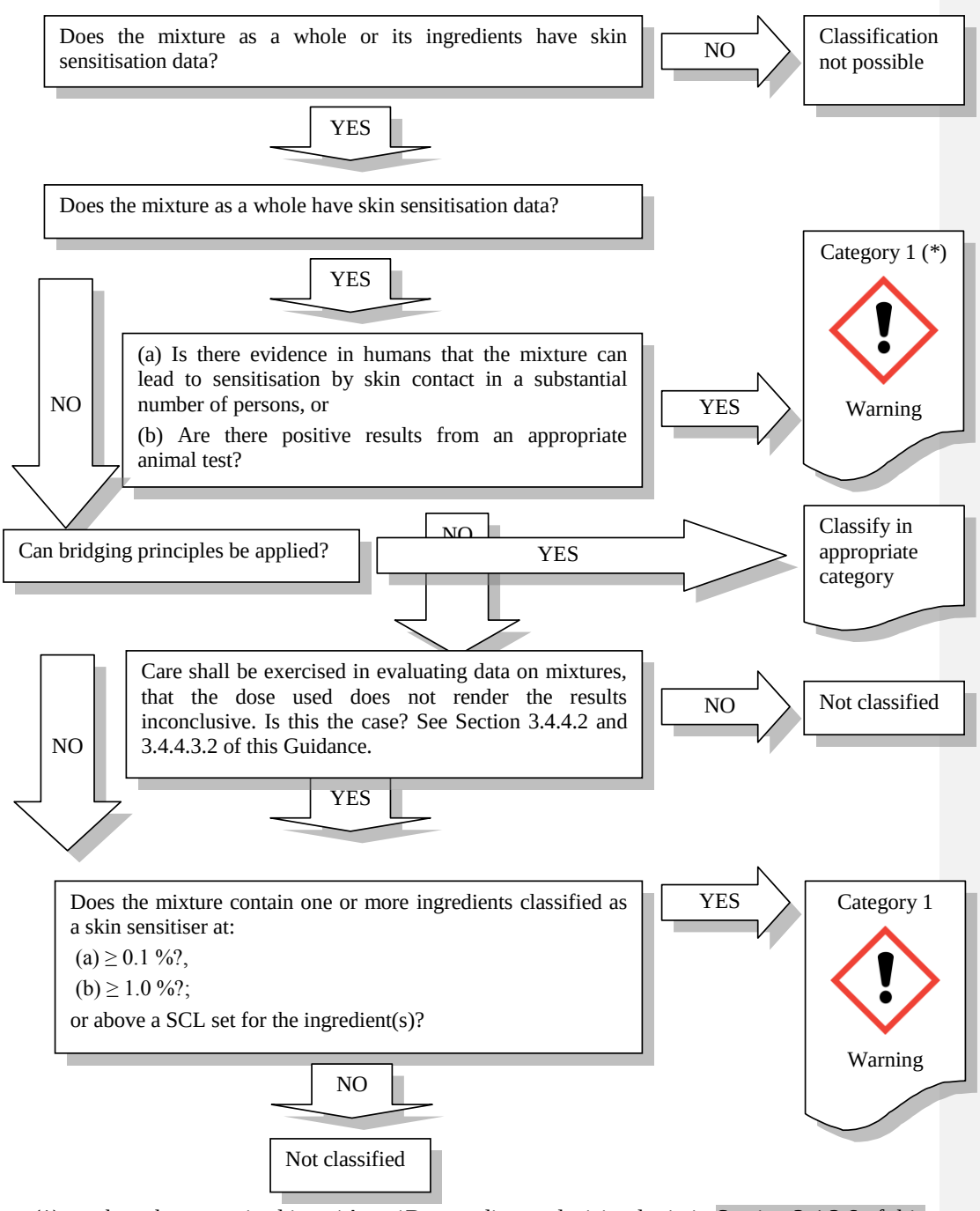
It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

3.4.4.4.1 Decision logic for respiratory sensitisation



(*) can be sub-categorised into 1A or 1B according to decision logic in Section 3.4.2.6 of this Guidance.

3.4.4.4.2 Decision logic for skin sensitisation



(*) can be sub-categorised into 1A or 1B according to decision logic in Section 3.4.3.6 of this Guidance.

3.4.5 Hazard communication for respiratory or skin sensitisation

3.4.5.1 Pictograms, signal words, hazard statements and precautionary statements

Guidance update to legal text "Annex I: Table 3.4.7" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.4.4.1. Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.4.7

Table 3.4.7

Respiratory or skin sensitisation label elements

Classification	Respiratory sensitisation	Skin sensitisation
	Category 1 and sub-categories 1A and 1B	Category 1 and sub-categories 1A and 1B
GHS Pictograms		
Signal Word	Danger	Warning
Hazard Statement	H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled	H317: May cause an allergic skin reaction
Precautionary Statement Prevention	P261 P285	P261 P272 P280
Precautionary Statement Prevention **4th ATP change**	P261 P284	P261 P272 P280
Precautionary Statement Response	P304 + P341 P342 + P311	P302 + P352 P333 + P313 P321 P363
Precautionary Statement Response **4th ATP change**	P304 + P340 P342 + P311	P302 + P352 P333 + P313 P321 P362 + P364
Precautionary Statement Storage		
Precautionary Statement Disposal	P501	P501

If the hazard pictogram "GHS08" applies for respiratory sensitisation, the hazard pictogram "GHS07" shall not appear for skin sensitisation or for skin and eye irritation (CLP, Article 26(1)(d)).

3.4.5.2 Additional labelling provisions

Annex II: 2.8. Mixtures containing at least one sensitising substance

The label on the packaging of mixtures not classified as sensitising but containing at least one substance classified as sensitising and present in a concentration equal to or greater than that specified in Table 3.4.6 of Annex I shall bear the statement:

EUH208 – 'Contains (name of sensitising substance). May produce an allergic reaction'.

Mixtures classified as sensitising containing other substance(s) classified as sensitising (in addition to the one that leads to the classification of the mixture) and present in a concentration equal to or greater than that specified in Table 3.4.6 of Annex I shall bear the name(s) of that/those substance(s) on the label.

3.4.6 Re-classification of substances and mixtures classified for respiratory or skin sensitisation according to DSD and DPD

3.4.6.1 Is direct "translation" of classification and labelling possible?

Direct translation from DSD to CLP Category 1 is possible for sensitising substances.

3.4.6.2 Re-evaluation of the skin sensitisation data

Re-evaluation of non-tested mixtures has to be done on the basis of any relevant new data that might have become available after the time of the latest classification or if an SCL has been set.

3.4.7 Examples of classification for skin sensitisation

3.4.7.1 Example of substances and mixtures fulfilling the criteria for classification for skin sensitisation

Example 1

Substance X gave a positive result in the LLNA with an EC3-value of 10.4%. As this EC3-value is above the cut-off of 2%, the substance is considered to be a moderate skin sensitiser, and should be classified as a Category 1 (Sub-category 1B) skin sensitiser. The GCL for classification of mixtures containing substance X is 1%.

Example 2

Substance Y tested positive in the LLNA with an EC3-value of 0.5%. In the GPMT a dermal induction concentration of 0.375% produced a positive response in 70% of the animals. On the basis of both these positive results, the substance is considered to be a strong sensitiser requiring classification as a Category 1 (Sub-category 1A) skin sensitiser. The GCL for classification of mixtures containing substance Y is 0.1%.

Example 3

Herby is a herbicide formulation containing 28 g/l substance X, a Sub-category 1B skin sensitiser (see example 1). There is no sensitisation data for the formulation itself. As Herby contains more than the GCL (1%) of this sensitising substance, and in the absence of any additional information, it should be classified as a Category 1 skin sensitiser.

Example 4

Substance Z being an extreme sensitiser, is classified as a Sub-category 1A. It has a specific concentration limit with regard to skin sensitisation of 0.001%, and due to this property any mixture containing the substance at a concentration ≥ 0.001% must be classified as Category 1 skin sensitiser.

Example 5

Woody is a wood preservative containing 2 strong sensitising substances (Sub-category 1A): substance A is present at 1% and substance B is present at 0.05%. There are no data for the formulation itself. The mixture will be classified as cat 1 H317, due to the content of substance A (present above the GCL of 0.1%). Substance B is present below the classification limit. The name of both substances should appear on the label, substance A because it determines the classification of the mixture, and substance B because it is present in a concentration above the elicitation level (1/10 of the GCL of 0.1%).

Example 6

Substance C was tested in a reduced LLNA test in accordance with OECD 429 using a concentration of 25%. This resulted in a stimulation index (SI) of 20 compared to the concurrent control. This is clearly above the SI of 3 required for classification. Therefore, classification as a skin sensitiser is required. However, the available information does not allow calculating an EC3 value required for determining the sub-categorisation. Although the substance was clearly positive at a high concentration of 25%, it cannot be excluded that also at a concentration of 2% or lower the SI will be 3. Therefore, there is not sufficient data for sub-categorisation. The substance is classified as Skin Sens Cat 1.

Example 7

Substance D gave a positive response in a guinea pig maximisation test with 90 % responding at 50 % intradermal induction dose. In a Buehler assay 70% responded at 30 % topical induction dose. The response in both GPMT and Buehler assay was > 60% and the substance was not tested at ≤ 1 % intradermal induction dose in the guinea pig maximisation test or at ≤ 20 % topical induction dose in the Buehler assay. Although the criteria for classification to subcategory 1B are fulfilled, the classification for subcategory 1A cannot be excluded and therefore the substance should be classified as a Category 1 skin sensitiser.

Example 8

If there are contradictory results from two or more skin sensitisation tests, the following examples will give guidance for the classification. Since these are ideal cases, the weight of evidence approach should be applied if studies indicate shortcomings/are not considered fully reliable.

- **8(a):** Substance E was tested in three separate animal tests performed with different test methods. In a Buehler assay no responses were observed with a topical induction

dose of 70%. In the LLNA the EC3 value was 0.8%, indicating classification for subcategory 1A. In GPMT, 30 % response was observed with an intradermal induction dose of 0,5 %, indicating classification for subcategory 1B. The substance should be classified for Skin Sens. Cat. 1A unless there is sufficient information to discount some of the results.

- **8(b):** Substance F is a skin sensitiser in humans indicating classification for sub-category 1A and in animals indicating classification for sub-category 1B. The substance should be classified for Skin Sens. Cat. 1A.
- **8(c):** Substance G is a skin sensitiser in animal test indicating classification for sub-category 1A and in humans indicating classification for category 1. The substance should be classified for Skin Sens. Cat. 1A.

3.4.7.2 Example of substances or mixtures not fulfilling the criteria for classification for skin sensitisation

Example 9

Substance H was tested at concentrations up to 50% in the LLNA using a recommended and appropriate vehicle. It gave a maximum stimulation index of 2.6 and evidence of a positive dose response. On the basis that the stimulation index was below 3 at a high dose, the substance does not require classification. However, had the highest concentrations been lower, e.g. 10%, and/or a non-standard vehicle used, then further information would be required before a classification decision could be reached.

Example 10

Insecto super is an insecticide formulation containing 9 g/l substance X (see Example 1). Substance X is a Sub-category 1B skin sensitiser (generic concentration limit in mixtures 1%). Based on the classification of substance X, the insecticide formulation shall not be classified as sensitising as the concentration of the substance is below the GCL of 1%. The label must bear the statement EUH208.

3.4.7.3 Examples of substances fulfilling the criteria for classification for respiratory sensitisation

Example 11

Five case studies describe that work-related exposure to substance P is associated with asthma or rhinitis. In all of these cases blinded specific bronchial challenge tests with substance P provoked the respiratory symptoms, confirming that substance P is the causal substance.

In a cohort of 51 workers exposed to substance P, 26 (51%) were diagnosed with occupational asthma and 12 of those also suffered from occupational rhinitis. The diagnosis was based on specific bronchial challenge tests with substance P.

There is sufficient human evidence to conclude that substance P should be classified as a category 1 respiratory sensitizer. Sub-categorization was not considered as there is currently no clear way to establish sub-categories .

Example 12

Work-related exposure to substance Q was associated with occupational asthma and rhinitis in several case studies. In those studies specific bronchial challenges were performed with substance Q and respiratory allergy symptoms could be reproduced, demonstrating that substance Q is the causal agent . In addition, a large retrospective analysis of nine longitudinal studies involving 2,689 persons exposed occupationally to substance Q in a period of 35 years, showed that the incidences of occupational asthma caused by substance Q were 2.7-5.5% in the earliest studies and decreased to 0.3-0.7% in the latest studies.

Guinea pigs were exposed to substance Q by inhalation for 3 hours a day for 5 consecutive days to concentrations of 4, 12, 24, and 48 mg/m^3. Three weeks after the first encounter with the inducing agent, animals were challenged with substance Q at a concentration of 2 mg/m^3. During challenge breathing patterns were affected already at the lowest test concentration in guinea pigs that were sensitized and challenged to substance Q and not in control animals. Additionally, pulmonary inflammation and increased specific IgG1 levels were observed in guinea pigs sensitized and challenged with substance Q.

On the basis of human evidence supported by data from an animal study, substance Q should be classified as a Category 1 respiratory sensitizer. Sub-categorization was not considered as there is currently no clear way to establish sub-categories .

3.4.8 References

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3.5 GERM CELL MUTAGENICITY

3.5.1 Definitions and general considerations for classification for germ cell mutagenicity

Annex I: 3.5.1.1. A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

3.5.1.2. The more general terms 'genotoxic' and 'genotoxicity' apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

Germ cell mutations are those that occur in the egg or sperm cells (germ cells) and therefore can be passed on to the organism's offspring. Somatic mutations are those that happen in cells other than the germ cells, and they cannot be transmitted to the next generation. This is an important distinction to keep in mind in terms of both the causes and the effects of mutation.

Annex I: 3.5.2.1 This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests *in vitro* and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances and mixtures within this hazard class.

Annex I: 3.6.2.2 *Specific considerations for classification of substances as carcinogens*

3.6.2.2.6. […] Mutagenicity: It is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

Hazard classification for germ cell mutagenicity primarily aims to identify substances causing heritable mutations or being suspected of causing heritable mutations. A secondary aim is that the hazard class germ cell mutagenicity offers supporting information with respect to the classification of carcinogenic substances. This is expressed by the broad meaning of the hazard statements "H340: May cause genetic defects" and "H341: Suspected of causing genetic defects" which comprises heritable genetic damage as well as somatic cell mutagenicity. Thus, classification as a germ cell mutagen (Category 1A, 1B, and 2) classifies for the hazard heritable genetic damage as well as providing an indication that the substance could be carcinogenic.

It is also warranted that where there is evidence of only somatic cell genotoxicity, substances are classified as suspected germ cell mutagens. Classification as a suspected germ cell mutagen may also have implications for potential carcinogenicity classification. This holds true especially for those genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, "site of contact" genotoxicants). This means that if positive results *in vitro* are supported by at least one positive local *in vivo*, somatic cell test, such an effect should be considered as enough evidence to lead to classification in Category 2. If there is also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

3.5.2 Classification of substances for germ cell mutagenicity

3.5.2.1 Identification of hazard information

3.5.2.1.1 Identification of human data

Occasionally, studies of genotoxic effects in humans exposed by, for example, accident, occupation or participation in clinical studies (e.g. from case reports or epidemiological studies) may be available. Generally, cells circulating in blood are investigated for the occurrence of various types of genetic alterations; see also the Guidance on IR/CSA, Section R.7.7.3.2.

3.5.2.1.2 Identification of non human data

Animal data

Some test methods have an officially adopted EU/OECD guideline for the testing procedure, although for many test methods this is not the case. Furthermore, modifications to OECD protocols have been developed for various classes of substances and may serve to enhance the accuracy of test results. Use of such modified protocols is a matter of expert judgement and will vary as a function of the chemical and physical properties of the substance to be evaluated. Commonly used non-guideline *in vivo* tests employ methods by which any tissue of an animal can be examined for effects on the genetic material, giving the possibility to examine site-of-contact tissues (*i.e.*, skin, epithelium of the respiratory or gastro-intestinal tract) in genotoxicity testing. In addition, test methods developed over the past decades in

Drosophila and in various species of plants and fungi are available; see also the Guidance on IR/CSA, Section R.7.7.3[8].

Other *in vivo* tests in somatic cells which provide supporting evidence on genotoxicity/mutagenicity may include, for example, a Comet single cell gel electrophoresis assay for DNA strand breaks, or a test for gene mutations in transgenic rodent models[9] using reporter genes.

With the exception of *in vivo* studies proving "site of contact" effects, genotoxicity data from such non-standard *in vivo* studies are not sufficient but may offer supporting information for classification.

In vitro data

Typically, *in vitro* tests are performed with cultured bacterial cells, human or other mammalian cells. The sensitivity and specificity of tests will vary with different classes of substances; see also the Guidance on IR/CSA, Section R.7.7.3.

Use of other data

See the Guidance on IR/CSA, Section R. 7.7.3.1.

Existing test methods

See the Guidance on IR/CSA, Section R. 7.7.3.1.

3.5.2.2 Classification criteria for substances

Annex I: 3.5.2.2. For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories as shown in Table 3.5.1.

Table 3.5.1
Hazard categories for germ cell mutagens

Categories	Criteria
CATEGORY 1:	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A:	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B:	The classification in Category 1B is based on: − positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or − positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the

[8] The Guidance on IR/CSA, Section R.7.7.3 is currently undergoing an update and consultation with publication foreseen for Dec 2013.
[9] OECD TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (28 July 2011)

	substance or its metabolite(s) to interact with the genetic material of germ cells; or − positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
CATEGORY 2:	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: − Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: − Somatic cell mutagenicity tests in vivo, in mammals; or − Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

3.5.2.3 Evaluation of hazard information

Annex I: 3.5.2.3.3 Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 ('Test Method Regulation') such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

3.5.2.3.1 Evaluation of human data

Human data have to be assessed carefully on a case-by-case basis. The interpretation of such data requires considerable expertise. Attention should be paid especially to the adequacy of the exposure information, confounding factors, co-exposures and to sources of bias in the study design or incident. The statistical power of the test may also be considered (see the Guidance on IR/CSA, Section R.7.4.4.2).

3.5.2.3.2 Evaluation of non human data

Evaluation of genotoxicity test data should be made with care. Regarding *positive* findings, responses generated only at highly toxic/cytotoxic concentrations should be interpreted with caution, and the presence or absence of a dose-response relationship should be considered. In case of *negativ*e findings *in vivo* toxicokinetic and other available information should be considered e.g. to verify whether the substance has reached the target organ (for detailed guidance see the Guidance on IR/CSA, Section R.7.7.4.1).

3.5.2.4 Decision on classification

Annex I: 3.5.2.3.1. To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in in vitro tests shall also be considered.

Annex I: 3.5.2.3.9. The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1). In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results. If

new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the most likely route of human exposure shall also be taken into account.

Classification as a Category 1A mutagen

Epidemiological studies have been to date unable to provide evidence to classify a substance as a Category 1A mutagen. Hereditary diseases in humans for the most part have an unknown origin and show a varying distribution in different populations. Due to the random distribution of mutations in the genome it is not expected that one particular substance would induce one specific genetic disorder. Therefore, it is unlikely that such evidence may be obtained by epidemiological studies to enable you to classify a substance as a Category 1A mutagen.

Classification as a Category 1B mutagen

Classification in Category 1B may be based on positive results of at least one valid *in vivo* mammalian germ cell mutagenicity test. In case there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

If there are only positive results of at least one valid *in vivo* mammalian somatic mutagenicity test but no respective data on mammalian germ cells are available, additional evidence is required to be able to classify as mutagen in Category 1B. Such additional data must prove that the substance or its metabolite(s) interacts *in vivo* with the genetic material of germ cells. It is also possible to obtain supporting evidence in an *in vivo* genotoxicity test with mammalian germ cells. In addition, genetic damage to germ cells in exposed humans proven to be caused by substance exposure may offer respective information. In case of other supporting evidence or where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

It could be argued that in a case where *in vivo* mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, then that substance should also be considered as a Category 1B mutagen. Germ cell mutagens as the spermatogonia are generally not protected from substance exposure by the blood-testes barrier formed by the Sertoli cells. In such circumstances the relevant criteria are as follows:

Annex I: 3.5.2.2. (extract from Table 3.5.1)

Category 1B
[…]
- positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells;

[…]

This wording expresses that supporting evidence in addition to an *in vivo* somatic cell mutagenicity test in mammals is needed to be able to classify a substance as a Category 1B mutagen. The second sentence in the green box above gives examples for such evidence, from these examples it is clear that such supporting evidence is experimental evidence. There has to be either data indicating that germ cell mutagenicity/genotoxicity is caused by the substance or data showing that the substance or its metabolite(s) interact with the genetic material of germ cells. Thus, in such circumstances, in addition to an *in vivo* somatic cell

mutagenicity test, further experimental evidence is needed to be able to classify a substance as a Category 1B mutagen.

Classification as a Category 2 mutagen

Classification in Category 2 may be based on positive results of a least one *in vivo* valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of a least one *in vivo* valid mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results. Genetic damage to somatic cells in exposed humans shown to be caused by substance exposure supported by positive *in vitro* mutagenicity results may also offer respective information warranting classification as a Category 2 mutagen. *In vitro* results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

In general, mutations can be differentiated into gene mutations (e.g. point or frame shift mutation), chromosome mutations (structural chromosome changes) and genome mutations (loss or gain of whole chromosomes). Different mutagenicity tests may detect different types of mutations and genotoxic effects which have to be taken into account in the weight of evidence determination. For instance, a substance which only causes chromosome mutations may be negative in a test for detecting point mutations. A complex data situation with positive and negative results might still lead to classification. This is because all tests detecting a certain type of mutation (e.g. point mutations) have been positive and all tests detecting chromosome mutations have been negative. Such circumstances clearly warrant classification although several tests have been negative which is plausible in this case.

A positive result for somatic or germinal mutagenicity in a test using intraperitoneal administration only shows that the tested substance has an intrinsic mutagenic property, and the fact that negative results are exhibited by other routes of dosage may be related to factors influencing the distribution/ metabolism of the substance which may be characteristic to the tested animal species. It cannot be ruled out that a positive test result in intraperitoneal studies in rodents only may be relevant to humans.

If there are positive results in at least one valid *in vivo* mutagenicity test using intraperitoneal application, or from at least one valid *in vivo* genotoxicity test using intraperitoneal application plus supportive *in vitro* data, classification is warranted. In cases where there are additional data from further *in vivo* tests with oral, dermal or inhalative substance application, a weight of evidence approach using expert judgement has to be applied in order to to come to a decision. For instance, it may be difficult to reach a decision on whether or not to classify in the case where there are positive *in vivo* data from at least one *in vivo* test using intraperitoneal application but (only) negative test data from (an) *in vivo* test(s) using oral, dermal, or inhalative application. In such a case, it could be argued that mutagenicity/genotoxicity can only be shown at internal body substance concentrations which cannot be achieved using application routes other than intraperitoneal. However, it also has to be taken into account that there is generally no threshold for mutagenicity unless there is specific proof for the existence of such a threshold as may be the case for aneugens. Thus, if mutagenicity/genotoxicity can only be demonstrated for the intraperitoneal route exclusively, then this may mean that the effect in the *in vivo* tests using application routes other than intraperitoneal may have been present, but it may not have been detected because it was below the detection limit of the oral, dermal, or inhalative test assays.

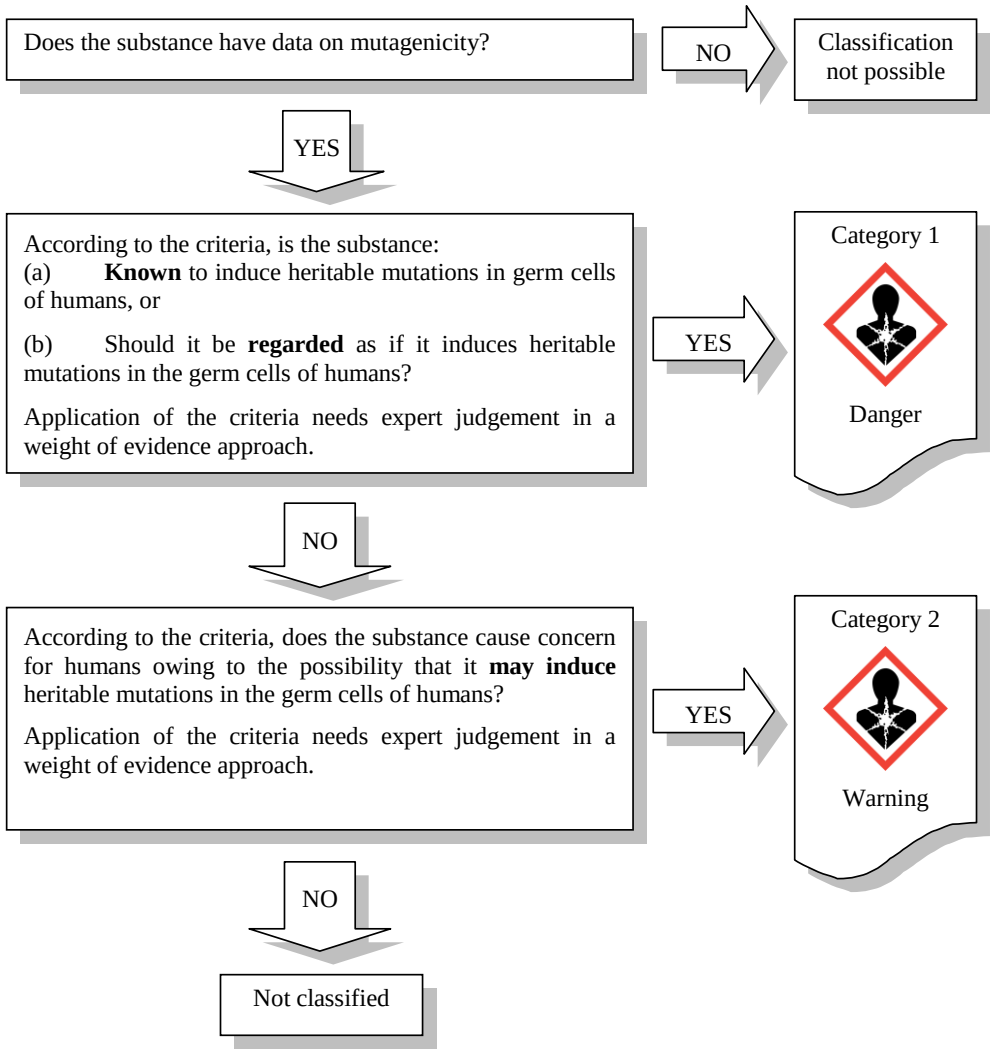
In summary, classification as a Category 2 mutagen would generally apply if only intraperitoneal *in vivo* tests show mutagenicity/genotoxicity and the negative test results from the *in vivo* tests using other routes of application are plausible. Factors influencing plausibility are e.g. the doses tested and putative kinetic data on the test substance. However, on a case-by-case analysis using a weight of evidence approach and expert judgement, non-classification may also result.

3.5.2.5 Setting of specific concentration limits

There is no detailed and accepted guidance developed for the setting of specific concentration limits (SCLs) for mutagenicity, as is the case for carcinogenic substances. Guidance such as the T_{25} concept for carcinogens covering all relevant aspects would need to be developed in order to derive SCLs for mutagens in a standardized manner. There are several reasons why it is considered impossible to set SCLs for mutagens without a comprehensive guidance, one of them being that mutagenicity tests have not been specifically developed for the derivation of a quantitative response. Moreover, different mutagenicity tests have different sensitivities in detecting mutagens. Thus, it is very difficult to describe the minimum data requirements which would allow a standardized SCL derivation. Another drawback in practice is that the results obtained for the most part do not offer sufficient information on dose-response, especially in the case for *in vivo* tests. In conclusion, the possibility to set SCL for germ cell mutagenicity is therefore not considered possible in the process of self-classification as there is no standardized methodical approach available which adequately takes into account all relevant information.

3.5.2.6 Decision logic for substances

The decision logic which follows is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logic.



3.5.3 Classification of mixtures for germ cell mutagenicity

3.5.3.1 Classification criteria for mixtures

Classification of mixtures will be based on the available test data for the individual ingredients of the mixture, using concentration limits for those ingredients. Under rare circumstances, the classification may be modified on a case-by-case basis based on the available test data for the mixture as a whole or based on bridging principles (see CLP Article 6(3) and CLP Annex I, 3.5.3.2 and 3.5.3.3).

3.5.3.1.1 When data are available for the complete mixture

Annex I: 3.5.3.2.1. Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients classified as germ cell mutagens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of germ cell mutagenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.5.3.1.1 When data are not available for the complete mixture: bridging principles

Annex I: 3.5.3.3.1. Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to paragraph 3.5.3.2.1), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

Bridging principles will only be used on a case by case basis (see section 3.5.4.1 of this guidance). Note that the following bridging principles are not applicable to this hazard class:

- concentration of highly hazardous mixtures
- interpolation within one hazard category

(see CLP Annex 1, 1.1.3.3 and 1.1.3.4)

3.5.3.2 Generic concentration limits for substances triggering classification of mixtures

Guidance update to legal text "Annex I: 3.5.3.1.1 Table 3.5.2" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.5.3.1.1. The mixture shall be classified as a mutagen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 mutagen and is present at or above the appropriate generic concentration limit as shown in Table 3.5.2 for Category 1A, Category 1B and Category 2 respectively.

Table 3.5.2

Generic concentration limits of ingredients of a mixture classified as germ cell mutagens that trigger classification of the mixture.

	Concentration limits triggering classification of a mixture as:

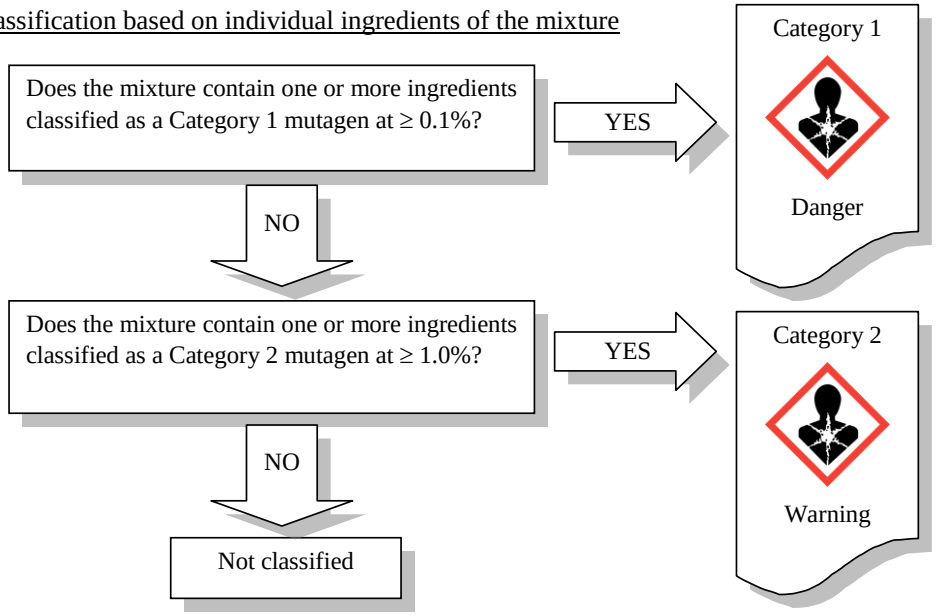
Ingredient classified as:	Category 1 mutagen		Category 2 mutagen
	Category 1A	Category 1B	
Category 1A mutagen	≥ 0,1 %	—	—
Category 1B mutagen	—	≥ 0,1 %	—
Category 2 mutagen	—	—	≥ 1,0 %
Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).			

The option to set SCL for germ cell mutagenicity is not considered possible in the process of self-classification as there is no standardized methodical approach available which adequately takes into account all relevant information (see Section 3.5.2.5 of this Guidance).

3.5.3.3 Decision logic for mixtures

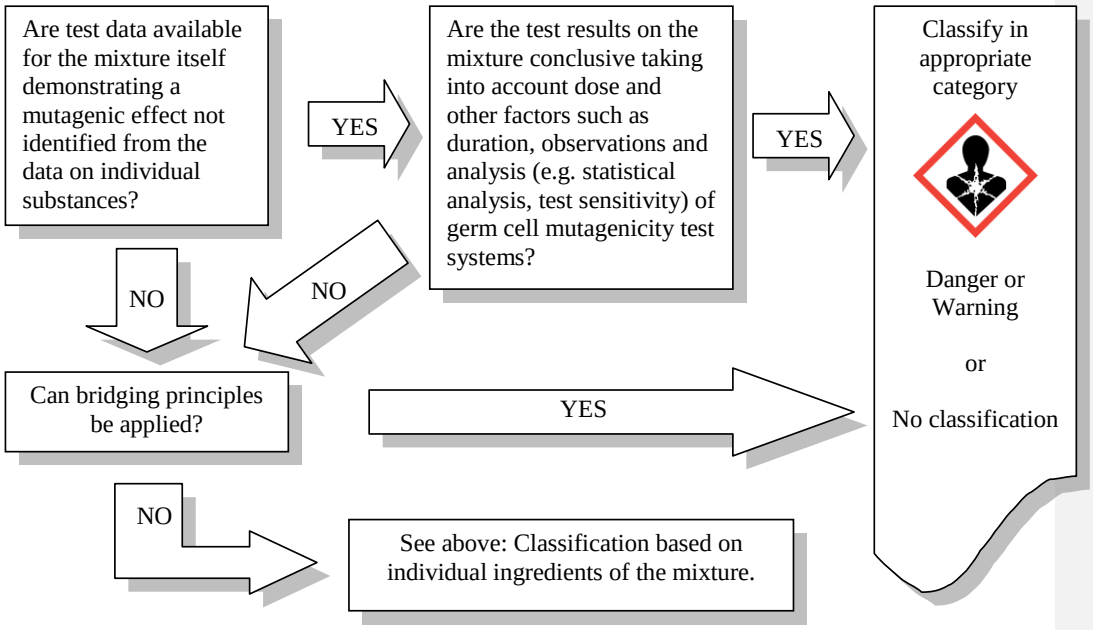
The decision logic which follows is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logic. This decision logic deviates (slightly) from the original GHS guidance, to meet CLP requirements.

Classification based on individual ingredients of the mixture



Modified classification on a case-by-case basis

Test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients (CLP Annex I, 3.5.3.2.1, see also CLP Article 6(3)).



3.5.4 Hazard communication in form of labelling for germ cell mutagenicity

3.5.4.1 Pictograms, signal words, hazard statements and precautionary statements

Guidance update to legal text "Annex I: Table 3.5.3" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.5.4.1. Label elements shall be used in accordance with Table 3.5.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.5.3

Label elements of germ cell mutagenicity

Classification	Category 1 (Category 1A, 1B)	Category 2
GHS Pictograms		
Signal Word	Danger	Warning
Hazard Statement	H340: May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281
Precautionary Statement Prevention **4th ATP change**	P201 P202 P280	P201 P202 P280
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

The hazard statement to be applied for the classification germ cell mutagenicity has to be amended to state the route of exposure if it is conclusively proven that no other routes of exposure will lead to the respective effect. A conclusive proof means that valid *in vivo* test data need to be available for all three exposure routes clearly indicating that only one exposure route leads to positive results. Moreover, such findings should be plausible with respect to the mode of action. It is estimated that such circumstances rarely, if ever, exist. Therefore, amending the hazard statement with the route of exposure generally does not have to be considered.

3.5.4.2 Additional labelling provisions

There are no additional labelling provisions for substances and mixtures classified for germ cell mutagenicity in CLP, however there are provisions laid out in Annex XVII to REACH. The packaging of substances with harmonised classification as germ cell mutagenicity Category 1A or Category 1B, and mixtures containing such substances at concentrations warranting classification of the mixture as germ cell mutagenicity Category 1A or Category 1B, "must be marked visibly**,** legibly and indelibly as follows: 'Restricted to professional users'." (REACH Annex XVII, point 29. Derogations from this obligation are outlined in the same provision).

3.5.5 Re-classification of substances/mixtures classified for germ cell mutagenicity according to DSD and DPD

Direct translation of classification and labelling is generally possible for substances and mixtures classified as germ cell mutagens.

In CLP, there is clear discrimination of *in vivo* mutagenicity tests and *in vivo* genotoxicity tests with respect to their relevance for classification. Moreover, in some circumstances which are assumed to occur very rarely if at all, a different classification may be the consequence if expert judgement is not applied.

For instance, positive results from studies showing mutagenic effects in germ cells of exposed humans can lead to classification as a Category 1B mutagen under CLP. However, using the criteria in DSD it is not clear how to classify in such a case. Moreover, *in vivo* somatic cell genotoxicity tests need to be supported by *in vitro* data in order to classify as a Category 2 mutagen under CLP. In such circumstances under DSD, *in vivo* data do not necessarily need to be supported by *in vitro* data. However, it has to be taken into account that such circumstances will rarely occur as the testing strategy uses *in vitro* tests as a starting point.

3.6 CARCINOGENICITY

3.6.1 Definitions and general considerations for classification for carcinogenicity

Annex I: 3.6.1.1. Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

More explicitly, chemicals are defined as carcinogenic if they induce tumours, increase tumour incidence and/or malignancy or shorten the time to tumour occurrence. Benign tumours that are considered to have the potential to progress to malignant tumours are generally considered along with malignant tumours. Chemicals can potentially induce cancer by any route of exposure (e.g. when inhaled, ingested, applied to the skin or injected), but carcinogenic potential and potency may depend on the conditions of exposure (e.g., route, level, pattern and duration of exposure).

Carcinogenic chemicals have conventionally been divided according to the presumed mode of action; genotoxic or non-genotoxic, see Section 3.6.2.3.2 (k) of this Guidance.

Classification of a substance as a carcinogen is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant

information (weight of evidence) being taken into account as appropriate. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. A number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans (weight of evidence determination). The list of factors for additional consideration is long and requires the most up-to-date scientific knowledge. It is recognised that, in most cases, expert judgement is necessary to be able to determine the most appropriate category for classification for carcinogenicity.

3.6.2 Classification of substances for carcinogenicity

3.6.2.1 Identification of hazard information

Carcinogens may be identified from epidemiological studies, from animal experiments and/or other appropriate means that may include (Quantitative) Structure-Activity Relationships ((Q)SAR) analyses and/or extrapolation from structurally similar substances (read-across). In addition some information on the carcinogenic potential can be inferred from *in vivo* and *in vitro* germ cell and somatic cell mutagenicity studies, *in vitro* cell transformation assays, and gap junction intercellular communication (GJIC) tests.

Extensive guidance on data requirements, information sources and strategies for the identification of potential carcinogens are given in the Guidance on IR/CSA, Section R.7.7.9 (Information requirements on carcinogenicity) and Section R.7.7.10 (Information and its sources on carcinogenicity) and for potential mutagens Section R.7.7.3 (Information and its sources on mutagenicity).

For more about non testing data see Section 3.6.2.3.4 of this Guidance.

3.6.2.2 Classification criteria for substances

Substances are classified according to their potential to cause cancer in humans. In some cases there will be direct evidence on the carcinogenicity to humans from epidemiological studies. However, in most cases the available information on carcinogenicity will be primarily from animal studies. In this case the relevance of the findings in animals to humans must be considered.

Annex I: 3.6.2.1. For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard.

Table 3.6.1

Hazard categories for carcinogens

Categories	Criteria

CATEGORY 1:	Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A:	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B:	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
	The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: − human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or − animal experiments for which there is sufficient ([1]) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
CATEGORY 2:	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited([1]) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
([1]) Note: See 3.6.2.2.4.	

3.6.2.3 Evaluation of hazard information

Annex I: 3.6.2.2.1. Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause cancer. The evaluations shall be based on all existing data, peer-reviewed published studies and additional acceptable data.

3.6.2.2.2. Classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories.

Classification of a substance as a carcinogen requires expert judgement and consideration of many different factors (weight and strength of evidence) included in the hazard information on carcinogenicity. The guidance provides an approach to data analysis rather than hard and fast rules. A stepwise approach to the classification can be taken where all the factors, both weight and strength of evidence, that may influence the outcome are considered systematically. Such approach, including consideration of these factors is outlined, in McGregor *et al*, 2009 and Boobis *et al*, 2006. Also the IPCS "Conceptual Framework for Evaluating a Mode of Action for Chemical carcinogenesis" (2001), ILSI "Framework for

Human Relevance Analysis of Information on Carcinogenic Modes of Action" (Meek *et al*., 2003; Cohen *et al*, 2003, 2004) and the International Agency for Research on Cancer (IARC, 2006 - Preamble Section B) provide a basis for systematic assessments which may be performed in a consistent fashion internationally; however they are not intended to provide lists of criteria to be checked off.

Specific considerations that are necessary are outlined in CLP Annex I, 3.6.2.2.3 (see Section 3.6.2.3.1 of this Guidance) and other important factors to consider in CLP Annex I, 3.6.2.2.6 (see Section 3.6.2.3.2 of this Guidance). Further guidance on these important factors is given in this document.

