3.4.3.3.1. 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 3.4.2.2.5 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.

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 3.4.4.1 of this Guidance.

3.4.3.3.2. 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 the case of skin sensitisation current test methods are based on application of a maximised dose, which can only 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 3.4.3.2 of this Guidance and CLP Annex I, 3.4.3.1.1.

3.4.3.3.3. 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 mixtures generated with current test methods as those described for any mixture in sections 3.4.3.2. 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

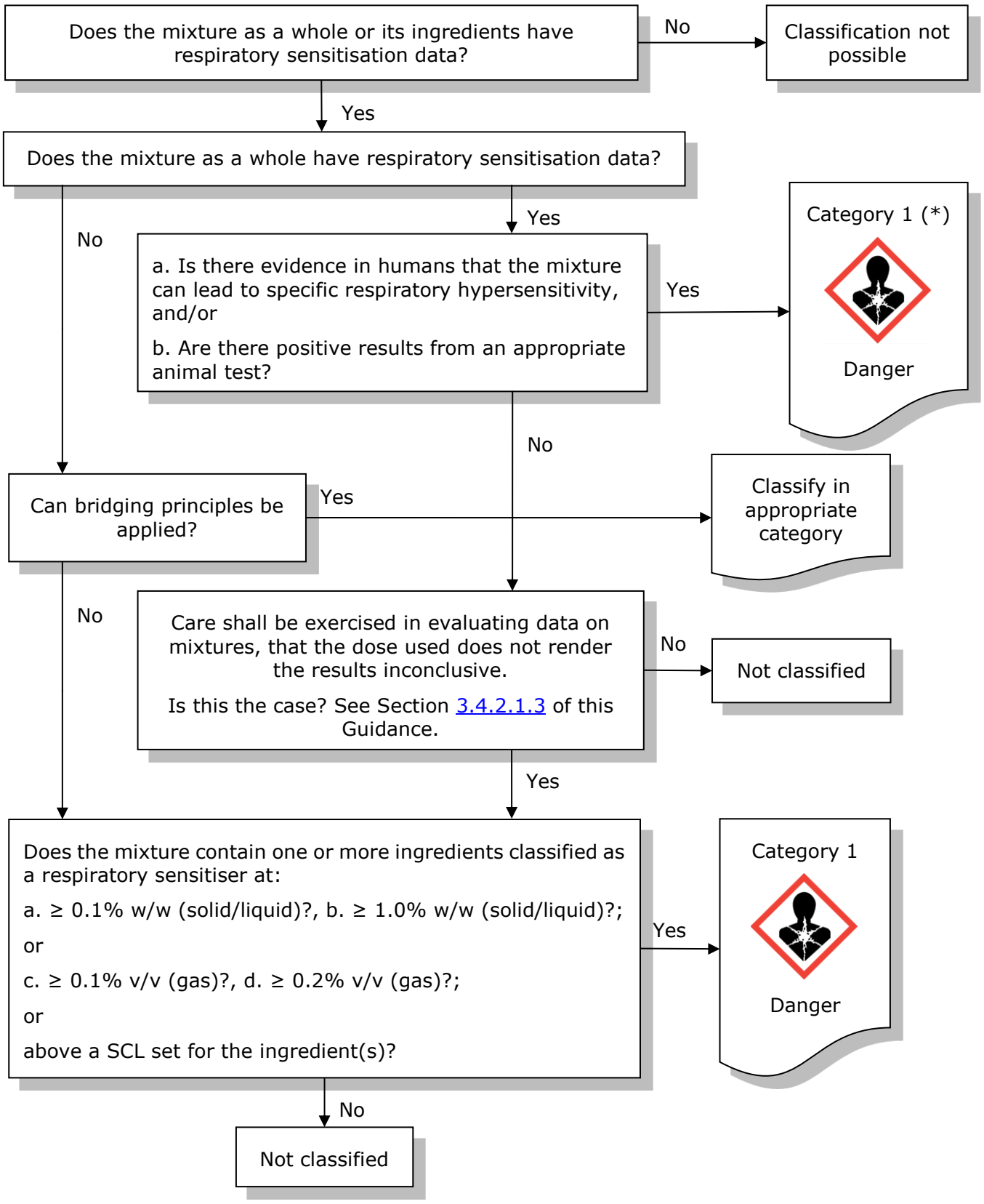
(see CLP Annex 1, 1.1.3.3 and 1.1.3.4).

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 3.4.3.3.3 of this Guidance.