3.6.2.3.1 Specific considerations for classification

There is a strong link between CLP and the IARC classification criteria. The definitions for sufficient and limited evidence as defined by IARC are part of the criteria (CLP Annex I, 3.6.2.2.3). IARC, however, understands the criteria of "sufficient" and "limited" as follows: 'It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.' (IARC 2006 preamble Section 6, Evaluation and rationale). This sentence emphasises that in certain circumstances expert judgement may overrule the strict interpretation of the IARC criteria for "sufficient" and "limited". These same limitations apply with the current criteria in that expert judgement is necessary and can override the strict interpretation of the definitions.

Annex I: 3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms 'sufficient' and 'limited' have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
- limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive

results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

For human studies, the quality and power of the epidemiology studies require expert consideration and would normally lead to a Category 1A classification if data of adequate quality shows causality of exposure and cancer development. The Guidance on IR/CSA, Section R.7.7.10.2, further discusses the types of human epidemiology data available and the limitations of the data. Where there is sufficient doubt in the human data then classification in Category 1B may be more appropriate. On the other hand epidemiological studies may fail, because of uncertainties in the exposure assessment and/or limited sensitivity and statistical power, to confirm the carcinogenic properties of a substance as identified in animal studies (WHO Working group, 2000).

3.6.2.3.2 Additional considerations for classification

Annex I: 3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are:

(a) tumour type and background incidence;

(b) multi-site responses;

(c) progression of lesions to malignancy;

(d)	reduced tumour latency;
(e)	whether responses are in single or both sexes;
(f)	whether responses are in a single species or several species;
(g)	structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
(h)	routes of exposure;
(i)	comparison of absorption, distribution, metabolism and excretion between test animals and humans;
(j)	the possibility of a confounding effect of excessive toxicity at test doses;
(k)	mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.
[…]	

As indicated above, the evaluation of animal carcinogenicity data requires consideration of a number of important additional factors which may increase or decrease the level of concern and the classification category. The list in CLP Annex I, 3.6.2.2.6 is not exhaustive. Each of these factors is discussed individually below.

(a) Tumour type and background incidence

Knowledge about the tumour type including its tumour biology is indispensable to decide on the relevance of observed tumours for humans.

By default, carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogens. Only when there is sufficient evidence showing that a certain type of tumour is not relevant to humans should this tumour type be excluded for classification.

Certain tumour types observed in animal carcinogenicity studies are of questionable or no relevance to humans. In case of multiple tumours anticipated to have no relevance for humans justification should be given for each tumour type. The justification for dismissing any particular tumour should be presented as a scientifically robust and transparent argument.

There are several reasons why a tumour observed in animals may be judged to be not relevant for humans or may be judged to be of lower concern. In most of these cases the tumour arises via a mode of action which does not occur in humans (see this Section part k). In some cases the tumour may arise in a tissue known to be overly susceptible in the species tested to development of certain tumours and consequently may be judged to be less relevant for humans. In a few cases a tumour may occur in a tissue with no equivalent in humans.

Tumours occurring in tissues with no human equivalent

Some of the commonly used animal species have some tissues with no equivalent in humans. Tumours occurring in these tissues include the following

– Forestomach tumours in rodents following administration by gavage of irritating or corrosive, non mutagenic substances. In rodents, the stomach is divided into two parts by the muco-epidermoid junction separating squamous from glandular epithelium. The proximal part, or forestomach, is non-glandular, forms a continuum with the oesophagus, and is lined by keratinized, stratified squamous epithelium. While humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. See also this Section (k), IARC (2003), and RIVM (2003).

– Tumours in the Zymbal's glands. Zymbal's glands are located beneath squamous epithelium at the anterior and posterior aspect of the ear canal. The external portion of the gland in rats is 3 to 5 millimetres in diameter.
– Tumours in the Harderian glands. Harderian glands are found in all vertebrates that possess a nictitating membrane, or third eyelid. They are located behind the eyeball in the orbit nictitating membrane, encircling the optic nerve. Humans have a rudimentary one.

Tumours occurring in such tissues indicate that the substance has the potential to induce carcinogenic effects in the species tested. It cannot automatically be ruled out that the substance could cause similar tumours of comparable cell/tissue origin (e.g. squamous cell tumours at other epithelial tissues) in humans. Careful consideration and expert judgement of these tumours in the context of the complete tumour response (i.e. if there are also tumours at other sites) and the assumed mode of action is required to decide if these findings would support a classification. However, tumours observed only in these tissues, with no other observed tumours are unlikely to lead to classification. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered.

Considering the background incidence and use of historical control data

Any statistically significant increase in tumour incidence, especially where there is a dose-response relationship, is generally taken as positive evidence of carcinogenic activity. However, in some cases the results involve an increase incidence of tumours in treated animals which lies at the borderline of biological and/or statistical significance or there is an increase in a spontaneous tumour type, then comparison of the tumour incidence with historical control tumour data is strongly encouraged.

Historical control data provide useful information on the normal pattern and range of tumour types and incidences for a particular strain/species, which may not be reflected by the tumour findings in the concurrent controls in any individual study. This can be particularly relevant for animal strains which have a propensity to develop a particular type of tumour spontaneously with variable and potentially high incidence. In such a case the tumour incidence in the treated group may be significantly above the concurrent control but could still be within the historical incidence range for that tumour type in that species and therefore may not be providing reliable evidence of treatment related carcinogenicity.

Some examples of animal tissues with a high spontaneous tumour incidence are:

– Adrenal pheochromocytoma in male F344 rats (NTP, 2007a), Sprague-Dawley rats (NTP, 2005; RIVM, 2001; Ozaki *et al*., 2002);
– Pituitary adenomas in F344 rats (NTP, 2007a), Sprague-Dawley rats (NTP 2005; RIVM 2005);
– Mammary gland tumours (adenomas and carcinomas) in female Sprague-Dawley rats (NTP, 2005);
– Mononuclear cell leukaemia in F344 rats (NTP, 2007a; RIVM, 2005);
– Liver tumours in B6C3F1 mice (NTP, 2007b; Haseman *et al*. 1998; Battershill, J.M. and Fielder, R.J., 1998);
– Leydig cell adenomas in male F344 rats (Cook *et al*., 1999; Mati *et al*., 2002; RIVM, 2004; EU Specialised Experts Report, 2004).

Historical control data can also be useful to judge the biological significance of marginal increases in uncommon tumours. If there is a small increase in a particular tumour type which

historical data shows to be very uncommon and unlikely to have occurred by chance then this may support a conclusion of carcinogenicity without the requirement for a statistically significant increase.

Use of historical control data should be on a case by case basis with due consideration of the appropriateness and relevance of the historical control data for the study under evaluation. In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. It is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). Historical data older than this should be used with caution and acknowledgement of its lower relevance and reliability. (RIVM, 2005; Fung *et al*, 1996; Greim *et al*, 2003).

Even when a particular tumour type may be discounted, expert judgment must be used in assessing the total tumour profile in any animal. However, appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification. Where the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories, (Battershill and Fielder, 1998). Expert judgment is required to evaluate the relevance of the results.

(b) Multi-site responses

In general, chemicals are evaluated for carcinogenic potential in two-year bioassays conducted in mice and rats. The chemicals produce a spectrum of responses ranging from no effects in either species to induction of malignant neoplasms in multiple tissues in both species. Between these two extremes, there are variable responses in tissues, sexes and species, which demonstrate that there are important differences among the carcinogens, as well as between the species in which they are tested. The tumour profile observed with a substance should be taken into account when considering the most appropriate classification.

Evidence shows that substances which cause tumours in either multiple sites and/or multiple species tend to be more potent carcinogens than those causing tumours at only one site in one species (Dybing *et al.*, 1997). This is often true for substances which are mutagenic. Also, where human carcinogens have been tested in two or more species, the majority have caused cancer in several species (Tennant, 1993). Thus, if a substance causes tumours at multiple sites and/or in more than one species then this usually provides strong evidence of carcinogenicity. Typically such a tumour profile would lead to a classification in category 1B.

(c) Progression of lesions to malignancy

In general, if a substance involves a treatment related increase in tumours then it will meet the criteria for classification as a carcinogen.

If the substance has been shown to cause malignant tumours this will usually constitute sufficient evidence of carcinogenicity supporting Category 1B (CLP Annex I, 3.6.2.2.3)

The induction of only benign tumours usually provides a lower strength of evidence for carcinogenicity than the induction of malignant tumours and will usually support Category 2

(CLP Annex I, 3.6.2.2.3). However, benign tumours may also be of significant concern and the strength of evidence for carcinogenicity that they provide should be considered using expert judgement. For instance, some benign tumours may have the potential to progress to malignant tumours and therefore any indication that the observed tumours have the potential to progress to malignancy may increase the level of concern. Also, some benign tumours, for example brain tumours, may be of concern in themselves.

(d) Reduced tumour latency

The latency of tumour development i.e. how quickly a substance induces tumours, often reflects the potency of a carcinogen. This is particularly true for mutagenic substances which often induce tumours with relatively short latency and usually more rapidly than non-genotoxic agents. Tumour latency is not generally investigated in detail in standard carcinogenicity studies, although some information may be provided if the study used serial sacrifices.

The latency of tumour formation does not materially affect the classification and hazard category. Any substance causing cancer will attract classification regardless of the latency for tumour development. This also includes tumour responses at late treatment/life periods if substance-related. However unusual tumour types or tumours occurring with reduced latency may add to the weight of evidence for the carcinogenic potential of a substance, even if the tumours are not statistically significant.

(e) Whether responses are in single or both sexes

In general, in standard carcinogenicity studies both male and female animals are tested. There may be cases where tumours are only observed in one sex.

Tumours in one sex only may arise for two broad reasons. The tumours may occur in a gender-specific tissue, for instance the uterus or testes (sex-specific tissue), or in a non sex-specific tissue, in one sex only. Tumours may also be induced by a mechanism that is gender (or sex) -specific, for instance a hormonally-mediated mechanism or one involving gender (or sex) -specific differences in toxicokinetics. As with all cases the strength of evidence of carcinogenicity should be assessed based on the totality of the information available using a weight of evidence type approach. A default position is that such tumours are still evidence of carcinogenicity and should be evaluated in light of the total tumorigenic response to the substance observed at other sites (multi-site responses or incidence above background) in determining the carcinogenic potential and the classification category.

If tumours are seen only in one sex of an animal species, the mode of action should be carefully evaluated to see if the response is consistent with the postulated mode of action. Effects seen only in one sex in a test species may be less convincing than effects seen in both sexes, unless there is a clear patho-physiological difference consistent with the mode of action to explain the single sex response. However, there is no requirement for a mechanistic understanding of tumour induction in order to use these findings to support classification. If there is clear evidence for induction of either a gender (or a sex)-specific tumour then classification in Cat 1B may be appropriate. However, it has to be taken into account that according to the criteria additional data are required to provide sufficient evidence for animal carcinogenicity (1B).

(f) Whether responses are in a single species or several species

The criteria indicate that carcinogenicity in a single animal study (both sexes, ideally in a GLP study) could be sufficient evidence and could therefore lead to a Category 1B

classification in the absence of any other data. This represents a change compared to the previous EU-system where such a study would rarely lead to the equivalent of a Category 1B classification. For classification as a Category 2 carcinogen under DSD either positive results in two animal species should be available or clear positive evidence in one species, together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

However, as defined under 'sufficient' evidence (CLP Annex I, 3.6.2.2.3 (b)), a single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites. Moreover a single study in one species and sex in combination with positive in-vivo mutagenicity data would be considered to provide sufficient evidence of carcinogenicity.

Positive responses in several species add to the weight of evidence, that a chemical is a carcinogen.

(g) Structural similarity or not to a chemical(s) for which there is good evidence of carcinogenicity

See Section 3.6.2.3.4 of this Guidance.

(h) Routes of exposure;

> **Annex I: 3.6.2.2.8.** The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity.

The classification for carcinogenicity generally does not specify specific routes of exposure. If a chemical has been shown to cause tumours by any route of administration then it may require classification, unless there is a robust justification for dismissing the findings from a particular route. However, under the previous EU system (Annex VI to DSD), classification specifically via inhalation was accepted by application of the risk phrase R49; May cause cancer by inhalation and a specific hazard statement has been established in CLP, H350i; May cause cancer by inhalation (CLP Annex VII, Table 1.1).

Most standard carcinogenicity studies use physiological routes of exposure for humans, namely inhalation, oral or dermal exposure. The findings from such routes are usually considered directly relevant for humans. Studies using these routes will generally take precedence over similar studies using other routes of exposure.

Sometimes other non-physiological routes are used, such as intra-muscular, sub-cutaneous, intra-peritoneal and intra-tracheal injections or instillations. Findings from studies using these routes may provide useful information but should be considered with caution. Usually dosing via these routes provides a high bolus dose which gives different toxicokinetics to normal routes and can lead to atypical indication of carcinogenicity. For instance, the high local concentration can lead to local tumours at the site of injection. These would not normally be considered reliable indications of carcinogenicity as they most likely arose from the abnormally high local concentration of the test substance and would lead to a lower category classification or no classification.

Where findings are available from studies using standard routes and non-physiological routes, the former will generally take precedence. Usually studies using non-standard routes provide supporting evidence only.

The hazard statement allows for identifying the route of exposure "if it is conclusively proven that no other routes of exposure cause the hazard" (CLP Annex I, Table 3.6.3). In this case the hazard statement may be modified accordingly. Genotoxic carcinogens are generally suspected to be carcinogenic by any route.

(i) Comparison of absorption, distribution, metabolism and excretion between test animals and humans;

> **Annex I: 3.6.2.2.9.** It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, i.e. structure activity relationship, is taken into consideration when undertaking classification.

Consideration of absorption, distribution, metabolism and excretion (toxicokinetics) of the substance in the test animal species and in humans is one important consideration, including where a substance is metabolised to an active carcinogenic metabolite. Toxicokinetic behaviour is normally assumed to be similar in animals and humans, at least from a qualitative perspective. On the other hand, certain tumour types in animals may be associated with toxicokinetics or toxicodynamics that are unique to the animal species tested and may not be predictive of carcinogenicity in humans. Where significant qualitative and quantitative differences in toxicokinetics exist between animals and humans this can impact on the relevance of the animal findings for humans and in certain instances may influence the category of classification. Where a carcinogenic metabolite identified in animals is demonstrated not to be produced in humans, no classification may be warranted where it can be shown that this is the only mechanism of action for carcinogenicity.

The use of physiologically-based pharmacokinetic (PB/PK) modelling requires more validation and while it may not lead directly to a modification of classification, however expert judgement in conjunction with PB/PK modelling may help to modify the concern for humans.

(j) The possibility of a confounding effect of excessive toxicity at test doses

In lifetime bioassays compounds are routinely tested using at least three dose levels to enable hazard identification and hazard characterisation as part of risk assessment. Of these doses, the highest dose needs to induce minimal toxicity, such as characterised by an approximately 10% reduction in body weight gain (maximal tolerated dose, MTD dose). The MTD is the highest dose of the test agent during the bioassay that can be predicted not to alter the animal's normal longevity from effects other than carcinogenicity. Data obtained from a sub-chronic or other repeated dose toxicity study are used as the basis for determining the MTD.

Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses.

Tumours occurring only at excessive doses associated with severe toxicity generally have a more doubtful potential for carcinogenicity in humans. In addition, tumours occurring only at sites of contact and/or only at excessive doses need to be carefully evaluated for human relevance for carcinogenic hazard. For example, as indicated in this Section (a) 'Tumour type and background incidence', forestomach tumours, following administration by gavage of an irritating or corrosive, non-mutagenic chemical, may be of questionable relevance, both due to the lack of a corresponding tissue in humans, but importantly, due to the high dose direct

effect on the tissue. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered.

The proceedings of a WHO/IPCS workshop on the Harmonization of Risk Assessment for Carcinogenicity and Mutagenicity (Germ cells) - A Scoping Meeting (IPCS, 1995; Ashby *et al*, 1996), points to a number of scientific questions arising for classification of chemicals, e.g. mouse liver tumours, peroxisome proliferation, receptor-mediated reactions, chemicals which are carcinogenic only at toxic doses and which do not demonstrate mutagenicity.

If a test compound is only found to be carcinogenic at the highest dose(s) used in a lifetime bioassay, and the characteristics associated with doses exceeding the MTD as outlined above are present, this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2 or no classification.

(k) Mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression

Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action; genotoxic or non-genotoxic. Genotoxic modes of action involve genetic alterations caused by the chemical interacting directly with DNA to possibly result in a change in the primary sequence of DNA after cell division. A chemical can also cause genetic alterations indirectly following interaction with other cellular processes (e.g. secondary to the induction of oxidative stress). Non-genotoxic modes of action include epigenetic changes, i.e. effects that do not involve alterations in DNA but that may influence gene expression, altered cell-cell communication, or other factors involved in the carcinogenic process. For example, chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced: the induction of urinary bladder tumours in rats may, in certain cases, be due to persistent irritation/inflammation, tissue erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones. Other modes of non-genotoxic action can involve specific receptors (e.g., peroxisome proliferator-activated receptor-alpha (PPARα) which is associated with liver tumours in rodents; or tumours induced by various hormonal mechanisms). More detail is given in the Guidance on IR/CIS Section R7.7.8.

Some modes of action of tumour formation are considered to be not relevant to humans. Where such a mechanism is identified then classification may not be appropriate. Only if a mode of action of tumour development is conclusively determined not to be operative in humans may the carcinogenic evidence for that tumour be discounted. However, a weight of evidence evaluation for a substance calls for any other tumorigenic activity to be evaluated as well. In addition, the existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation) may lead to a downgrading of a Category 1 to Category 2 classification.

The various international documents on carcinogen assessment all note that mode of action in and of itself, or consideration of comparative metabolism, should be evaluated on a case-by-case basis and are part of an analytic evaluative approach. One must look closely at any mode of action in animal experiments taking into consideration comparative toxicokinetics/toxicodynamics between the animal test species and humans to determine the relevance of the results to humans. This may lead to the possibility of discounting very specific effects of certain types of chemicals. Life stage-dependent effects on cellular differentiation may also lead to qualitative differences between animals and humans.

To establish a mode of action will usually require specific investigative studies over and above the standard carcinogenicity study. All available data must be considered carefully to judge if it can be concluded with confidence that the tumours are being induced through that specific mechanism. The IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans (2007) can be a useful way to construct and present a robust and transparent assessment of such data.

Some mechanisms of tumour formation considered not relevant for humans:

- Kidney tumours in male rats associated with substances causing α2μ-globulin nephropathy (IARC, 1999)
- Pheochromocytomas in male rats exposed to particulates through inhalation secondary to hypoxemia (Ozaki *et al*, 2002)
- Leydig cell adenomas induced by dopamine antagonists or gonadotropin-releasing hormone (GnRH) (EU Specialised Experts, 2004; RIVM, 2004)
- Urinary bladder tumours due to crystals in the bladder (IARC, 1999)
- Forestomach tumours in rodents following administration by gavage of irritating or corrosive, non-genotoxic substances (RIVM, 2003; IARC 2003)
- Certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999; EU Specialised Experts, 1999)
- Liver tumours in rodents conclusively linked to peroxisome proliferation (IARC, 1994)

3.6.2.3.3 Consideration of mutagenicity

Annex I: 3.6.2.2.6. […] Mutagenicity: It is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

As indicated in Section 3.6.2.1 of this Guidance and above, carcinogenic chemicals have conventionally been divided according to the presumed mode of action; genotoxic or non-genotoxic. Evidence of genotoxic activity is gained from studies on mutagenic activity.

It should be noted that in general if a substance is mutagenic then it will be considered to be potentially carcinogenic in humans however mutagenicity data alone are insufficient information to justify a carcinogen classification. In some cases where only *in vitro* and *in vivo* mutagenicity are present without carcinogenicity data, a Category 2 classification can be considered when all factors have been considered such as type and quality of the mutagenicity data, structure activity relationships etc. A single positive carcinogenicity study in one species and sex in combination with positive *in-vivo* mutagenicity data would be considered to provide sufficient evidence of carcinogenicity.

Lack of genotoxicity is an indicator that other mechanisms are in operation as indicated in Section 3.6.2.3.2(k) of this Guidance. Thus careful analysis based on all available information is required to identify the mechanism and derive a classification category taking into account the factors leading to the tumours observed, in the animals.

3.6.2.3.4 Non testing data

Annex I: 3.6.2.2.7. A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors

such as formation of common significant metabolites, e.g. for benzidine congener dyes.

A chemical that has not been tested for carcinogenicity may in certain instances be classified as a carcinogen based on tumour data from a structurally similar chemical with which it is predicted to have similar carcinogenic activity. Such an approach must always be based on a robust and transparent argument to support this supposition. There may also be evidence demonstrating similarity in terms of other important factors such as toxicokinetics or mutagenic activity etc. (OECD 2004, 2005, 2007; Guidance on IR/CSA, Section R.6, QSARs and grouping of chemicals).

In the absence of carcinogenicity data, read-across can be used to support a classification for carcinogenicity when the chemical in question is similar to a known or suspected carcinogen (Category 1A, 1B or 2). The similarity between chemicals is considered in terms of structural features, physico-chemical properties and overall toxicological profile.

In general the chemicals will share a common structural element or functional group (*i.e.*, a toxophore) that has been shown to be integral to the underlying mechanism of carcinogenicity for chemicals with this toxiphore in well conducted studies. These toxiphores can be identified through expert judgement or through automated systems such as (Q)SARs. The read-across should also consider the physico-chemical properties of the chemical and data from other toxicity studies to judge the similarity between the chemicals in terms of bioavailability by relevant routes of exposure and toxicokinetics. The toxicity profile from other studies should also be compared (*e.g.*, acute and repeated-dose toxicity and mutagenicity) and should share similarities in nature and severity. Data from shorter term toxicity studies may be useful, particularly for non-genotoxic carcinogens, to indicate that the chemicals cause the same underlying pathological changes (*e.g.*, hyperplasia), and act via a common mode of action. Any predictions made on the basis of read-across should take into account the totality of data on the chemicals in question, including the physico-chemical properties, toxicological profile, toxicokinetics, structural analogy and the performance of any (Q)SAR models used, in a weight of evidence approach driven by expert judgement. The final decision must be clear, scientifically defensible and transparent.

The specific category depends on the category of the known carcinogen and the degree of confidence in the robustness of the read-across prediction. The category will not be higher than the chemical used to read-across from, but normally may be the same. However a lower category may be applied if the read-across highlights a possible carcinogenic hazard, and thus supports a classification, but there is uncertainty as to the robustness of the read-across prediction or there is evidence, for instance from mechanistic or other studies, that the chemical may be of lower concern for carcinogenicity.

If a chemical is similar to a substance known to be carcinogenic and shares the toxiphore that is considered to be causally related to carcinogenicity, then it is unlikely that there will be sufficient confidence in a prediction of no hazard (for instance based on arguments relating to differences in physico-chemical or steric properties), to justify no classification in the absence of supporting negative experimental data. However, the bioavailability of the toxiphore will need evaluation (Guidance on IR/CSA R.6).

3.6.2.4 Decision on classification

As mentioned throughout, classification as a carcinogen is based on consideration of the strength of evidence with additional considerations (weight of evidence) being taken into account as appropriate. It is recognised that, in most cases, expert judgment is necessary to determine the classification category.

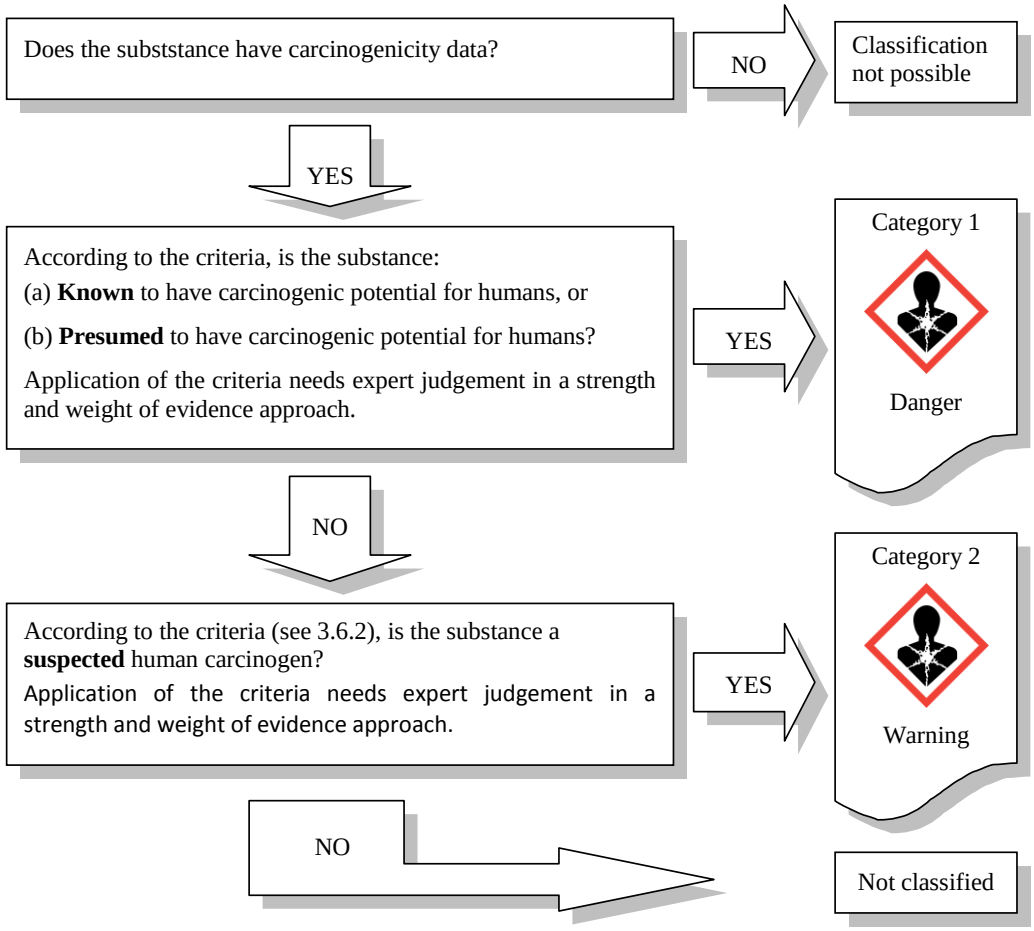
3.6.2.5 Setting of specific concentration limits

Experimental studies have revealed large variations in the doses of various carcinogenic substances needed to induce tumours in animals. Thus, the amounts of chemical carcinogens required to induce tumours vary with a factor of up to 10^8-10^9 for different compounds. It is reasonable to assume that there is similar variation in the potency of substances carcinogenic to humans (Sanner and Dybing, 2005).

The carcinogenic properties of mixtures are normally not tested. The classification and labelling of mixtures for carcinogenicity is therefore based on the classification of the ingredients and the percentage of each ingredient in the mixture. As indicated in Section 3.6.3 of this Guidance, the criteria contain default percentages for classification of mixtures with carcinogenic properties but CLP, Article 10.1 allows the use of specific concentration limits (SCL) based on the potency of the carcinogen(s). The EU has adopted the T25 concept for carcinogenicity (Dybing *et al*., 1997) with additional considerations as a measure for intrinsic potency and a guidance document (EC, 1999) to assist in establishing SCLs for carcinogens. By using this approach the SCL may occasionally be reduced or raised from the default generic concentration limits.

3.6.2.6 Decision logic for classification of substances

The decision logic which follows is taken from the GHS Guidance. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.



3.6.3 Classification of mixtures for carcinogenicity

3.6.3.1 Classification criteria for mixtures

Classification of mixtures will be based on the available test data for the **individual ingredients** of the mixture, using cut-off values/concentration limits for those ingredients and taking into account potency consideration. The classification may on **a case-by-case basis** be based on the available test data for the mixture as a whole (see Section 3.6.3.1.2 of this Guidance) or based on bridging principles (see Section 3.6.3.1.3 of this Guidance).

3.6.3.1.1 When data are available for all ingredients or only for some ingredients

Guidance update to legal text "Annex I: 3.6.3.1.1 Table 3.6.2" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.6.3.1.1. The mixture will be classified as a carcinogen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 carcinogen and is present at or above the appropriate generic concentration limit as shown in Table 3.6.2 below for Category 1A, Category 1B and Category 2 respectively.

Table 3.6.2

Generic concentration limits of ingredients of a mixture classified as carcinogen that trigger classification of the mixture

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:		
	Category 1 carcinogen		Category 2 carcinogen
	Category 1A	Category 1B	
Category 1A carcinogen	≥ 0,1 %	—	—
Category 1B carcinogen	—	≥ 0,1 %	—
Category 2 carcinogen	—	—	≥ 1,0 % [Note 1]

Note

The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

Note 1

If a Category 2 carcinogen is present in the mixture as an ingredient at a concentration ≥ 0,1% a SDS shall be available for the mixture upon request.

In case a SCL has been established for one or more ingredients these SCLs have precedence over the respective GCLs. See Section 3.6.2.5 of this Guidance for the setting of SCLs for substances.

3.6.3.1.2 When data are available for the complete mixture

Annex I: 3.6.3.2.1. Classification of mixtures will be based on the available test data for the

individual ingredients of the mixture using concentration limits for the ingredients classified as carcinogens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of carcinogenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.6.3.1.3 When data are not available for the complete mixture: bridging principles

Annex I: 3.6.3.3.1. Where the mixture itself has not been tested to determine its carcinogenic hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to the provisions of paragraph 3.6.3.2.1) to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

Bridging principles will only be used on a case by case basis (see section 3.6.3.1 of this guidance). Note that the following bridging principles are not applicable to this hazard class:

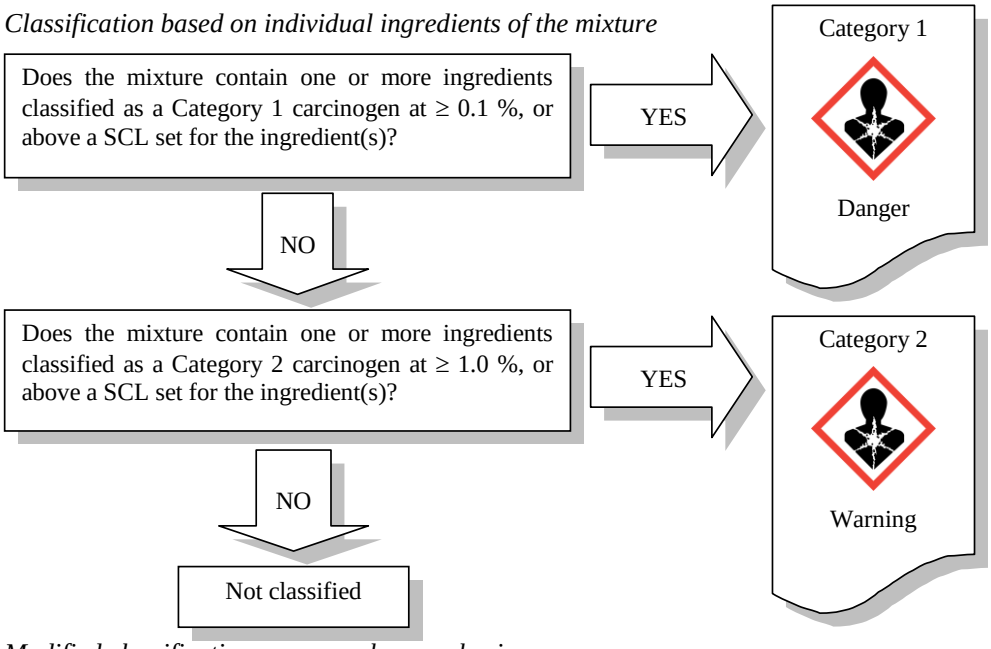
- concentration of highly hazardous mixtures
- interpolation within one hazard category

(see CLP Annex 1, 1.1.3.3 and 1.1.3.4)

3.6.3.2 Decision logic for classification of mixtures

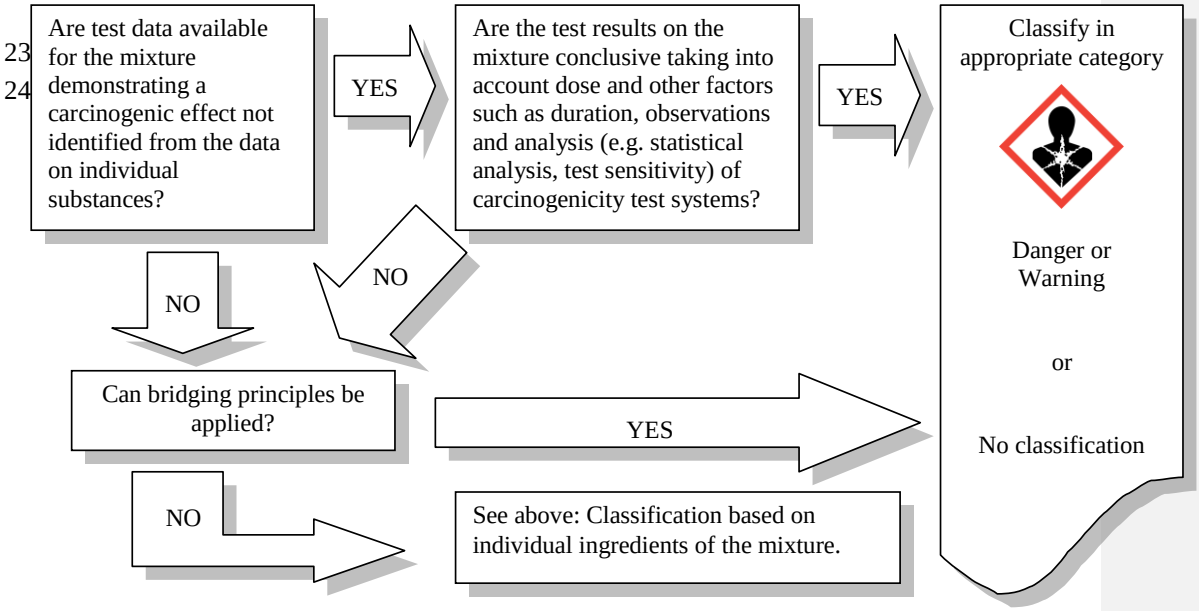
The decision logic which is based on the GHS Guidance is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.

Classification based on individual ingredients of the mixture



Modified classification on a case-by-case basis

Test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients (CLP Annex I, 3.6.3.1.1, see also CLP Article 6(3)).



3.6.4 Hazard communication in form of labelling for carcinogenicity

3.6.4.1 Pictograms, signal words, hazard statements and precautionary statements

Annex I; 3.6.4.1 Label elements shall be used in accordance with Table 3.6.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table3.6.3

Label elements for carcinogenicity

Classification	Category 1 (Category 1A, 1B)	Category 2
GHS Pictograms		
Signal Word	Danger	Warning
Hazard Statement	H350: May cause cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H351: Suspected of causing cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281
Precautionary Statement Prevention **4th ATP change**	P201 P202 P280	P201 P202 P280
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

The wording of the Precautionary Statements is found in CLP Annex IV, Part 2.

Where there is conclusive proof that cancer is caused only by certain route(s), then this route may be stated in the hazard statement. In case of Category 1 carcinogens where there is conclusive proof that cancer is caused only by inhalation, the hazard phrase "H350i: May cause cancer by inhalation" applies (CLP Annex VII, Table 1.1).

3.6.4.2 Additional labelling provisions

There are no additional labelling provisions for carcinogenic substances and mixtures in CLP, however there are provisions laid out in Annex XVII to REACH. The packaging of

substances with harmonised classification as carcinogenic Category 1A or Category 1B, or mixtures containing such substances at concentrations warranting classification of the mixture as carcinogenic Category 1A or Category 1B, "must be marked visibly**,** legibly and indelibly as follows: 'Restricted to professional users'." (REACH, Annex XVII, point 28. Derogations from this obligation are outlined in the same provision).

3.6.5 Re-classification of substances and mixtures classified for carcinogenicity according to DSD and DPD

3.6.5.1 Is direct "translation" of classification and labelling possible?

A direct translation as indicated in the translation table in Annex VII to CLP is generally possible. Translation from classification according to DSD and DPD to the classification according to CLP is as follows:

Carc. Cat. 1 is translated into Carc. 1A;

Carc. Cat. 2 is translated into Carc. 1B, and

Carc. Cat. 3 is translated into Carc. 2, respectively.

3.6.5.2 Some additional considerations for re-classification

There are only few situations where the direct translation may lead to different results, however, these are likely to be very rare.

The first difference in applying the CLP criteria is that sufficient evidence (Carc. 1B) for carcinogenicity in animals can also be derived from two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. The second difference applying the CLP criteria is that sufficient evidence (Carc. 1B) for carcinogenicity in animals can be derived from an increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP. The criteria according to DSD allowed classification in Carc. Cat. 2 (analogous to CLP Carc. 1B) where there were positive results in two animal species or clear positive evidence in one species, together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Another difference can be derived from the IARC classification as '*possibly carcinogenic to humans (IARC 2B)'.* This category is used for substances for which there is less than *sufficient evidence of carcinogenicity* in experimental animals. According to IARC, classification as '*possibly carcinogenic to humans'* may be derived from solely strong evidence from mechanistic and other relevant data. This means that no *in vivo* carcinogenicity nor (Q)SAR data need to be available to arrive at classification for limited evidence of carcinogenicity.

3.6.6 Examples of classification for carcinogenicity

Classification for carcinogenicity involves the consideration of many different factors, as outlined above, and is a complex task which needs expert judgement. Therefore no examples of classification for carcinogenicity are included in this guidance document.

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3.7 REPRODUCTIVE TOXICITY

3.7.1 Definitions and general considerations for reproductive toxicity

Annex I: 3.7.1.1. Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document N°225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) Adverse effects on sexual function and fertility;

(b) Adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2. For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation

3.7.1.3. *Adverse effects on sexual function and fertility*

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4. *Adverse effects on development of the offspring*

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.1 Special considerations on effects on or via lactation

This classification is intended to indicate when a substance may cause harm due to its effects on or via lactation. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

Annex I: 3.7.1.5. Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately. This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

Therefore, if the adverse effects that lead to impaired development in the offspring also occur after *in utero* exposure then the substance would also be classified for developmental toxicity. In other words, the classification for effects on or via lactation is independent of consideration of the reproductive toxicity of the substance, and a substance can be classified

for effects on or via lactation whether or not the substance is also classified for reproductive toxicity.

Classification for effects on or via lactation alone is not sufficient for a substance to be subject to harmonised classification and labelling in accordance with CLP Article 36 (1).

3.7.2 Classification of substances for reproductive toxicity

3.7.2.1 Identification of hazard information

3.7.2.1.1 Identification of human data

Epidemiological studies as well as clinical data and case reports may be available as stated in CLP Annex I, 3.7.2.2.3 and further in the Guidance on IR/CSA, Section R.7.6.3.2.

3.7.2.1.2 Identification of non human data

In-vitro animal data and non-testing information used for classification is outlined in CLP Annex I, 3.7.2.5. and further specific references to different testing methods are listed in the Guidance on IR/CSA, Section R.7.6.3.1.

3.7.2.2 Classification criteria

Annex I: 3.7.2.1.1. For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1 (a)

Hazard categories for reproductive toxicants

Categories	Criteria
CATEGORY 1	Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Category 1A	Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans.
Category 1B	Presumed human reproductive toxicant The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of

	the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2	Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

3.7.2.2.1 Classification in the presence of parental toxicity

3.7.2.2.1.1 Effects to be considered in the presence of marked systemic effects

In general all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of parental toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be performed.

Fertility effects

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

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

Developmental effects:

Annex I: 3.7.2.4. *Maternal toxicity*

3.7.2.4.1. Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2. Based on pragmatic observation, maternal toxicity may, depending on severity, influence

development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3. Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

Adverse effects on postnatal survival and growth seen only at dose levels causing maternal toxicity may be due to lack of maternal care or other causes such as adverse effects on or via lactation or developmental toxicity. In case post-natal effects are caused by lack of maternal care classification for developmental effects may not be warranted.

3.7.2.2.1.2 Relevance of specific effects in the parent

All types of reproductive toxic effects may be considered as secondary to parental toxicity. With current knowledge it is not possible to identify specific effects indicating toxicity in parental animals which do not have any relevance to reproductive toxicity (e.g. peroxisome proliferation). However parental toxicity that is less than marked should not influence the classification for reproductive toxicity independent of the specific parental effects observed.

In general it is very difficult to prove a causal relationship between a parentally mediated mechanism and adverse effects in the offspring. Usually data are insufficient to conclude if an effect on the offspring is a direct effect or secondary to parental toxicity. In order to determine whether a reproductive toxic effect is independent or secondary to a parental effect, it would be most appropriate to correlate individual data for offspring and their parents. Nevertheless, associations between parental and offspring effects do not by default prove a causal relationship.

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

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

Annex I: 3.7.2.4.4. Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated x 100)([1])

Fertility index

(no. animals with implants/no. of matings x 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calculation of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

([1]) It is recognised that the Mating index and the Fertility index can also be affected by the male.

1 #### 3.7.2.2.2 Substances causing effects on or via lactation

Annex I*: Table 3.7.1 (b)*

Hazard category for lactation effects
EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

There are the two general criteria for this classification.

(i) …are absorbed by women and have been shown to interfere with lactation.

This relates to effects in the mother that impact adversely on the breast milk, either in terms of the quantity produced or the quality of the milk produced (i.e. the composition). Any effect on the quantity or quality of the breast milk is likely to be due to systemic effects in the mother. However, overt maternal toxicity may not be seen (e.g. the substance may just affect the transfer of a nutrient into the milk with no consequence for the mother). The type and magnitude of the maternal effects and their potential influence on lactation/milk production need to be considered on a case-by-case basis to determine whether classification for effects on or via lactation is necessary.

If a substance causes marked overt systemic toxicity in the mother at the same dose level then it is possible that this may indirectly impair milk production or impair maternal care as a non-specific secondary effect. The type and magnitude of the maternal effects and their potential influence on lactation/milk production needs to be considered on a case-by-case basis using expert judgment. If there is robust evidence to indicate that the effects on lactation are not caused directly by the substance then it should not be classified as such.

A substance which does not cause overt toxicity in the mother but which interferes with milk production or quality will normally be classified for effects on or via lactation because in this case the effect on lactation is most likely a direct substance-related effect.

(ii) … may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

This relates to the ability of the substance (including metabolites), to enter the breast milk in amounts sufficient to cause a concern. When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification. In general, positive data should usually be available to show that a substance leads to an adverse effect in offspring due to effects on lactation to support classification. However, in exceptional circumstances, if there are substantiated grounds for concern that the substance may have an adverse effect via lactation then it may be classified as such in the absence of direct evidence. This should be based on a quantitative comparison of the estimated transfer via the milk and the threshold for toxicity in the pups. This might apply in cases where the substance has the capacity to bioaccumulate which would lead to a

potentially higher burden in the offspring, or where there is evidence that the offspring may be more sensitive to the substance's toxicity than adult.

The mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation.

3.7.2.3 Evaluation of hazard information

Appropriate classification will always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach. Individual datasets should be analysed case by case using expert judgment.

3.7.2.3.1 Use of data from standard repeat dose tests

Fertility effects:

Toxicological effects, including marked effects, observed in a standard repeat dose study could be considered valid for the pre-mating phase for adult females and the pre- and post-mating phase for adult males. However in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant.

For pregnant and lactating females and juveniles data from standard repeat dose studies cannot easily be extrapolated.

Developmental effects:

A detailed assessment of toxicity in pregnant animals cannot be extrapolated from studies with non-pregnant animals. However information from general toxicity studies might give an indication of the maternal toxicity that could be anticipated in a subsequent developmental toxicity study.

3.7.2.3.2 Study design

Assessment of the dose-response relationships of parental and reproductive toxicity end points and their possible interrelationship require study designs where the dose intervals are not too far apart. This will improve dose-response assessment and will also reduce the chance of masking malformations by severe toxicity (e.g. resorptions, lethality) at high dose levels. This may lead to experimental designs in which more than the standard three dose groups and a control are tested. Endpoints from repeat dose toxicity studies may be considered useful for inclusion in subsequent reproductive toxicity studies. These endpoints should be evaluated both in parental animals and in offspring.

3.7.2.3.3 Evaluation of evidence relating to effects on or via lactation

(a) Human evidence indicating a hazard to babies during the lactation period;

This criterion acknowledges that human data, e.g. from epidemiological studies or case reports, indicating a hazard to babies during the lactation period can also be used to support classification for effects on or via lactation. The use of human data is self-explanatory and any study should be assessed on its merits for which expert judgment may be required. Observations in humans that give evidence of adverse effects in breastfed babies of mothers exposed to the chemical in question should be taken to provide clear evidence supporting classification. Such studies which do not show an adverse effect need to be considered carefully. Human studies investigate the risk under the specific conditions of exposure, and a

negative finding may just reflect inadequate methods to detect effects or insufficient exposures rather than prove the absence of a hazard.

In practice, useful human data are likely to be rare due to the nature of the endpoint. More likely are survey type studies which measure the levels of the chemical in breast milk. Such studies may provide useful information on the potential for maternal exposure to lead to the presence of the chemical in the breast milk and so they may be of use in assessing the need for classification for effects on or via lactation.

(b) Results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk;

Ideally, studies will be available which inform directly on whether the substance causes adverse effects in the offspring due to an adverse effect on lactation. One generation or multi-generation reproductive toxicity studies, which involve direct exposure or exposure via the milk of the offspring postnatally, usually provide information on this. The most common study performed today is the two-generation study, but one-generation studies with new study designs, like the screening study OECD TG 421/422 or the developmental neurotoxicity study OECD TG 426, also exist. The value of these studies is that they directly observe the pups during lactation and any adverse effects, such as deaths, decreased viability, clinical signs such as reduced bodyweight gain etc, can be directly observed and quantified. However, expert judgement is required to decide whether these effects in pups are due to a direct adverse effect on lactation, or are due to impaired nursing behaviour which is a non specific secondary consequence of maternal toxicity. If the impaired nursing behaviour is proven to be a substance related specific effect on behaviour, then classification for effects on or via lactation may be appropriate. It should also be noted that some developmental effects resulting from exposure in utero would only manifest post-natally and those should not be used for classification for effects on or via lactation. Cross-fostering studies, where available, may help establish whether effects are due to in utero or lactational exposure. If there is sufficient data that animal results are not relevant to humans, they should not be taken into account.

(c) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk;

The criterion indicates that toxicokinetic studies showing that the substance can be present at potentially toxic levels in breast milk can support classification. The implicit assumption behind this clause is that the pups may receive a body burden of the toxic entity through suckling that is sufficient to cause toxicity when the level of the toxic entity in the milk is above a certain threshold level ("a level to cause concern"). There is no robust way to estimate what this threshold is, although the likely body burden expected in the breastfed child may be compared to the toxicity data in adults (e.g. an appropriate NOAEL or BMD) to indicate whether toxicity is likely. The mere presence of a substance in the milk, without a robust argument that these levels may be potentially toxic to offspring would not normally support classification.

The toxicokinetics of a substance and the likelihood that it will enter the breast milk may be predicted on the basis of the physico-chemical properties of the chemical (e.g. using pKa, logP, water solubility, and molecular weight etc) and this information could be used as part of the argumentation outlined above. The potential of a substance to bioaccumulate following repeated exposure may also be an important factor to consider as this may contribute to the body burden reaching a potentially toxic level in the offspring. Studies where the

offspring/neonates have extended exposure, such as multi-generation studies, implicitly allow for bioaccumulation and so findings from these studies can, in themselves, be taken to provide information on the potential effects of bioaccumulation. Where these types of studies are not available, potential bioaccumulation can be taken into consideration as part of the toxicokinetic assessment using expert judgement.

There may be toxicokinetic and toxicodynamic reasons why neonates may potentially be more or less vulnerable to a particular adverse effect than adults due to the fact that certain systems (e.g. the immune and metabolic systems) and tissues/organs are immature and are still developing. Whether the neonate is more or less vulnerable than adults will depend on the specific chemical and will be determined by factors such as the hazardous properties of the chemical, its' physico-chemical properties and how it is metabolised. Therefore, the relative sensitivity of neonates and adults to a substance must be judged on a case by case basis using expert judgement. In the absence of any reliable and robust information to inform on this, it should be assumed that neonates and adults are equivalent in terms of sensitivity to the substance.

Overall, classification for effects on or via lactation can be assigned on the basis of toxicokinetic data or a well substantiated estimate of the exposure through the milk alone provided that it is supported by an argument clearly justifying that the level present in the breast milk would be likely to harm developing offspring.

3.7.2.4 Decision on classification

According to CLP Annex I, Section 3.7.2.1.1, reproductive toxic substances are allocated to either Category 1A, 1B or 2. Effects on lactation are allocated to a separate hazard category and should be ascribed to a substance irrespective if it classified in any other category for reproductive toxicity or not.

3.7.2.5 Setting of specific concentration limits

Article 10(1) Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous.

Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.

In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.

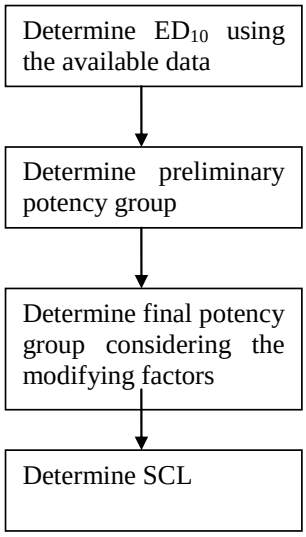
3.7.2.5.1 Procedure

The available data from animal and human studies are evaluated to establish the reproductive toxicity dose descriptor, ED_{10} (effective dose with a 10% effect level above the background), as described below. A preliminary conclusion as to whether the substance shows high,

medium or low potency is taken based on the ED_{10} data. The preliminary potency evaluation may be modified after due consideration of a number of modifying factors as described in chapter 3.7.2.5.5. This results in the final potency group. Each final potency group is connected with a generic concentration limit (GCL) or a specific concentration limit (SCL). In this way SCLs are then set taking into account all relevant considerations. See figure 3.7.2.5.1. A background document containing the justification of the boundaries of the potency groups and the SCLs is available in Annex VI to this document.

It is noted that there may be alternative approaches to assess potency, such as basing it on the BMD Methodology (Bench Mark Dose). However such alternative methods are not elaborated in this current guidance, although this does not exclude their use. If alternative approaches are used, they have to be clearly justified from a scientific and regulatory point of view (see Article 10, CLP) and they must be able to provide robust scientific proposals and justifications.

Figure 3.7.2.5.1 Procedure for setting SCL for reproductive toxicity



3.7.2.5.2 Cases where potency evaluation is difficult or unfeasible

The process for evaluating potency assumes the availability of certain types of data. However, these data may not always be available. Also, the classification of substances as reproductive toxicants may be based on information such as grouping, read-across and the use of QSARs (Guidance IR/CSA, sections R.6 and R.7.2.3.1). In such cases, no direct estimate of the reproductive toxicity potency based on an ED_{10} value is possible. While there are often good reasons for extrapolation of the hazardous properties from one or more substances to another, the expected potency of the individual substances within the group may vary. In these cases a potency evaluation may be difficult or impossible. However, determination of the classification and the potency using non-testing methods is possible in some cases. These cases could include interpolation of an ED_{10} within a group of substances with comparable structures and effects or correction for molecular weight in case of extrapolation between different salts with comparable availability. If the classification of a substance in Category 2 is done on the basis of "limited evidence", the quality of the

available data will in such cases determine whether a potency assessment is possible. In cases where no further evaluation is possible, the generic concentration limits of CLP apply. In general, more conclusive evidence is required when moving a substance to a lower potency group than to a higher potency group.

3.7.2.5.3 Determination of the ED_{10} value

The ED_{10} value (as used for reprotoxicity SCLs) is the lowest dose which induces reproductive toxic effects which fulfil the criteria for classification for reproductive toxicity with an incidence or magnitude of 10% after correction for the spontaneous incidence (see in 3.7.2.5.3.2).

Determining exactly which effect or combination of effects is the one that fulfils the classification criteria may seem difficult. However, for the majority of substances in the database, the developmental effect(s) observed at the lowest dose level was(/were) an increase in malformations and/or lethalities of the offspring. The ED_{10} for effects on sexual function and fertility is mainly based on effects on fertility and histopathological changes of the reproductive organs. These effects clearly fulfil the classification requirements. Also, allocation to the final SCLs is based on a limited number of potency groups and not on the exact ED_{10} value. Therefore, in practice, it is likely that the ED_{10} values for several different effects fall into the same potency grouping, resulting in the same SCL.

The ED_{10} may be obtained either directly or by linear interpolation from experimental data or estimated using Bench Mark Dose (BMD) software. The use of BMD software will result in a more precise estimate of the ED_{10} because all data from the dose-response curve are used. The use of BMD software is needed when an ED_{10} cannot be determined using linear interpolation due to the absence of a NOAEL when the LOAEL has an effect size above 10%. In general, however, the use of BMD software is not required because of the wide potency groups used for setting the SCLs. However, it could be important for substances which are close to the boundary of a potency group. When an ED_{10} cannot be calculated by direct or linear interpolation from experimental data or by the use of BMD software, interpolation between the control group and the LOAEL should be used to determine the ED_{10}. In such cases, only SCLs below the GCL can be determined and not those above the GCL, if no other reliable information is available, because it may be difficult in these cases to prove the absence of effects at lower dose levels.

3.7.2.5.3.1 Determination in practice

In practice, often several effects on reproduction are observed in various studies, and the classification is based on the weight of evidence of all results. As a first step, it should be determined whether the classification is for effects on development, for effects on sexual function and fertility or both. The effects used for classification for developmental toxicity should be used to determine the potency for developmental toxicity only. The same applies to effects on sexual function and fertility. This means that for substances fulfilling the criteria for classification for both developmental effects and effects on sexual function and fertility, two ED_{10} values are derived which may differ and lead eventually to different SCLs. For both developmental effects and effects on sexual function and fertility, the lowest ED_{10} for the effect(s) that fulfil the criteria for classification in the different studies, is then used as the ED_{10} that determines the potency of that substance. Where there are doubts as to whether a specific effect fulfils the classification criteria, ED_{10} values for different effects could be taken forward to the next step, when modifing factors are considered, to determine the impact.

The calculation of the ED_{10} by linear interpolation requires a different approach depending on whether the effect is measured as an incidence (quantal data, non-parametric data), a magnitude (continuous data, parametric data) or both.

3.7.2.5.3.2 Quantal or non-parametric data

For effects that are measured as changes in incidence, such as an increase in the number of malformations or resorptions, the ED_{10} is defined as the dose level at which 10% of the test population above the incidence in the concurrent control shows the effect. There may be occasions where the historical control data have to be taken into account (for example when the concurrent control data are atypical and close to the extremes of the historical data). In the example in Table 3.7.2.5.1, the ED_{10} is 90 mg/kg bw/day because at this dose level 12% - 2% (control) = 10% of the test population shows the effect above the incidence in the control group.

Table 3.7.2.5.1 Example of the calculation of the ED_{10}

Dose	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Malformations	2%	3%	7%	12%

For some effects the results of the calculation of the ED_{10} based on the incidence in pups may be different from that based on the incidence in litters. Scientific evidence may indicate which parameter is more appropriate, but in the absence of such information it is not possible to estimate which ED_{10} is more appropriate for a specific effect. In such cases, both the incidence in offspring and the incidence in litters should be calculated, and the lower ED_{10} value should be used.

3.7.2.5.3.3 Continuous or parametric data

For effects that are measured as changes in magnitude such as mean pup weight or testis weight, the ED_{10} is defined as the dose at which a change of 10%, compared to the concurrent control group, is observed. In the example in Table 3.7.2.5.2, the ED_{10} is 19.3 mg/kg bw/day because at this dose level the mean foetal bodyweight is calculated to be 90% of the control value. A 10% reduction of the control value of 6.2 g gives 5.58 g. Interpolation between 10 and 30 mg/kg bw/day to a dose level which would be expected to result in a foetal bodyweight of 5.58 g gives a value of 19.3 mg/kg bw/day.

Calculations: (30 – 10)/ (6 - 5.1) = 22.2 ; 6.0 – 5.58 = 0.42 ; 0.42 x 22,2 = 9.3 ; 10 + 9.3 = 19.3 mg/kg bw/day.

Table 3.7.2.5.2 Example on the calculation of the ED_{10}

dose	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Mean foetal bodyweight (g)	6.2	6.0	5.1	4.5
		NOAEL	LOAEL	

3.7.2.5.3.4 Data combining incidence and magnitude

Some effects such as histopathological changes in the testis are a combination of effects on incidence and magnitude (grading of the effect by a pathologist). However, calculation of an

ED_{10} taking both the incidence and the magnitude into account is not possible or at least more complex. The ED_{10} should therefore be based on the incidence of the effect below or above a certain magnitude. The magnitude of the effects that will be selected as a starting point has to be chosen carefully. Normally the particular effect size would be the lowest relevant for the respective classification. The ED_{10} is then determined as the dose level at which the incidence, of effects with a magnitude above that of the starting point, is 10% above the incidence in the control group. In practice this means that the grading system is converted into a simplified system where only percentages of animals in each dose group with an effect with a magnitude above the starting point are regarded as positive. However, it is recognised that this approach uses only a part of the actual data and is imprecise, and it may be appropriate that other effects also be considered in determining the ED_{10}.

Table 3.7.2.5.3 Example on the calculation of the ED_{10} for testicular effects (N=10)

	Dose (mg/kg)	**Testicular degeneration (n)**				
		none	slight	moderate	marked	severe
	0	4	5	1	0	0
	10	5	5	0	0	0
NOAEL	30	5	4	1	0	0
LOAEL	90	0	0	4	2	4

For the example in Table 3.7.2.5.3, the effects observed in the 10 mg/kg and 30 mg/kg dose groups have to be considered as equivalent to the effects of the control group so the NOAEL is 30 mg/kg. The magnitude of the testicular effect in the control group and the 10 and 30 mg/kg bw/day groups is slight or less. Because of the incidence observed in these three groups, the level of damage estimated as the starting point magnitude is 'slight'. The ED_{10} is then defined as a 10% increase of moderate effects or more above the control. In this example the incidences for moderate testicular degeneration or more are 10%, 0%, 10% and 100% at respectively 0, 10, 30 and 90 mg/kg bw/day. The ED_{10} is then defined as the dose level with 20% (control plus 10%) of moderate testicular effects. The ED_{10} would be 36.6 mg/kg bw/day based on interpolation between 30 and 90 mg/kg bw/day to a dose with 20% animals with moderate testicular degeneration or higher.

3.7.2.5.3.5 Specific data types

Non-oral studies

In most cases only oral studies will be available and used for determination of the potency. However, if the classification is based on the effects seen in non-oral studies or only non-oral studies are available, then these data should also be used to determine the potency. This requires route-to-route extrapolation of the external dermal or inhalatory dose to a corresponding oral dose. This should be done as described in the ECHA guidance on information requirements and chemical safety assessment in REACH (IR/CSA, section R.8).

Extrapolation from dermal exposure to oral exposure should only be done when there are sufficient kinetic data on dermal availability because assuming a high dermal availability is not a worst case assumption. In cases where such data are not available a direct comparison of the dermal dose with the oral potency ranges could be performed in exceptional cases. However, such comparison should not result in moving the substance to a lower potency

group (higher ED_{10}) – only moving the substance to a higher potency group (lower ED_{10}) should be considered.

Extrapolation from inhalatory exposure to oral exposure can only be done when there are sufficient kinetic data on inhaled availability because assuming a high inhaled availability is not a worst case assumption. If no inhalatory information on availability is available then it should be assumed that the inhalation and oral availability are comparable. However, such comparison should not result in moving the substance to a lower potency group (higher ED_{10}) – only moving the substance to a higher potency group (lower ED_{10}) should be considered.

Human data

The use of human data for ED_{10} calculation has several drawbacks including limited data on exposure, limited data on the size of the exposed population and limited information on whether the exposure included the window of sensitivity. For all these reasons, it is difficult to determine an ED_{10} based on human data. Therefore, and because in most instances animal data are also available for determining an ED_{10}, these data are evaluated together on a case by case basis. Guidance on the use of human data for the derivation of DNELs and DMELs has been developed by ECHA and is available at the ECHA website, see http://guidance.echa.europa.eu/guidance4_en.htm

3.7.2.5.4 Provisional evaluation of the potency classification

A preliminary potency evaluation applying the ED_{10} value is made at this stage.

ED_{10} values can be used to place substances classified as a reproductive toxicant into selected ranges that define potency groups. In this way, it is possible to identify reproductive toxicants of high, medium and low potency. For the purpose of determining the preliminary potency group, the boundaries in Table 3.7.2.5.4 are used.

Table 3.7.2.5.4 Boundaries of the potency groups[10].

Potency group	**Boundaries**
High potency group	ED_{10} value $\leq$ 4 mg/kg bw/day
Medium potency group	4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day
Low potency group	ED_{10} value $\geq$ 400 mg/kg bw/day.

3.7.2.5.5 Modifying factors

Modifying factors are a means to account for case-specific data situations which indicate that the potency group for a substance as obtained by the preliminary assessment, should be changed. While most modifying factors would result in a higher potency group than the preliminary one, also the opposite could occur: If substance-specific knowledge is available (such as e.g. toxicokinetic information on a higher bioavailability in test animals vs. humans), also a lower potency class might be assigned.

While some modifying factors should always be taken into account, other modifying factors could be more relevant when the potency is close to the boundary between two groups (see Table 3.7.2.5.4 above).

[10] see Annex VI of this guidance document for more details

Some modifying factors are of a more qualitative nature. When applied, they will simply point to a potency group different from the one resulting from the preliminary assessment. Other modifying factors might be quantifiable, at least on a semi-quantitative scale. In such cases, a potency group higher (or lower) than the preliminary one should be chosen if the estimated size of the modifying factor exceeds the distance of the preliminary ED10 to the border of the relevant (higher or lower) adjacent potency group.

Furthermore, for some substances more than one modifying factor will apply. It will then take expert judgement to decide on how to reasonably combine all of these individual factors into one overall modifying factor. In exceptional cases, such a combination of individual factors might even result in a change of two potency classes (e.g. assignment of the high potency class, where the preliminary assessment had resulted in the low potency class).

In this context, it should be noted that several of the modifying factors may be interrelated. Moreover, some factors may have already been taken into account in deciding on the classification as a reproductive toxicant. Where such considerations have been made, care should be taken not to use that information again when determining the potency. For example, when the effects determining the ED_{10} were observed at dose levels also causing maternal toxicity, this should already have been taken into consideration during the classification and should not be used again to set a higher SCL.

3.7.2.5.5.1 Type of effect / severity

The type of effect(s) resulting in the same classification as reproductive toxicant differs between substances. Some effects could be considered as more severe than others, however, ranking different effects based on their severity is controversial and difficult to establish criteria. Further, the effects of a developmental toxicant can differ between dose levels from variations via malformations to death of the foetuses. The adverse effects on fertility and sexual function of a substance can differ between dose levels from small changes in testes histopathology through effects on fertility to an irreversible and complete absence of fertility. As the difference between the dose levels is often smaller than the proposed potency groups (factor 10-100) this will make no difference in most cases. Also classification is in most cases based on severe effects like malformations or death of the foetuses for developmental toxicants and effects on fertility or histopathological changes of the reproductive organs for fertility toxicants. For most classified substances such severe effects were already observed at the lowest dose with reproductive effects [(Muller et al, 2012)]. Therefore, differentiation between types of effect is considered to have limited added value. Exceptions can be dealt with on a case by case basis.

For example, if the ED_{10} results in a preliminary conclusion for the medium potency group but is close to the border for the high potency group and the ED_{10} is based on a severe effect like malformations or irreversible effects on sexual function and fertility then using the higher potency group (lower ED_{10}) for that substance should be considered. To determine what is "close to the border" is to compare the distance to the next category border with the significance of modifying factors.

3.7.2.5.5.2 Data availability

There are several aspects to this modifying factor, some of which are:

- limited data availability where certain test protocols are lacking and therefore certain parameters have not been evaluated,

- limited data availability where the spectrum of evaluated parameters is sufficient, but only studies with limited duration are available, and
- limited data availability where only a LOAEL, but no NOAEL could be identified.

Where only limited data are available, such as a screening study (OECD 421 and 422), a 28-day repeated dose toxicity study or non-OECD studies which do not exclude the presence of reproductive effects at lower dose levels, the calculated ED_{10} should not be used to set a SCL above the GCL.

Furthermore it should be considered to assign a modifying factor accounting for the limitations in the database in a similar approach to the one used in deriving DNELs under REACH. Guidance regarding the potential size of such a factor can be obtained from ECHA's Guidance on IR/CSA R.8 ('Characterisation of dose [concentration]-response for human health'). Section R.8.4.3.1 'Assessment of factors relating to extrapolation', gives recommendations on how to set factors for extrapolating to longer study durations as well as for compensation of the lack of a NOAEL or of the generally poor quality of a database.

If there are only limited data which result in an ED_{10} in the medium potency group which is close to the border for the high potency group, then using the higher potency group should be considered. For example an ED_{10} of 8 mg/kg bw/day might have been estimated based on a LOAEL for malformations in the absence of a NOAEL, This ED_{10} is only higher by a factor of 2 (i.e 2 times the border of the high potency group of 4 mg/kg bw/d : see. Table 3.7.2.5.4 above), and assigning the high potency group should be considered until additional data at lower dose levels are available. Thus, there is uncertainty, if the ED_{10} based on extrapolation from and below the LOAEL in the absence of a NOAEL and a correction may be justified. The size of this uncertainty could be determined by the BMDL (Benchmark dose lower 95%-confidence bound). In such cases, the BMDL could be used as a potency estimate instead of the ED_{10}.

3.7.2.5.5.3 Dose-response relationship

The ED_{10} will in most cases probably be in the same range as the NOAEL and LOAEL. However, in cases of a shallow dose effect relationship curve, the LOAEL may sometimes be clearly below the ED_{10}. In such situations, if a substance would fall into a lower potency group based on the ED_{10} but into a higher potency group based on the LOAEL then the higher potency group should be used for that substance.

3.7.2.5.5.4 Mode or mechanism of action

It is assumed that effects observed in animal studies are relevant to humans. Where it is known that the mode or mechanism of action is not relevant for humans or is of doubtful relevance to humans, this should have been taken into account in the classification and should not be used again as a modifying factor for potency. However, quantitative differences in toxicodynamics can be taken into account when not already taken into account in the classification. In cases where mechanistic information shows a lower sensitivity in humans than in experimental animals, this may move substances which are close to the potency boundaries to a lower potency group. In cases where mechanistic information indicates a higher sensitivity in humans than in experimental animals, this may move substances near the potency boundaries to a higher potency group. In general, more conclusive evidence is required when moving a substance to a lower potency group than to a higher potency group.

3.7.2.5.5.5 Toxicokinetics

The toxicokinetics of a substance can differ between the tested animal species and humans. Where a difference is known this should be taken into account when determining the potency group of a substance. This should be based on a comprehensive knowledge of all involved toxicokinetic factors and not only on a single parameter. Also differences in kinetics between pregnant and non-pregnant animals and transport to the foetus should be taken into account. Based on the available data, quantification of this modifying factor has to be performed on a case by case basis. This modifying factor can work in both directions, as e.g. bioavailability in humans might be known to be lower or higher than in the animal species tested.. In general, more conclusive evidence is required when moving a substance to a lower potency group than to a higher potency group.

3.7.2.5.5.6 Bio-accumulation of substances

The study design of, for example, developmental studies is aimed at exposure only during development. For substances which bio-accumulate, the actual exposure in the time window of sensitivity for some developmental effects may therefore be much lower than when exposure at the same external dose level would have started long before the sensitivity window. Furthermore, human exposure may occur for a long period before the sensitive window. This should be taken into account when determining the potency group. For substances for which no experimental data are available with respect to their potential for accumulation, section R.7.12 of ECHA's IR/CSA Guidance R.7c ('Endpoint specific guidance') provides some hints on how to make an informed estimate about a respective concern.

"Suspected" bio-accumulating substances should be considered as to whether they should be moved into the next higher potency group (lower ED_{10}). However this should be considered on a case by case basis and the "suspected" bio-accumulation ability should be justifed. In the case that the following evidence should be available, the higher potency group would not be necessary:

- the relevant studies used for the ED_{10} were performed in a way that internal doses could have been expected to have reached a steady state during a sufficiently long part of the study time, and in particular with developmental studies during critical time windows of development, or

- the increase in the internal dose caused by the accumulation versus that following a single administration, is smaller than the distance between the ED_{10} and the border to the next higher potency group.

For example, if a substance preliminarily assigned to the medium potency group is known or suspected to be bio-accumulative and the ED_{10} for development has been obtained from a pre-natal developmental study in rats without any significant pre-treatment of the dams before mating, assignment to the high potency category should be considered. Conversely, if reliable toxicokinetic data demonstrate that steady state plasma levels after prolonged repeated administration do not exceed those after single exposure by more than a factor of 2, while the preliminary ED_{10} is 20 mg/kg bw/d (i.e. factor 5 from the border to the high potency category) changing the potency class might not appear necessary.

3.7.2.5.6 Assigning specific concentration limits (SCLs)

Based upon the preliminary potency evaluation using only the ED_{10} and applying the modifying factors, a substance can be placed in the final potency group using the table below. The GCL or SCL of that substance can then be found in the same table.

Table 3.7.2.5.5 SCLs for substances in each potency group and classification category

	Category 1		Category 2	
	Dose	SCL	Dose	SCL
Group 1 high potency	ED_{10} below 4 mg/kg bw/day	0.03% (factors of 10 lower for extremely potent substances [B])	ED_{10} below 4 mg/kg bw/day	0.3% (factors of 10 lower for extremely potent substances [B])
Group 2 medium potency	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	0.3% (GCL)	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	3% (GCL)
Group 3 low potency	ED_{10} above 400 mg/kg bw/day	3%	ED_{10} above 400 mg/kg bw/day	3-10% [A]

[A] The limit of 10% may be considered in certain cases, such as for substances with a ED_{10} value above 1000 mg/kg bw/day and a NOAEL below 1000 mg/kg bw/day.

[B] For substances with an ED10 more than 10 fold below 4 mg/kg bw/day, meaning an ED10 below 0.4 mg/kg bw/day, a 10-fold lower SCL should be used. For even more potent substance the SCL should be lowered with a factor of 10 for every factor of 10 the ED10 is below 4 mg/kg bw/day.

3.7.2.5.6.1 Assigning two SCLs to a substance

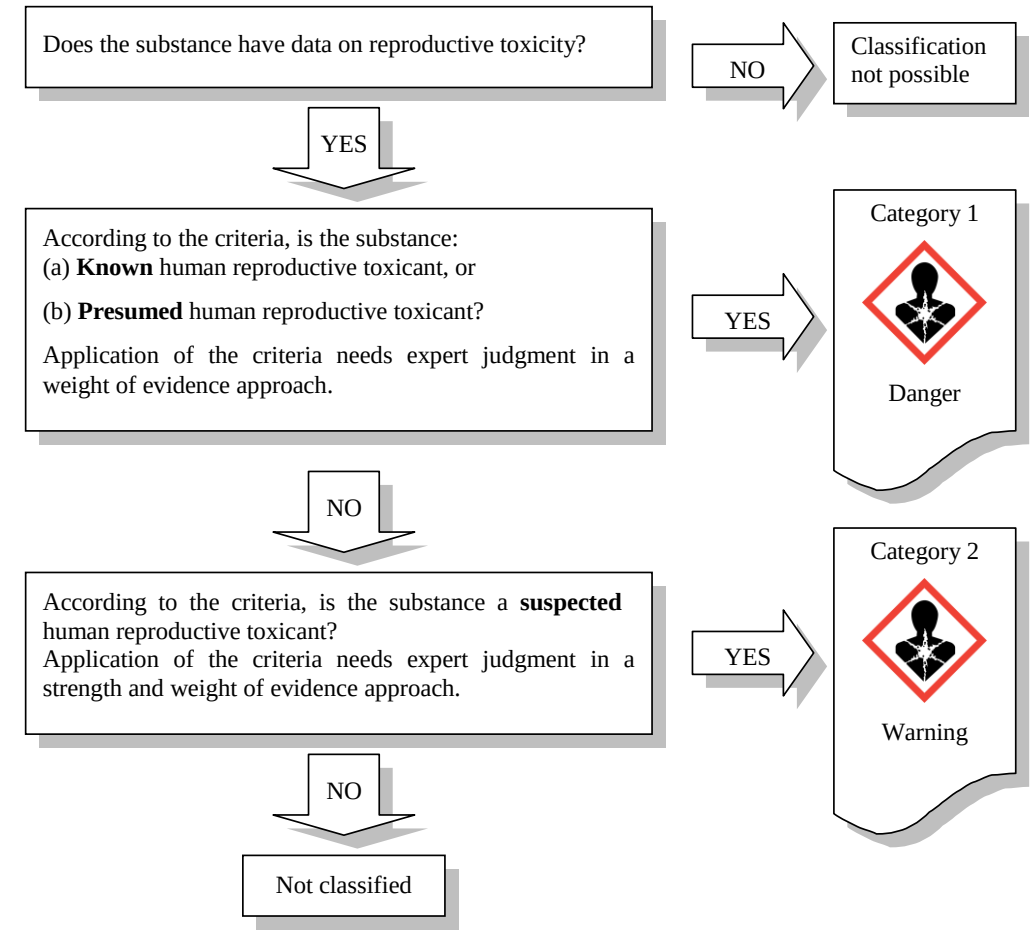
A substance toxic to reproduction is classified in one category for both effects on development and on sexual function and fertility. Within each category effects on development and on sexual function & fertility are considered separately. The potency and resulting concentration limits have to be determined separately for the two main types of reproductive toxic effects. In case the potency and resulting specific concentration limits are different for sexual function/fertility and development for a substance, the substance needs to be assigned one SCL for developmental toxicity and another SCL for effects on sexual

function and fertility. These concentration limits will in all cases trigger different specifications of the hazard statements for the two main types of effects, to be applied to mixtures containing the substance (see also 3.7.4.1, Annex I, CLP)

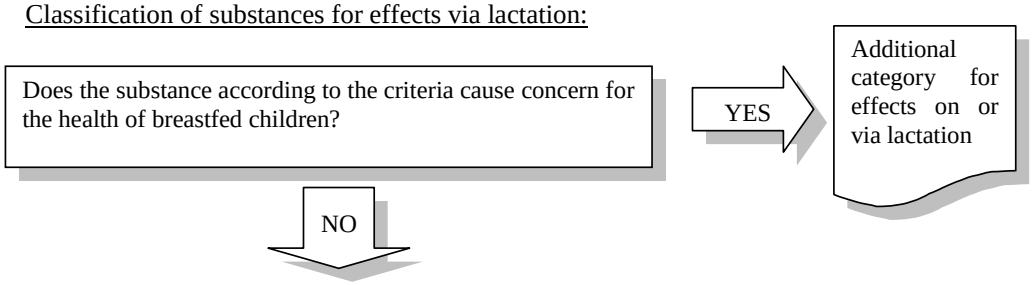
3.7.2.6 Decision logic

The decision logic which follows is provided here as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logic.

Classification of substances for fertility or developmental effects:



Classification of substances for effects via lactation:



Not classified

3.7.3 Classification of mixtures for reproductive toxicity

3.7.3.1 Classification criteria for mixtures

Reproductive toxicity classification of mixtures is based on the presence of an ingredient classified for reproductive toxicity (see CLP Article 6(3) and Annex I, 3.7.3). Only in case there is data available for the mixture itself which demonstrate effects not retrieved from the ingredients, this data might be used for classification. If such data is not available for the mixture itself, data on a similar mixture can be used in accordance to the bridging principle (see CLP Annex I, 1.1.3).

Guidance update to legal text "Annex I: Table 3.7.2" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: *Table 3.7.2*

Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or for effects on or via lactation that trigger classification of the mixture

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1 reproductive toxicant		Category 2 reproductive toxicant	Additional category for effects on or via lactation
	Category 1A	Category 1B		
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note

The concentration limits in Table 3.7.2 apply to solids and liquids (w/w units) as well as gases (v/v units).

Note 1

If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration at or above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.1.1 When data are available for the individual ingredients

Annex I: 3.7.3.1.1. The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 below for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2. The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

3.7.3.1.2 When data are available for the complete mixture

Annex I: 3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.1.3 When data are not available for the complete mixture: bridging principles

Annex I: 3.7.3.3.1 Subject to the provisions of paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

Bridging Principles will only be used on a case by case basis (see section 3.7.3.1 of this guidance). Note that the following bridging principles are not applicable to this hazard class:

- concentration of highly hazardous mixtures
- interpolation within one hazard category

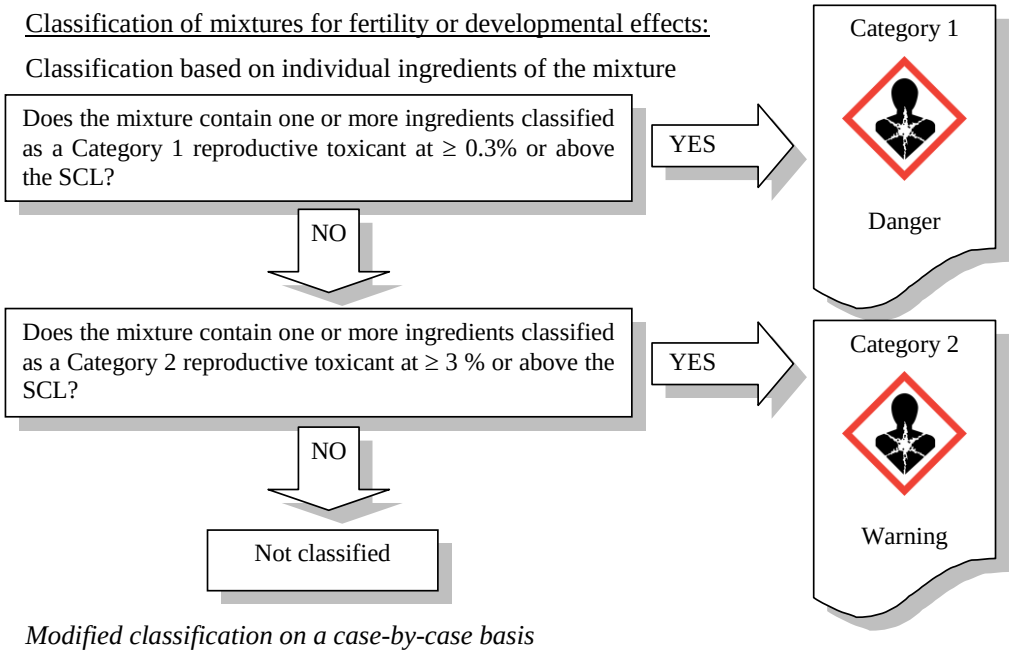
(see CLP Annex 1, 1.1.3.3 and 1.1.3.4)

3.7.3.2 Decision logic

The decision logic which follows is provided here as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logic.

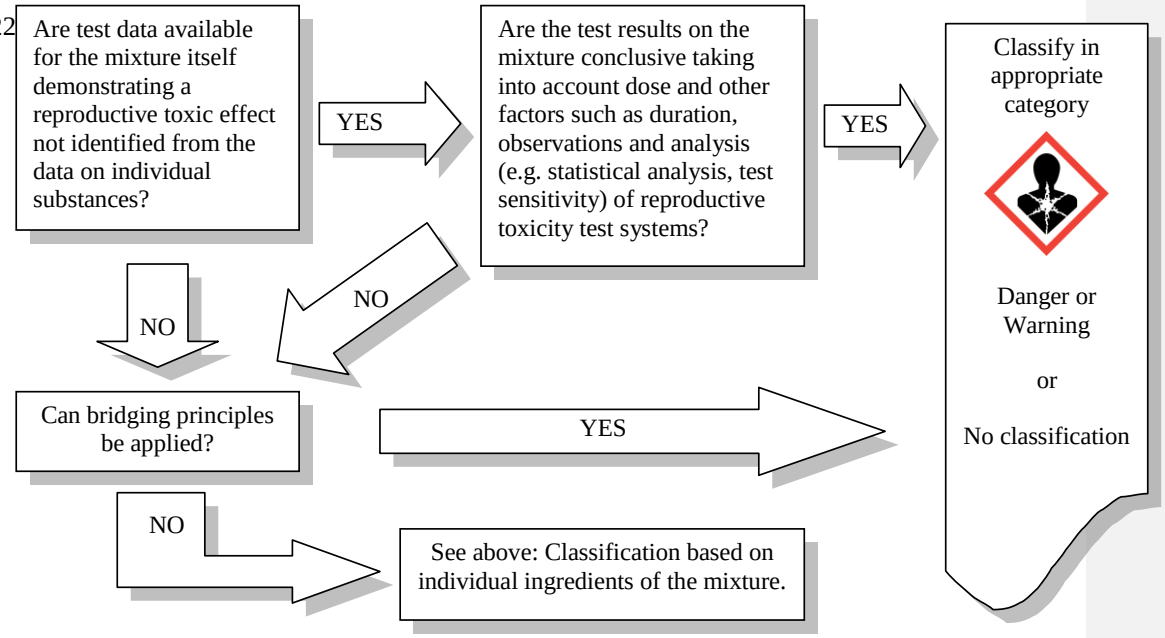
<u>Classification of mixtures for fertility or developmental effects:</u>

Classification based on individual ingredients of the mixture



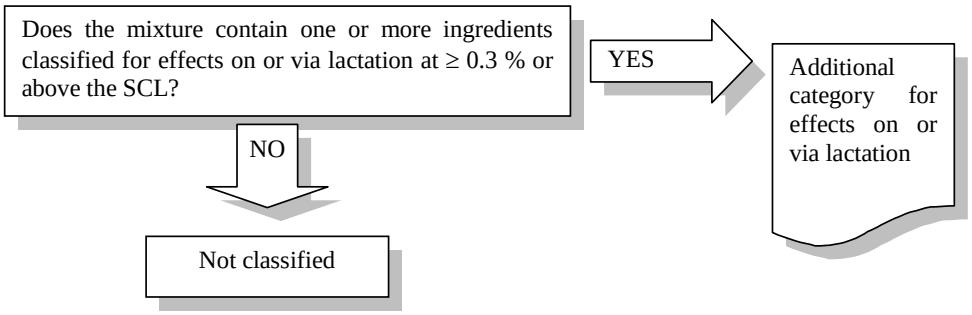
Modified classification on a case-by-case basis

Test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients (CLP Annex I, 3.7.3.1.1, see also CLP Article 6(3)).