3.4.3.4. Decision logic for classification of mixtures

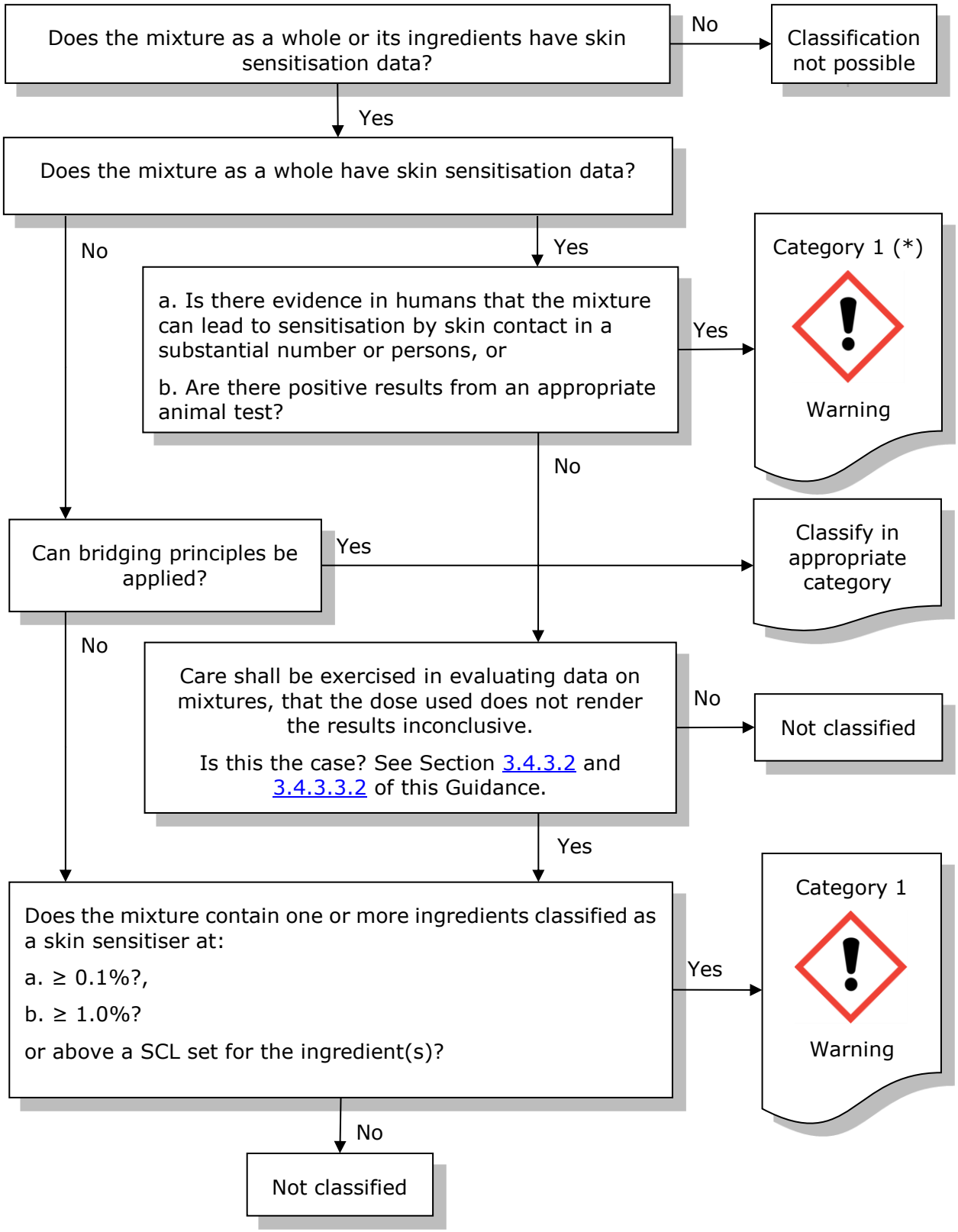
It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

3.4.3.4.1. Decision logic for classification of mixtures for respiratory sensitisation



(*) can be sub-categorised into 1A or 1B according to decision logic in Section 3.4.2.1.6 of this Guidance.

3.4.3.4.2 Decision logic for classification of mixtures for skin sensitisation



(*) can be sub-categorised into 1A or 1B according to decision logic in Section 3.4.2.2.6 of this Guidance.

3.4.4. Hazard communication for respiratory or skin sensitisation

3.4.4.1. Pictograms, signal words, hazard statements and precautionary statements

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	*P261* *P284*	*P261* *P272* *P280*
Precautionary Statement Response	*P304 + P341* *P342 + P311*	*P302 + P352* *P333 + P313* *P321* *P363*
Precautionary Statement Response	*P304 + P340* *P342 + P311*	*P302 + P352* *P333 + P313* *P321* *P362 + P364*
Precautionary Statement Storage		

Precautionary Statement Disposal	*P501*	*P501*

Article 26 1 (d)

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.

3.4.4.2. 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.

Where a mixture is labelled in accordance with section 2.4 or 2.5, the statement EUH208 may be omitted from the label for the substance concerned.

3.4.5. Examples of classification for skin sensitisation

3.4.5.1. Example of substances and mixtures fulfilling the criteria for classification for skin sensitisation

3.4.5.1.1. 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%.