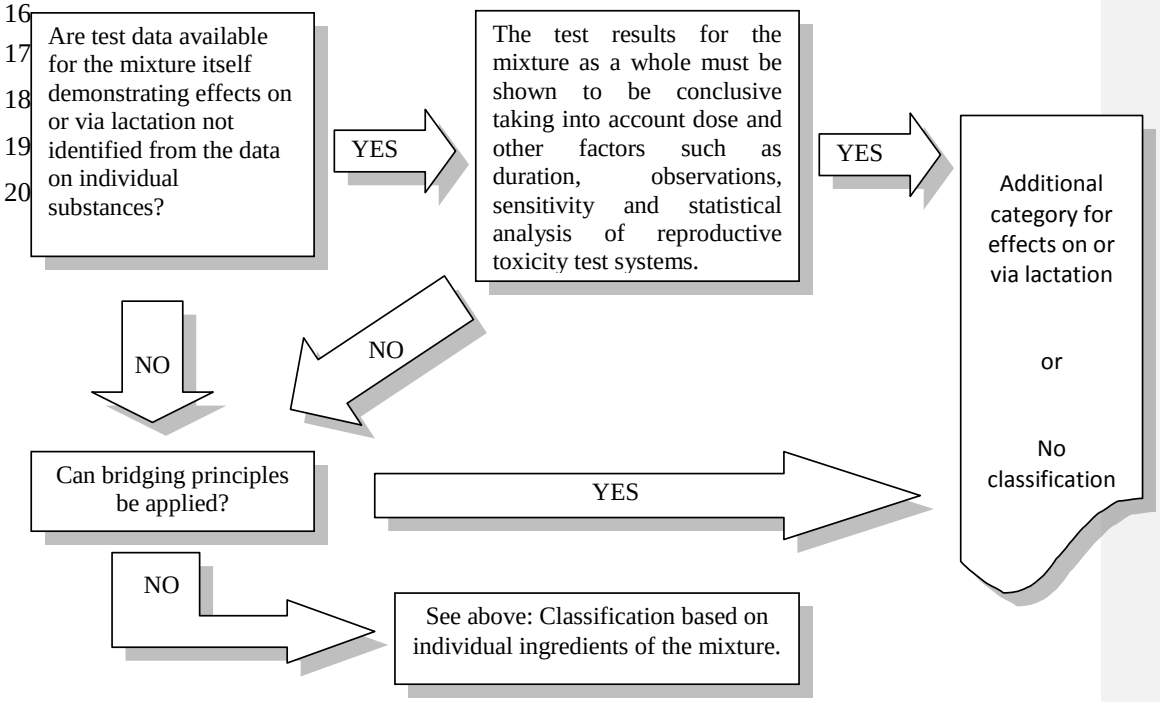
Classification of mixtures for effects via lactation:

Classification based on individual ingredients of the mixture



Modified classification on a case-by-case basis

Test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients (CLP Annex I, 3.7.3.1.1, see also CLP Article 6(3)).



3.7.4 Hazard communication in form of labelling for reproductive toxicity

3.7.4.1 Pictograms, signal words, hazard statements and precautionary statements

Annex I: 3.7.4.1. Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3.

Table 3.7.3

Label elements for reproductive toxicity

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

As shown in CLP Annex I, Table 3.7.3, a substance classified as reproductive toxicant in Category 1A or 1B must be assigned the hazard statements H360 and a substance classified in Category 2 must be assigned H361. Each of these two hazard statements includes reference to the adverse effects on sexual function and fertility or adverse effects on development of the offspring.

Depending on the data available, the hazard statement H360 or H361 must e.g. be assigned a reproductive toxic substance: in the case the criteria for Category 1A/1B or 2 are fulfilled, for *either* sexual function or fertility *or* developmental toxicity and when the other reproductive effect cannot be excluded.

In case reliable and adequate data are available on reproductive toxicity, (so that it is possible to ascribe one category for the fertility effects and one category for developmental toxic effects); it is possible to specify the hazard in the hazard statement.

The resulting different variants of H360 and H361 are shown in the table below, which also provides some examples when they should be assigned a substance.

Table 3.7.4.1: *Hazard statements for reproductive toxicity: H360 and H361, and their specifications*

H360	"May damage fertility or the unborn child" *Examples:* *1) a substance classified in Repr Cat 1A/B because of adverse effects on fertility and for which developmental toxic effects cannot be excluded* 2) *a substance classified in Repr Cat 1 A/B but the effects cannot be specified with respect to fertility or developmental toxicity*
H361	"Suspected of damaging fertility or the unborn child" *Example:* *1) a substance classified in Repr. Cat 2 on the basis of effects on developmental toxicity and for which fertility effects cannot be excluded* *2) a substance classified in Repr. Cat 2 but the effects cannot be specified with respect to fertility or developmental toxicity*
H360F	"May damage fertility." *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects and effects on developmental toxicity can be excluded according to reliable and adequate data*
H360D	"May damage the unborn child." *Example: a substance classified in Repr Cat 1A/B on the basis of developmental toxicity and effects on fertility can be excluded according to reliable and adequate data*
H361f	"Suspected of damaging fertility". *Example: a substance classified in Repr Cat 2 on the basis of fertility effects and effects on developmental toxicity can be excluded according to reliable and adequate data*
H361d	Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 2 on the basis of fertility effects and effects on developmental toxicity can be excluded according to reliable and adequate data*
H360FD	May damage fertility. May damage the unborn child. *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects and developmental toxicity.*

H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 2 on the basis of fertility effects and developmental toxicity.*
H360Fd	May damage fertility. Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects and in Repr Cat 2 on the basis of developmental toxicity.*
H360Df	May damage the unborn child. Suspected of damaging fertility. *Example: a substance classified in Repr Cat 1A/B on the basis of developmental toxicity and classified in Repr Cat 2 on the basis of fertility effects.*

According to CLP Annex I, Section 3.7.4.1, the hazard statements must be amended by specifying the route of exposure if it is conclusively proven that no other routes of exposure will lead to an adverse effect on sexual function or fertility or development of the offspring. When conclusively proven, it is meant that valid *in vivo* test data need to be available for all three exposure routes clearly indicating that only one exposure route has caused positive results i.e. adverse effects on the reproduction. Moreover, such a finding should be considered plausible with respect to the mechanism or mode of action. It is estimated that such a situation would rarely occur. Thus, amendment of the hazard statement with the route of exposure generally does not have to be considered

Guidance update to section 3.7.4.1 of the Guidance in accordance with the 4th ATP: to be applied from 1 December 2014 for substance and 1 June 2015 for mixtures.

3.7.4.1 Pictograms, signal words, hazard statements and precautionary statements

Comment [SJ1]: Replacement section 3.7.4.1 for 4th ATP changes

Annex I: 3.7.4.1. Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3.

Table 3.7.3

Label elements for reproductive toxicity

Classification	Category 1 (Category 1A, 1B)	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of	H362: May cause harm to breast-fed children.

	hazard)	exposure cause the hazard)	
Precautionary Statement Prevention **4th ATP change**	P201 P202 P280	P201 P202 P280	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex VII: Note 4 under Table 1.1

Note 4

Hazard statements H360 and H361 indicate a general concern for ~~both the reproductive properties related to fertility and developmental effects~~;effects on fertility and/or development: "May damage/Suspected of damaging fertility or the unborn child". According to the ~~classification~~ criteria, ~~(Annex I, section 3.7)~~ the general hazard statement can be replaced by the hazard statement indicating ~~only the property~~ the specific effect of concern ~~in case either fertility or developmental effects are proven to be not relevant~~ in accordance with section 1.1.2.1.2. of Annex VI. When the other differentiation is not mentioned, this is due to evidence proving no such effect, inconclusive data or no data and the obligations in Article 4(3) shall apply for that differentiation.

Annex VI: 1.2.3 Hazard statements for reproductive toxicity

[…]

According to the criteria, the general hazard statement can be replaced by the hazard statement indicating the specific effect of concern in accordance with section 1.1.2.1.2. When the other differentiation is not mentioned, this is due to evidence proving no such effect, inconclusive data or no data and the obligations in Article 4(3) shall apply for that differentiation.

[…]

Hazard statements H360 and H361 indicate a general concern for effects on fertility and/or development. As shown in CLP Annex I, Table 3.7.3, a substance classified as reproductive toxicant in Category 1A or 1B must be assigned the hazard statements H360 and a substance classified in Category 2 must be assigned H361. Each of these two hazard statements includes the mentioning of the adverse effects on sexual function and fertility or adverse effects on development of the offspring.

The effects of concern should be specified in the hazard statement. Where the effect cannot be specified with respect to fertility or development the general statement must be applied.

When the other differentiation is not mentioned in the CLP Annex VI, this can be due to one of the reasons listed in Note 4 under Table 1.1 in CLP Annex VII (see above). In this case the obligations under Article 4(3) CLP must apply, i.e. classification under Title II shall be carried out for this differentiation.

Self classification must take into account all available relevant data including published RAC documents for Harmonised Classification and Labelling (RAC opinions, background documents and responses to comments as available on ECHA website in section Risk Assessment Committee http://echa.europa.eu).

The resulting different variants of H360 and H361 are shown in the table below, which also provides some examples when they can be assigned.

Table 3.7.4.1: *Hazard statements for reproductive toxicity: H360 and H361, and their specifications*

H360	"May damage fertility or the unborn child" *Example:* *a substance classified in Repr Cat 1 A/B but the effects cannot be specified with respect to fertility and/or developmental toxicity.*
H361	"Suspected of damaging fertility or the unborn child" *Example:* *a substance classified in Repr Cat 2 but the effects cannot be specified with respect to fertility and/or developmental toxicity.*
H360F	"May damage fertility." *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects. For the effects on developmental toxicity there is evidence providing no such effect, inconclusive data or no data.*
H360D	"May damage the unborn child." *Example: a substance classified in Repr Cat 1A/B on the basis of developmental toxicity. For the effects on fertility there is evidence providing no such effect, inconclusive data or no data.*
H361f	"Suspected of damaging fertility". *Example: a substance classified in Repr Cat 2 on the basis of fertility effects. For the effects on developmental toxicity there is evidence providing no such effect, inconclusive data or no data.*
H361d	Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 2 on the basis of developmental toxicity. For the effects on fertility there is evidence providing no such effect, inconclusive data or no data.*
H360FD	May damage fertility. May damage the unborn child. *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects and developmental toxicity.*
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 2 on the basis of fertility effects and developmental toxicity.*
H360Fd	May damage fertility. Suspected of damaging the unborn child. *Example: a substance classified in Repr Cat 1A/B on the basis of fertility effects and*

	which fulfills the criteria for Repr Cat 2 on the basis of developmental toxicity.
H360Df	May damage the unborn child. Suspected of damaging fertility. *Example: a substance classified in Repr Cat 1A/B on the basis of developmental toxicity and which fulfills the criteria for Repr Cat 2 on the basis of fertility effects.*

According to CLP Annex I, Section 3.7.4.1, the hazard statements must be adapted by specifying the route of exposure if it is conclusively proven that no other routes of exposure will lead to an adverse effect on sexual function or fertility or development of the offspring. When conclusively proven, it is meant that valid *in vivo* test data need to be available for all three exposure routes clearly indicating that only one exposure route has caused positive results i.e. adverse effects on the reproduction. Moreover, such a finding should be considered plausible with respect to the mechanism or mode of action. It is estimated that such a situation would rarely occur.

3.7.4.2 Additional labelling provisions

There are no additional labelling provisions for reproductive toxic substances and mixtures in CLP, however there are provisions laid out in Annex XVII to REACH. The packaging of substances with harmonised classification for reproductive toxicity Category 1A or Category 1B, and mixtures containing such substances at concentrations warranting classification of the mixture for reproductive toxicity Category 1A or Category 1B, "must be marked visibly, legibly and indelibly as follows: 'Restricted to professional users'." (REACH Annex XVII, point 30).

3.7.5 Re-classification of substances and mixtures classified for reproductive toxicity according to DSD and DPD

3.7.5.1 Is direct "translation" of classification and labelling possible?

Generally yes. In case there is no re-evaluation of the data, the hazard statement specifying both 'damage to fertility' and 'damage to the unborn child' should be assigned. It is possible to omit the hazard statement specifying fertility or developmental effects; in case there are clearly negative results (see Section 3.7.4.1 of this Guidance).

However, in some very rare situations, a reproductive toxicant classified with Repr. Cat. 3; R62 may need classification with Repr. Cat. 1B H360 under CLP. According to Annex VI to DSD, for the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known anti-fertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. According to CLP, such supporting evidence is not needed.

Classification for effects on or via lactation according to CLP is directly equivalent to assignment of R64 according to DSD as the criteria are essentially the same. Therefore, direct translation of R64 to H362 is possible.

Guidance update to section 3.7.5.1 of the Guidance in accordance with the 4th ATP: to be applied from 1 December2014 for substance and 1 June 2015 for mixtures

3.7.5.1 Is direct "translation" of classification and labelling possible?

Comment [SJ2]: Replacement section 3.7.5.1 for 4th ATP changes

Generally yes (see Section 3.7.4.1 of this Guidance).

However, in some very rare situations, a reproductive toxicant classified with Repr. Cat. 3; R62 may need classification with Repr. Cat. 1B H360F under CLP. According to Annex VI to DSD, for the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known anti-fertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. According to CLP, such supporting evidence is not needed.

Classification for effects on or via lactation according to CLP is directly equivalent to assignment of R64 according to DSD as the criteria are essentially the same. Therefore, direct translation of R64 to H362 is possible.

3.7.6 Examples

3.7.6.1 Examples of the determination of SCLs

Four examples are given below:

3.7.6.1.1 Example 1

1.Identification

Substance Name:	XXXXXX

2. EU CLP classification

Repro	1B
H	360D

3. ED_{10} in animals

Brief summary

OECD 414, Wistar rats, GD 6-19, 0, 20, 60, 180 mg/kg bw. The number of live foetuses per litter was significantly reduced and the postimplantation loss was 43 % at the high dose compared to only 8 % in the control being statistically significant.
The mean foetal body weight was reduced by 14 %. Further, the incidence of external malformations (anasarca and/or cleft palate) was significantly increased. About 10 % of the high dose foetuses were affected (13/132 foetuses; in 7/22 litters) while no such changes were observed in the control.
Skeletal malformations were also statistically significantly increased: 7.8 % affected foetuses per litter (7/73 foetuses in 5/21 litters) were noted in the high dose group compared to 1.1 % in the control. The incidences of shortened scapula (4/73 foetuses), bent radius/ulna (2/73 foetuses), malpositioned and bipartite sternebrae (2/73 foetuses) were statistically significantly increased. Soft tissue variations (dilated renal pelvis and ureter) were significantly increased in foetuses from high dose dams compared to controls (27.1 % vs. 6.4 %).
At 0, 20, 60, 180 mg/kg 7.9, 14.8, 9.6, 43 % postimplantation loss was found, respectively.

Remarks on the study used for the determination of the ED_{10}

Species, strain, sex:	Female Wistar rat
Study type:	OECD 414
Route of administration:	Oral gavage
Effect descriptor for LOAEL:	Post-implantation loss, anasarca, cleft palate
Mode of action:	Not known
Genotoxicity classification:	None
Potential to accumulate:	No data. not known

Determination of the ED_{10} value

Control resorption rate (= postimplantation loss) is 7.9%. ED_{10} rate would be 17.9%. Interpolation between NOAEL (classification) (9.6% at 60 mg/kg) and LOAEL (classification) (43% at 180 mg/kg) leads to an ED_{10} of 89.8 mg/kg bw/d.

Calculation:

(180 – 60) / (43 – 9.6) = 3.593 mg/kg per % (steepness). Going from 9.6% to 17.9% requires addition of 8.3%. This equals 8.3% * 3.593 mg/kg per % = 29.8 plus 60 as the starting point = 89.8 mg/kg bw/day.

The ED_{10} for other relevant effects was above 89.8 mg/kg bw/day.

Preliminary potency group

medium

4. Elements that may modify the preliminary potency evaluation

4.1. Dose-response relationship

Not relevant as ED_{10} not borderline.

4.2. Type of effect / severity

Not relevant as ED_{10} not borderline.

4.3. Data availability

Not relevant. Only one valid study available.

4.4. Mode of action

No data.

4.5. Toxicokinetics

No data.

4.6. Bio-accumulation

Little information, only environmental. Accumulation in organisms is not to be expeceted due to the calculated BCF at 3.16. The substance tends not to accumulate in biota due to the low calculated BCF (<<500) and low measured log Kow (<<4).

5. Allocation of potency group and SCL

medium potency, GCL

6.References

Confidential

3.7.6.1.2 Example 2 (developmental part only)

1. Identification

Substance Name :	XXXXXX

2. EU CLP classification

Repro	1B
H	360 FD

3. ED_{10} in animals

Brief summary

Study used for the determination of the ED_{10}:

Pregnant females received daily gavage doses of 0, 25, 50, 100 or 175 mg/kg during the gestation period (GD 6-19).

LOAEL effect	0 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	175 mg/kg
Skeletal malformations	2/22 (9 %)	2/17 (12 %)	5/15 (33%)	10/19 (53%)	6/12 (50%)

Clear maternal toxicity was evident only at the highest dose level.

Remarks on the study used for the determination of the ED_{10}

Species, strain, sex:	Rabbit, New Zealand White, female
Study type:	Developmental 6-19
Route of administration:	Gavage
Effect descriptor for LOAEL:	Skeletal malformations (axial skeleton, ribs)
Mode of action:	Substance is metabolised to a substance which causes the developmental effect
Genotoxicity classification:	None
Potential to accumulate:	Unknown

1 **Determination of the ED_{10} value**

ED_{10} was determined as 33 mg/kg.

Control skeletal malformations is 9%. ED_{10} rate would be 19%. Interpolation between NOAEL (classification) (12% at 25 mg/kg) and LOAEL (classification) (33% at 50 mg/kg) leads to an ED_{10} of 33.3 mg/kg bw/day.

Calculation:

(50– 25) / (33 – 12) = 1.19 mg/kg per % (steepness). Going from 12% to 19% requires addition of 7%. This equals 7% * 1.19 mg/kg per % = 8.3 plus 25 as the starting point = 33.3 mg/kg bw/day.

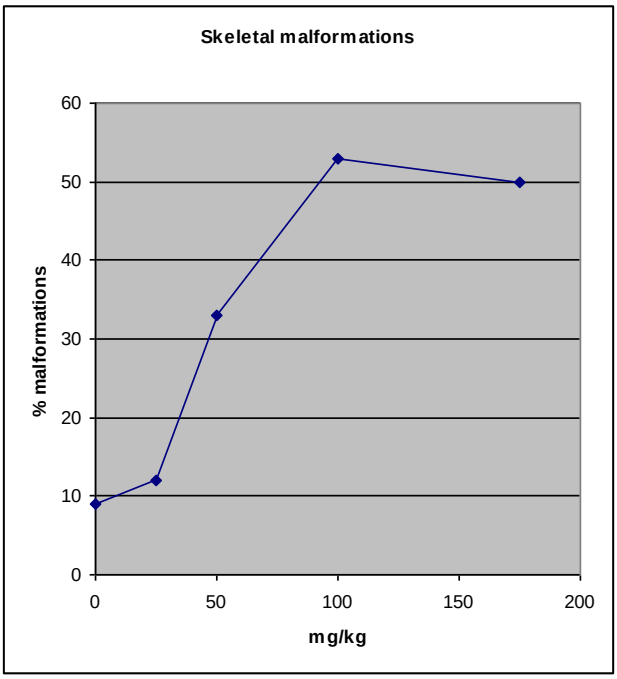
2 **Preliminary potency group**

Medium potency group.

3 **4. Elements that may modify the preliminary potency evaluation:**

4 **4.1 Dose-response relationship**

The effect on which the classification is based is the occurrence of malformations. As the lowest ED_{10} was the ED_{10} for skeletal malformations, this ED_{10} was chosen as the basis for the SCL. The dose effect relationship is clear. The ED_{10} (33 mg/kg) is not borderline with the LOAEL. There is no reason to consider the dose-response relationship to modify the potency of the substance.



4.2 Type of effect / severity

The effect on which the classification is based is the occurrence of malformations, which is a severe effect. Moving the substance to a higher potency group should be considered.

4.3 Data availability

Not relevant. Different studies are available showing a developmental effect on different species (rat, mouse, rabbit).

4.4 Mode of action

The toxic metabolite has been extensively investigated and established as a strong embryotoxicant and teratogen. There is no mechanistic information showing a higher or a lesser sensitivity in humans than in experimental animals.

4.5 Toxicokinetics

Human and rat liver microsomal preparations (mixtures) have been shown to produce qualitatively and quantitively similar oxidative metabolic products suggesting that the human pathways for this substance may be similar to those observed in experimental animals.

4.6 Bio-accumulation

Unknown

5. Allocation of potency group and SCL

The effect on which the classification is based is the occurrence of malformations. This is a severe effect. Due to the fact that the ED_{10} (33 mg/kg) is not a borderline case, it is not justified to move the substance to the highest potency group although the ED_{10} is based on a severe effect like malformations. Medium potency, GCL.

6. References

Confidential

3.7.6.1.3 Example 3 (limited to developmental toxicity)

1. Identification

Substance Name :	XXXXXX

2. EU CLP classification

Repro	1B
H	360 fD

1 **3. ED_{10} in animals**

2 **Brief summary**

Several studies in rats were available for the evaluation of the developmental effect of this substance. These included 2-generation studies, developmental toxicity studies, and studies with exposure in sensitive periods during gestation. The most relevant study for the evaluation of potency was considered to be a two-generation study performed according to the revised OECD Test Guideline 416. In this study the substance was administered in the diet. Developmental toxicity was evident as reduced absolute and adjusted AGD in F1 and F2 offspring as well as and reduced foetal and testicular weight in offspring. The NOAEL was 50 mg/kg bw/day based on reduced AGD from 250 mg/kg bw/day. These effects were reported in the absence of marked maternal toxicity. Effects on the reproductive organs were also reported in male offspring in the developmental toxicity studies at higher doses.

3 **Remarks on the study used for the determination of the ED_{10}**

Species, strain, sex: Study type:	CD(Sprague-Dawley) rats male and female: 2-generation according to OECD 416
Route of administration:	Oral in feed
Effect descriptor for LOAEL:	Overall: reduced anogenital distance Classification: increase in areolae in males
Mode of action:	Antiandrogenic effect, mechanism relevant for humans
Genotoxicity classification:	Not classified for germ cell mutagenicity
Potential to accumulate:	No

4 **Determination of the ED_{10} value**

Calculation of the ED_{10} value: 416 mg/kg bw/day

Dose (mg/kg bw/day)	% male F1 with areola
0	2.63
50	0.0
250 (NOAEL)	0.76
750 (LOAEL)	32.3

The ED_{10} is calculated by interpolation between 250 and 750 mg/kg bw/day to a dose level with 10% above control level. Roughly, an increase of 30% above control was found at 750 mg/kg bw/day. Interpolation between 250 and 750 mg/kg bw/day results in a dose of 16.67 mg/kg bw/day for each % of increase in areola ((750-250)/30). A 10% increase (ED_{10}) is expected at 250 + 10 * 16.67 = 416 mg/kg bw/day.

5 **Preliminary potency group**

Low potency

4. Elements that may modify the preliminary potency evaluation

4.1 Dose-response relationship

A dose-response relationship on decreased AGD was evident for decrease in AGD in the two-generation study. (AGD was decreased in male offspring in a dose-related pattern from 250 mg/kg bw/day (1. 89 mm at 250 mg/kg bw/day and 1.70 mm at 750 mg/kg bw/day (control: 2.06 mm)).

4.2 Type of effect / severity

Development: reduced anogenital distance (absolute and adjusted) from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day.

Maternal toxicity: organ weight changes, and histopahological lesions in the liver graded as minimal in females at 750 mg/kg bw/day.

NOAEL for developmental effects: 50 mg/kg bw/day based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring.

NOAEL for maternal toxicity: 250 mg/kg bw/day.

4.3 Data availability

A two-generation study is considered relevant for the assessment of development toxicity.

4.3 Mode of action

The mechanism (antiandrogen activity) is considered relevant for humans.

4.5 Toxicokinetics

When metabolites are measured in urine, they are related to the day before exposure. The metabolites of the substance in rats differ quantitatively from those in humans. In several studies the pattern of malformations induced by some of the metabolites were similar to that produced by the substance, suggesting that the metabolic products may be responsible for the developmental toxicity.

Although there is a difference in toxicokinetics between rats and humans, this difference is not expected to result in a difference in potency between rats and humans as the available data indicate comparable effects and potency of the metabolites.

4.6 Bio-accumulation

Low to medium bioaccumulation

5. Allocation of potency group and SCL

The ED_{10} was 416 mg/kg bw/day. The elements that may modify the potency evaluation were considered to not modify the potency. This substance is shown to have a low potency. Therefore an SCL of 3 % should be applied.

6. References

Confidential.

1

2 **3.7.6.1.4 Example 4**

3 **1 Identification**

Substance Name :	XXXXXX

4 **2 EU CLP classification**

Repro	2
H	361f

5 **3 ED_{10} in animals**

6 **Brief summary:**

Only two repeated dose studies are available for this substance and no fertility studies. In the inhalatory repeated dose study testicular lesions were observed after exposure to 2.87 mg/l for 4 exposures of 16 to 20 hours per week during 11 weeks. Other dose levels were not tested. In the oral 90 day study, effects on the testes were observed after exposure to 660 mg/kg bw/day. Other dose levels were not tested.

7 **Remarks on the study used for the determination of the ED_{10}**

Species, strain, sex:	Rats, CD(SD)BR males
Study type:	90 days, 5 days per week, 120 day observation period
Route of administration:	gavage
Effect descriptor for LOAEL:	testicular atrophy in 50% of the animals
Mode of action:	A metabolite is assumed to be causing the testicular effects. A direct effect of this metabolite on the Sertoli cells is postulated.
Genotoxicity classification:	none
Potential to accumulate:	unknown

8 **Determination of the ED_{10} value**

The dose level of 660 mg/kg bw/day is considered as the LOAEL but in the absence of a NOAEL an ED_{10} cannot be determined by interpolation or the BMD approach because only one dose level was tested. An ED_{10} can be estimated based on interpolation between 660 mg/kg bw/day (50% of the animals affected) and the control (0 % of the animals affected). This results in an ED_{10} of 132 mg/kg bw/day by interpolation.

9 **Preliminary potency group**

Medium potency group

4 Elements that may modify the preliminary potency evaluation

4.1 Dose-response relationship

There is no data available on the dose response relationship.

4.2 Type of effect / severity

There are clear testicular effects. It is unknown whether these effects will result in functional effects on fertility as this has not been tested.

4.3 Data availability

There is only limited data available at one exposure level.. A LOAEL can be determined but it in the absence of a NOAEL it cannot be excluded that effects on sexual organs occur at levels below the LOAEL. The available data are considered as limited.

4.4 Mode of action

A metabolite is assumed to be the cause of the testicular effects. A direct effect of this metabolite on the Sertoli cells is postulated.

4.5 Toxicokinetics

Unknown

4.6 Bio-accumulation

Unknown

5 Allocation of potency group and SCL

An ED_{10} can only be estimated using interpolation between the only dose tested and the controls.. The resulting ED10 indicates medium potency. However, there is only very limited data. As there is only an LOAEL and no NOAEL, it cannot be excluded that testicular effects can be induced at lower levels. However, there is no evidence that this substance can induce testicular effects at dose levels below 4 mg/kg bw/day. Therefore, a medium potency is considered the best estimate based on the available data.

6 References

Confidential

3.8 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT-SE)

3.8.1 Definitions and general considerations for STOT-SE

Annex 1: 3.8.1.1. Specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically addressed in Chapters 3.1 to 3.7 and 3.10 are included (see also 3.8.1.6).

There are two hazard classes for single exposure toxicity: "Acute toxicity" and "STOT-SE". These are independent of each other and both may be assigned to a substance or a mixture if the respective criteria are met. Acute toxicity refers to lethality and STOT-SE to non lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such a case the most appropriate class should be assigned.

Acute toxicity classification is generally assigned on the basis of evident lethality (e.g. an LD_{50}/LC_{50} value) or where the potential to cause lethality can be concluded from evident toxicity (e.g. from fixed dose procedure). STOT-SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality.

Furthermore, specific toxic effects covered by other hazard classes are not included in STOT-SE. STOT-SE should only be assigned where the observed toxicity is not covered more appropriately by another hazard class. For example, specific effects caused after a single exposure like corrosion of skin or effects on the reproductive organs should be used for classification for skin corrosion or reproductive toxicity, respectively, but not for STOT-SE.

Annex 1: 3.8.1.4. Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs.

3.8.1.5. Specific target organ toxicity can occur by any route that is relevant for humans, i.e. principally oral, dermal or inhalation.

3.8.1.7. The hazard class Specific Target Organ Toxicity – Single Exposure is differentiated into:

Specific target organ toxicity – single exposure, Category 1 and 2;

Specific target organ toxicity – single exposure, Category 3.

The hazard class STOT-SE has 3 categories, with Categories 1 and 2 being distinct from Category 3 in terms of the toxicity they cover and the criteria. Categories 1 and 2 for non lethal "significant and/or severe toxic effects" are the basis for classification with the category reflecting the dose level required to cause the effect. Category 3 covers "transient effects" occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE). The relationship between Categories 1/2 vs. Category 3 is discussed in Section 3.8.2.4.3 of this Guidance.

3.8.2 Classification of substances for STOT-SE

3.8.2.1 Identification of hazard information

Annex 1: 3.8.2.1.5. The information required to evaluate specific target organ toxicity comes either from single exposure in humans, such as: exposure at home, in the workplace or environmentally, or from studies conducted in experimental animals.

CLP does not require testing of substances or mixtures for classification purposes. The assessment is based on the respective criteria together with available adequate and robust test data/information. Generally, information relevant to STOT-SE can be obtained from human experience or acute toxicity studies in animals.

3.8.2.1.1 Identification of human data

Relevant information with respect to toxicity after single exposure may be available from case reports, epidemiological studies, medical surveillance and reporting schemes and national poisons centres.

Data on sensory irritation of the airways may be available from volunteer studies including objective measurements of RTI such as electrophysiological responses, data from lateralization threshold testing, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids (Guidance on IR/CSA, Section 7.2.3.2). For more details see the Guidance on IR/CSA, Section 7.4.3.2 and R.7.2.

3.8.2.1.2 Identification of non human data

ANNEX 1: 3.8.2.1.5 The standard animal studies in rats or mice that provide this information are acute toxicity studies which can include clinical observations and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Results of acute toxicity studies conducted in other species may also provide relevant information.

Annex 1: 3.8.2.1.7.3. Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, must be taken into consideration in the classification process, …

Non-testing data

Physicochemical data

Physicochemical properties, such as pH, physical form, solubility, vapour pressure, particle size, can be important parameters in evaluating toxicity studies and in determining the most appropriate classification especially with respect to inhalation where physical form and particle size can have a significant impact on toxicity.

(Q)SAR models, Read across

"Non-testing" data (i.e. data not obtained from experimental methods) can be provided by the use of techniques such as grouping/category formation, Quantitative and qualitative Structure Activity Relationship (Q)SAR models and expert systems, which generally relate physico-chemical properties and chemical structure to toxicity. The use of these methods is described in more detail in Section 2.3.2 of this Guidance and in the Guidance on IR/CSA, Section R.7.4.4.1.

The potential use of (Q)SAR models for predicting effects relevant to STOT-SE Categories 1/2 is currently quite limited and may only be applicable in specific cases. However, they may be somewhat more useful for STOT-SE Category 3 where there are some well established relationships between physicochemical properties or chemical structure and effects such as narcosis and respiratory tract irritation. For instance substances such as aldehydes, unsaturated carbonic esters and reactive inorganic compounds are generally found to be respiratory tract irritants.

In addition, there are systems which can predict the metabolism of substances. These can be useful in providing information on the potential for the substance to be metabolised to substances with known toxicity. An example is certain esters, which after enzymatic cleavage to carbonic acids and alcohols in the nasal region, cause respiratory irritation.

For more details see the Guidance on IR/CSA, Section 7.4.3.1.

Testing data

Animal data

The standard tests on acute toxicity are listed in the Guidance on IR/CSA, Section R.7.4.3.1.

For **Category 1 and 2**, in general terms, most studies involving single exposure via any relevant route of exposure, such as acute toxicity studies, can be used for classification purposes. Older acute toxicity studies which tended to only measure lethality as an observational endpoint (e.g. to determine LD_{50}/LC_{50}) will generally not provide useful information for STOT-SE. However, newer acute toxicity test protocols, such as the fixed-dose and up-down procedures, have a wider range of observations on signs of toxicity and therefore may provide information relevant for STOT-SE. Other standard studies, e.g. neurotoxicity tests, or ad-hoc studies designed to investigate acute toxicity, can also provide valuable information for STOT-SE.

Care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at relevant doses then a classification for acute toxicity would take precedence and STOT-SE would not be assigned.

Although classification in **Category 3** is primarily based on human data, if available, animal data can be included in the evaluation. These animal data on RTI and NE will generally come from standard acute inhalation studies, although it is possible that narcosis could be observed in studies using other routes. Standard acute toxicity tests are often more useful for Category 3 than for STOT-SE Categories 1/2 because overt findings of narcosis and RTI are more often reported in clinical observations.

The Alarie test gives specific information on the potential for sensory irritation. Further, information on this test and its limitations can be found in the Guidance on IR/CSA, Section R.7.2.

Furthermore the Inhalation Hazard Test (Annex to OECD TG 403) might give information on the potential for RTI of volatile substances. Though the focus of STOT-SE is on effects caused by single exposure, data from studies with repeated exposure might give additional valuable information, especially with respect to the underlying mode of action of RTI.

In vitro data

Since there are currently no *in vitro* tests that have been officially adopted by the EU or OECD for assessment of acute toxicity, there are also no useful test systems for STOT-SE (see the Guidance on IR/CSA, Section R.7.4.3.1). Any available studies should be assessed using expert judgement.

3.8.2.2 Classification criteria for Categories 1 and 2

Annex I: 3.8.2.1.1. Substances are classified for immediate or delayed effects separately, by the use of expert judgement (see 1.1.1) on the basis of the weight of all evidence available, including the use of recommended guidance values (see 3.8.2.1.9). Substances are then placed in Category 1 or 2, depending upon the nature and severity of the effect(s) observed (Table 3.8.1).

Table 3.8.1

Categories for specific target organ toxicity-single exposure

Categories	Criteria
Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the

	potential to produce significant toxicity in humans following single exposure Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: (a) reliable and good quality evidence from human cases or epidemiological studies; or (b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2 (see 3.8.2.1.6).
Note: Attempts shall be made to determine the primary target organ of toxicity and to classify for that purpose, such as hepatotoxicants, neurotoxicants. The data shall be carefully evaluated and, where possible, secondary effects should not be included (e.g. a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems). **3.8.2.1.2.** The relevant route or routes of exposure by which the classified substance produces damage shall be identified (see 3.8.1.5).	

STOT-SE Category 1 and 2 is assigned on the basis of findings of "significant" or "severe" toxicity. In this context "significant" means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound or serious than "significant" effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

3.8.2.2.1 Guidance values

Annex 1: 3.8.2.1.9.1 In order to help reach a decision about whether a substance shall be classified or not, and to what degree it shall be classified (Category 1 or Category 2), dose/concentration 'guidance values' are provided for consideration of the dose/concentration which has been shown to produce significant health effects.

Annex 1: 3.8.2.1.9.3. The guidance value (C) ranges for single-dose exposure which has produced a significant non-lethal toxic effect are those applicable to acute toxicity testing, as indicated in Table 3.8.2.

Table 3.8.2

Guidance value ranges for single-dose exposures [a]

			Guidance value ranges for:*	
Route of exposure	Units	Category 1	Category 2	Category 3
Oral (rat)	mg/kg body weight	C ≤ 300	2000 ≥ C > 300	Guidance values do not apply [b]
Dermal (rat or rabbit)	mg/kg body weight	C ≤ 1000	2000 ≥ C > 1000	
Inhalation (rat) gas	ppmV/4h	C ≤ 2500	20000 ≥ C > 2500	
Inhalation (rat) vapour	mg/l/4h	C ≤ 10	20 ≥ C > 10	
Inhalation (rat) dust/mist/fume	mg/l/4h	C ≤ 1.0	5,0 ≥ C >1,0	

Note

(a) The guidance values and ranges mentioned in Table 3.8.2 above are intended only for guidance purposes, i.e. to be used as part of the weight of evidence approach, and to assist with decision about classification. They are not intended as strict demarcation values.

(b) Guidance values are not provided for Category 3 substances since this classification is primarily based on human data. Animal data, if available, shall be included in the weight of evidence evaluation.

* Note: There is a misprint in Annex I, Table 3.8.2; the heading 'Guidance value ranges for:' should also belong to the column 'Category 1'.

Where significant or severe toxicity has been observed in animal studies, the dose/exposure level causing these effects is compared to the guidance values provided to determine if classification in Category 1 or 2 is most appropriate.

In cases of inhalation studies with exposure times different to 4 hours an extrapolation can be performed similar to the one described in Section 3.1 of this Guidance for Acute Toxicity.

3.8.2.3 Classification criteria for Category 3: Transient target organ effects

Currently, the criteria for classification in Category 3 only cover the transient effects of "respiratory tract irritation" and "narcotic effects".

Annex I: *Table 3.8.1 (continued)*

Categories for specific target organ toxicity-single exposure

Categories	Criteria
Category 3	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter 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. Substances are classified specifically for these effects as laid down in 3.8.2.2

Annex 1: 3.8.2.2.1 Criteria for respiratory tract irritation

The criteria for classifying substances as Category 3 for respiratory tract irritation are:

(a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.

(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids).

(c) he symptoms observed in humans shall 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" shall 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 irritation.

(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.

(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

It is clearly indicated in the CLP that there are currently no validated animal tests that deal specifically with RTI, but that animal studies can be used as a part of weight of evidence evaluation (CLP Annex I, 3.8.2.2.1.2(d)). However when there are no data in human and animal data suggesting RTI effects, expert judgement is needed to estimate the severity of the effects observed in animals, the conditions of the test, the physical-chemical properties of the substance and whether those considerations alone might be sufficient for a classification in Category 3 for RTI.

The generic term RTI covers two different effects: "sensory irritation" and "local cytotoxic effects". Classification in STOT-SE Category 3 for respiratory tract irritation is generally limited to local cytotoxic effects.

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. It helps to minimize exposure by decreasing the respiration-time-volume and inducing the exposed to leave the areas of irritant concentrations, if possible. Sensory irritation-related effects are fully reversible given that its biological function is to serve as a warning against substances that could damage the airways.

Local cytotoxic irritant effects induce tissue changes at the site of contact which can be detected by clinico-pathological or pathological methods. Such effects may induce long lasting functional impairment of the respiratory system.

The basic mechanisms underlying morphological changes comprise cytotoxicity and induction of inflammation. Based on the quality and severity of morphological changes, the function of the respiratory system could be impaired, which may lead to the development of

consequential systemic effects, i.e. there might be consequences on distal organs by a diminution of the oxygen supply. As the functional impairment is seldom evaluated by experimental inhalation studies in animals, data on functional changes will mainly be available from experience in humans.

Further see the Guidance on IR/CSA, Section R.7.2.

Annex 1: 3.8.2.2.2. Criteria for narcotic effects

The criteria for classifying substances as Category 3 for narcotic effects are:

(a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness.

(b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

3.8.2.4 Evaluation of hazard information on STOT-SE for substances

3.8.2.4.1 Evaluation of human data

Annex I: 3.8.2.1.6. In exceptional cases, based on expert judgement, it is appropriate to place certain substances with human evidence of target organ toxicity in Category 2:

(a) when the weight of human evidence is not sufficiently convincing to warrant Category 1 classification, and/or

(b) based on the nature and severity of effects.

Dose/concentration levels in humans shall not be considered in the classification and any available evidence from animal studies shall be consistent with the Category 2 classification. In other words, if there are also animal data available on the substance that warrant Category 1 classification, the substance shall be classified as Category 1.