3.4.5.1.2. 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%.

3.4.5.1.3. 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.

3.4.5.1.4. 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 a Category 1 skin sensitiser.

3.4.5.1.5. Example 5

Woody is a wood preservative containing two 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%).

3.4.5.1.6. 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 calculation of an EC3 value required to determine 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.

3.4.5.1.7. 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.

3.4.5.1.8. 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. 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. 1A.

8(c): Substance G is a skin sensitiser in animal tests indicating classification for sub-category 1A and in humans indicating classification for category 1. The substance should be classified for Skin Sens. 1A.

3.4.5.2. Example of substances or mixtures not fulfilling the criteria for classification for skin sensitisation

3.4.5.2.1. 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 have been required before a classification decision could be reached.

3.4.5.2.2. 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.

3.4.5.3. Examples of substances fulfilling the criteria for classification for respiratory sensitisation

3.4.5.3.1. Example 11

Five case studies describe the fact 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.

3.4.5.3.2. 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.

3.4.6. 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.*

Annex I*: 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*

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.*

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

There is a number of *in vivo* assays for genotoxicity/mutagenicity testing, with or without OECD TGs. 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 *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[66]. These latter tests have, however, been deleted as OECD TGs as of 2014.

In vivo tests in somatic cells which provide information on genotoxicity include, for example, the Comet single cell gel electrophoresis assay[67] for DNA strand breaks. Assays such as gene mutations in transgenic rodent (TGR) models[68] using reporter genes or mammalian erythrocyte micronucleus test for chromosome aberrations can be used for mutagenicity assessment. Please note that of these assays TGR is suitable for germ cells.

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.

[66] The Guidance on IR/CSA, Chapter R.7a (version 4.1).

[67] OECD TG 489 In Vivo Mammalian Alkaline Comet Assay (26 September 2014).

[68] OECD TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (26 July 2013).

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 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 *negative* 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).

Read-across and (Q)SARs can be used as part of a WoE approach for germ cell mutagenicity classification. If there are positive *in vitro* data from mammalian mutagenicity assays, structural similarities not sufficient for grouping/read-across may still warrant classification.

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 classification of 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.

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 such 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;*

[…]

Supporting evidence in addition to positive results of a valid *in vivo* somatic cell mutagenicity test in mammals is needed to be able to classify a substance as a Category 1B mutagen when no data on mammalian germ cells are available. In the examples provided in the second sentence in the green box, mutagenicity/genotoxicity in germ cells or data showing that the substance or its metabolite(s) interact with the genetic material of germ cells is mentioned. Moreover, genetic damage to germ cells in exposed humans, related to substance exposure, may offer additional information. 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 by application of a WoE approach using expert judgement.

Classification as a Category 2 mutagen

Classification in Category 2 may be based on positive results of at 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 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 may be relevant to humans. Note that intraperitoneal injection is since 2016 generally not recommended for new testing without specific scientific justification because it is not an intended route of human exposure. However, existing studies with intraperitoneal injection should be assessed as described in this and the next paragraph

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 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. Classification of substances containing CMR constituents, additives or impurities

From a compositional and a toxicological point of view the situation for substances containing CMR constituents, additives or impurities is the same as for mixtures containing components classified for these endpoints. For this reason the classification procedure for CMR endpoints that is foreseen by CLP for mixtures containing CMR components, is considered applicable also to substances containing CMR constituents, additives or impurities (see Section 1.1.6.1). As discussed in Section 3.5.3 below, mixtures containing components classified as germ cell mutagens shall be normally classified using only the relevant available information for the individual substances in the mixture. Further, in cases where the available test data on the mixture itself demonstrate CMR effects which have not been identified from the information on the individual substances, those data shall also be taken into account. For CMR endpoints the lowest incidence possible to detect in the tests may be by far unacceptable in humans. Thus a dose as high as possible (such as maximal tolerated dose, MTD dose) is needed to be able to detect CMR hazards. Dilution, as would be the case if mixtures or substances containing CMR constituents were tested, would increase the risk that CMR hazards would not be detected.

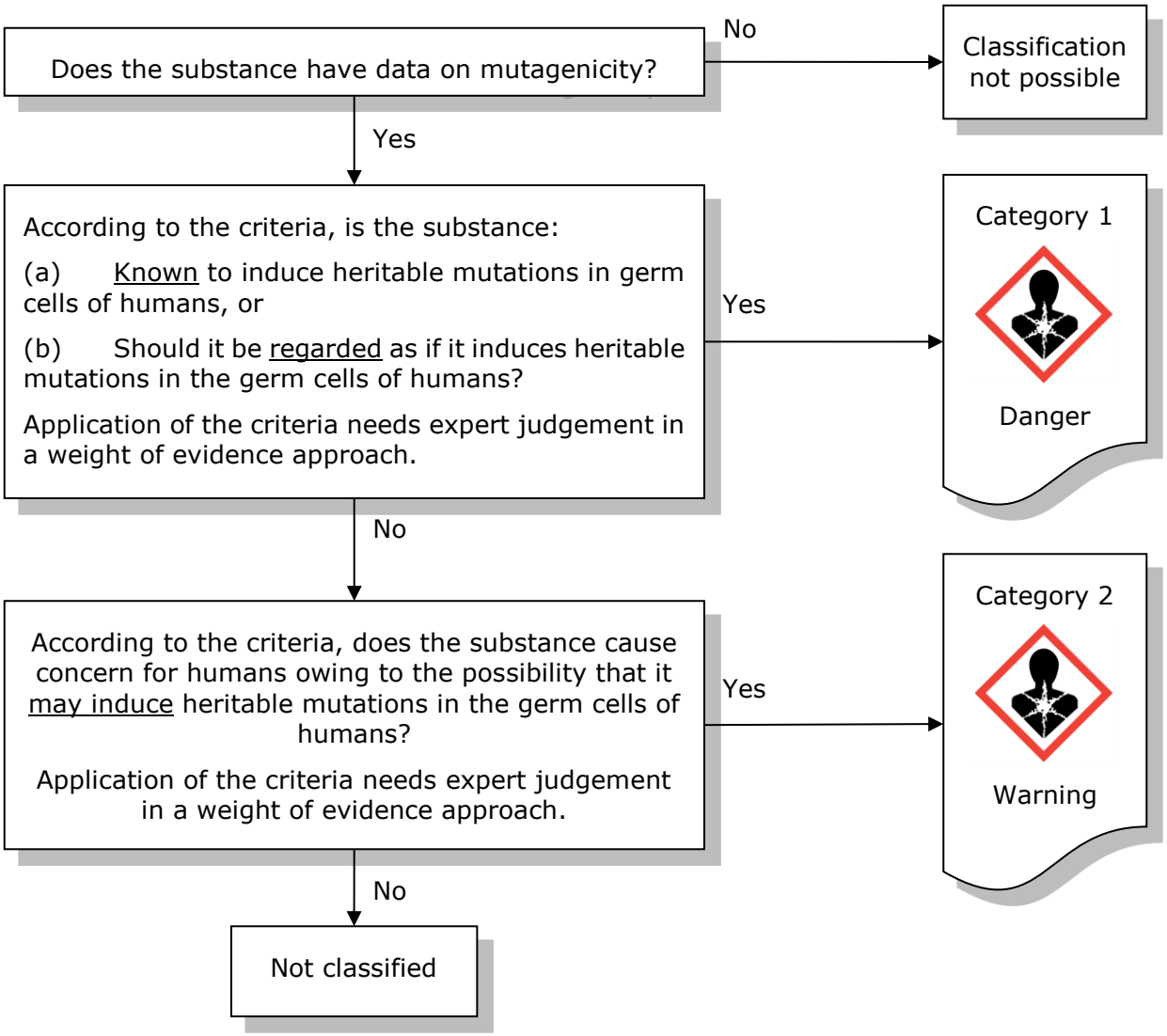
According to article 10 (1) substances in other substances and substances in mixtures are treated in the same way regarding the use of GCLs and SCLs.

3.5.2.6. 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 and substances toxic to reproduction. 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.

3.5.2.7. Decision logic for classification of 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.*

3.5.3.1.2. 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. 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)

Note that the bridging priciples are relevant only in case of comparable tested mixtures showing mutagenic effects not established from the evaluation of the individual ingredients. Classification for CMR hazards is based on tests with the ingredients.

3.5.3.2. Generic concentration limits for substances triggering classification of 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).*			