Annex 1: 3.8.2.1.7.2. Evidence from human experience/incidents is usually restricted to reports of adverse health consequence, often with uncertainty about exposure conditions, and may not provide the scientific detail that can be obtained from well-conducted studies in experimental animals.

Annex 1: 3.8.2.1.10.2. When well-substantiated human data are available showing a specific target organ toxic effect that can be reliably attributed to single exposure to a substance, the substance shall normally be classified. Positive human data, regardless of probable dose, predominates over animal data. Thus, if a substance is unclassified because specific target organ toxicity observed was considered not relevant or significant to humans, if subsequent human incident data become available showing a specific target organ toxic effect, the substance shall be classified.

Human data are potentially very valuable for determining an appropriate classification as they provide direct evidence on the effects of a substance in humans. However, the evaluation of human data is often made difficult by various limitations frequently found with the types of studies and data highlighted in Section 3.8.2.4.1 of this Guidance. These include uncertainties relating to exposure assessment (i.e. unreliable information on the amount of a substance the subjects were exposed to or ingested) and confounding exposures to other substances. As a result it should be acknowledged that human data often do not provide sufficiently robust evidence on their own to support classification but may contribute to a weight of evidence assessment with other available information such as animal studies.

Categories 1 and 2

In general, where reliable and robust human data are available showing that the substance causes significant target organ toxicity these take precedence over other data, and directly support classification in Category 1. Available animal data may support this conclusion but do not detract from it (e.g. if the same effect is not observed in animals).

In exceptional cases, where target organ toxicity is observed in humans but the data reported are not sufficiently convincing to support Category 1 because of the lack of details in the observations or in the exposure conditions, and/or with regard to the nature and the severity of the effects observed, then classification in Category 2 could be justified (CLP Annex I, 3.8.2.1.6). In this case, any animal data must also be consistent with Category 2 and not support Category 1 (see below). In this case, if the animal data support Category 1, they will take precedence over the human data. This is because the reliability of the human data in this case is probably lower than the reliability of data from standard well conducted animal studies and should accordingly have less weight in the assessment.

When using human data, there is no consideration of the human dose/exposure level that caused those effects.

Category 3

Respiratory Tract Irritation

Human evidence for RTI often comes from occupational case reports where exposure is associated with signs of RTI. Such reports should be interpreted carefully using expert judgement to ensure that they provide reliable information. For instance, there should be a clear relationship between exposure and the development of signs of RTI, with RTI appearing relatively soon after the start of exposure. A solid substance which causes RTI due to physical/mechanical irritation when inhaled as a dust should not be classified. For more details on RTI, see the Guidance on IR/CSA Chapter R7a.7.2.1, and example n° 3 for sulfur dioxide.

Narcotic Effects

Narcotic effects may range from slight dizziness to deep unconsciousness and may be caused by several mechanisms:

- pharmaceutical drugs (designed effect; often receptor-mediated; effective dose usually low; patient under professional observation; limited importance for industrial chemicals and their safety assessment.)
- unspecific effects of many organic industrial chemicals on CNS-membranes at high dose levels (often solvent vapours, ≥ 6000 ppm in respired air volume). Such effects can be expected at high exposure levels due to otherwise low toxicity.

- organic chemicals with similarities to and interference with CNS-transmitters; often metabolic transformation necessary; certain solvents, e.g. butandiol, butyrolactone, methoxyethanol; medium levels of effective dose. Children may be considerably more susceptible than adults.
- chemicals with high specific CNS toxicity; narcotic effects usually close to near-lethal doses (example: H_2S).

Narcotic effects are usually readily reversible on cessation of exposure with no permanent damage or changes.

Human evidence relating to narcosis should be evaluated carefully. Often the reporting of clinical signs is relatively subjective and reports of effects such as severe headache and dizziness should be interpreted carefully to judge if they provide robust evidence of narcosis. Where relevant human data do not mirror realistic exposure conditions, for instance in case reports from accidental over-exposure situations, supportive information may be needed to corroborate the observed effects. A single case report from accidental or deliberate exposure (i.e. abuse) is unlikely to provide sufficiently robust evidence to support classification without other evidence. For more details on evaluation of available human information see also Section 3.1.2.3.1 of this Guidance and the Guidance on IR/CSA, Section R.7.4 (especially R.7.4.4.2). Example n° 4 for toluene illustrates the procedure.

3.8.2.4.2 Evaluation of non human data

Annex 1: 3.8.2.1.5. The standard animal studies in rats or mice that provide information are acute toxicity studies which can include clinical observations and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/ organs to be identified. Results of acute toxicity studies conducted in other species may also provide relevant information.

Annex 1: 3.8.2.1.10.1. When a substance is characterised only by use of animal data (typical of new substances, but also true for many existing substances), the classification process includes reference to dose/concentration guidance values as one of the elements that contribute to the weight of evidence approach.

Annex 1: 3.8.2.1.10.3. A substance that has not been tested for specific target organ toxicity may, where appropriate, be classified on the basis of data from a validated structure activity relationship and expert judgement-based extrapolation from a structural analogue that has previously been classified together with substantial support from consideration of other important factors such as formation of common significant metabolites.

The type of evidence mentioned in CLP Annex I, 3.8.2.1.7 and 3.8.2.1.8 to support or not to support classification (e.g. clinical biochemistry, changes in organ weights with no evidence of organ dysfunction) is rarely obtained from animal tests designed to measure acute lethality/toxicity (see Section 3.8.2.1.2 of this Guidance).

Categories 1 and 2

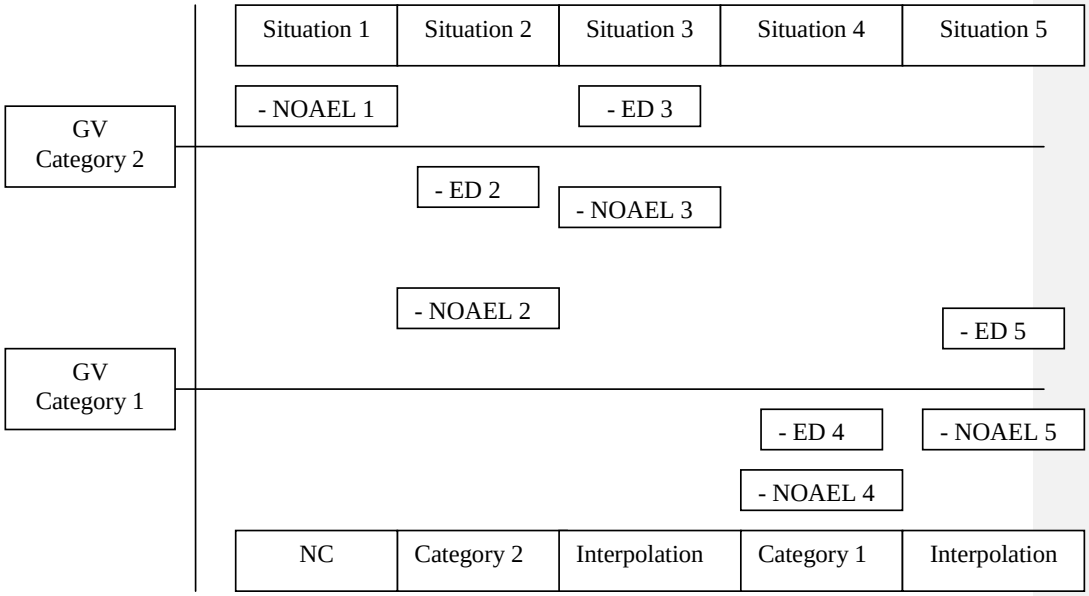
Generic guidance on data evaluation is presented in the Guidance on IR/CSA, Sections R.7.4 and R.7.4.4.2. All available animal data which are of acceptable quality should be used in a weight of evidence approach based on a comparison with the classification criteria described above. The assessment should be done for each route of exposure.

For each study the effects seen in each sex at or around the guidance values (GV) for Category 1 and Category 2 should be compared with the effects warranting classification in

Category 1 and 2. In general findings in the most sensitive sex would be used to determine the classification. If the NOAEL from the study is above the GV, the results of that study do not indicate classification for that category (situations 1 and 2 in Figure 1). If the NOAEL is below the GV then the effective dose (ED) level, the lowest dose inducing significant/severe target organ toxicity as defined in Section 3.8.2.2.1 of this Guidance should be determined based on the criteria described above. If the ED is below the GV then this study indicates that classification is warranted (situations 2 and 4 in Figure 1).

In a case where the ED is above a GV but the NOAEL is below the GV (situations 3 and 5) then interpolation between the ED and the NOAEL is required to determine whether the effects expected at or below the GV would warrant classification .

Figure 3.8.2.4.2 *Comparison between the NOAEL and the ED versus the guidance values*



Where a number of studies are available these should be assessed using a weight of evidence approach to determine the most appropriate classification. Where the findings from individual studies would lead to a different classification then the studies should be assessed in terms of their quality, species and strain used, nature of the tested substance (including the impurity profile and physical form) etc to choose the most appropriate study to support classification. In general, the study giving the most severe classification will be used unless there are good reasons that it is not the most appropriate. If the effects observed in animals are not considered relevant for humans then these should not be used to support classification. Similarly, if there is robust evidence that humans differ in sensitivity or susceptibility to the effect observed in the study then this should be taken into account, possibly leading to an increase or decrease in the classification assigned. The final classification based on non human data will be the most severe classification of the three exposure routes.

Category 3

There are no similar guidance values for Category 3. Therefore, if the study shows clear evidence for narcotic effects or respiratory tract irritation at any dose level then this could support classification with Category 3.

In evaluating inhalation studies a differentiation of respiratory tract effects and systemic effects should always be attempted. In addition, the region in the respiratory tract and the qualitative nature of observed effects is pivotal. Often, the lesions observed are representing stages of a reaction pattern leading to severe and irreversible functional and structural alterations. Therefore reversibility of effects is a significant discriminator. For further details see also Section 3.8.2.3 of this Guidance.

3.8.2.4.3 Evaluation of non-testing and *in vitro* data

Non-testing and *in vitro* data can contribute to the weight of evidence supporting a classification. As described in Annex XI of REACH approaches such as (Q)SAR, grouping and read-across can provide information on the hazardous properties of substances in place of testing and can be used for classification purposes. Also see the Guidance on IR/CSA R7.4.4.1.

3.8.2.4.4 Conversions

The guidance values are given in mg/kg bodyweight. Where the doses in a study are given in different units they will need to be converted as appropriate. For instance the dosages in feeding and drinking water studies are often expressed in ppm, mg test substance/ kg (feed) or mg (test substance)/l (drinking water).

The conversion from mg/l to ppm assuming an ambient pressure of 1 at 101.3 kPa and 25°C is ppm = 24,450 x mg/l × 1/MW.

3.8.2.4.5 Weight of evidence

Annex 1: 3.8.2.1.6. In exceptional cases, based on expert judgement, it is appropriate to place certain substances with human evidence of target organ toxicity in Category 2:

1) when the weight of evidence is not sufficiently convincing to warrant Category 1 classification, and/or

2) based on the nature and severity of effects.

Dose/concentration levels in humans shall not be considered in the classification and any available evidence from animal studies shall be consistent with the Category 2 classification. In other words, if there are also animal data available on the substance that warrant Category 1 classification, the substance shall be classified as Category 1.

The available information should be considered using expert judgement and a weight of evidence assessment, as described in CLP Annex I, 1.1.1 and Module 1 and in the approach described in Section 3.8.2.3 of this Guidance.

If there are no human data then the classification is based on the non-human data. If there is human data indicating no classification but there is also non-human data indicating

classification then the classification is based on the non-human data unless it is shown that the human data cover the exposure range of the non-human data and that the non-human data are not relevant for humans. If the human and non-human data both indicate no classification then classification is not required.

3.8.2.5 Decision on classification of substances

Decision on classification for STOT-SE is based on the results of weight of evidence approach described in 2.3.

STOT-SE and acute toxicity are independent of each other and both may be assigned to a substance if the respective criteria are met. However, care should be taken not to assign each class for the same effect, in other words a double classification for the same effect has to be avoided. STOT-SE will be considered where there is clear evidence for a specific organ toxicity especially in absence of lethality, see examples no 1 and no 3 (methanol and tricresylphosphate).

If no classification has been warranted for acute toxicity despite significant toxic effect, the substance should be considered for classification as STOT-SE.

Normally, the assignment of STOT-SE Category 1 or 2 is independent to the assignment of Category 3. Therefore, a substance may be classified in both Category 1/2 and Category 3 if the respective criteria are met, for instance, in the case of a neurotoxic substance that also causes transient narcotic effects. If Category 1/2 is assigned on the basis of effects in the respiratory tract then Category 3 should not be assigned as this would provide no additional information.

Classification as acutely toxic and/or corrosive is considered to cover and communicate the specific toxicological effect(s) adequately. An additional classification as specific target organ toxicant (single exposure, Category 1 or 2) is not indicated if the severe toxicological effect is the consequence of the local (i.e. corrosive) mode of action.

It is a reasonable assumption that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing frank respiratory tract corrosion. If there is evidence from animal studies or from human experience to support this then Category 3 may be appropriate. In general, a classification for corrosivity is considered to implicitly cover the potential to cause RTI and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed.

Category 3 effects should be confined to changes, whether functional or morphological, occurring in the upper respiratory tract (nasal passages, pharynx and larynx). Localized irritation with associated adaptive responses (e.g., inflammation, epithelial metaplasia, goblet cell hyperplasia, proliferative effects) may occur and are consistent with Category 3 responses. Injury of the olfactory epithelium should be distinguished in terms of irritation-related (non-specific) and metabolic/ non-irritant (specific).

3.8.2.6 Setting of specific concentration limits for STOT-SE

Article 10(1) Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the

classification of the substance or mixture as hazardous.

Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.

In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.

Specific concentration limits (SCLs) for STOT-SE may be set by the supplier in some situations according to Article 10 of CLP. For STOT-SE, this may only be done for substances inducing STOT-SE Category 1 at a dose level or concentration clearly (more than one magnitude) below the guidance values according to Table 3.8.2, e.g. below 30 mg/kg bodyweight from the oral single exposure study. This will be mainly based on data in experimental animals but can also be based on human data if reliable exposure data are available. The SCL (SCL Cat. 1) for a Category 1 substance triggering classification of a mixture in Category 1 can be determined using the following formula:

$$SCLCat.1 = \frac{ED}{GV1} \times 100\% \qquad \textbf{EQUATION 3.8.2.6(A)}$$

SCL Cat 1: 0.7 mg/kgbw/300 mg/kgbw x 100%=0.23% --> 0.2%

In this formula the ED is the dose of the Category 1 substance inducing significant specific target organ toxicity and GV1 is the guidance value for Category 1 according to Table 3.8.2 of Annex I. The resulting SCL is rounded down to the nearest preferred value[11] (1, 2 or 5).

Example of determining STOT-SE SCL for a Category 1 substance:

$$= \frac{0.7 mg/kgbw}{300 mg/kgbw} \times 100\% \qquad = 0.23\% \text{ --> } 0.2\%$$

Though classification of a mixture in Category 1 is not triggered if a Category 1 constituent is present in lower concentrations than the established SCL, a classification in Category 2 should be considered.

The SCL (SCL Cat. 2)for a Category 1 substance triggering classification of a mixture in Category 2 can be determined using the following formula:

$$SCLCat.2 = \frac{ED}{GV2} \times 100\% \qquad \textbf{EQUATION 3.8.2.6(B)}$$

In this formula the ED is the dose of the Category 1 substance inducing specific target organ toxicity and GV2 is the upper guidance value for Category 2 according to Table 3.8.2 of Annex I. The resulting SCL is rounded down to the nearest preferred values (1, 2 or 5). However, if the calculated SCL for classification in Category 2 is above 1%, which is the Generic Concentration Limit, then no SCL should be set.

[11] This is the "preferred value approach" as used in EU and are values to be established preferentially as the numerical values 1.2 or 5 or multiples by powers of ten.

Example for a substance in SCL Category 2:

$$= \frac{0.7 mg/kgbw}{2000 mg/kgbw} \times 100\% \qquad = 0.035 \text{ --> } 0.02\% \text{ (rounded down)}$$

For example, a Category 1substance inducing specific target organ toxicity at 0.7 mg/kg bw/day in an acute oral study would generate an SCL for classification of mixtures in Category 1 at 0.2% and in Category 2 at 0.02% (Cat1: $C \geq 0.2\%$; Cat 2: $0.02\% \leq C < 0.2\%$).

It is not appropriate to determine SCLs for substances classified in Category 2 since ingredients with a higher potency (i.e. lower effect doses than the lower guidance values of Category 2) will be classified in Category 1; substances with higher effect doses than the upper guidance value of Cat2 will generally not be classified.

Classification in STOT-SE Category 3 for RTI and narcotic effects does not take potency into account and consequently does not have any guidance values. A pragmatic default GCL of 20% is suggested, although a lower or higher SCL may be used where it can be justified. Therefore, an SCL can be determined on a case-by-case basis for substances classified as STOT-SE Category 3 and expert judgement shall be exercised.

Specific concentration limits for each of the hazard classes skin and eye irritation, and STOT-SE Category 3 for respiratory tract irritation need to be addressed separately, while unjustified read-across of SCLs from one hazard class to another is not acceptable.

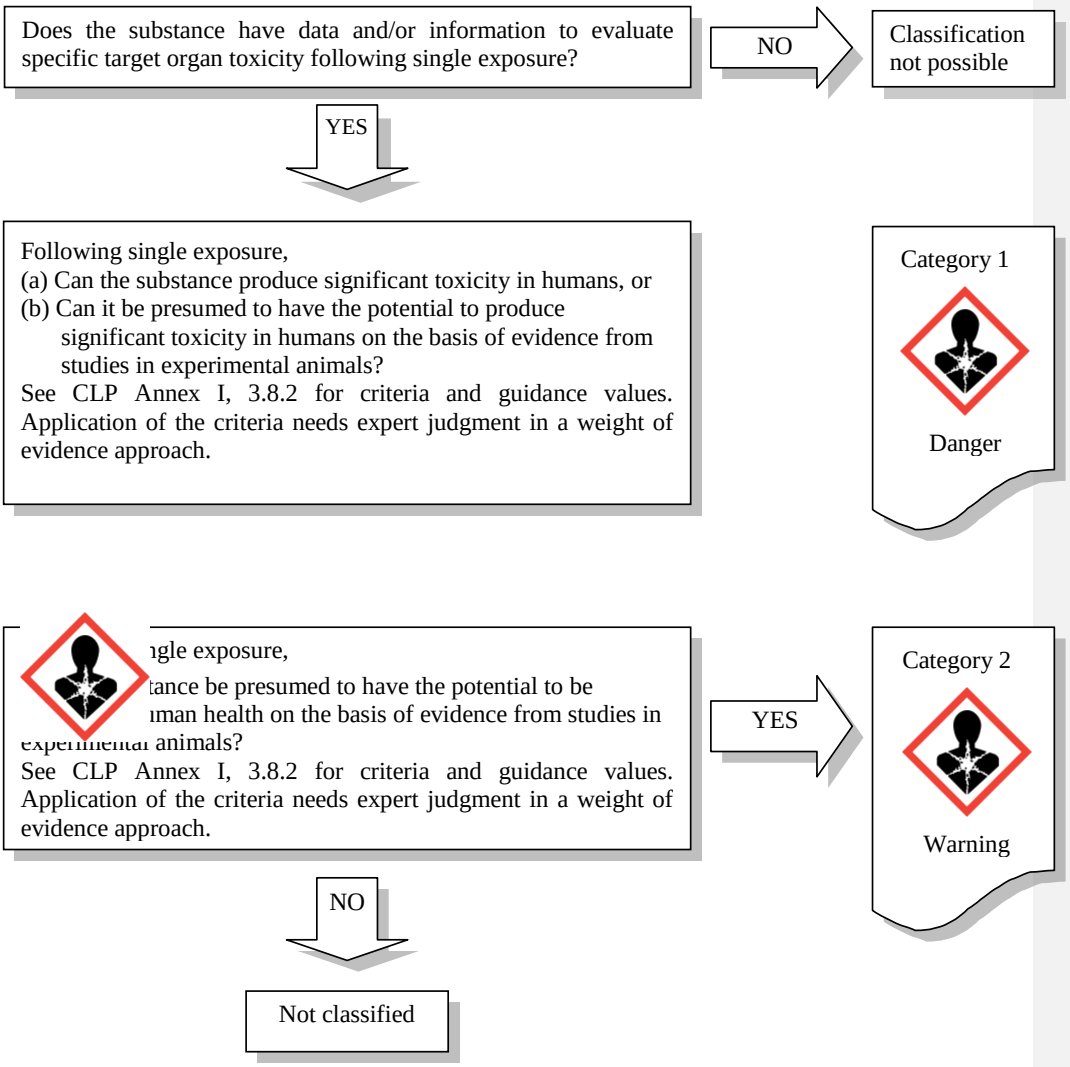
For narcotic effects, the factors to be taken into consideration in order to set lower or higher SCLs are the effective dose/concentration, and in addition for liquids, the volatility (saturated vapour concentration) of the substance.

3.8.2.7 Decision logic

The decision logic is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

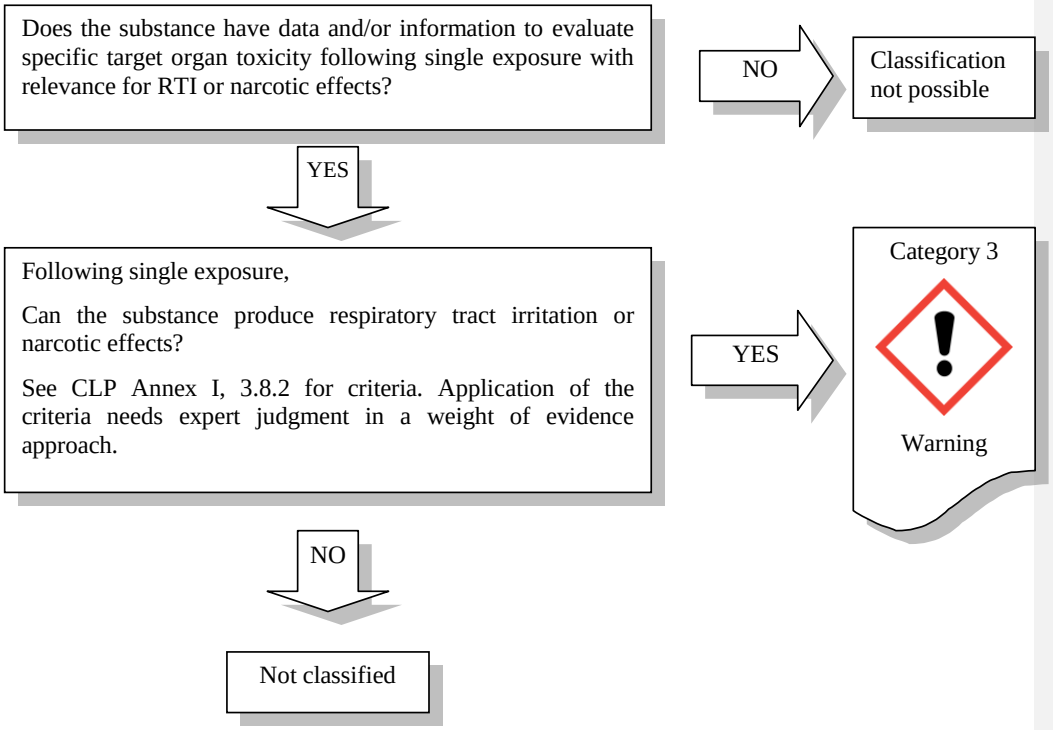
This decision logic deviates slightly from the original UN GHS in separating the connection between Category 2 and Category 3, since, different from the procedure in other hazard classes, they have to be regarded as independent.

Classification in Category 1 and Category 2



1 Classification in Category 3

2



3.8.3 Classification of mixtures for STOT-SE

3.8.3.1 Identification of hazard information

Where toxicological information is available on a mixture this should be used to derive the appropriate classification. Such information may be available from the mixture manufacturer. Where such information on the mixture itself is not available information on similar mixtures and/or the component substances in the mixture must be used, as described below.

3.8.3.2 Classification criteria for mixtures

Annex 1: 3.8.3.1. Mixtures are classified using the same criteria as for substances, or alternatively as described below.

3.8.3.2.1 When data are available for the complete mixture

Annex 1: 3.8.3.2.1. When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture shall be classified by weight of evidence evaluation of these data (see 1.1.1.3). Care shall be exercised in evaluating data on mixtures, that the dose, duration, observation or analysis, do not render the results inconclusive

In cases where test data for mixtures are available, the classification process is exactly the same as for substances.

3.8.3.2.2 When data are not available for the complete mixture: bridging principles

Annex 1: 3.8.3.3.1. Where the mixture itself has not been tested to determine its specific target organ toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures toadequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging principles set out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture(see Section 1.6.3 of this Guidance).

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified using the calculation method or concentration thresholds as described in Sections 3.8.3.2.3, 3.8.3.2.4 and 3.8.3.3 of this Guidance.

3.8.3.2.3 When data are available for all ingredients or only for some ingredients of the mixture

Annex 1: 3.8.3.4.1. Where there is no reliable evidence or test data for the specific mixture itself, and the bridging principles cannot be used to enable classification, then classification of the mixture is based on the classification of the ingredient substances. In this case, the mixture shall be classified as a specific target organ toxicant (specific organ specified), following single exposure, when at least one ingredient has been classified as a Category 1 or Category 2 specific target organ toxicant and is present at or above the appropriate generic concentration limit as mentioned in Table 3.8.3 below for Category 1 and 2 respectively.

A mixture not classified as corrosive but containing a corrosive ingredient should be considered for classification in Category 3 RTI on a case-by-case basis following the approach explained above (see Section 3.8.2.3 of this Guidance). More information on classification of mixtures into Category 3 is provided below (Section 3.8.3.3 of this Guidance).

3.8.3.2.4 Components of a mixture that should be taken into account for the purpose of classification

Components with a concentration equal to or greater than the generic concentration limits (1% for Category 1 components and 10% for Category 2. See CLP Annex I, Table 3.8.3), or with a Specific Concentration Limit (see Section 3.8.2.6 of this Guidance) will be taken into account for classification purposes. For Category 3, the GCL is 20%. Specific concentration limits have preference over the generic ones.

3.8.3.3 Generic concentration limits for substances triggering classification of mixtures for STOT-SE

The STOT-SE hazard class does not foresee summation of Category 1 or 2 substances in the classification process of a mixture. Furthermore, as Category 1 and 2 depict different hazards than Category 3 the assessment must be done independently from each other.

Annex 1: *Table 3.8.3*

Generic concentration limits of ingredients of a mixture classified as a specific target organ toxicant that trigger classification of the mixture as Category 1 or 2

INGREDIENT CLASSIFIED AS:	Generic concentration limits triggering classification of the mixture as :	
	Category 1	Category 2
Category 1 Specific Target Organ Toxicant	Concentration ≥ 10%	1.0% ≤ concentration < 10%
Category 2 Specific Target Organ Toxicant		Concentration ≥ 10% [(Note 1)]

Note 1:

If a Category 2 specific target organ toxicant is present in the mixture as an ingredient at a concentration ≥ 1.0% a SDS shall be available for the mixture upon request.

3.8.3.4.4. Care shall be exercised when toxicants affecting more than one organ system are combined that the potentiation or synergistic interactions are considered, because certain substances can cause target organ toxicity at < 1% concentration when other ingredients in the mixture are known to potentiate its toxic effect.

3.8.3.4.5. Care shall be exercised when extrapolating toxicity of a mixture that contains Category 3 ingredient(s). A generic concentration limit of 20% is appropriate; however, it shall be recognised that this concentration limit may be higher or lower depending on the Category 3 ingredient(s) and that some effects such as respiratory tract irritation may not occur below a certain concentration while other effects such as narcotic effects may occur below this 20% value. Expert judgement shall be exercised. Respiratory tract irritation and narcotic effects are to be evaluated separately in accordance with the criteria given in section 3.8.2.2. When conducting classifications for these

hazards, the contribution of each component should be considered additive, unless there is evidence that the effects are not additive.

Categories 1 and 2

Each single classified component in a concentration range given in CLP Annex I, Table 3.8.3 triggers the classification of the mixture, i.e. additivity of the concentrations of the components is not applicable.

Category 3

When a mixture contains a number of substances classified with Category 3 and present at a concentration below the GCL (i.e. 20%), an additive approach to determine the classification of the mixture as a whole should be applied unless there is evidence that the effects are not additive. In the additive approach the concentrations of the individual substances with the same hazard (i.e. RTI or narcotic effects) are totalled separately. If each individual total is greater than the GCL then the mixture should be classified as Category 3 for that hazard. A mixture may be classified either as STOT-SE 3 (RTI) or STOT-SE 3 (narcotic effects) or both.

Example

The following example shows whether or not additivity should be considered for Specific Target Organ Toxicity – Single Exposure (STOT-SE) Category 3 transient effects.

Ingredient information:

Ingredient	Wt%	Classification
Ingredient 1	0.5	-
Ingredient 2	3.5	Category 3 – Respiratory Tract Irritation
Ingredient 3	15	Category 3 - Narcotic effects
Ingredient 4	15	Category 3 - Narcotic effects
Ingredient 5	66	-

Answer**:**

Mixture is Category 3 – Narcotic effects

∑%Category 3 – Narcotic effects = 15% + 15% = 30% which is > 20%, therefore classify as Category 3 – Narcotic Effects

∑%Category 3 – Respiratory Irritation = 3.5%, which is < 20%, not classified for Respiratory Irritation

Rationale:

(a) Classification via application of substance criteria is not possible since test data was not provided for the mixture (CLP Annex I, 3.8.3.2);

(b) Classification via the application of bridging principles is not possible since data on a similar mixture was not provided (CLP Annex I, 3.8.3.3.1);

(c) Application of CLP Annex I, 3.8.3.4.5 is used for classification. Expert judgement is necessary when applying this paragraph. CLP Annex I, 3.8.3.4.5 notes that a cut-off value/concentration limit of 20% has been suggested, but that the cut-off

value/concentration limit at which effects occur may be higher or less depending on the Category 3 ingredient(s). In this case, the classifiers judged that 30% is sufficient to classify.

SCLs

In the case where a specific concentration limit has been established for one or more ingredients these SCLs have precedence over the generic concentration limit.

3.8.3.4 Decision logic for mixtures

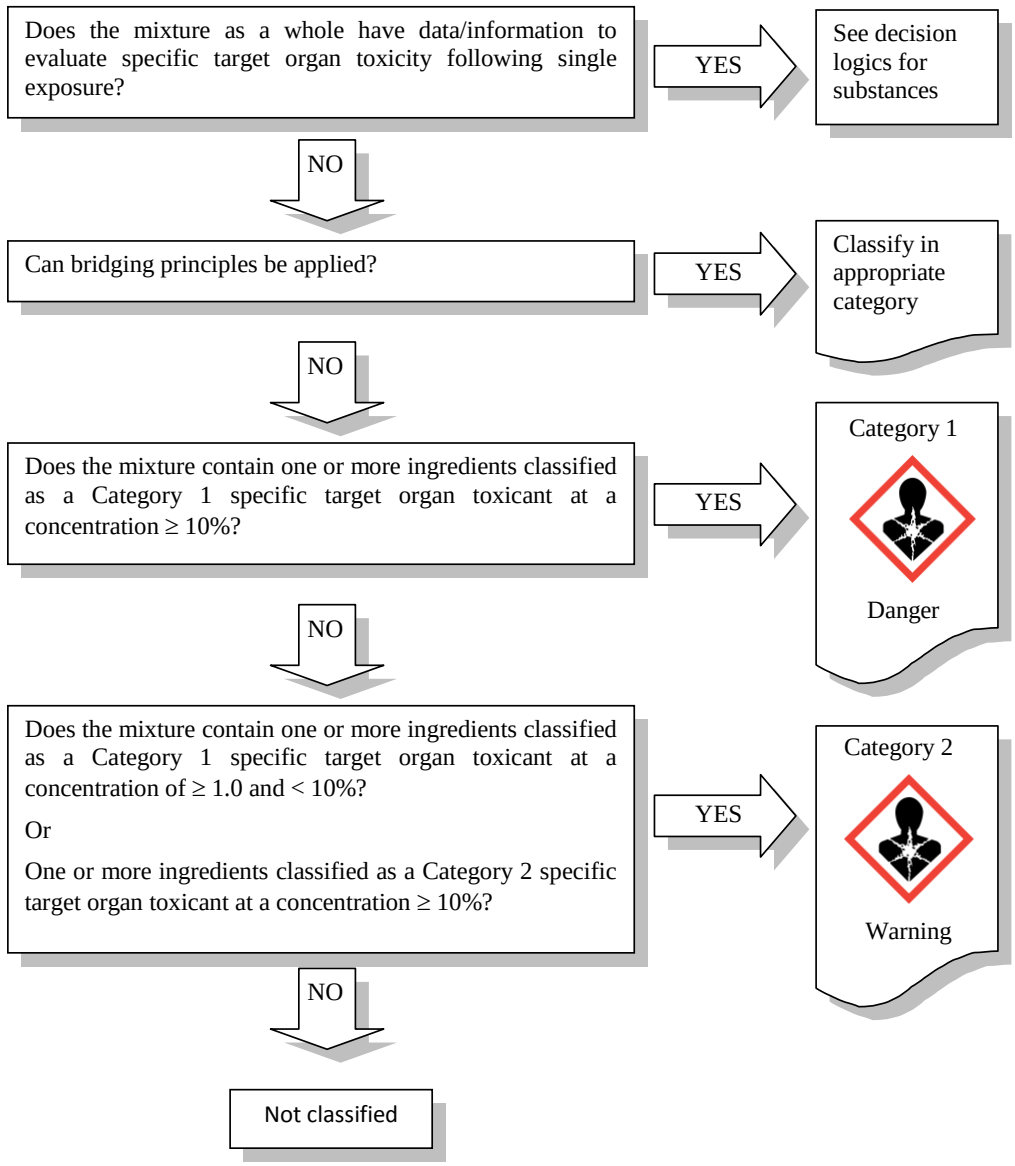
A mixture should be classified either in Category 1 or in Category 2, according to the criteria described above. The corresponding hazard statement (H370 for Category 1 or H371 for Category 2) should be used without specifying the target organs, except if the classification of the mixture is based on data available for the complete mixture, in which case the target organs may be given. In the same way, the route of exposure should not be specified, except if data are available for the complete mixture and it is conclusively demonstrated that no other routes of exposure cause the hazard.

If the criteria are fulfilled to classify also the mixture in Category 3 for respiratory irritation or narcotic effects, only the corresponding hazard statement (H335 and/or H336) will be added in hazard communication.

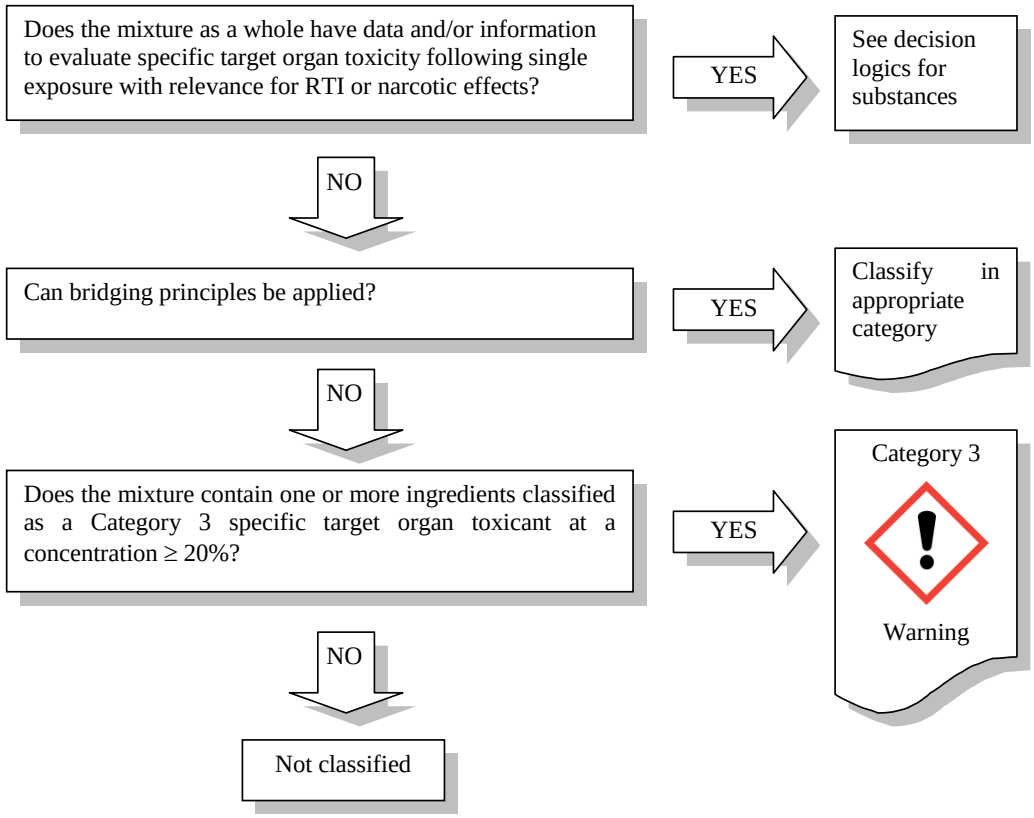
The decision logic is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

This decision logic deviates slightly from the original UNGHS in separating the connection between Category 2 and Category 3, since different from the procedure in other hazard classes they have to be regarded as independent.

Classification in Category 1 or 2



Classification in Category 3



3.8.4 Hazard communication in form of labelling for STOT-SE

3.8.4.1 Pictograms, signal words, hazard statements and precautionary statements

Guidance update to legal text "Annex I: Table 3.8.4" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex I: 3.8.4.1. Label elements shall be used in accordance with Table 3.8.4., for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.8.4

Label elements for specific target organ toxicity after single exposure

Classification	Category 1	Category 2	Category 3
GHS Pictograms			
Signal word	Danger	Warning	Warning
Hazard statement	H370: Causes damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H371: May cause damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H335: May cause respiratory irritation; or H336: May cause drowsiness or dizziness
Precautionary statement Prevention	P260 P264 P270	P260 P264 P270	P261 P271
Precautionary Statement Response	P307 + P311 P321	P309 + P311	P304 + P340 P312
Precautionary Statement Response **4th ATP change**	P308 + P311 P321	P308 + P311	P304 + P340 P312
Precautionary Statement Storage	P405	P405	P403 + P233 P405
Precautionary Statement Disposal	P501	P501	P501

The hazard statement should include the primary target organ(s) of toxicity. Organs in which secondary effects were observed should not be included. The route of exposure should not be specified, except if it is conclusively demonstrated that no other routes of exposure cause the hazard. When a mixture is classified for STOT-SE on basis of test data, the hazard statement will specify the target organs, in the same way as for a substance. If a mixture is classified on

basis of the ingredients, the hazard statement (H370 for Category 1 or H371 for Category 2) may be used without specifying the target organs, as appropriate.

In the same way, the route of exposure should not be specified, except if data are available for the complete mixture and if it is conclusively demonstrated that no other routes of exposure cause the hazard. It is recommended to include no more than three primary target organs for practical reasons and because the classification is for specific target organ toxicity. If more target organs are effected it is recommended that the overall systemic damage should be reflected by using the phrase "damage to organs".

3.8.4.2 Additional labelling provisions

ANNEX I: 3.8.2.1.10.4
Saturated vapour concentration shall be considered, where appropriate, as an additional element to provide for specific health and safety protection.

According to CLP Annex I, 3.8.2.1.10.4 the saturated vapour concentration shall be considered as an additional element for providing specific health and safety protection. Thus if a classified substance is highly volatile a supplementary precautionary advice (e.g. "Special/additional care should be taken due to the high saturated vapour pressure") might be given in order to emphasize the hazard in case it is not already covered by the general precautionary statements. (As a rule, the supplementary precautionary advice would normally be given for substances for which the ratio of the effect concentration at ≤ 4h to the SVC at 20° C is ≤1/10).

Diluted corrosive substances (may) exhibit an irritation potential with respect to the respiratory tract if they have a sufficient saturated vapour concentration. Expert judgement is needed for a decision with respect to a classification in STOT-SE Category 3. In these cases a switch from one hazard class (skin corrosion/irritation) to another (STOT-SE) would be justified.

3.8.5 Re-classification of substances and mixtures classified for STOT-SE according to DSD and DPD

Classification with STOT-SE 1 and 2 according to CLP is comparable to the classification with R39/X and R68/X according to DSD. Classification with R39 – 41 has been used occasionally for substances inducing mortality in eye irritation studies. This classification should not be translated to STOT-SE but will result in additional labelling with EUH070. Classification with STOT-SE 3 according to CLP is comparable to the classification with R37 and R67 according to DSD.