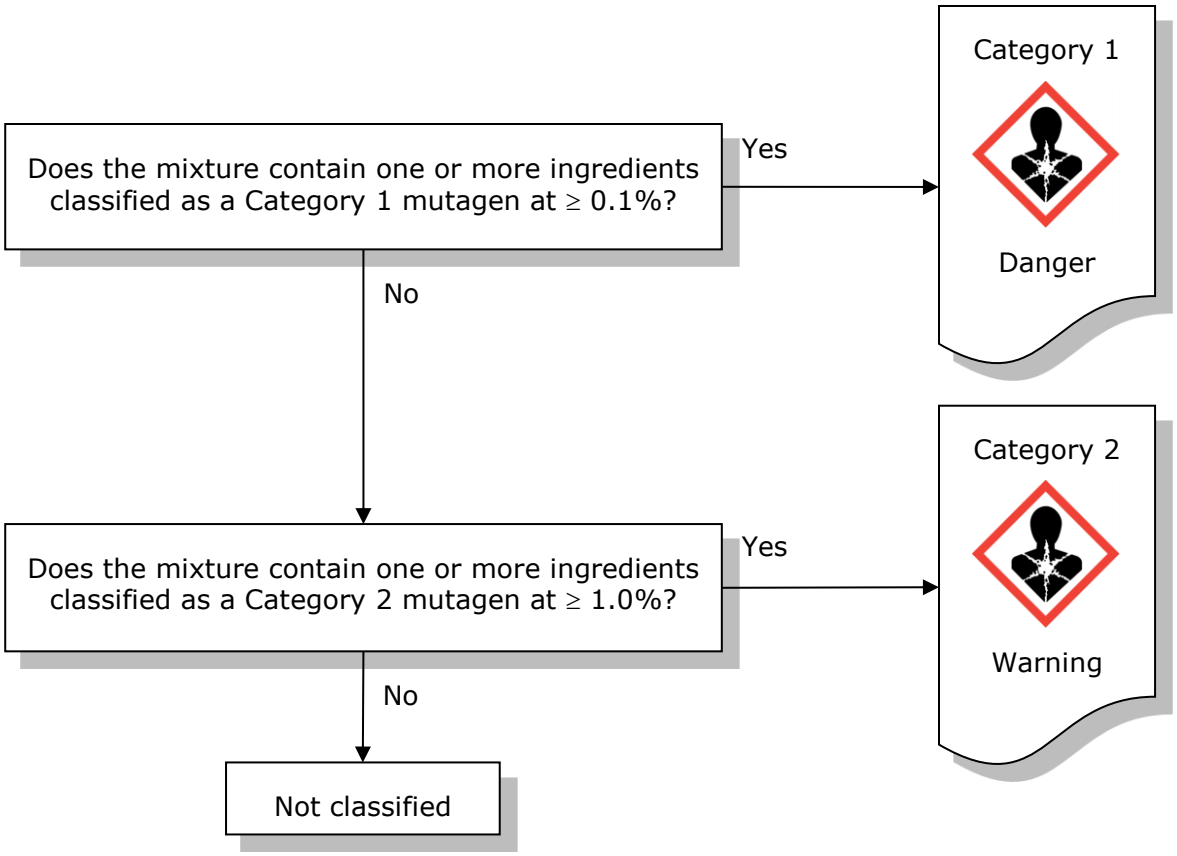
The option to set SCL for germ cell mutagenicity is not considered possible in the process of self-classification as there is no standardized methodical approach available which adequately takes into account all relevant information (see Section 3.5.2.6 of this Guidance).

For germ cell mutagenicity it is reasonable to assume additivity for mutagens, unless there are specific reasons not to do so.

3.5.3.3. Decision logic for classification of mixtures

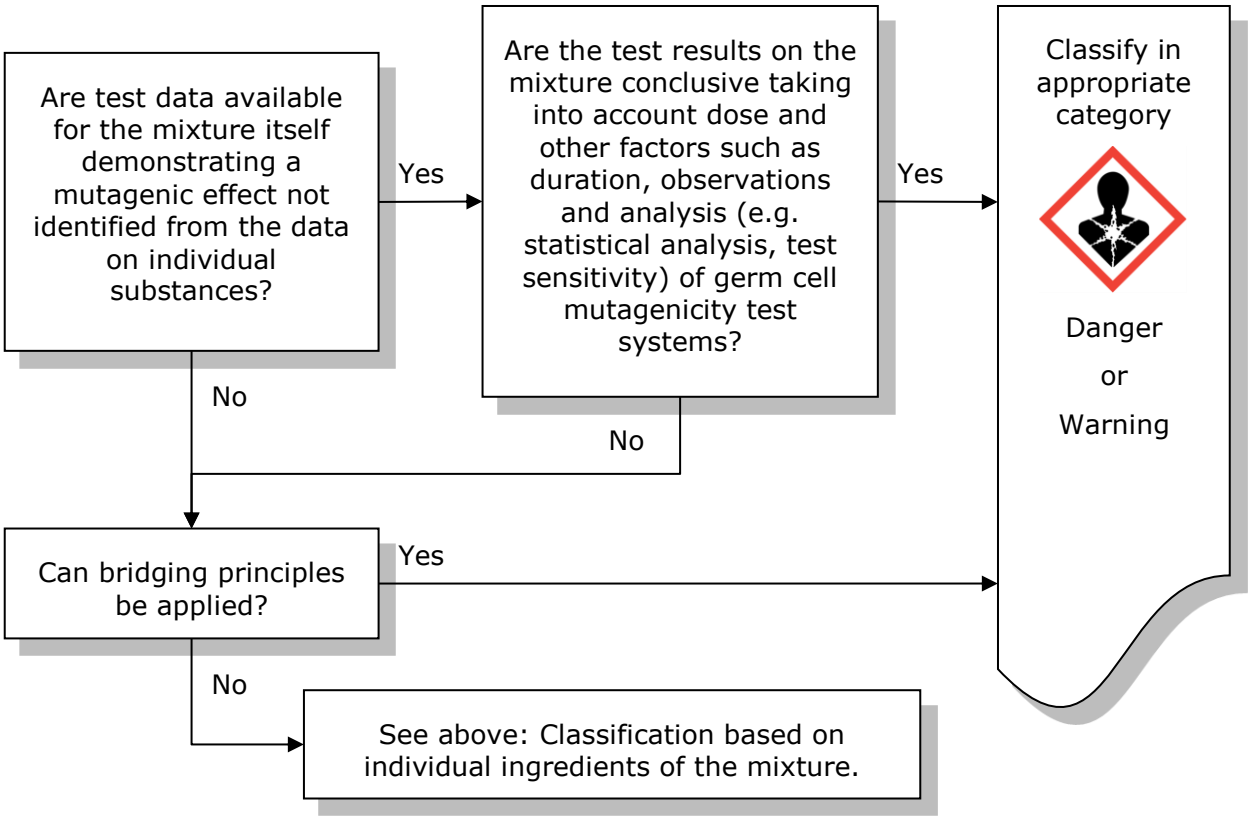
The decision logic which follows is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logic. This decision logic deviates (slightly) from the original GHS guidance, to meet CLP requirements.

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)).


Are test data available for the mixture itself demonstrating a mutagenic effect not identified from the data on individual substances?
Yes
Are the test results on the mixture conclusive taking into account dose and other factors such as duration, observations and analysis (e.g. statistical analysis, test sensitivity) of germ cell mutagenicity test systems?
Yes
Classify in appropriate category
Danger
or
Warning
No
No
Can bridging principles be applied?
Yes
No
See above: Classification based on individual ingredients of the mixture.

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

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* *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 under the CLP Regulation. However entry 29 of Annex XVII to REACH addresses such substances and mixtures. The packaging of substances with a harmonised classification as

Muta 1A or 1B and that are included in Appendices 3 and 4 of Annex VII of REACH,as well as the packaging of mixtures containing those substances above the concentration limits leading to the classification of the mixture, 'must be marked visibly, legibly and indelibly as follows: "Restricted to professional users".' Derogations from this obligation are outlined in the same provision.

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.*

Annex I: *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.*

Annex I*: 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.*

Annex I*: 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 single species or several species

The criteria indicate that carcinogenicity in a single animal species (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.

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, a specific hazard statement has been established in CLP, H350i; May cause cancer by inhalation.

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. Classification of substances containing CMR constituents

From a compositional and a toxicological point of view the situation for substances containing CMR constituents, additives or impurities is the same as for mixtures containing components classified for these endpoints. For this reason the classification procedure for CMR endpoints that is foreseen by CLP for mixtures containing CMR components, is considered applicable also to substances containing CMR constituents, additives or impurities (see Section 1.1.6.1). As discussed in Section 3.6.3 below, mixtures containing components classified as carcinigenic shall be normally classified using only the relevant available information for the individual substances in the mixture. Further, in cases where the available test data on the mixture itself demonstrate CMR effects which have not been identified from the information on the individual substances, those data shall also be taken into account. For CMR endpoints the lowest incidence possible to detect in the tests is by far unacceptable in humans. Thus a dose as high as possible (such as maximal tolerated dose, MTD dose) is needed to be able to detect CMR hazards. Dilution, as would be the case if mixtures or substances containing CMR constituents were tested, would increase the risk that CMR hazards would not be detected.

According to article 10 (1) substances in other substances and substances in mixtures are treated in the same way regarding the use of GCLs and SCLs.