3.8.5.1 Is direct "translation" of Classification and Labelling possible for STOT-SE substances or mixtures?

Direct translation of substances or mixtures classified with R39/X is possible but the category may change. All substances or mixtures classified with R39/24, R39/25, R39/27, R38/28 and/or vapours and dusts/mists/fumes classified with R39/26 or R39/23 shall be classified as STOT-SE 1 because less adverse effects and higher guidance values are required for classification according to CLP compared to DSD. Setting of SCLs may be considered for substances showing STOT-SE at levels clearly below the guidance values (see Section 3.8.2.6 of this Guidance).

All substances or mixtures classified with R68/22, R68/21 and/or R68/20 (for vapours) shall be classified at least as STOT-SE 2. However, due to the higher guidance values, the requirement for less severe effects, and because STOT-SE in humans always leads to classification in Category 1, this is a minimal classification and may not adequately convey the seriousness of the toxicity. Therefore, classification in Category 1 should be considered. Dusts/mists/fumes classified with R68/20 can be directly translated into STOT-SE 2 because the guidance values are the same. Gases classified with R68/20 should be re-evaluated because of the change from guidance values in mg/L into ppm.

If translation results in a classification in STOT-SE 1 for one route and in STOT-SE 2 for another route only classification in Category 1 is required (for both routes).

Classification as STOT-SE is not route specific as it was for classification with R39/X and R68/X. The route specificity of STOT-SE is included in the hazard statement and includes route-to-route extrapolation by default unless conclusively shown otherwise. Therefore, the route specific data on STOT-SE should be re-evaluated. A re-evaluation is also necessary because the primary target organs for STOT-SE should be stated in the hazard statement.

All substances or mixtures classified with R67 shall be classified as STOT-SE Category 3 H336.

All substances or mixtures classified with R37 shall be classified as STOT-SE Category 3 H335. Also additional labelling with EUH071 (Corrosive to the respiratory tract) shall be considered.

3.8.5.2 Re-evaluation of the STOT-SE data

Gases classified with R39/23 or R39/26 should be re-evaluated because of the change from guidance values in mg/L into ppmV.

Substances or mixtures not classified for STOT-SE, should be considered for re-evaluation because less adverse effects and higher guidance values are required for classification according to CLP compared to DSD. Also, effects in humans are now considered for classification without restrictions to the exposure level.

3.8.6 Examples of classification for STOT-SE

3.8.6.1 Examples of substances fulfilling the criteria for classification

3.8.6.1.1 Example 1: Methanol

Application	Use of adequate and reliable human data, where animal data are not appropriate. Independent classification for STOT-SE and Acute toxicity due to different effects		
	Test Data	**Classification**	**Rationale**
Available information	*Animal data:* LD_{50} rat > 5,000 (mg/kg bw) No specific target organ toxicity (impairment of seeing ability) observed in rats, even in high doses.	Classification not possible	The rat is known to be insensitive to the toxicity of methanol and is thus not considered to be a good model for human effects (different effect/mode of action)
	Human experience: Broad human experience from many case reports about blindness following oral intake. Methanol is known to cause lethal intoxications in humans (mostly via ingestion) in relatively low doses: " …minimal lethal dose in the absence of medical treatment is between 300 and 1000 mg/kg bw" (IPCS)	STOT-SE Category 1	The classification criteria for Category 1 are fulfilled: clear human evidence of a specific target organ toxicity effect which is not covered by Acute toxicity.
Remarks	The standard animal species for single exposure (acute) tests, the rat, is not sensitive, i.e. no appropriate species for this specific target organ effect. Methanol is classified independently for acute toxicity, since the impairment of vision is not causal for the lethality, i. e. there are different effects. Labelling: Pictogram GHS 08; Signal word: Danger; Hazard statement: H370 Causes damage to the eye.		

1 **3.8.6.1.2 Example 2: Tricresyl phosphate**

Application	Use of valid human evidence supported by animal data		
	Test Data	**Classification**	**Rationale**
Available information	**Human experience:** There are well documented case reports about severe neurotoxic effects **Animal experiments:** Severe neurotoxic effects (Paralysis) were observed after single exposure of doses < 200 mg/kg bw LD_{50} rat oral 3000 - 3900 mg/kg bw	STOT-SE Category 1	The classification criteria are clearly fulfilled based on human experience as well as on results of animal studies
Remarks	**Labelling:** Pictogram GHS 08; Signal word: Danger; Hazard Statement: H370 Causes damage to the central nervous system.		

2 **3.8.6.1.3 Example 3: Sulfur dioxide**

Application	Use of valid human evidence		
	Test Data	**Classification**	**Rationale**
Available information	**Human experience:** Broad, well documented human experience on irritating effect to respiratory system.	STOT-SE Category 3	The classification criteria for Category 3 (Respiratory Tract Irritation) are fulfilled based on well documented experience in humans
Remarks	Labelling: Pictogram GHS 07; Signal word: Warning; Hazard statement: H335 May cause respiratory irritation		

3 **3.8.6.1.4 Example 4: Toluene**

Application	Use of valid animal data		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data:** In valid animal experiments narcotic effects (transient effect on nervous system) at ≥ 8 mg/l were observed.	STOT-SE Category 3	The classification criteria for Category 3 (Narcotic Effects) are fulfilled based on well documented results in animal experiments
Remarks	Labelling: Pictogram GHS 07; Signal word: Warning; Hazard statement: H336 May cause drowsiness and dizziness		

4

3.8.6.2 Examples of substances not fulfilling the criteria for classification

3.8.6.2.1 Example 5: ABC

Application	No classification for STOT-SE in case same effect leading to Acute toxicity classification		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data:** In a study in rats after single exposure at 2,000 mg/kg bw severe damage in liver (macroscopic examination) and mortality in 6/10 animals were observed	No classification in STOT- SE	Though a specific organ is damaged, the substance will be classified in Acute Toxicity (Category 4), since lethality was observed which was due to the liver impairment. It is assumed that the LD_{50}=ATE is ≤ 2,000 mg/kg bw. There should be no double classification for the same effect/mechanism causing lethality by impairment of a specific organ, thus no classification for STOT-SE

3.8.6.2.2 Example 6: N,N-Dimethylaniline

Application	No classification for STOT-SE in case same effect leading toAcute toxicity classification		
	Test Data	**Classification**	**Rationale**
Available information	**Animal data:** Acute oral toxicity: LD_{50} values > 1,120-1,300 mg/kg bw oral rat and 1,690 mg/kg bw dermal rabbit; ca. 50 mg/kg are lethal in cats due to high Met HB formation; no specific target organ toxicity (blood toxicity) observed in rats.	No classification in STOT-SE	The criteria for STOT-SE classification are not fulfilled despite a clear specific target organ effect in humans and in a relevant animal species. The substance is classified in Category 3 Acute Toxicity since the Met HB formation is causative for the lethality in humans and in animals (cats) in low doses.
	Human experience: Broad human experience from many case reports about lethal intoxications caused by methemoglobinemia following oral/dermal/inhalation exposure to aromatic amines	No classification in STOT-SE	
Remarks	The standard animal species for single exposure (acute) tests, the rat, is not sensitive, i.e. no appropriate species for this specific effect.		

3.9 SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE (STOT-RE)

3.9.1 Definitions and general considerations for STOT-RE

Annex I: 3.9.1.1. Specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. However, other specific toxic effects that are specifically addressed in Chapters 3.1 to 3.8 and Chapter 3.10 are not included here.

According to CLP Annex I, 3.9.1.1, specific toxic effects covered by other hazard classes are not included in STOT-RE. STOT-RE should only be assigned where the observed toxicity is not covered more appropriately by another hazard class. For example specific effects like tumours or effects on the reproductive organs should be used for classification for carcinogenicity or reproductive toxicity, respectively, but not for STOT-RE.

Annex I: 3.9.1.3. These adverse health effects include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health.

3.9.1.4. Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs.

3.9.1.5. Specific target organ toxicity can occur by any route that is relevant for humans, i.e. principally oral, dermal or inhalation.

Annex I: 3.9.2.2. The relevant route or routes of exposure by which the classified substance produces damage shall be identified.

The purpose of STOT-RE is to identify the primary target organ(s) of toxicity (CLP Annex I, 3.9.1.4) for inclusion in the hazard statement. Where possible secondary effects are observed in other organs, they should be carefully considered for the classification. The STOT-RE classification should identify those routes by which the substance causes the target organ toxicity (CLP Annex I, 3.9.1.5 and 3.9.2.2). This is usually based on the available evidence for each route. There are no compelling reasons to do route-to-route extrapolation to attempt to assess the toxicity by other routes of exposure for which there are no data.

Annex I: 3.9.1.6. Non-lethal toxic effects observed after a single-event exposure are classified as described in Specific target organ toxicity — Single exposure (section 3.8) and are therefore excluded from section 3.9.

Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.

3.9.2 Classification of substances for STOT-RE

3.9.2.1 Identification of hazard information

Annex 1: 3.9.2.5. The information required to evaluate specific target organ toxicity comes either from repeated exposure in humans, such as exposure at home, in the workplace or environmentally, or from studies conducted in experimental animals.

CLP does not require testing of substances and mixtures for classification purposes. The assessment is based on the respective criteria and consideration of all available adequate and reliable information, primarily such relating to repeated-dose exposures but also taking into account the general physico-chemical nature of the substance. The most useful information is generally from human epidemiology, case studies and animal studies, but information obtained using read-across from similar substances and from appropriate *in vitro* models can also be used, where appropriate.

3.9.2.1.1 Identification of human data

Relevant information with respect to repeated dose toxicity may be available from case reports, epidemiological studies, medical surveillance and reporting schemes, and national poisons centres.

Details are given in the Guidance on IR/CSA, Section 7.5.3.2.

3.9.2.1.2 Identification of non human data

Annex 1: 3.9.2.5. …. The standard animal studies in rats or mice that provide this information are 28 day, 90 day or lifetime studies (up to 2 years) that include haematological, clinicochemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species shall also be used, if available. Other long-term exposure studies, such as on carcinogenicity, neurotoxicity or reproductive toxicity, may also provide evidence of specific target organ toxicity that could be used in the assessment of classification.

Non-testing data

Physico-chemical data

Physicochemical properties, such as pH, physical form, solubility, vapour pressure, and particle size, can be important parameters in evaluating toxicity studies and in determining the most appropriate classification especially with respect to inhalation where physical form and particle size can have a significant impact on toxicity.

(Q)SAR models

Structurally or mechanistically related substance(s), read-across/grouping/chemical category and metabolic pathway approach: A (Q)SAR analysis for a substance may give indications for a specific mechanism of action and identify possible organ or systemic toxicity upon repeated exposure. Overall, (Q)SAR approaches are currently not well validated for repeated dose toxicity. (Guidance on IR/CSA, Section R7.5.4.1). Data on structurally analogous substances may be available and add to the toxicity profile of the substance under investigation. The concept of grouping, including both read-across and the related chemical category concept has been developed under the OECD HPV chemicals program. For certain substances without test data the formation of common significant metabolites or information with those of tested substances or information from precursors may be valuable information.

(For more details see the Guidance on IR/CSA, Sections R.6.1 and R.6.2.5.2 and OECD (2004)). OECD Principles for the Validation, for Regulatory Purposes, of (Quantitative) Structure-Activity Relationship Models)

Testing data

Animal data

"The most appropriate data on repeated dose toxicity for use in hazard characterisation and risk assessment are primarily obtained from studies in experimental animals conforming to internationally agreed test guidelines. In some circumstances repeated dose toxicity studies not conforming to conventional test guidelines may also provide relevant information for this endpoint" (Guidance on IR/CSA, Section R.7.5.3.1). Studies not performed according to Standard Test Guidelines and/or GLP have to be evaluated on case by case basis by expert judgement and in the context of a total weight of evidence assessment if there are more data (for more information see Section 3.9.2.3.4 of this Guidance and the Guidance on IR/CSA, Section R.7.5.4.1.

The standard test guidelines are described in the Gudiance on IR/CSA, Section R.7.5.4.1. There may also be studies employing different species and routes of exposure. In addition, special toxicity studies investigating further the nature, mechanism and/or dose relationship of a critical effect in a target organ or tissue may also have been performed for some substances. Other studies providing information on repeated dose toxicity: although not aiming at investigating repeated dose toxicity per se and other available EU/OECD test guideline studies involving repeated exposure of experimental animals may provide useful information on repeated dose toxicity, e.g reproduction toxicity or carcinogenicity studies. For more details see the Guidance on IR/CSA, Section R .7.5.4.1 (ECHA, 2008).

In vitro data

At present available *in vitro* data is not useful on its own for regulatory decisions such as classification and labelling. However, such data may be helpful in the assessment of repeated dose toxicity, for instance to detect local target organ effects and/or to clarify the mechanisms of action. Since, at present, there are no validated and regulatory accepted *in vitro* methods, the quality of each of these studies and the adequacy of the data provided should be carefully evaluated" (Guidance on IR/CSA, Section R.7.5.4.1).

3.9.2.2 Classification criteria for substances

Annex 1: 3.9.2.1. Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement (see 1.1.1), on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), (see 3.9.2.9), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed (Table 3.9.1).

Table 3.9.1

Categories for specific target organ toxicity-repeated exposure

Categories	Criteria
Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on

	the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).
Note Attempts shall be made to determine the primary target organ of toxicity and classify for that purpose, such as hepatotoxicants, neurotoxicants. One shall carefully evaluate the data and, where possible, not include secondary effects (a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems).	

In the Note above "classify" would mean to identify the primary target organ.

STOT-RE is assigned on the basis of findings of “significant” or “severe” toxicity. In this context “significant” means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. “Severe” effects are generally more profound or serious than “significant” effects and are of a considerably adverse nature which significantly impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

Annex 1: 3.9.2.9.4. The decision to classify at all can be influenced by reference to the dose/concentration guidance values at or below which a significant toxic effect has been observed.

Annex 1: 3.9.2.9.6. Thus classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values (C) as indicated in Table 3.9.2 below:

Table 3.9.2

Guidance values to assist in Category 1 classification

Route of exposure	Units	Guidance values (dose/concentration)
Oral (rat)	mg/kg body weight/day	C ≤ 10
Dermal (rat or rabbit)	mg/kg body weight/day	C ≤ 20
Inhalation (rat) gas	ppmV/6h/day	C ≤ 50
Inhalation (rat) vapour	mg/litre/6h/day	C ≤ 0,2

Inhalation (rat) dust/mist/fume	mg/litre/6h/day	C ≤ 0,02

Annex 3.9.2.9.7. Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in Table 3.9.3 below:

Table 3.9.3

Guidance values to assist in Category 2 classification

Route of Exposure	Units Guidance	Value Ranges: (dose/concentration)
Oral (rat)	mg/kg body weight/day	10 < C ≤ 100
Dermal (rat or rabbit)	mg/kg body weight/day	20 < C ≤ 200
Inhalation (rat) gas	ppmV/6h/day	50 < C ≤ 250
Inhalation (rat) vapour	mg/litre/6h/day	0,2 < C ≤ 1,0
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	0,02 < C ≤ 0,2

Annex 1 3.9.2.9.8. The guidance values and ranges mentioned in paragraphs 3.9.2.9.6 and 3.9.2.9.7 are intended only for guidance purposes, i.e., to be used as part of the weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values.

Annex 1 3.9.2.9.5. The guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of greater or lesser duration, using dose/exposure time extrapolation similar to Haber's rule for inhalation, which states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure. The assessment shall be done on a case-by-case basis; for a 28-day study the guidance values below is increased by a factor of three.

Haber's rule is used to adjust the standard guidance values, which are for studies of 90-day duration, for studies of longer or shorter durations. It should be used cautiously with due consideration of the nature of the substance in question and the resulting value produced.

In particular, care should be taken when using Haber's rule to assess inhalation data on substances which are corrosive or local active or have the potential to accumulate with repeated exposure.

One particular problem to note is that when adjusting the guidance value for very short study durations this can lead to very high guidance values which are not appropriate. For instance, for a 4 day exposure a guidance value of 2250 mg/kg bw/day for classification as STOT-RE category 2 could potentially be produced. This is above the limit for acute toxicity of 2000 mg/kg bw and it does not make sense to have a guidance value for repeated dose toxicity that is above the guidance value for mortality after acute exposure. To address this problem a pragmatic approach is proposed. For studies with exposure durations shorter than 9 days (i.e 10% of the 90 days to which the default general guidance value applies) the guidance value used should be no greater than 10 times the default guidance value. For example, the effects

in an oral range-finding study of 9 days or less should be compared with a guidance value of 1000 mg/kg bw/day for STOT-RE Category 2.

Expert judgement is needed for the establishment of equivalent guidance values because one needs to know about the limitations of the applicability of the proportionality. In the following table the equivalents for 28-day and 90-day studies according to Haber's rule are given:

Table 3.9.2.2 *Equivalent guidance values for 28-day and 90-day studies*

Study type	Species	Unit	Category 1 90-day	Category 1 28-day	Category 2 90-day	Category 2 28-day
Oral	Rat	mg/kg bw/d	≤ 10	≤ 30	≤ 100	≤ 300
Dermal	Rat	mg/kg bw/d	≤ 20	≤ 60	≤ 200	≤ 600
Inhalation, gas	Rat	ppmV/6 h/d	≤ 50	≤ 150	≤ 250	≤ 750
Inhalation, vapor	Rat	mg/l/6 h/d	≤ 0.2	≤ 0.6	≤ 1	≤ 3
Inhalation, dust/mist/fume	Rat	mg/l/6 h/d	≤ 0.02	≤ 0.06	≤ 0.2	≤ 0.6

Guidance update to legal text "Annex I: 3.9.2.9.9" in accordance with the 4th ATP: to be applied from 1 December 2014 for substances and 1 June 2015 for mixtures

Annex 1: 3.9.2.9.9. Thus it is feasible that a specific profile of toxicity occurs in repeat-dose animal studies at a dose/concentration below the guidance value, such as < 100 mg/kg bw/day by the oral route, however the nature of the effect, such as nephrotoxicity seen only in male rats of a particular strain known to be susceptible to this effect may result in the decision not to classify. Conversely, a specific profile of toxicity may be seen in animal studies occurring at or above a guidance value, such as ≥ 100 mg/kg bw/day by the oral route, and in addition there is supplementary information from other sources, such as other long-term administration studies, or human case experience, which supports a conclusion that, in view of the weight of evidence, classification is the prudent action to take.

3.9.2.3 Evaluation of hazard information

Annex 1: 3.9.2.4. […] Evaluation shall be based on all existing data, including peer-reviewed published studies and additional acceptable data.

3.9.2.3.1 Evaluation of human data

Annex 1: 1.1.1.4. For the purpose of classification for health hazards (Part 3) established hazardous effects seen in appropriate animal studies or from human experience that are consistent with the criteria for classification shall normally justify classification. Where evidence is available from both humans and animals and there is a conflict between the findings, the quality and reliability of the evidence from both sources shall be evaluated in order to resolve the question of classification. Generally, adequate, reliable and representative data on humans (including epidemiological studies, scientifically valid case studies as specified in this Annex or statistically backed experience) shall have precedence over other data. However, even well-designed and conducted epidemiological studies may lack a sufficient number of subjects to detect relatively rare but still significant effects, to assess potentially confounding factors. Therefore, positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness, quality and statistical power of both the human and animal data.

Annex 1 3.9.2.7.2. Evidence from human experience/incidents is usually restricted to reports of adverse health consequence, often with uncertainty about exposure conditions, and may not provide the scientific detail that can be obtained from well-conducted studies in experimental animals.

Where relevant human data do not mirror realistic exposure conditions, supportive information may be needed to corroborate the observed effects. A single case report from deliberate exposure (i.e. abuse) is unlikely to provide sufficiently robust evidence to support classification without other evidence.

The Guidance on IR/CSA, Section R.7.5.4.2 gives a detailed description on the use of human hazard information

3.9.2.3.2 Evaluation of non human data

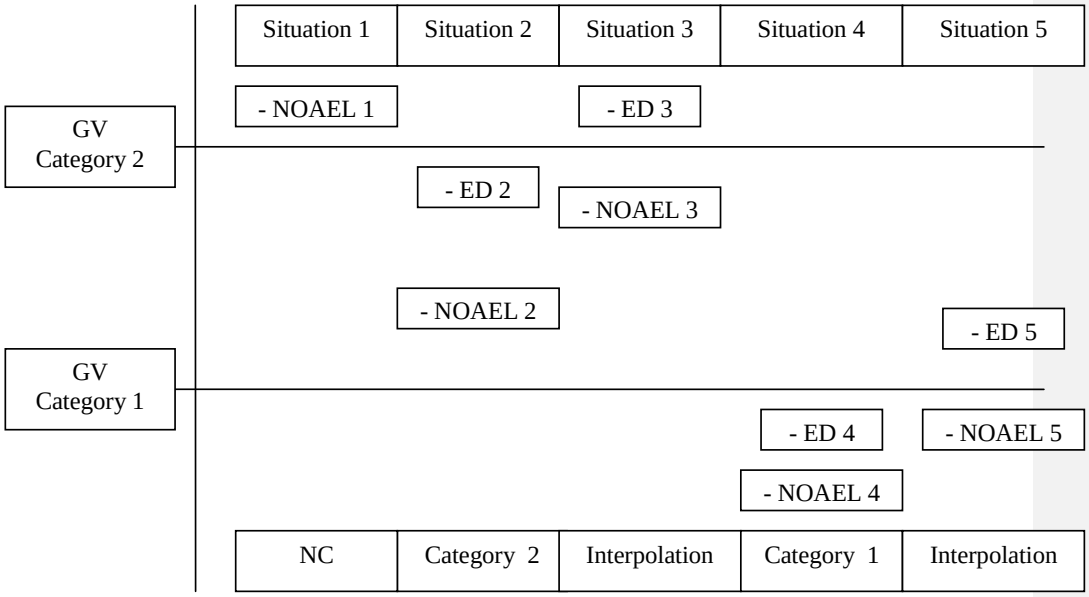
Annex 1 3.9.2.7.3. Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment.

All available animal data which are of acceptable quality should be used in a weight of evidence approach based on a comparison with the classification criteria described above. This should be done separately for each route for which data are available.

For each study the effects seen in each sex at or around the guidance values for Category 1 and Category 2 should be compared with the effects warranting classification in Category 1 and Category 2. In general findings in the most sensitive sex would be used to determine the classification. If the NOAEL from the study is above the guidance value (GV), the results of that study do not indicate classification for that category (situations 1 and 2 in Figure 3.9.2.3.2 below). If the NOAEL is below the GV then the effective dose level (ED), i.e. the lowest dose inducing significant/severe target organ toxicity as defined in Section 3.9.2.2 of this Guidance, should be determined based on the criteria described above. If the ED is below the GV then this study indicates that classification is warranted (situations 2 and 4 in Figure 1).

In a case where the ED is above a GV but the NOAEL is below the GV (situations 3 and 5) then interpolation between the ED and the NOAEL is required to determine whether the effects expected at or below the GV would warrant classification .

Figure 3.9.2.3.2 *Comparison between the NOAEL and the ED versus the guidance values*



Where a number of studies are available these should be assessed using a weight of evidence approach to determine the most appropriate classification. Where the findings from individual studies would lead to a different classification then the studies should be assessed in terms of their quality, species and strain used, nature of the tested substance (including the impurity profile and physical form) etc to choose the most appropriate study to support classification. In general, the study giving the most severe classification will be used unless there are good reasons that it is not the most appropriate. If the effects observed in animals are not considered relevant for humans then these should not be used to support classification. Similarly, if there is robust evidence that humans differ in sensitivity or susceptibility to the effect observed in the study then this should be taken into account, possibly leading to an increase or decrease in the classification assigned.

If there are differences in effects at the GV between studies with different duration then more weight is usually given to studies of a longer duration (28 days or more). This is because animals may not have fully adapted to the exposure in studies of shorter durations and also because longer duration studies tend to include more thorough and extensive investigations (e.g. in terms of detailed pathology and haematological effects etc) which can generally give more substantial information compared to shorter duration studies. If a 90-day as well as a 28-day study are available expert judgement has to be used and not just Haber's rule.

If there are differences in effects between good quality data in the same sex, species and strain then other variables such as particle size, vehicle, substance purity and impurities and concentration should be considered. If the results are considered to be depending on a specific impurity then different classifications depending on the concentration of the impurity could be considered.

Any information pertaining to the relevance of findings in animals to humans must be taken into account and may be used to modify the classification from how it would be if based on the available animal data. For instance, it may be shown that the findings in animals are not

relevant for humans, for example if the toxicity in animals is mediated by a mode of action that does not occur in humans. This would potentially provide a supporting case for no classification. Similarly, evidence may suggest that the potency of the substance may be higher or lower in humans than in animals, for example because of differences in toxicokinetics/toxicodynamics between the species. Such evidence could be used to increase or decrease the severity of the classification as appropriate. It should be noted that such arguments for modifying the classification must be robust and transparent (see Section 3.9.2.3.4 of this Guidance).

The final classification based on non human data will be the most severe classification of the three routes. If it is shown that classification for this endpoint is not required for a specific route then this can be included in the hazard statement (see Section 3.9.2.4 of this Guidance). Evaluation of non human data can result in no classification, STOT RE 1 or STOT RE 2. The results of the evaluation in non human data should be used in combination with the results of the evaluation of human data.

3.9.2.3.3 Conversions

The guidance values are giving in mg/kg bw. Where the doses in a study are given in different units they will need to be converted as appropriate. For instance the dosages in feeding and drinking water studies are often expressed in ppm, mg test substance/ kg (feed) or mg (test substance)/l (drinking water).

Where insufficient information is reported in the study to perform the conversion, Table 3.9.2.3.3.1 and Table 3.9.2.3.3.2 can be used as "Approximate relations". These tables are derived from the following documents: Guidance on IR/CSA, Chapter 8, Table 17; and OECD ENV/JM/MONO (2002)19, 04-Sep-2002, Table 1; L.R. Arrington (Introductory Laboratory Animal Science, 1978).

Table 3.9.2.3.3(a) *Food conversion*

Animal	Weight (kg)	Food consumed per day (g)	Factor 1mg/kgbw/d equivalent to ppm in diet
Rat, young	0.10	10	10
Rat, older	0.40	20	20
Mouse	0.02	3	7
Dog	10	250	40

Table 3.9.2.3.3(b) *Conversion drinking water*

Animal	Weight (kg)	Drinkingwater consumed per day(g)	Factor 1mg/kgbw/d equivalent to ppm in drinking water
Rat, young	0.25	28 (25-30)	9
Rat, older	0.40	28 (25-30)	14
Mouse	0.025	5 (4-7)	8
Dog	13	350	37

The conversion is performed according to the following simple equation:

$$\text{mg/kg bw} = \text{ppm/factor}$$

Example: In a 4 week study rats received the 1000 ppm test substance in feed

Dosage (mg/kg bw): 1000:10= 100 mg/kg bw.

In any case a calculation of the average substance intake based on measured bodyweight and consumption data is preferable and should be performed where possible.

Gases: mg/l into ppm:

Effect doses from gases given in the unit mg/l have to be converted into the unit ppm as used by the CLP via the following simplified formula assuming values for ambient pressure of 1 atm = 101.3 kPa and 25 ° c:

$$\text{mg/l} = \text{ppm} \times \text{MW} \times 1/24{,}450$$

3.9.2.3.4 Weight of evidence

Annex 1: 3.9.2.3. Classification is determined by expert judgment (see section 1.1.1), on the basis of the weight of all evidence available including the guidance presented below.

3.9.2.4. Weight of evidence of all data (see section 1.1.1), including human incidents, epidemiology, and studies conducted in experimental animals, is used to substantiate specific target organ toxic effects that merit classification. This taps the considerable body of industrial toxicology data collected over the years. Evaluation shall be based on all existing data, including peer-reviewed published studies and additional acceptable data.

Annex 1: 3.9.2.10.2. When well-substantiated human data are available showing a specific target organ toxic effect that can be reliably attributed to repeated or prolonged exposure to a substance, the substance shall normally be classified. Positive human data, regardless of probable dose, predominates over animal data. Thus, if a substance is unclassified because no specific target organ toxicity was seen at or below the dose/concentration guidance value for animal testing, if subsequent human incident data become available showing a specific target organ toxic effect, the substance shall be classified.

3.9.2.10.3. A substance that has not been tested for specific target organ toxicity may, where appropriate, be classified on the basis of data from a validated structure activity relationship and expert judgment-based extrapolation from a structural analogue that has previously been classified together with substantial support from consideration of other important factors such as formation of common significant metabolites.

In cases where there is sufficient human evidence that meets the criteria given in CLP Annex I, Table 3.9.1 to support classification then this will normally lead to classification in Category 1, irrespective of other information available.

Where human evidence does not meet this criterion, for example when the weight of evidence is not sufficiently convincing (limited number of cases or doubt on causal relationship) or because of the nature and severity of the effects (CLP Annex I, 3.9.2.7.3 and 3.9.2.8.1), then classification is based primarily on the non-human data

If there are no human data then the classification is based on the non-human data. If there is human data indicating no classification but there is also non-human data indicating classification then the classification is based on the non-human data unless it is shown that the human data cover the exposure range of the non-human data and that the non-human data are not relevant for humans. If the human and non-human data both indicate no classification then classification is not required.

3.9.2.4 Decision on classification

Annex 1: 3.9.2.7.1. Reliable evidence associating repeated exposure to the substance with a consistent and identifiable toxic effect demonstrates support for the classification.

Annex 1: 3.9.2.7.3. Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites.

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters.

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at

microscopic examination.

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

Annex 1: 3.9.2.8. Effects considered not to support classification for specific target organ toxicity following repeated exposure

3.9.2.8.1. It is recognised that effects may be seen in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

(a) Clinical observations or small changes in bodyweight gain, food consumption or water intake that have toxicological importance but that do not, by themselves, indicate "significant" toxicity.

(b) Small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance

(c) Changes in organ weights with no evidence of organ dysfunction.

(d) Adaptive responses that are not considered toxicologically relevant.

(e) Substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification.

If the evaluation of available data on a substance shows that the criteria for classification in a category are fulfilled then the substance shall be classified in that category for STOT-RE. If the data show that classification is warranted in Category 1 for one route and in Category 2 for another route then the substance shall only be classified in Category 1.

Hazard statements are provided in Section 3.9.4.1 of this Guidance and can specify the route(s) of exposure according to Table 3.9.2.4.1 below. If only data is available for one route showing that classification is warranted then no route should be stated in the hazard statement. If the data conclusively show that no classification for STOT-RE is warranted for a specific route then the remaining routes should be stated. If the data show that classification is warranted in Category 1 for one route and in Category 2 for another route then the hazard statement for Category 1 should include both routes because substances are placed in one of two categories.

Table 3.9.2.4.1 *Inclusion of route of exposure in Hazard statement*

Route 1	Route 2	Route 3	H-statement H372
Category 1	Category 2	unknown	Causes damage to organs through prolonged or repeated exposure
Category 1	Category 2	NC	Causes damage to organs via route 1 and 2
Category 1	NC	unknown	Causes damage to organs through prolonged or repeated exposure
Category 1	unknown	unknown	Causes damage to organs through prolonged or repeated exposure
Category 1	NC	NC	Causes damage to organs via route 1

3.9.2.5 Additional considerations

In the following sections some special aspects in the decision process on classification are described in more detail.

3.9.2.5.1 Irritating/corrosive substances

Substances (or mixtures) classified as corrosive may cause severe toxicological effects following repeated exposure, especially in the lungs following inhalation exposure. In such cases, it has to be evaluated whether the severe effect is a reflection of true repeated exposure toxicity or whether it is in fact just acute toxicity (i.e. corrosivity). One way to distinguish between these possibilities is to consider the dose level which causes the toxicity. If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity. In this case, classification as specific target organ toxicant (repeated exposure) would be warranted even if the substance (or mixture) is also classified as acutely toxic and/or corrosive.

In assessing non systemic effects caused by irritating/corrosive substances it should be kept in mind, that the guidance values /criteria for R48 in the DSD and later on those for STOT-RE of the CLP were derived from acute toxicity criteria (lethality based) assuming that systemic effects show a time dependent increase of severity due to accumulation of toxicity and taking also adaptive and detoxification processes into account. The effect considered in this context was lethality. This indicates that classification was intended for the presence of severe health damage, only. (see ECBI/67/00)

3.9.2.5.2 Hematotoxicity

Methaemoglobin generating agents

Methaemoglobinemia has often been regarded as an acute clinical symptom resulting from the action of methemoglobin-generating agents. If lethality is observed in humans or in animals[12] or can be predicted (QSAR), methemoglobin generating substances should be classified in the Acute Toxicity Hazard Class. Since this effect is difficult to detect in rodents, expert judgement should be used (cf. Guidance on Acute toxicity, Example2). If methemoglobinemia does not result in lethality but exposure to methaemoglobin generating agents results in signs of damage to the erythrocytes and haemolysis, anaemia or hypoxemia,

[12] Observation of lethality following methemoglobin formation is not usual, as several animals are more tolerant to it. Extrapolation to the human situation must be the critical decision key.

the formation of methaemoglobin shall be classified accordingly either in STOT-SE or STOT-RE (Muller A. *et al*., 2006).

Haemolytic anaemia

The guidance developed for classification of substances inducing haemolytic anaemia according to 67/548/EEC (Muller A. *et al.*, 2006) cannot directly be used under CLP because of the changes in criteria (see CLP Annex I, 3.9.2.7.3 c and 3.9.2.8.b, d). The major criterion for haemolytic anaemia changed:

- From "Any consistent changes in haematology which indicate severe organ dysfunction."
- To "Any consistent and significant adverse changes in haematology."

This indicates that less adverse effects are considered for classification according to CLP. This is consistent with the changes in the other criteria for classification for repeated exposure.

Adaptation towards the criteria according to CLP results in the following guidance:

It is evident that anaemia describes a continuum of effects, from sub-clinical to potentially lethal in severity. Overall, the interpretation of study findings requires an assessment of the totality of findings, to judge whether they constitute an adaptive response or an adverse toxicologically significant effect. If a haemolytic substance induces one or more of the serious health effects listed as examples below within the critical range of doses, classification is warranted. It is sufficient for classification that only one of these criteria is fulfilled.

Annex I: 2.9.2.7.3.

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

Example:

- Premature deaths in anaemic animals that are not limited to the first three days of treatment in the repeated dose study (Mortality during days 0–3 may be relevant for acute toxicity).
- Clinical signs of hypoxia, e.g. cyanosis, dyspnoea, pallor, in anaemic animals that are not limited to the first three days of treatment in the repeated dose study.

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

(c) any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis parameters;

Examples:

- Reduction in Hb at ≥20%.
- Reduction in functional Hb at ≥20% due to a combination of Hb reduction and MetHb increase.

- Haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).
- Haemosiderinuria supported by relevant histopathological findings in the kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multifocal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

Example:

- Multifocal or diffuse fibrosis in the spleen, liver or kidney.

(f) morphological changes that are potentially reversible but are clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver)

Example:

- Tubular nephrosis

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

In the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs." (CLP Annex I, 3.9.1.4).

Example:

- Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%) in a 28 day study.
- Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis.

Annex I: 3.9.2.8.1. It is recognised that effects may be seen in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

(a) clinical observations or small changes in bodyweight gain, food consumption or water intake that have toxicological importance but that do not, by themselves, indicate 'significant' toxicity;

(b) small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;

Example:

- Significant decrease in Hb without any other significant indicators of haemolytic anaemia.
- Minimal to slight increase in MetHb formation without any other indications of significant haemolytic anaemia.

(c) changes in organ weights with no evidence of organ dysfunction;

(d) adaptive responses that are not considered toxicologically relevant.

Example:

- Only adaptive or compensating effects without significant signs of haemolytic anaemia.

(e) substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification.

3.9.2.5.3 Mechanisms not relevant to humans (CLP Annex I, 3.9.2.8.1. (e))

In general, valid data from animal experiments are considered relevant for humans and are used for hazard assessment/classification. However, it is acknowledged that there are cases where animal data are not relevant for humans and should not be used for that purpose. This is the case when there is clear evidence that a substance – induced effect is due to a species-specific mechanism which is not relevant for humans. Examples for such species differences are described in this section.

<u>α-2-μ globulin nephropathy in male rats</u>

The protein α-2-μ globulin, which is primarily synthesized in male rats, has the capability to bind to certain chemicals. The resultant adducts accumulate as droplets in the kidneys and causes progressive renal toxicity within a few weeks which can ultimately lead to kidney tumours. This specific mechanism is unique to male rats and has no relevance for humans. Examples of chemicals causing α-2-μ globulin nephropathy are: unleaded gasoline, chlorinated paraffins, isophorone, d-limonene.

<u>Specific thyroid toxicity via liver enzyme induction</u>

Certain chemicals cause induction of liver enzymes and are interfering with the regulation of thyroid hormones. An increase in the activity of hepatic UDPG-transferase results in increased glucuronidation of thyroid hormones and increased excretion. It is known that rodents are highly sensitive to a reduction in thyroid hormone levels (T4), resulting in thyroid toxicity (e.g. hypertrophy, hyperplasia) after repeated stimulation / exposure of this organ. This in turn is related to an increase in the activity of hepatic UDPG-transferase. Humans, unlike rodents, possess a T_4 binding protein that greatly reduces susceptibility to plasma T_4 depletion and thyroid stimulation. Thus, such a mechanism/effect cannot be directly extrapolated to humans, i.e. these thyroid effects observed in rodents caused by an increase in hepatic UDPG-transferase are therefore considered of insufficient concern for classification.

<u>Peroxisome induction/proliferation</u>

Peroxisomes are cell-organelles which can be induced to a specifically high level in rats and mice under certain conditions, e.g. by repeated exposure to long chain and branched fatty acids. Peroxisome proliferation which is especially occurring in the liver causes liver toxicity (e.g. hyperplasia, oxidative stress) and can ultimately after long-term exposure also may lead to tumours. There is no evidence of e.g. hepatomegaly from clinical studies in humans treated with peroxisome proliferators (I.H.F. Purchase, Human & Experimental Toxicology (1994), 13, Suppl. 2 S47-S48). Examples are Clofibrat and Diethylhexylphthalate (DEHP).

<u>Lung Overload</u>

The relevance of lung overload in animals to humans is currently not clear and is subject to continued scientific debate.

3.9.2.5.4 Adaptive responses (CLP Annex I, 3.9.2.8.1. (d))

Adaptive (compensatory) changes generally constitute a normal biochemical or physiological response to a substance or to the effect of the substance (e.g. in response to methaemoglobin formation), usually manifested as an increase in background processes such as metabolism or erythropoiesis etc, which are generally reversible with no adverse consequences on cessation of exposure. In some cases the adaptive response may also be associated with pathological changes which reflect the normal response of the target tissue to substances: for example, liver hypertrophy in response to enzyme induction, increase in alveolar macrophages following inhalation of insoluble particles that must be cleared from the lungs, or development of epithelial hyperplasia and metaplasia in the rat larynx in response to inhalation of irritants.

Determination of whether adaptive changes support a classification requires a holistic assessment of the nature and severity of the observations and their dose-response relationship using expert judgement. Exposure to a substance can lead to a spectrum of effects which vary in incidence and severity with dose. At lower doses there may be adaptive changes which are not considered to be toxicologically significant or adverse, whereas at higher doses these changes may become more severe and/or other effects may occur which together constitute frank toxicity. Also, sometimes the adaptive effect is observed but the primary effect is not because the relevant parameter is not determined or not determined at the right time. For example, irritation of the larynx after inhalation of irritants is not observed at the end of a repeated dose study because of the quick response. The adaptive effect can then be used as an indication of the primary effect. It is often difficult to clearly distinguish between changes which are adaptive in nature and those which represent clear overt toxicity and this assessment requires expert judgement. Where the response to a substance is considered to be purely adaptive at dose levels relevant for classification then no classification would be appropriate.

3.9.2.5.5 Post-observation periods in 28 day and 90 day studies

For subacute/subchronic testing protocols, the usual guideline procedure is to sacrifice the exposed animals immediately after the end of the exposure period (d 29 or 91).

Japanese agencies often require a 14 days postobservation period for 28 day studies (OECD TG 407). This means that 10 more animals in the top dose and 10 more animals as an additional control group are then necessary.

The reversibility of organotoxic effects can often be estimated by the pathologist from histologic findings without a post-observation period.

- Certain effects are entirely reversible such as simple irritation or many forms of liver, testicular and hematotoxicity.
- Other effects may be reversible in morphological terms but the reserve capacity of the organism may be irreversibly compromised (such as in the case of kidney toxicity with a persistent loss in kidney nephrons).
- Some forms of tissue toxicity may be fundamentally irreversible, such as CNS- and neuro-toxicity with specific histological findings, cardiac toxicity and lung toxicity. Often, such effects do not return to normal morphology and may deteriorate even after the end of exposure.