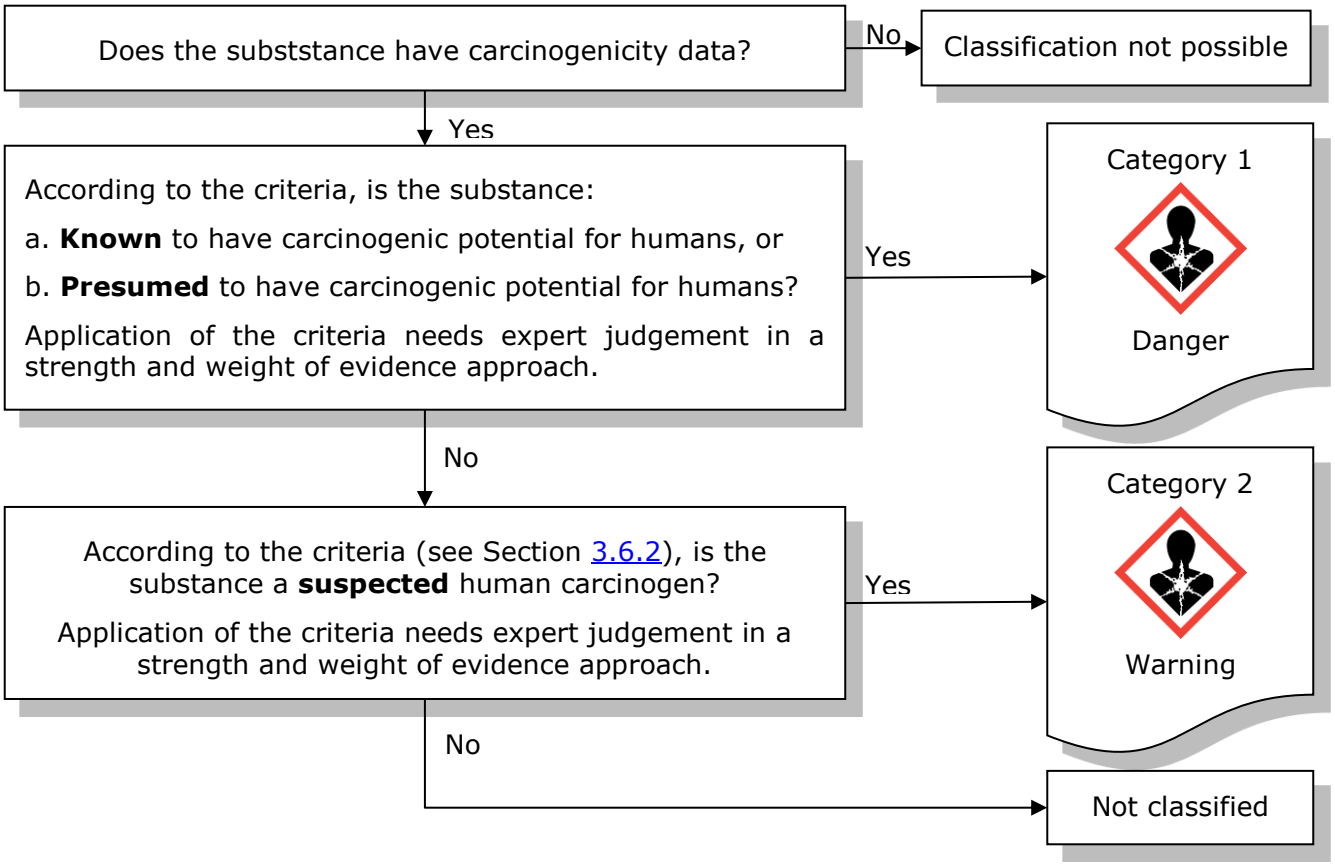
3.6.2.6. Setting of specific concentration limits

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.

3.6.2.7. 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

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 3.6.2.6 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:

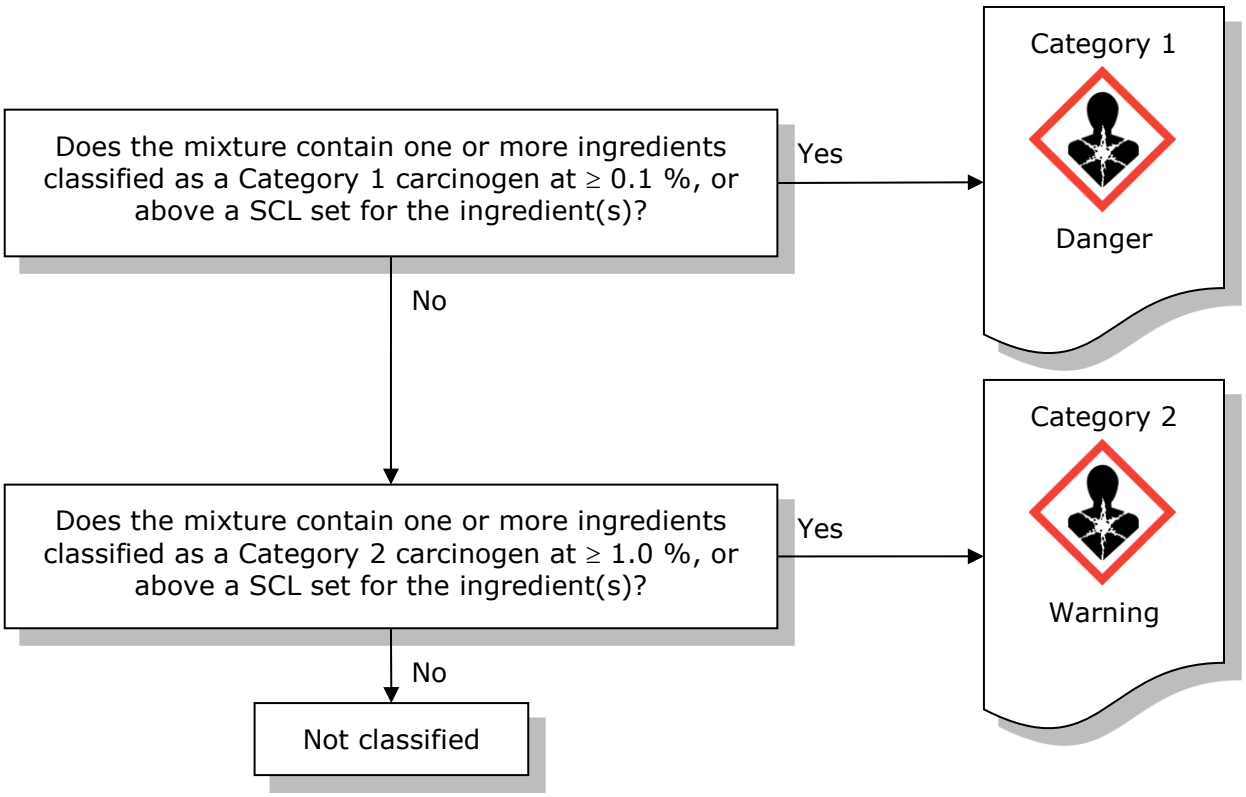
- concentration of highly hazardous mixtures
- interpolation within one hazard category