3.9.2.6 Setting of specific concentration limits

Specific concentration limits (SCLs) for STOT-RE may be set by the supplier in some situations according to Article 10.1 of CLP. For STOT-RE, this may only be done for substances inducing target organ toxicity at a dose level or concentration clearly (more than

one magnitude) below the guidance values according to CLP Annex I, Table 3.9.2, that corresponds to ED below 1 mg/kg bw from the 90-day oral study. Where the exposure duration is not 90 days the ED has to be adjusted to an equivalent for 90 days using Haber's law and expert judgement (as described above). This will be mainly based on data in experimental animals but can also be used for human data if reliable exposure data are available. Setting of SCLs above the GCL is not applicable for STOT-RE because classification for STOT-RE is based on potency. Substances with a low potency do not require classification for this hazard class and substances with a medium or high potency are classified in a category defined by the GV.

The SCL for a Category 1 substance (*SCL Cat.1*) can be determined using the following formula:

$$SCLCat.1 = \frac{ED}{GV1} \times 100\% \qquad \text{Equation 3.9.2.6(a)}$$

SCL Cat 1: 0.12 mg/kg bw/10 mg/kg bw x 100%= 1.2% --> 1%

ED (effective dose) is the dose inducing specific target organ toxicity and GV1 is the guidance value for Category 1 according to CLP Annex I, Table 3.9.2 of Annex I corrected for the exposure duration. The resulting SCL is rounded down to the nearest preferred value (1, 2 or 5).

Though classification of a mixture in Category 1 is not triggered if a Category 1 constituent is present in lower concentrations than the established SCL, a classification in Category 2 should be considered. The SCL for classification of a mixture in Category 2 (*SCLCat. 2*) based on substances classified in Category 1 can be determined using the following formula:

$$SCLCat.2 = \frac{ED}{GV2} \times 100\% \qquad \text{Equation 3.9.2.6(b)}$$

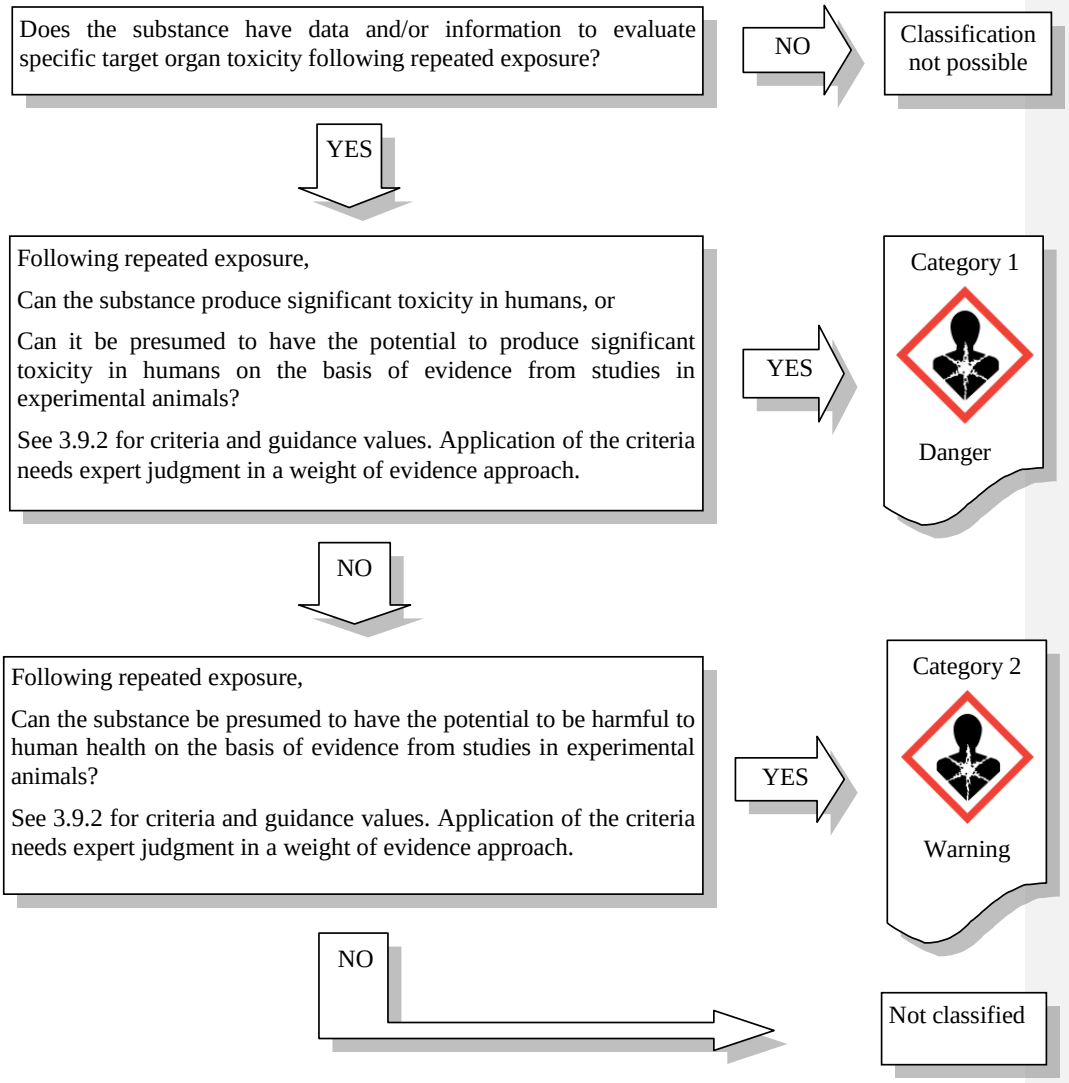
SCL Cat 2: 0.12 mg/kg bw/100 mg/kg bw x 100%=0.12% --> 0.1%

In this formula the ED (effective dose) is the dose inducing specific target organ toxicity and GV2 is the upper guidance value for Category 2 according to CLP Annex I, Table 3.9.3 corrected for the exposure duration. The resulting SCL is rounded down to the nearest preferred values (1, 2 or 5).

It is not appropriate to determine SCLs for substances classified in Category 2 since ingredients with a higher potency (i.e. lower effect doses than the guidance values of Category 2) will be classified in Category 1 and substances with respective higher effect doses will generally not be classified. For example, a substance inducing significant specific target organ toxicity at 0.12 mg/kg bw/day in a 90-day oral study would require a SCL for Category 1 of 1% and for Category 2 of 0.1%.

3.9.2.7 Decision logic for classification of substances

The decision logic which follows is provided as additional guidance to the criteria. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.



3.9.3 Classification of mixtures for STOT-RE

3.9.3.1 Identification of hazard information

Where toxicological information is available on a mixture this should be used to derive the appropriate classification. Such information may be available from the mixture manufacturer. Where such information on the mixture itself is not available information on similar mixtures and/or the component substances in the mixture must be used, as described below.

Further, the hazard information on all individual components in the mixture could be identified as described in Section 3.9.3.3.2 of this Guidance.

3.9.3.2 Classification criteria for mixtures

Annex 1: 3.9.3.1. Mixtures are classified using the same criteria as for substances, or alternatively as described below. As with substances, mixtures shall be classified for specific target organ toxicity following repeated exposure.

3.9.3.3 When data are available for the complete mixture

Annex 1: 3.9.3.2.1. When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture (see 1.1.1.3), then the mixture shall be classified by weight of evidence evaluation of these data. Care shall be exercised in evaluating data on mixtures, that the dose, duration, observation or analysis, do not render the results inconclusive.

In cases where test data for mixtures are available, the classification process is exactly the same as for substances.

3.9.3.3.1 When data are not available for the complete mixture: bridging principles

Annex 1: 3.9.3.3.1. Where the mixture itself has not been tested to determine its specific target organ toxicity, but there are sufficient data on the individual ingredients *and* similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging principles set out in section 1.1.3.

In order to apply bridging principles, there needs to be sufficient data on similar tested mixtures as well as the ingredients of the mixture. (see Section 1.6.3 of this Guidance).

When the available identified information is inappropriate for the application of the bridging principles then the mixture should be classified based on its ingredients as described in Sections 3.9.3.3.2, 3.9.3.3.3 and 3.9.3.4 of this Guidance.

3.9.3.3.2 When data are available for all ingredients or only for some ingredients of the mixture

Annex 1: 3.9.3.4.1. Where there is no reliable evidence or test data for the specific mixture itself, and the bridging principles cannot be used to enable classification, then classification of the mixture is based on the classification of the ingredient substances. In this case, the mixture shall be classified as a specific target organ toxicant (specific organ specified), when at least one ingredient has been classified as a Category 1 or Category 2 specific target organ toxicant and is present at or above the appropriate generic concentration limit as laid out in Table 3.9.4 below for Category 1

and 2 respectively.

3.9.3.3.3 Components of a mixture that should be taken into account for the purpose of classification

Components with a concentration equal to or greater than the generic concentration limits (see CLP Annex I, Table 3.9.4) or with a specific concentration limit (see also Section 3.9.3.5 of this Guidance) will be taken into account for classification purposes. Specific concentration limits have preference over the generic concentration limits.

3.9.3.4 Generic concentration limits for substances triggering classification of mixtures

Annex 1: *Table 3.9.4*

Generic concentration limits of ingredients of a mixture classified as a specific target organ toxicant that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of the mixture as:	
	Category 1	Category 2
Category 1 Specific Target Organ Toxicant	Concentration ≥ 10%	1.0% ≤ concentration < 10%
Category 2 Specific Target Organ Toxicant		Concentration ≥ 10% (Note 1)

Note 1
If a Category 2 specific target organ toxicant is present in the mixture as an ingredient at a concentration ≥ 1,0 % a SDS shall be available for the mixture upon request.

Annex 1: 3.9.3.4.4. Care shall be exercised when toxicants affecting more than one organ system are combined that the potentiation or synergistic interactions are considered, because certain substances can cause target organ toxicity at < 1% concentration when other ingredients in the mixture are known to potentiate its toxic effect.

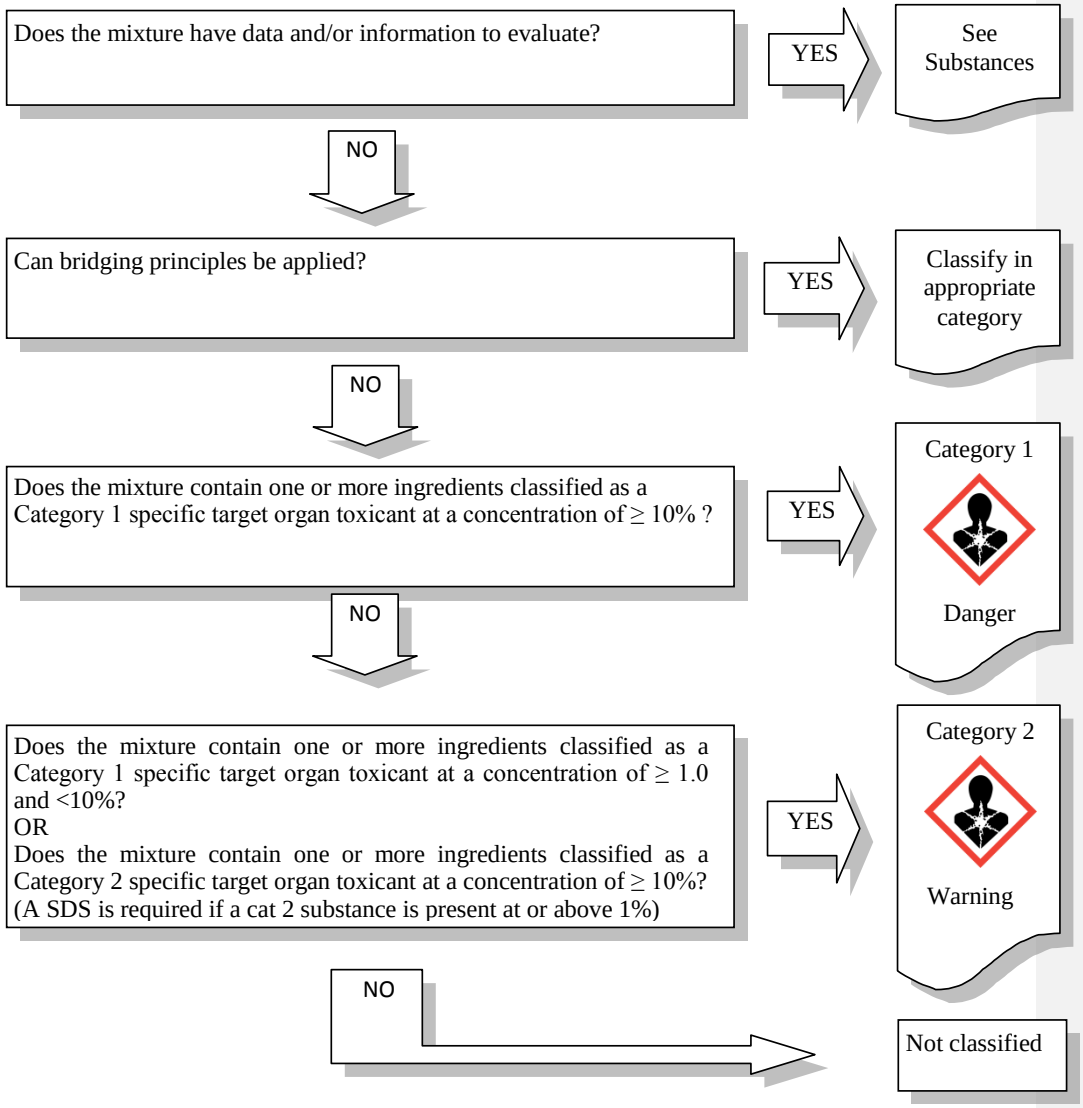
In the case a specific concentration limit has been established for one or more ingredients these SCLs have precedence over the respective generic concentration limit.

When classifying a mixture for STOT-RE the additive approach, where the concentrations of individual components with the same hazards are summed, is not used. If any individual component is present at a concentration higher than the relevant generic or specific concentration limit then the mixture will be classified.

3.9.3.5 Decision logic for mixtures

A mixture should be classified either in Category 1 or in Category 2, according to the criteria described above. When a mixture is classified for STOT-RE on the basis of test data, the hazard statement will specify the target organs, in the same way as for a substance. If a mixture is classified on basis of the ingredients, the hazard statement (H372 for Category 1 or H373 for Category 2) may be used without specifying the target organs, as appropriate. In the same way, the route of exposure should not be specified, except if data are available for the complete mixture and if it is conclusively demonstrated that no other routes of exposure cause the hazard.

The decision logic which follows is provided as additional guidance to the criteria**.** It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.



3.9.4 Hazard communication in form of labelling for STOT-RE

3.9.4.1 Pictograms, signal words, hazard statements and precautionary statements

Annex I: 3.9.4.1. Label elements shall be used in accordance with Table 3.9.5 for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.9.5

Label elements for specific target organ toxicity after repeated exposure

Classification	Category 1	Category 2
GHS Pictograms		
Signal word	Danger	Warning
Hazard statement	H372: Causes damage to organs (state all organs affected, if known) through prolonged or repeated exposure (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H373: May cause damage to organs (state all organs affected, if known) through prolonged or repeated exposure (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary statement prevention	P260 P264 P270	P260
Precautionary statement response	P314	P314
Precautionary statement storage		
Precautionary statement disposal	P501	P501

The hazard statement should include the primary target organ(s) of toxicity. Organs in which secondary effects were observed should not be included. The route of exposure should not be specified, except if it is conclusively demonstrated that no other routes of exposure cause the hazard.

When a mixture is classified for STOT-RE on basis of test data, the hazard statement will specify the target organs, in the same way as for a substance. If a mixture is classified on basis of the ingredients, the hazard statement (H372 for Category 1 or H373 for Category 2) may be used without specifying the target organs, as appropriate.

In the same way, the route of exposure should not be specified, except if data are available for the complete mixture and if it is conclusively demonstrated that no other routes of exposure cause the hazard.

It is recommended to include no more then three primary target organs for practical reasons and because the classification is for specific target organ toxicity. If more target organs are affected it is recommended that the overall systemic damage should be reflected by using the more general term “damage of organs”.

3.9.4.2 Additional labelling provisions

Annex 1: 3.9.2.10.4 Saturated vapour concentration shall be considered, where appropriate, as an additional element to provide for specific health and safety protection

According to CLP Annex I, 3.9.2.10.4 the saturated vapour concentration shall be considered as an additional element for providing specific health and safety protection. Thus if a classified substance is highly volatile a supplementary precautionary advice (e.g. "Special/additional care should be taken due to the high saturated vapour pressure") might be given in order to emphasize the hazard in case it is not already covered by the general **P** statements. (As a rule substances for which the ratio of the effect concentration at ≤ 4h to the SVC at 20° C is ≤ 1/10).

Although not according to the criteria of STOT-RE, the following EU-special hazard statement "Repeated exposure" may be used when appropriate:

EUH066- "Repeated exposure may cause skin dryness or cracking" (see Section 3.2 of this Guidance on Skin Corrosion/Irritation).

3.9.5 Re-classification of substances and mixtures classified for STOT-RE according to DSD and DPD

Classification with STOT-RE according to CLP is comparable to the classification with R48/X according to DSD. Also substances and mixtures currently classified with R33 should be considered because there is no corresponding classification in CLP. However, differences are present regarding the approach to route-to-route extrapolation.

3.9.5.1 Is direct "translation" of classification and labelling possible for STOT-RE substances and mixtures?

Direct translation of substances or mixtures classified with R48/X is possible because classification criteria are based on the dose and the severity of a toxic effect and are comparable in both, CLP and DSD. However, in some cases a change in the Category may result by reviewing the data.

Substances or mixtures classified with R48/23, R48/20 (for vapour), R48/24 and/or R48/25 shall be classified as STOT-RE Category 1 because less adverse effects and higher guidance values are required for classification according to CLP compared to DSD. Notable, there is one exception: dust/mist/fume with an ED > 0.02 and ≤ 0.025 mg/l/6h which are classified according to DSD with R48/23 might not be classified in Category 1 according to CLP. Setting of SCL may be considered for substances showing STOT-RE at levels clearly below the guidance values (see Section 3.9.2.6 of this Guidance).

All substances or mixtures classified with R48/20 (for dust/mist/fume), R48/21 and/or R48/22 shall be classified generally at least as STOT-RE Cat 2. Again, dust/mist/fume with an ED > 0.2 and ≤ 0.25 mg/l/6h which are classified according to DSD with R48/20 might not be classified according to CLP. However, due to the general increase in guidance values, the requirement for less severe effects classification in Category 2 should also be considered.

If translation results in a classification in STOT-RE Category 1 for one route and in STOT-RE Category 2 for another route only classification in Category 1 is required (for both routes). In contrast to DSD where the route of exposure is included in the classification and correlates with the routes tested (or extrapolated), according to CLP the exposure route should be specified only when it is conclusively proven that no other routes of exposure cause the hazard. Therefore, the route specific data on STOT-RE should be re-evaluated. A

re-evaluation is also necessary because the primary target organs for STOT-RE should be stated in the hazard statement.

3.9.5.2 Re-evaluation of the STOT-RE data

Gases classified with R48/20 or R48/23 should be re-evaluated because the guidance values changed from general guidance values in mg/L for dusts and mists, vapours and gases to a specific guidance value for gases in ppm.

Substances or mixtures not classified for, STOT-RE including substances or mixtures classified with R33, should be re-evaluated because less adverse effects and higher guidance values are required for classification according to CLP compared to DSD. Also, effects in humans are now considered for classification generally without restrictions to the exposure level.

3.9.6 Examples of classification for STOT-RE

Remarks:

The classification proposals for the examples refer only to STOT-RE.

Labelling is done only with respect to hazard statements (statement with respect of organs affected = target organs).

3.9.6.1 Examples of substances fulfilling the criteria for classification

3.9.6.1.1 Example 1: Hydroxylamine / Hydroxylamonium salts (CAS no. 7803-49-8)

Application of criteria for evaluation/classification and decision on classification: Use of studies with different duration; Haber's rule; Expert judgement

Available information:

1) Human experience: No information available

2) Animal data:

Background:

Hydroxylamine and its salts are direct MetHb producers in contrast to aromatic amines, which require metabolic activation (XI/484/92).

Several studies are available for the assessment of the toxicity after repeated administration:

- 4-week drinking water study (BASF, 1989)
- 3-month drinking water study (BASF, 1989)
- Combined chronic/carcinogenicity study in drinking water in rats (BASF, 2001)

Though not explicitly stated in the criteria the "... study with the longest duration should normally be used".

- In the 3-month-study at the dose level of 21 mg/kg bw only "slight to moderate hematotoxic effects" were observed. Thus this dose would not be a sufficient ED causing "significant/severe" effects, but it can be concluded that via interpolation an ED would result within the Guidance Value Range for Cat 2 (10-100 mg/kg bw).
- A classification in Category 2 would be warranted based on the 3-month-study.

In the combined chronic/carcinogenicity study (BASF, 2001), the effects observed after 12 and 24 months are to be considered separately:

12 month study:

- 0 ppm (control): hemosiderin storage of low degree in males and females (spleen)
- 5 ppm (males 0.3 mg and females 0.4 mg/kg bw/day): No substance-induced effects; hemosiderin storage of low degree in males and females, comparable to controls.
- 20 ppm (males 1.1 mg and females 1.6 mg/kg bw/day): Here, hemosiderin deposits with the gradation of moderate was observed in the spleens of the males; hemosiderin storage of low degree in females comparable to controls. This effect is not to be regarded as serious since hematology did not reveal any findings whatsoever with regard to anemia. This is supported by the fact that no substantial (1/10 moderate, but 1/10 severe in the male control group) extramedullary hematopoiesis was observed in this group. In the histopathological examination, the spleen was not found to be impaired morphologically. Thus, this dose is to be regarded as the NOAEL for males whereas it is the NOEL for females.
- 80 ppm (males 4.5 mg and females 6.2 mg/kg bw/day): The clinicochemical findings are assessed as mild anemia in the males (e.g. decrease of RBC, HB and HT (< 10%); MCV increased at the beginning and compensatory normalization later) and, also as mild anemia in the females (decrease in RBC $\leq$ 12%, HB < 10% and HT < 10%). The increase of MCV, PLT and RET and of Howell-Jolly bodies is regarded as a compensatory effect, and the bone marrow still reacts, i.e. it does not demonstrate "... decreased bone marrow production of red blood cells" within the meaning of the criteria. The only slight increase of the Heinz bodies is considered to be a sign of a weak hematotoxic effect. From the point of view of histopathology, the effects (hemosiderin storage, extramedullary hematopoesis) can be regarded as signs of anemia, but not within the meaning of "serious" (the effect was more pronounced in the females than in the males). The extramedullary hematopoiesis observed is thus again compensatory in the sense of a functional counterreaction.

Assessment:

For a 12-month study, cut-off values of 25 and 2.5 mg/kg bw/day (100 mg/kg bw/day : 4) have to be regarded for STOT-RE Category 1 vs. Category 2 respectively. At the dose level of 1.1 (m) or 1.6 mg/kg bw/day (f), no hematotoxic effects whatsoever or extramedullary hematopoiesis were observed, nor substantial hemosiderin deposits. The effects at 4.5 (f) and 6.2 (m) mg/kg bw/day are regarded as mild anemia; however, more distinct effects may be expected to occur up to the cut-off value (25 mg/kg bw/day). Therefore, a classification in Category 2 seems justified.

24-month study:

In contrast to the 12-month study, no complete hematological examination was carried out, i.e. only morphological parameters were evaluated, yet full histopathology. The following findings relevant to classification – with the exception of the neoplasias – were obtained:

- 5 ppm (males 0.2 mg and females 0.4 mg/kg bw/day): No non-neoplastic effects
- 20 ppm (males 1 mg and females 1.6 mg/kg bw/day): Increased proportion of hemosiderin deposits in the spleens of the females, but no extramedullary hematopoiesis, which demonstrates that there was no clear anemia before.

Remark:
The fact that, at this dose level, hemosiderin was detected only in the males in the 12-month study and an increased proportion of it only in the females in the 24-month study shows that this effect was only borderline.

– 80 ppm (males 3.7 mg and females 6.2 mg/kg bw/day): Again hemosiderin storage and extramedullary hematopoesis were observed, yet no serious effects in hematology nor histopathology. Furthermore, the results of the study do not indicate that any animal died prematurely as a result of the anemia.

Remark:
No effects were observed neither in kidneys nor in liver in the 12-month study. In the 3 month study only in the highest dose the relative liver weights were increased in the males; in the 3 month as well as in the 24-month study only marginal effects (diffuse hemosiderin storage in the liver) in both sexes was observed in the highest dose.

Assessment:

The results of the 24 month study show that effects as seen after 12 month exposure are not substantially increased.

Classification:

Based on the evaluation of the 3-month-study and the more relevant 12-month-study by expert judgement a classification in Category 2 is warranted.

Labelling:

Hazard statement: H373 May cause damage to blood system through prolonged or repeated exposure

3.9.6.1.2 Example 2: But-2-yn-1,4-diol (EC No 203-788-6; CAS No 110-65-6)

Current classification according to DSD: Xn; R48/22

Application of criteria for evaluation/classification and allocation of hazard statements with respect to specific target organs and route of exposure

Available information:

1) Human experience: no information available

2) Animal data:

– 28d oral study
– 28d inhalation study
– Acute oral toxicity: LD_{50} rat 132 (males) and 176 (females) mg/kg bw -> Category 3
– Acute dermal toxicity: LD_{50} 424 (males) and 983 (females) mg/kg bw-> Category 3
– Acute inhalation toxicity: LC_{50} rat 0.69 mg/l -> Category 2
– Corrosivity in animal experiments (Category 1)

STOT-RE oral:

28d rat oral (gavage): doses 0; 1; 10; 50 mg/kg bw/d

- 1 mg/kg bw: NOEL
- 10 mg/kg bw: LOEL

- Increased liver weight (not statistically significant)
- Hepatic and spleenic changes (no clear desription of severity given)
- Diminished RBC counts in females, yet no other changes in blood chemistry
- Histopathology: in 2/10 males and 3/10 females swelling of parenchymal cells and increased polymorphism of the hepatocyte nuclei and the nuclear cells. These effects are regarded as not "significant/severe toxic effects"
- 50 mg/kg bw: mortality (3/8 males; 3/8 females); hepato- and nephrotoxicity responsible for mortality; no distinct hepato- and nephrotoxicity described for survivors
- Hematology: decrease in RBC count ca. 20% and 21% in HB both in males and females; decrease in Hematocrite 11%. These effects are regarded as "moderate hematotoxicity".

Conclusion for the highest dose group: severe effects.

Assessment:

The substance has a high acute toxicity (s.a.). Since the factor between the acute LD_{50} and the subacute lethal dose (20 applications) is only 2-3, it can be assumed that the substance has a low cumulative potential. On the other hand there is a steep dose response in the 4 week study, thus it can be concluded by interpolation that at 30 mg/kg bw moderate but no "significant/severe" toxicity could be expected; 30 mg/kg bw is the guidance value for Category 1 in a 4 week study according to Haber's rule: 10 mg/kg bw x 3)

STOT-RE inhalation

In a valid 4 week inhalation study (vapour) rats were exposed to 0.5; 5; and 25 mg/m^3/6h/d.

- 0.5 mg/m^3: NOAEC for local effects in the respiratory tract
- 5 mg/m^3: minimal-slight focal squamous metaplasia and inflammation in the larynx
- 25 mg/m^3: minimal-slight focal squamous metaplasia and inflammation in the larynx
- 25 mg/m^3: NOAEC for systemic effects including hematology, clinical chemistry, histopathology and neuropathology examinations

Assessment:

Up to the highest concentration tested there were no systemic effects. Since the substance is classified as corrosive an irritation of the respiratory tract by the vapour could be expected and has been observed in minimal-slight degree at 5-25 mg/m^3. It is assumed that the irritation would increase with higher concentrations. The corrosive/irritation potential is covered by the classification as "corrosive" Category 1, thus no classification as STOT-RE with respect to the inhalation route would result.

Classification:

Category 2 for the oral route is proposed since within the guidance values of 30-300 mg/kg bw in a 4 week study serious effect occurred. According to a total weight of evidence approach it is concluded that these significant effects would not be observed below 30 mg/kg bw, the concentration limit for Category 1.

Classification via the inhalation route is not warranted, since at the highest concentration tested only local effects, but no systemic effects, were observed. The local effects (corrosivity/irritancy) are covered by the respective classification.

Labelling:

Hazard statement: H373 May cause damage to liver and kidney through prolonged or repeated exposure.

Remark: Since the substance is classified as STOT-RE via the oral route and specific toxicity has not been conclusively excluded for the dermal route (rather it can be expected due to high dermal absorbtion in acute toxicity, Category 3) the Hazard statement for STOT-RE in total without specifying a route has to be applied based on the classification via the oral route.

(See also Risk assessment report BUT-2YNE-1,4-DIOL; EC 2005. Available at ECHA website: http://echa.europa.eu/documents/10162/49324502-03ba-4005-8800-b2bebf924d2d)

3.9.6.1.3 Example 3: XYZ

Application of criteria for evaluation/classification and allocation of hazard statements with respect to specific target organs and route of exposure.

Available information:

1) Human experience: No information available
2) Animal data:

Key chronic toxicity data (underlined for EU classification)			CLP Repeated Exposure (STOT) classification
Type of study - Effects	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	
mouse, oral 28 days 0, 300, 600, 1200 ppm (M: 0, 51-58, 101-115, 177-226 mg/kg bw/d, F: 0, 59-66, 111-127, 221-281 mg/kg bw/d) hematological changes in M (↓ RBC count, Hb, Ht)	M: no NOAEL F: 300 (59-66)	M: 300 (51-58) F: 600 (111-127)	Category 2 based on the effects on blood
rat, oral 13 weeks 0, 50, 500, 1000 ppm (M: 0, 3.5, 38, 67 mg/kg bw/d, F: 0, 4, 38, 80 mg/kg bw/d) hematological changes in F (↓ RBC count, Hb, Ht)	50 (M: 3.5, F: 4)	500 (M: 38, F: 38)	Category 2 based on the effects on blood
male rat, oral 30, 60, 90 days 0, 5, 10, 25 mg/kg bw/d (by gavage) (open literature) mortality at 5 (5/25), 10 (7/25) & 25 (8/25) mg/kg bw			No classification is proposed on the basis of this study because the mortality observed in the 3 groups are in contradiction with the other relevant experiments in this species (mortality not dose related, some animals (2/6) already died after

			30 days at 5 mg/kg bw)
rat, oral 2 years 0, 30, 150, 300 ppm (M: 0, 1.46, 7.31, 14.66 mg/kg bw/d, F : 0, 1.8, 8.86, 18.57 mg/kg bw/d) eyelid masses: 1 F/50 at 150 ppm, 5 M/50 & 3 F/49 at 300 ppm changes in erythroid parameters (↓ RBC count, ↑ MC Hb, ↑ MCV in F at 300 ppm) extramedullary hemopoiesis in liver (M: 150 & 300 ppm, F: 300 ppm), spleens ↑ myeloid hyperplasia in BM, in femur & sternum of F at 300 ppm ↑ i. hemorrhages w/i mesenteric lymph nodes at 150 & 300 ppm	30 (M: 1.46, F: 1.8)	150 (M: 7.31, F: 8.86)	Category 2 based on the effects on blood (haemolytic anaemia accompanied by compensatory mechanisms)
rat, oral 80 weeks M: 0, 5, 20, 52 mg/kg bw/d F: 0, 6, 26, 67 mg/kg bw/d (open literature) ataxic syndrom in F at 67 mg/kg bw/d (unusual gait). The condition of these rats worsened, leading to paralysis posterior to the lumbar region, atrophy of the hing legs. No specific hystopathological lesion of CNS or PNS.			No classification (effects above the cut-off values)
rat, oral, 104 weeks 0, 3, 30, 300 ppm (M: 0, 0.1, 1.2, 11.6 mg/kg bw/d, F: 0, 0.1, 1.4, 13.8 mg/kg bw/d) (open literature) anemia in 300 ppm (F) (not in 30 ppm) regressive changes of sciatic nerve (degeneration) + atrophy of calf muscle in F at 300 ppm, but no neurologcal signs progression of myocardial lesions at 300 ppm			Category 2 based on the effects on blood and nervous system
mouse, oral, 97/98 weeks	15		Category 2 based

M : 0, 15, 150, 300 ppm (0, 3, 24, 50 mg/kg bw/d) F : 0, 15, 300, 600 ppm (0, 3, 57, 112 mg/kg bw/d) retinal atrophy at ≥ 150 ppm (↓ or absence of outer nuclear cell layer of retina) ↑ turnover of erythrocytes	(M: 5.2, F: 3.1)		on the effects on blood. Category 2 based on the effects on the retina

Classification for XYZ : STOT-RE Category 2

Labelling :

Symbol: GHS08

Signal word: Warning

Hazard statement: H373 May cause damage to the blood and nervous systems through prolonged or repeated exposure.

Justification :

The effects on blood are reported in the 2 species (mouse, rat), at doses low enough to justify Category 2. The effects on NS are reported in the rat at doses low enough to justify Category 2.

3.9.6.2 Examples of substances not fulfilling the criteria for classification

3.9.6.2.1 Example 4: MCCPs (Medium Chain Chlorinated Paraffins) = Alkanes, C_{14-17}, Chloro- (EC No 287-477-0; CAS No 85535-85-9)

Application of criteria for evaluation/classification with regard to mechanisms not relevant to humans (see Section 3.9.2.5.3 of this Guidance)

Available information:

1) Human experience: No information available

2) Animal data: see summary

Key chronic toxicity data: *Summary of data for repeated exposure*
The only available data relate to a number of oral dosing studies (up to 90 days duration) that have investigated the repeated dose toxicity of MCCPs (C_{14-17}, 40% or 52% chlorinated paraffins) in rodents. However, only two studies emerge as providing helpful dose-response information in respect of classification and labelling (IRDC 1984, Poon *et al.* 1995). The others, all presented in more detail in the ESR RAR, were generally mechanistic studies on the interplay between liver and thyroid and the relevance of effects on these organs to human health, conducted at relatively high exposure levels. In rats, the liver, thyroid and kidney are the target organs for repeated dose toxicity of MCCPs. For the liver, increases in weight and changes in enzyme activity are seen in rats at exposure levels of 36 mg/kg bw/day or more (Poon *et al.*, 1995). These effects are considered part of an adaptive response to an increase in metabolic demand. There is also the possibility that peroxisome proliferation plays a role. These findings were not considered to justify classification. At higher exposure levels (around 360 mg/kg bw/day), single cell necrosis was observed in rats (Poon *et al.*, 1995), but this is above the cut-off level for classification.

Increased thyroid weight was observed in a 90-day study only at the highest exposure level tested, 625 mg/kg bw/day (IRDC 1984). Histopathologically, lesions such as hyperplasia have been observed down to the lowest exposure levels tested (eg. 0.4 mg/kg bw/day by Poon *et al.*, 1995) with an exposure-related increase in severity. However, the severity only ranged from "mild" to "moderate" even with an increase in exposure of 3 orders of magnitude. The thyroid changes (increased weight and follicular hypertrophy and hyperplasia) are considered to occur as a result of repeated stimulation of this organ caused by the well-characterised negative feedback control effect arising from plasma T_4 depletion. This in turn is related to an increase in the activity of hepatic UDPG-transferase. Humans, unlike rodents, possess a T_4 binding protein that greatly reduces susceptibility to plasma T_4 depletion and thyroid stimulation. The thyroid effects observed in rats are therefore considered of insufficient concern for classification.

No adverse renal effects were seen in males and female rats at 0.4 mg/kg bw/day in a 90-day study (Poon *et al.*, 1995). Inner medullary tubular dilatation was seen at 4 mg/kg bw/day in the kidneys of females only. These lesions were slight, with changes increasing only marginally in severity and incidence at higher levels (up to 420 mg/kg bw/day for females). An exposure-related increase in the incidence and severity of a mixed population of interstitial inflammatory cells, tubular regeneration and minimal degenerative changes in the tubular epithelium was seen in treated males and females at 10 mg/kg bw/day or more. At 10 mg/kg bw/day the severity of these changes was graded as 'trace', and even at the highest exposure level, 625 mg/kg bw/day it was only 'mild'. As the effects observed in the highest dose group do not seem to be severe, no classification is proposed for repeated-exposure effects.

Mechanistic studies conducted using short-chain chlorinated paraffins (SCCPs, C_{10-13}) indicate deposition of β2μ-globulin in proximal convoluted tubules and this may be the primary mechanism for renal toxicity in male rats.

Classification for MCCP's: No classification for STOT-RE

Justification:

Effects on the liver: the effects justifying the classification (necrosis) are above the cut-off limit values.

Effects on the thyroid: the effects observed are specific for the rat and do not justify classification.

Effects on the kidneys: the data are not detailed enough to have an idea what are effectively the effects around the cut-off values (10-100 mg/kg bw) instead of 50 mg/kg bw (DSD cut-off value) but probably we could come to the same conclusion, i.e. the effect is not enough to justify the classification in any category.

3.9.6.3 Examples of mixtures fulfilling the criteria for classification

3.9.6.3.1 Example 5:

Application of criteria for mixture classification: 'When data are available for the complete mixture' (see Section 3.9.3.3 of this Guidance).

Available information:

A mixture with a suspect ingredient (8%) has been tested in a valid 90-day oral study according to TG OECD 408 and GLP. At the dose of 90 mg/kg bw/day severe liver damage (necrosis) has been observed, at 30 mg/kg bw/day slight-moderate liver impairment. The NOAEL was 9 mg/kg bw/day.

Classification: STOT-RE Category 2

Justification:

The classification is based on data of a valid, appropriate animal study for the complete mixture. Therefore the criteria for substances (CLP Annex I, Table 3.9.3) are applied.

3.9.6.3.2 Example 6

Application of criteria for mixture classification: 'When data are available for all components' (see Section 3.9.3.3 of this Guidance). Components of a mixture that should be taken into account are listed below together with their concentrations. Generic concentration limits should be used, non-additivity is applied.

Available information:

Ingredient	% w/w	Classification
1	39	NC
2	5.5	STOT-RE Category 1
3	54	NC
4	1.5	STOT-RE Category 2

Classification of the mixture: STOT-RE Category 2

Justification:

No test data with respect to STOT-RE are available for the complete mixture. Bridging principles can not be applied since no respective test data on a similar mixture are available. The classification of the mixture will be based on the classified ingredients (CLP Annex I, Table 3.9.4).

There is one STOT-RE Category 1 ingredient in a concentration of <10%. Therefore the mixture is not classified in STOT-RE Category 1. There is one STOT-RE Category 1 ingredient in a concentration of ≥ 1% and <10%, therefore STOT-RE Category 2 is warranted. The STOT-RE Category 2 ingredient with 1.5% is not taken into account at all, since the concentration is < 10%.

3.9.6.3.3 Example 7

Application of criteria for mixture classification 'When data are available for all components' (Section 3.9.3.3 of this Guidance). Components of a mixture that should be taken into account are listed below together with their concentrations. Generic concentration limits should be used, specific concentration limits should take precedence over generic concentration limits when available, and non-additivity applies.

Available information:

Ingredient	Classification	Concentration (% w/w)	Mixture Classification	Remarks
A	STOT-RE Category 1	0.1		SCL 0.2%
B	STOT-RE Category 1	9		

Classification of the mixture: STOT-RE Category 2 based on 9% of B, which is ≥ 1% and < 10%; A does not contribute to the classification of the mixture, as the concentration of A is < 0.2% (the SCL) and additivity of the two ingredients is not foreseen.

3.9.6.3.4 Example 8

Application of criteria for mixture classification 'When data are available for all components' (Section 3.9.3.3 of this Guidance). Components of a mixture that should be taken into account are listed below together with their concentrations. Generic concentration limits should be used, specific concentration limits should take precedence over generic concentration limits when available, and non-additivity applies.

Available information:

Ingredient	Classification	Concentration (% w/w)	Remarks
A	STOT-RE Category 1	0.3	SCL 0.2%
C	STOT-RE Category 2	9	

Classification of the mixture: STOT-RE Category 1 since the concentration of A, even if being lower than the generic concentration limit, is higher than the SCL; C does not contribute to the classification.

3.9.6.4 Example of mixtures not fulfilling the criteria for classification

3.9.6.4.1 Example 9

Application of criteria for mixture classification: 'When data are available for all components' (Section 3.9.3.3 of this Guidance); components of a mixture that should be taken into account are listed below together with their concentrations. Generic concentration limits should be used, non-additivity is applied:

Available information:

Ingredient	Concentration (% w/w)	Classification
1	39	NC
2	9	STOT-RE Category2
3	49.5	NC
4	2.5	STOT-RE Category 2

Classification of the mixture: NC (no classification).

Justification:

No test data with respect to STOT-RE are available for the mixture as a whole. Bridging principles can not be applied, since no respective test data on a similar mixture are available (CLP Annex I, Table 3.9.4).

The classification of the mixture is based on the classified ingredients. No ingredient is classified in STOT-RE Category 1. Therefore the mixture cannot be classified in STOT-RE Category 1. Though the sum of the STOT-RE Category 2 ingredients (11.5 %) is above the generic concentration limit of 10%, the mixture is not classified. This is because for STOT-RE the no additivity approach applies and no individual ingredient ≥ 10% is present in the mixture.

3.9.7 References

Muller, A. et al (2006) Regulatory Toxicology and Pharmacology 45, 229-241

VI ANNEX VI: BACKGROUND DOCUMENT TO THE GUIDANCE FOR SETTING SPECIFIC CONCENTRATION LIMITS FOR SUBSTANCES CLASSIFIED FOR REPRODUCTIVE TOXICITY ACCORDING TO REGULATION (EC) NO 1272/2008

1 Executive summary

Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation or CLP) contains rules including criteria for the classification of substances and mixtures. While the classification of substances for human health hazards is based on specific criteria for each hazard class, the classification of mixtures is mainly based on the concentration and the classification of the substances contained in the mixture. CLP includes generic concentration limits (GCLs) which are specific for a hazard class and category and which indicate a threshold above which the presence of a substance in a mixture leads to classification of the mixture. However, under certain conditions specific concentration limits (SCLs) must or may be used . As the Regulation itself does not provide any further guidance on when and how to set SCLs, guidance has been developed for certain hazard classes (see the respective chapters on setting SCLs in Part 3 of the Guidance on the Application of the CLP Criteria).

This Annex provides a background to the method for the determination of SCLs for substances classified as reproductive toxicants as outlined in the guidance in Part 3.

The potency, expressed as the dose for the induction of reproductive effects was identified as the best determinant for setting SCLs. The ED_{10} for effects warranting classification was selected as the most appropriate parameter for estimating the potency. The ED_{10} is the dose level which induces reproductive effects in 10% of the animals above the control group or a change of 10% in the effect compared to the control group. Based on the ED_{10} the substance is placed in a potency group. However, modifying factors can alter the potency group, especially when the potency estimate is close to the boundary between two groups.

The distribution of the potency of a large number of substances classified in Annex VI to CLP as developmental toxicants and/or substances affecting sexual function and fertility was determined by means of establishing two databases. In line with other methods for setting SCLs for other hazard classes, it is proposed to define three potency groups. The boundaries for the potency groups were determined in line with the provisions outlined in Article 10(1) of CLP, the results of the database analyses and policy considerations. Most substances are foreseen to fall into the medium potency group which is linked to the GCL. For substances in the high and low potency group, the SCLs included in the table below are proposed.

	Category 1		Category 2	
	Dose	SCL	Dose	SCL
High potency group	ED_{10} below 4 mg/kg bw/day	0.03% (factors of 10 lower for extremely potent substances[B])	ED_{10} below 4 mg/kg bw/day	0.3% (factors of 10 lower for extremely potent substances[B])

Medium potency group	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	0.3% (GCL)	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	3% (GCL)
Low potency group	ED_{10} above 400 mg/kg bw/day	3%	ED_{10} above 400 mg/kg bw/day	3-10% [A]

[A] The limit of 10% may be considered in certain cases, such as for substances with a ED_{10} value above 1000 mg/kg bw/day and a NOAEL below 1000 mg/kg bw/day

[B] For substances with an ED_{10} more than 10 fold below 4 mg/kg bw/day, meaning an ED_{10} below 0.4 mg/kg bw/day, a 10-fold lower SCL should be used. For even more potent substance the SCL should be lowered with a factor of 10 for every factor of 10 the ED_{10} is below 4 mg/kg bw/day.

2 Introduction

2.1 General description of the classification system for reprotoxic substances and mixtures

Regulation (EC) No 1272/2008 (CLP) contains rules for the classification of substances and mixtures. In chapter 3.7 of Annex I to this Regulation, criteria are given for the classification of substances as reprotoxicants in one of the following categories:

Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data must provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to

place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Effects on or via lactation are also part of the hazard class reproductive toxicity. Classification for these effects is independent of the classification in the classes 1A, 1B or 2 as described above. Development of a method for the determination of SCLs for substances with effects on or via lactation is outside the scope of this document. Therefore, these effects and this classification are not further considered in this document.

The classification of mixtures containing substances classified for reproductive toxicity and of substances containing impurities, additives or constituents classified for reproductive toxicity is based on the concentration of the reproductive toxic component(s). Table 3.7.2 of Annex I to CLP contains GCLs above which classification for reproductive toxicity is required. The GCL is 0.3% for reprotoxicants Category 1A and 1B and 3.0% for Category 2. However, a GCL for all substances may not be protective for high potency substances and may be overprotective for substances with a low potency. Therefore, SCLs may be needed for such substances.

According to CLP Article 10, SCLs shall be set where adequate and reliable scientific information shows that the hazard of a substance is evident at a level below the GCL. This results in SCLs below the GCLs. SCLs above the GCLs may be set in exceptional circumstances where adequate, reliable and conclusive scientific information shows that a hazard of a substance is not evident at a concentration above the GCL. Normally, substances that fulfil the criteria for reproductive toxicity are subject to a harmonised classification and labelling and included in Annex VI to CLP. In such cases, SCLs are set via the procedure for harmonisation of classification and labelling of substances in line with CLP Article 37. When there is no such harmonised entry in Annex VI to CLP, a manufacturer, importer or downstream user must self-classify reproductive toxic substances and must set lower or may set higher SCLs than the GCLs if justified according to CLP Article 10(1). He may also provide a proposal for a harmonised classification (CLP Article 37(2)), including an SCL where appropriate.

2.2 Description of the process for the development of a method to set SCLs for reproductive toxic substances

There are no hazard specific criteria for the setting of SCLs in CLP . According to CLP Article 10 (7), the European Chemicals Agency (ECHA) is required to provide further guidance on the setting of SCLs. A working group was established to develop such guidance for the hazard class reproductive toxicity, with the exception of the effects on or via lactation.

The work on the proposal for guidance on the determination of SCLs for reproductive toxicants was initiated by an EU working group of the TC C&L (Technical Committee on Classification and Labelling of Dangerous Substances), continued under the REACH Implementation Project (RIP) 3.6 and subsequently under the auspices of ECHA.

To get an impression of the possible parameters for potency and their distribution, two databases were compiled, containing several parameters for a large number of substances classified for developmental toxicity and impaired fertility. Based on the compiled data choices were made for the most appropriate parameter, the boundaries of the potency groups and the associated SCLs.

In the course of the guidance development, three documents have been produced. The first document is the actual guidance chapter included in the Guidance on the Application of the CLP Criteria. The second document is this annexed background document, describing the process and considerations and providing the rationale for the proposed guidance. The third document is a publication of the databases of parameters for developmental toxicants and substances with an effect on sexual function or fertility and the analyses of the databases [(Muller et al., 2012)]

Chapter 2 of this document describes potency parameters and contains a number of theoretical considerations on the determination of the most appropriate parameter and the SCLs. A description of the databases and the analyses is also provided in this chapter. Chapter 4 is dedicated to the non-modifying factors. Chapter 5 describes and justifies the potency boundaries and corresponding SCLs.

2.3 Considering potency in setting specific concentration limits for various health hazards

The criteria for classification for reproductive toxicity are based on the strength of scientific evidence that the substance can cause reproductive toxicity. In general, no specific considerations are given to the potency of the substance to induce reproductive toxicity.

On the other hand, classification for several other health hazard classes is based on potency. Substances with different potency are classified in different categories within the hazard class. The classification of mixtures for that hazard class is then based on the concentration of the substance in the mixture and the hazard category or the potency (for acute toxicity) of the substance.

For acute toxicity, the potency is based on the acute toxicity estimate (ATE). The ATE is the dose level which induces 50% mortality in a acute toxicity study (LD_{50} or LC_{50}) or the estimated LD_{50} or LC_{50} using fixed dose procedure or the acute toxic class method. This value is used to classify a substance into one of several categories. For mixtures, the ATE value is used to estimate the potency of a mixture by calculation. The estimated potency is then used to classify the mixture into a hazard category.

For specific target organ toxicity (STOT) after single and repeated exposure, the potency is defined as the dose at which the substance shows significant toxic effects in a study. Based on the potency, a substance is either classified for STOT into one of two hazard categories or not classified. The classification of a mixture containing a substance classified for STOT depends on the percentage of the substance in the mixture and the hazard category of the substance. A minimal percentage is included in the criteria. SCLs have to be determined for substances with a very high potency.

Classification for carcinogenicity is, as for reproductive toxicity, based on the strength of scientific evidence and again no specific consideration is given to the potency. The classification of mixtures containing a carcinogenic substance is based on the GCL unless a SCL has been allocated for that substance as provided in Annex VI to CLP. SCLs for carcinogenic substances are determined based on the potency for carcinogenic effects based on the T25. The T25 is defined as the daily dose (in mg/kg bw) inducing a tumour incidence of 25% upon lifetime exposure after correction for the spontaneous incidence. This is mainly based on animal studies. Substances are divided into 3 groups based on the T25. High potency substances have a T25 $\leq$ 1mg/kg bw/ day, medium potency substances have a T25 between 1 -100 mg/kg bw/day, and T25> 100 mg/kg bw/day for low potency substances. Besides the T25, other elements were included that modify the potency evaluation

(Commission Working Group, date unknown). This method has been included in the Guidance on the Application of the CLP Criteria.

The use of potency for the classification into different categories for several other hazard classes and the use of the potency to set SCLs for carcinogenic substances, justifies the use of potency as a first approach also for setting SCLs for reproductive toxic substances. As no definition of potency for reproductive toxicants was available, the following definition is used as a working definition:

Reproductive toxicity potency is defined as the dose which induces reproductive toxic effects with a specific type, incidence and magnitude, considering the study design in terms of species and strain, exposure route, exposure duration, exposure window in the life cycle, and possible concomitant parental toxicity.

According to this definition 'Potency' is primarily based on applied *dose* and can be modified by consideration of 'severity'. Within this definition the dose is defined as the amount of substance to which the animals or humans that showed the effect (meaning type, incidence and magnitude) were exposed on an mg/kg bw/day basis. The incidence is the proportion of animals or humans that showed the effect. The type of effect describes which property of an organ or system of the animal or human is affected and the magnitude describes the level of change compared to the control. Together, the incidence, type and magnitude describe the 'severity' of the effect, meaning how adverse the effect or combination of effects is. With specific incidence, type and magnitude (together specific severity) a comparable level of severity is indicated for different effects.

The working definition above allows potency to be defined at different levels of specific severity, for example at the ED_{10} and the LOAEL (Lowest Observed Adverse Effect Level), and for different type of effects. Therefore, several possible estimates for potency were investigated.

2.4 Parameters for potency for reproductive toxicity

A consistent database to derive potency estimates for reproductive toxicity was lacking. Therefore, data on substances classified for effects on reproduction were collected and analysed. This was done separately for substances with an effect on development and substances with an effect on sexual function and fertility because the types of effects clearly differ between these two main types of reproductive effects. Therefore, this chapter falls into two parts, namely one for parameters for potency of substances with developmental effects (chapter 2.3.1) and one for parameters for potency of substances with effects on sexual function and fertility (chapter 2.3.2). As potency is primarily based on the dose in mg/kg bw/day at which different adverse effects are observed, a number of parameters/dose descriptors (e.g. NOAEL[13], LOAEL[14], ED_{10} etc.) exist for each type of adverse effect. The collected data included the NOAEL, LOAEL and ED_{10} (effective dose with a 10% incidence or effect level above the background) as parameters for the effect on reproduction of each substance. They were further divided into effects fulfilling the criteria for classification (named "LOAEL (classification)" for example) and any effects on reproduction (named "NOAEL (overall)" for example). Together, this sub-division results in 6 different potency parameters, see Table 1. Other data, e.g. a mutagenicity classification of a substance, the type of effect at the LOAEL and species used in the test, were also collected. These parameters were analysed and the results tabulated and plotted graphically. The results are published by

[13] NOAEL means No Observed Adverse Effect Level
[14] LOAEL means Lowest Observed Adverse Effect Level

Muller et al., 2012. As the data for these two main types of reproductive toxicity were analysed separately, the results are provided separately.

2.4.1 Potency parameters for developmental toxicants (Muller et al, 2012)

Data for one or more of the parameters for development were available for 99 substances classified for developmental toxicity when the work on this guidance development started. For almost all substances a LOAEL is available but a NOAEL and ED_{10} were sometimes missing. The absence of a NOAEL is mostly caused by the absence of a dose level without an effect in the study or database of a substance. The absence of an ED_{10} value is mainly caused by the absence of a NOAEL and in most of those cases an ED_{10} could only be derived by a benchmark dose (BMD) approach to avoid interpolation between the LOAEL and the vehicle control. Another cause for the absence of ED_{10} values is the limited reporting of effect levels in the consulted study summaries or study reports.

The difference in the average value between the highest and lowest of the 6 parameters for potency is a factor of 4 or less. This is very small compared to the difference in potency between substances for each parameter of up to 1,000,000 fold (Table 1). The potency difference is more pronounced for a NOAEL or LOAEL compared to an ED_{10} mainly because for most potent substances only a NOAEL and/or a LOAEL was available but not an ED_{10}. The available data indicate that there is a close relation between the NOAEL, LOAEL and ED_{10} for most substances. The average LOAEL is between a factor of 2 and 3 above the average NOAEL. The fact that it is not closer to the factor of 3 to 4 that is normally used between dose levels is probably due to the absence of a NOAEL for a number of substances. The average ED_{10} (classification), is slightly higher than the average LOAEL (classification). The difference is more pronounced for the "overall" values, namely approximately a factor of 2. These findings are caused by both the dose spacing in the studies and the limited discriminative power of the NOAEL approach.

Table 1. Average values (assuming log/normal distribution) (in mg/kg bw/day) and potency differences for parameters for all developmental toxicants of the database (Muller et al, 2012)

Parameter	N	Average	Standard deviation	Lowest value	Highest value	Potency difference
NOAEL (overall)	68	12	10	0.002	684	342000
LOAEL (overall)	98	25	13	0.002	2281	1140500
ED_{10} (overall)	59	43	6	0.3	785	2617
NOAEL (classification)	76	18	11	0.002	1100	550000
LOAEL (classification)	97	40	13	0.002	2281	1140500
ED_{10} (classification)	63	48	6	0.3	933	3110

A part of the differences in average values and potency between the different parameters in Table 1 is probably caused by the difference in the number of substances for which a particular variable is present. When only substances are used for which all 6 parameters were present, this reduces the database to 44 substances (Table 2). A part of the difference between the parameters in potency difference can be explained by the unusual dose levels (NOAEL

0.026 mg/kg bw/day and LOAEL 0.26 mg/kg bw/day) used in the study for the substance that had the lowest values for all parameters (cadmium oxide).

Table 2. Average values (assuming log/normal distribution) (in mg/kg bw/day) and potency differences for parameters for developmental toxicants (N=44) with all 6 parameters (Muller et al, 2012)

Parameter	Average	Standard deviation	Lowest value	Highest value	Potency difference
NOAEL (overall)	19	7	0.026	684	26308
LOAEL (overall)	58	7	0.260	2281	8773
ED_{10} (overall)	44	5	0.300	570	1900
NOAEL (classification)	25	7	0.026	684	26308
LOAEL (classification)	71	6	0.260	2281	8773
ED_{10} (classification)	49	6	0.300	933	3110

Comparing Tables 1 and 2 indicates no major changes in average, standard deviation and highest value for each parameter. However, the lowest value changes for several parameters. The resulting potency difference becomes much more comparable between the parameters. This indicates that the difference between the parameters in potency difference in Table 1 is mainly due to the absence of an ED_{10} for some very potent substances.

2.4.2 Potency parameters for substances with an adverse effect on sexual function and fertility (Muller et al, 2012)

Data for one or more of the potency parameters were available for 93 substances classified for adverse effects on sexual function and fertility (hereafter called fertility toxicants) when the work with the guidance development started. For all substances, an LOAEL was available but a NOAEL and an ED_{10} were sometimes missing. The absence of a NOAEL is mostly caused by the absence of a dose level without an effect in the study or database of a substance. The absence of an ED_{10} value is mainly caused by the absence of a NOAEL and in most of those cases an ED_{10} could only be derived by a Benchmark Dose (BMD) approach to avoid interpolation between the LOAEL and the vehicle control. Another cause for the absence of an ED_{10} values is the limited reporting of effect levels in the consulted study summaries or study reports.

The difference in the average values between the highest and lowest of the 6 parameters for potency is less than a factor of 4. This is small compared to the difference in potency between substances for each parameter of up to 30,000 (Table 3). The difference in potency within the parameters is more pronounced for the NOAEL values than for the values of LOAEL and ED_{10}, which is mainly due to one substance with a NOAEL of 0.032 mg/kg bw/day but an LOAEL of 10 mg/kg bw/day. The available data indicate that there is a close relation between the NOAEL, LOAEL and ED_{10} for most substances. The average LOAEL is between a factor 2 and 3 above the average NOAEL. The fact that it is not closer to the factor of 3 to 4 that is normally used between dose levels is probably due to the absence of an NOAEL for a number of substances. The average ED_{10} is between the average NOAEL and LOAEL.

Table 3. Average values (assuming log/normal distribution) (in mg/kg bw/day) and potency differences for parameters for all fertility toxicants of the database

Parameter	N	Average	Standard deviation	Lowest value	Highest value	Potency difference
NOAEL (overall)	68	20	7	0.032	635	19844
LOAEL (overall)	93	54	7	0.25	2060	8240
ED_{10} (overall)	37	31	5	0.6	1065	1775
NOAEL (classification)	70	24	7	0.032	940	29375
LOAEL (classification)	93	62	7	0.33	2060	6242
ED_{10} (classification)	37	33	6	0.6	1065	1775

A part of the differences in the average values and in potency between the different parameters in Table 3 is probably caused by the difference in the number of substances for which a particular parameter is present. When only substances are used for which all 6 parameters were present, this reduces the database to 34 substances (Table 4).

Table 4. Average values (assuming log/normal distribution) (in mg/kg bw/day) and potency differences for parameters for fertility toxicants (N=34) with all 6 parameters

Parameter	Average	Standard deviation	Lowest value	Highest value	Potency difference
NOAEL (overall)	19	6	0.3	250	833
LOAEL (overall)	72	6	0.7	1000	1429
ED_{10} (overall)	35	5	1.3	1065	819
NOAEL(classification)	24	6	0.3	940	3133
LOAEL(classification)	89	6	0.7	1580	2257
ED_{10} (classification)	39	5	1.3	1065	819

Comparing Tables 3 and 4 indicates no major changes in average, standard deviation and highest value for each parameter. However, the lowest value changes for some parameters. The resulting potency difference becomes much more comparable between the parameters. This indicates that part of the differences between the parameters in potency difference in Table 3 is due to the absence of an ED_{10} for some very potent substances.

2.4.3 Conclusions on the most appropriate parameter for potency

As LOAELs are available for almost all substances, this could be considered the most useful informed parameter on which to base potency. However, in the absence of a NOAEL, a LOAEL is not a suitable parameter for potency because there is no indication to what extent the real LOAEL could be lower than the LOAEL observed. The lower number of substances for which an ED_{10} is available is probably due to the limitations of the available study summaries for several substances. Use of the ED_{10} requires access to a detailed summary of

the study or the study report itself which was not available for several substances in the database.

However, this guidance will be applied by both industry and Member State Competent Authorities when preparing proposals for harmonised classification and labelling, and by industry in case of self-classification of a reproductive toxic substance for which there is no entry in Annex VI to CLP.

Companies have access to their own studies. It is expected that by the completion of the REACH registration deadlines, more detailed information including ED_{10} will be available for more substances than in this database used to develop this guidance.

Member States will have access to the study summaries in the registrations. The full studies could be requested by ECHA or by a Member State Competent Authority, according to CLP Article 49(3).

It should be noted that in the absence of a NOAEL, an ED_{10} cannot be determined by interpolation, in case the size of the effect at the LOAEL is more than 10%. However, an ED_{10} can be estimated using bench mark dose (BMD) software when sufficient data are available. A NOAEL and LOAEL cannot be estimated using the BMD approach. In addition, a fixed level of effect of e.g. 10% (ED_{10}) is considered to be more representative for the potency and facilitates comparisons of relative potency between substances to a greater extent, than a LOAEL which is a chosen dose level.

For most other hazard classes, the SCLs are based on effect levels. For carcinogenicity the T25 is used, and for skin sensitisation the EC_3 value or the dose level with a certain level of responders is used. Therefore, the LOAEL or ED_{10} is considered a more appropriate parameter for determination of an SCL than the NOAEL.

For substances where there is a difference in the LOAEL overall (lowest dose with any effect on reproduction) versus the LOAEL classification (lowest dose with an effect on reproduction fulfilling the classification criteria), this is in most cases due to non-significant increases in lethalities or malformations or decreases in foetal body weight at the LOAEL overall versus significant increases in lethalities or malformations at the LOAEL classification. The difference between significant and non-significant effects will disappear if the ED_{10} is used as parameter for potency.

The difference in parameters between “overall” and “classification” was sometimes due to limited effects that normally do not warrant classification such as a small increase in variations at the LOAEL and to more severe effects warranting classification at a higher dose level. To have a more consistent parameter for potency, it was preferred to use the parameters for effects warranting classification.

Overall, the use of the ED_{10} for effects warranting classification is proposed as the most appropriate estimate for the potency. The advantage of this parameter is that it is a dose level with a specified level of effects of at least a certain severity. This is in line with most classification criteria and with other methods for the determination of SCLs.

Furthermore, not all aspects included in the working definition of reproductive potency are fully taken into account in the ED_{10}. Therefore, certain additional parameters should be considered which can change the potency group as determined by using the ED_{10}, resulting in the setting of lower or higher concentration limits. See chapter 4 for such modifying factors.

3 Modifying factors

Several possible elements of reproductive toxicity were considered as elements which should also be taken into account when determining the potency group for reproductive toxicity of a substance (modifying factors). Modifying factors may change the potency group for a substance. While some modifying factors should always be taken into account, other modifying factors could be more relevant when the potency is close to the boundary between two groups (see Table 8 above). It should be noted that several of the elements may be interrelated.

Some factors may have already been taken into account in deciding on the classification as a reproductive toxicant. Where such considerations have been made, care should be taken not to use that information again when determining the potency. For example, when the effects determining the ED_{10} were observed at dose levels also causing maternal toxicity, this should already have been taken into consideration during the classification and should not be used again to set a higher SCL. Factors considered not to be used as modifying factors are included in section 4.7 of this Annex. The following factors are used as modifying factors:

- Type of effect / severity
- Data availability
- Dose-response relationship
- Mode or mechanism of action
- Toxicokinetics
- Bio-accumulation of substances

The justification of the use of these modifying factors is provided in the guidance (see section 3.7.2.5.5).

4 Non-modifying factors

A wide range of parameters were considered as possible modifying factors for the determination of reproductive potency. Parameters selected as modifying factors are included above. Parameters or factors considered but not included as modifying factors are listed below:

4. 1 Species and strains

The species used to determine the ED_{10} could be considered as a modifying factor if it is shown that a certain species is generally more sensitive to reproductive toxicants, meaning showing effects at a lower exposure level, and this can be considered relevant to humans. However, comparison of the different parameters between the two most used species for developmental effects, rats and rabbits, did not indicate a difference in average NOAEL, LOAEL or ED_{10} in this analysis. Furthermore, almost all studies that were determinative for the classification for fertility were studies in rats. Therefore, species is not regarded as a modifying factor. The most sensitive species for each substance has to be used to determine the potency parameter unless there is clear evidence that the observed effects are not relevant to humans or when there is good evidence for a difference in sensitivity between humans and the test species. This also applies to different strains.

4. 2 Systemic or maternal toxicity

Adverse effects on fertility and sexual function may be caused as a secondary effect of systemic toxicity to other organs. Developmental effects may be caused as a secondary effect of maternal toxicity. However, this should have already been taken into account for

classifying a substance in a specific category. Therefore, this should not also be used for modifying the concentration limit.

4. 3 Mutagenicity

Analyses of the databases [(Muller et al., 2012)] indicate that substances classified both for reproductive toxicity and mutagenicity have a higher potency (lower ED_{10}) than substances classified for reproductive toxicity only. However, as this higher potency is already included in the lower ED_{10}, there is no need to use mutagenicity as a modifying factor.

4.4 Volatility

Volatility is a physical property related to exposure rather than to the intrinsic hazardous potency of a substance. However, the exposure level to a substance in a mixture is not only influenced by the concentration but also by the volatility of the substance. The higher the volatility of a substance the higher the inhalation exposure may be when handling such a substance in a mixture. Inhalation exposure to vapours are not covered by the experimental oral testing limit of 1000 mg/kg bw/day as the exposure at workplaces can be more than one order of magnitude above the extrapolated exposure level covered by the limit dose (Schneider et al., 2007). This is probably the reason why no limit dose for classification is included in the classification criteria (see appendix I, 3.7.2.5.4). Therefore, volatility could be considered as a modifying factor.

However this argument is not specific for reproductive toxicity and should then apply to all relevant hazard classes. In methods for setting SCLs for other hazard classes such as carcinogenicity, the volatility is not used as a modifying factor, although it is suggested to be a factor to take into consideration when setting SCLs for narcotic effects (STOT-SE 3). Further, volatility is not specifically mentioned in the criteria for classification for any other hazard class other than STOT-SE and -RE (3.8.2.1.10.4 and 3.9.2.10.4) for which the guidance recommends a specific precautionary statement on the label for highly volatile substances.

However for some hazard classes, volatility is taken into account in the classification of substances and mixtures by using different numeric criteria (acute toxicity, table 3.1.1) or guidance values (STOT-SE table 3.8.2 and STOT-RE, table 3.9.2 and 3.9.3) for vapours than for dusts and mists. For STOT-SE and STOT-RE, the method for setting SCLs is directly depending on these guidance values.

It was decided not to include volatility as a modifying factor because it is a physical property that depends also on other factors (e.g. temperature and composition of the mixture) and is therefore more related to exposure rather that to the intrinsic hazardous potency of the substance.

5 Potency groups and specific concentration limits

5.1 Justification of the proposed potency boundaries and specific concentration limits

In the following some general considerations on potency groups are first provided, followed by justifications for the approach taken and for the suggested boundaries of the potency groups and the corresponding concentration limits.

5.1.1 General considerations on potency groups

5.1.1.1 Legal requirements

According to the second subparagraph of CLP Article 10(1)

"Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I."

According to the third subparagraph of CLP Article 10(1)

"In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.".

5.1.1.2 Scientific results of the database analysis

The databases with ED_{10} values for substances (Category 1 and 2) with an effect on development and with an effect on sexual function and fertility were compared to determine whether there is a difference in potency between Category 1 and Category 2 substances [(Muller et al, 2012)]. The results should be carefully interpreted because of the limitations of the database: the database is based on a limited number of substances and the available data per substance is reduced to a single number (ED_{10}) and some modifying factors. Reducing the data in the database would have included removal of differences in effects and doubts between Category 1 and Category 2. In any case, the comparisons indicate that the average potency of substances with an effect on development and with an effect on sexual function and fertility are comparable and that also the average potencies of Category 1 and 2 substances are comparable and certainly do not differ by a factor of 10.

5.1.1.3 Policy related considerations and proposed method

Data derived from an insensitive test method could in some cases not be regarded as adequate, reliable and conclusive evidence, as mentioned in Article 10 (1) (3rd para). For example, a screening assay which only uses a limited number of animals and studied endpoints, cannot be used to set higher SCLs (but can be used to set lower SCLs). Also a study resulting in an LOAEL without an NOAEL cannot be used to set higher SCLs.

Determination of the boundaries of the potency groups (see Table 8) and the SCL or GCL for each group is a policy related issue. CLP Article 10, the criteria in Annex I to CLP and the available data do not give a clear direction. Therefore, a simple system was developed. Furthermore, the approach taken is similar to the one developed for other hazard classes such as skin sensitization and carcinogenicity, which should be an appropriate justification for the current method.

Determination of the potency for reproductive toxicity will in most cases be based on limited data from one or a few studies. It was recognised that an exact SCL for each substance that also differs for each substance would indicate a precision that is not realistic or scientifically justified. Also, Janer (2007) has shown that the variation in the NOAELs of 2-generation studies for one substance is considerable. Therefore, it is proposed to divide the substances into large potency groups with associated SCLs as it is done for other hazard classes. Three potency groups are proposed. As shown in Table 10 below, substances with the lowest potency (highest ED_{10}) fall in a group with an SCL above the GCL. Most substances should

fall in the group with the GCL. Only substances with a very high potency (low ED_{10}) should fall in the group with a SCL below the GCL. It is proposed to include approximately 70 – 80% in the GCL potency group and 5 to 15% in the low and high potency groups. Further, as the average potency of developmental toxicants and substances affecting sexual function and fertility are comparable, it is proposed to use the same boundaries for both types of effect. Also, the database shows there is no difference in potency between substances in Category 1 and Category 2. Therefore it is proposed to use the same boundaries for Category 1 and 2 substances.

5.1.1.4. Other methods considered

Several other options for a method for determining SCLs were discussed including a method that was used by the TC C&L in a limited number of cases in the past. This method is based on the limit dose of 1000 mg/kg bw/day, as described in the test guideline OECD 414 and 416.

The concentration limit expressed as a % in mixtures is derived by dividing the NOAEL by the limit dose followed by multiplication by 100 (see ECBI/47/02 Add.7). This method would result in an individual SCL for each substance. This would indicate a precision that cannot be expected from standard reproduction studies. Also this would result in an SCL for most substances and in a GCL for only some substances. Therefore, this method was not considered. Potency groups are used in the proposed method because this does not give the impression of a high precision and allow the placing of many substances in the medium potency group with the connected GCL.

5.1.2 Justification of the boundaries between the three potency groups.

The estimated percentages of already classified substances in each group for both Category 1 and 2 substances with an effect on development or an adverse effect on fertility and sexual function are provided in the tables below. They are based on the distribution of potencies of known developmental toxicants and of known fertility toxicants [(Muller et al., 2012)]. Several possible values of the boundaries between the three groups are tested. The estimations are based on counting the number of substances above or below a number of possible boundaries and applying some of the modifying factors such as the presence of a NOAEL and considering also the saturated vapour concentration for substances in the low potency group. However, the saturated vapour concentration, reflecting volatility, is not proposed as a modifying factor in the guidance.

Taking into account all modifying factors for all substances would imply a full assessment of the potency for all substances. This was not possible within the available resources. As most modifying factors result in a shift from the low potency group into the medium potency group and from the medium potency group into the high potency group, it is likely that the percentages in the low potency group may decrease and the percentages in the high potency group may increase. (Thus, the effect of volatility on the frequencies in Table 9 should be marginal.)

Based on the ED_{10} distribution a rough estimate was made by the Working group of the optimal boundaries using a range of a factor of 100 for the medium potency group. Then the number of substances falling into several combinations of boundaries was estimated.

Table 9. Percentages of substances in the three potency groups using the ED_{10} and some of the modifying factors for different boundaries of the potency groups and considering the saturated vapour concentration of low potency substances.

			Boundaries of the high and low potency groups					
			<2 mg/kg	**<3 mg/kg**	**<4 mg/kg**	**<5 mg/kg**	**<6 mg/kg**	**<7 mg/kg**
Type of effect	**Classification**	**Potency group**	**>200 mg/kg**	**>300 mg/kg**	**>400 mg/kg**	**>500 mg/kg**	**>600 mg/kg**	**>700 mg/kg**
Development	Cat 1A/1B	High potency	12,1	13,8	17,2	20,7	20,7	20,7
	H360D	Medium potency	75,9	77,6	79,3	77,6	79,3	79,3
		Low potency	12,1	8,6	3,4	1,7	0,0	0,0
		% with SCL	24,1	22,4	20,7	22,4	20,7	20,7
	Cat 2	High potency	10,3	13,8	13,8	17,2	17,2	20,7
	H361d	Medium potency	72,4	72,4	79,3	75,9	82,8	79,3
		Low potency	17,2	13,8	6,9	6,9	0,0	0,0
		% with SCL	27,6	27,6	20,7	24,1	17,2	20,7
Fertility	Cat 1A/1B	High potency	3,4	3,4	3,4	6,9	10,3	13,8
	H360F	Medium potency	89,7	93,1	96,6	93,1	89,7	86,2
		Low potency	6,9	3,4	0,0	0,0	0,0	0,0
		% with SCL	10,3	6,9	3,4	6,9	10,3	13,8
	Cat 2	High potency	6,3	9,4	10,9	15,6	15,6	17,2
	H361f	Medium potency	71,9	76,6	81,3	78,1	79,7	79,7
		Low potency	21,9	14,1	7,8	6,3	4,7	3,1
		% with SCL	28,1	23,4	18,8	21,9	20,3	20,3
All		avg high potency	8.0	10.1	11.3	15.1	16.0	18.1
		avg medium potency	77.5	79.9	84.1	81.2	82.9	81.1
		avg low potency	14.5	10.0	4.5	3.7	1.2	0.8
		avg % with SCL	22,5	20,1	15,9	18,8	17,1	18,9

As shown in Table 9 boundaries of 4 to 400 mg/kg bw/day would result in the maximum number of substances being included in the medium potency range for most types of effects and classifications and for both type of effects and classifications combined. For developmental effects Category 1 and 2 the percentage of substances in the medium potency group is within the target of ca. 70-80%. For effects on sexual function and fertility Category 2 this is almost the case. Only for Category 1 is this not the case. The percentage of substances in the medium potency group could be reduced by reducing the factor of 100 between the boundaries. However, because of the large difference in potency of the substances classified for reproductive toxicity of up to a million, this was not considered necessary. The percentage of substances in the high potency group is higher than the percentage in the lower potency group for the boundaries of 4 to 400 mg/kg bw/day. However, the percentage of substances in the high potency group was above 15% for substances classified for an effect on development in Category 1.

Following the PEG consultation, it was agred that volatility was not considered a modifying factor and thus, the ED_{10} distribution changes as shown in table 10. Borders of 4 to 400 mg/kg bw/day would result in the maximum number of substances being included in the medium potency range for most type of effects and classifications and for both type of effects and classifications combined. However, the same value also applies to some of the other borders. For developmental effects Category 1 and 2 the percentage of substances in the medium potency group is within the target of ca. 70-80%. For effects on sexual function and fertility Category 2 this is not the case. The percentage of substances in the medium potency group could be reduced by reducing the factor of 100 between the borders. However, because of the large difference in potency of the substances classified for reproductive toxicity of up to a million, this was not considered necessary. The percentage of substances in the high potency group is approximately the same as the percentage in the lower potency group for the borders of 4 to 400 mg/kg bw/day.

Table 10. Percentages of substances in the three potency groups using the ED_{10} and some of the modifying factors but not volatility for different borders of the potency groups.

			Borders of the high and low potency groups					
			≤2 mg/kg	**≤3 mg/kg**	**≤4 mg/kg**	**≤5 mg/kg**	**≤6 mg/kg**	**≤7 mg/kg**
Type of effect	**Classification**	**Potency group**	**≥200 mg/kg**	**≥300 mg/kg**	**≥400 mg/kg**	**≥500 mg/kg**	**≥600 mg/kg**	**≥700 mg/kg**
Development	Cat 1A/1B	High potency	12.1	13.8	17.2	20.7	20.7	20.7
	H360D	Medium potency	67.2	74.1	77.6	75.9	79.3	79.3
		Low potency	20.7	12.1	5.2	3.4	0	0
		% with SCL	32.8	25.9	22.4	24.1	20.7	20.7
	Cat 2	High potency	7.3	9.8	9.8	12.2	12.2	14.6
	H361d	Medium potency	68.2	65.8	70.7	70.7	75.6	78.1
		Low potency	24.4	24.4	19.5	17.1	12.2	7.3
		% with SCL	31.7	34.2	29.3	29.3	24.4	21.9
Fertility	Cat 1A/1B	High potency	3.4	3.4	3.4	6.9	10.3	13.8
	H360F	Medium potency	86.3	89.7	93.2	89.7	86.3	86.2
		Low potency	10.3	6.9	3.4	3.4	3.4	0
		% with SCL	13.7	10.3	6.8	10.3	13.7	13.8
	Cat 2	High potency	6.3	9.4	10.9	15.6	15.6	17.2
	H361f	Medium potency	68.7	73.4	78.2	75.0	76.6	76.5
		Low potency	25.0	17.2	10.9	9.4	7.8	6.3
		% with SCL	31.3	26.6	21.8	25.0	23.4	23.5
All		avg high potency	7.3	9.1	10.3	13.9	14.7	16.6
		avg medium potency	72.6	75.7	79.9	77.8	79.4	80.0
		avg low potency	20.1	15.2	9.8	8.3	5.9	3.4
		avg % with SCL	27.4	24.3	20.1	22.2	20.6	20.0

On average, combining both effect types and both classification categories, the goal of 70-80% of the substances in the medium potency group and 5 -15% of the substances in the low and high potency group was fulfilled with boundaries of 4 and 400 mg/kg bw/day. However, other combinations of boundaries such as 3 and 300 and 5 to 500 mg/kg bw/day also fulfill these requirements. Using these boundaries would result in a change of potency group for 10 to 14 substances (5 – 7%). Further it could be considered to lower the factor of 100 between the borders to increase the number of substances. For example, using boundaries of 5 to 300 mg/kg bw/day would result in 13.9% high potency substances, 15.2% low potency substances and 71% substances in the medium potency group. Also, the percentages provided in the tables 9 and 10 are calculated not using every modifying factor. Therefore, it can be stated that the choice of the boundaries is arbitrary. However, based on the available information, the boundaries of 4 to 400 mg/kg bw/day seem to be reasonable.

5.1.3 Concentration limits for Category 1 and Category 2 substances

The generic concentration limit (GCL) from the respective categories will be used for medium potency substances (group 2). As mentioned earlier the GCL is 0.3% for reproductive toxicants Category 1A and 1B and 3.0% for Category 2.

Category 1A and 1B

Different concentration limits have to be used for the different potency groups. Substances classified in Category 1 in the low potency group (group 3) can have a SCL above the GCL of 0.3%. We propose to use an SCL of 3% which is tenfold of the GCL. A factor of 10 is used often in CLP as difference in GCL between hazard categories. This factor is also used in the guidance for setting SCLs for carcinogens. For substances in group 1 (high potency), it is proposed to use a SCL of 0.03%. For extremely potent reproductive toxicants with an ED_{10} (classification) of more than 10 fold below the boundary limit of 4 mg/kg bw/day it is proposed to use even lower SCLs. For every factor of 10 below the upper limit the SCL is reduced with a factor of 10.

Category 2

Substances classified in Category 2 in the low potency group (group 3) can have a SCL above the GCL of 3%. We propose to use an SCL of 3-10% which is one to 3-fold of the GCL. An SCL above 10% was considered too high. The upper SCL of 10% can only be used in exceptional cases (NOAEL below 1000 mg/kg bw/day but ED_{10} above 1000 mg/kg bw/day). This would account for none of the substances in the database. For high potency substances (group 1), it is proposed to use an SCL of 0.3%. For extremely potent reproductive toxicants with an ED_{10} (classification) of more than 10-fold below the boundary limit of 4 mg/kg bw/day it is proposed to use even lower SCLs. For every factor of 10 below the upper limit, the SCL is reduced by a factor of 10.

The resulting SCLs for each potency group are presented in Table 11.

Table 11. SCLs for substances in each potency group and classification category

	Category 1		Category 2	
	Dose	SCL	Dose	SCL
Group 1 high potency	ED_{10} (classification) below 4 mg/kg bw/day	0.03% (factors of 10 lower for extremely	ED_{10} (classification) below 4 mg/kg bw/day	0.3% (factors of 10 lower for extremely potent

		potent substances[B])		substances)
Group 2 medium potency	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	0.3% (GCL)	$ED_{10} \geq 4$ mg/kg bw/day, and $\leq$ 400 mg/kg bw/day	3% (GCL)
Group 3 low potency	ED_{10} (classification) above 400 mg/kg bw/day	3%	ED_{10} (classification) above 400 mg/kg bw/day	3-10% [A]

[A] The limit of 10% may be considered in certain cases, such as for substances with an ED_{10} value above 1000 mg/kg bw/day and a NOAEL below 1000 mg/kg bw/day.

[B] For substances with an ED_{10} more than 10 fold below 4 mg/kg bw/day, meaning an ED_{10} below 0.4 mg/kg bw/day, a 10-fold lower SCL should be used. For even more potent substance the SCL should be lowered with a factor of 10 for every factor of 10 the ED_{10} is below 4 mg/kg bw/day.

Assigning two SCLs to a substance

A reproductive toxic substance is classified in one category for both effects on development and on sexual function and fertility. Within each category effects on development and on sexual function & fertility are considered separately. The potency and resulting concentration limits have to be determined separately for the two main types of reproductive toxic effects. In case the potency and resulting specific concentration limits are different for sexual function/fertility and development for a substance, the substance needs to be assigned one SCL for developmental toxicity and another SCL for effects on sexual function and fertility. These concentration limits will in all cases trigger different specifications of the hazard statements for the two main types of effects, to be applied to mixtures containing the substance (see also 3.7.4.1, Annex I, CLP).

5.2 Assigning SCLs

The SCL or GCL for each substance can be determined using the final potency group of the substance using Table 9.

6 References

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