(see CLP Annex 1, 1.1.3.3 and 1.1.3.4)

3.6.3.2. Decision logic for classification of mixtures

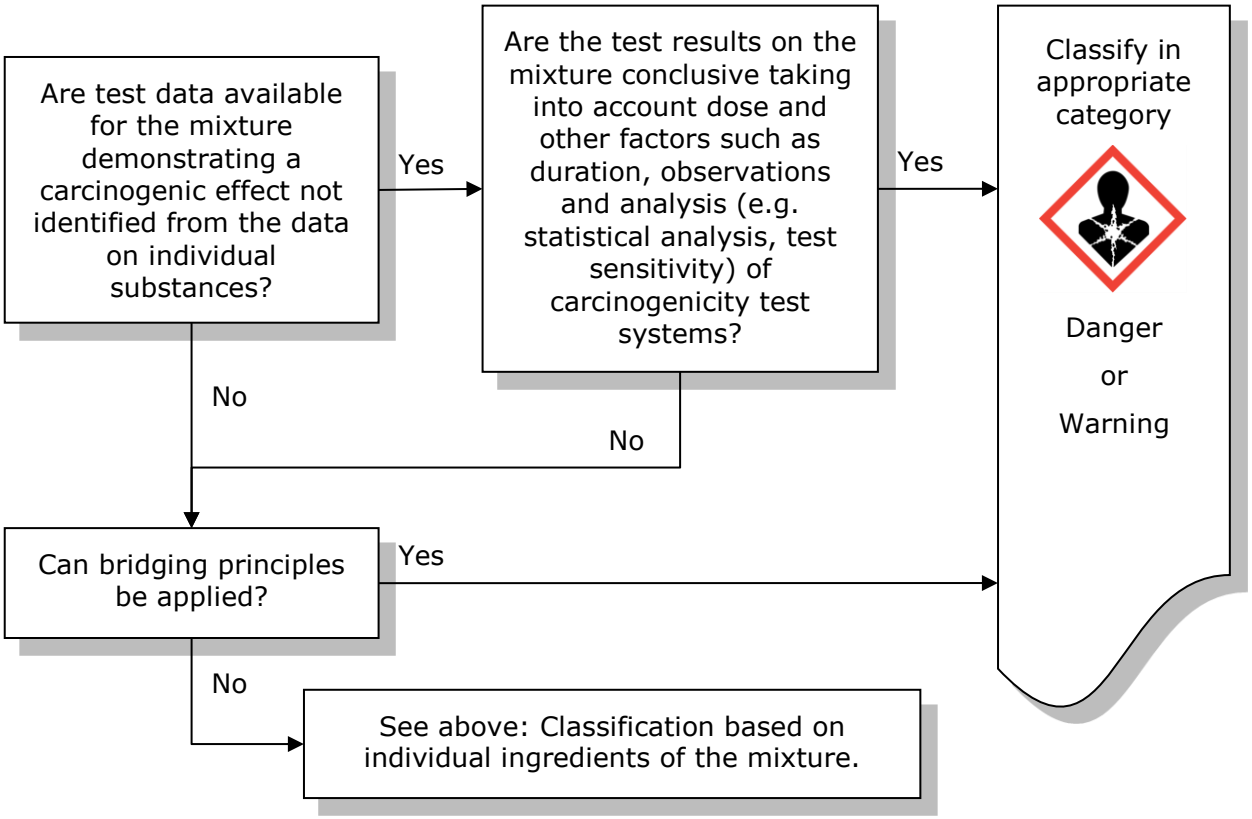
The decision logic which is based on the GHS Guidance is revised to meet CLP requirements. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.

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.*

Table 3.6.3

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	*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).

3.6.4.3. 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.

3.6.5. 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.

Annex I: *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*

Annex I*: 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.

Annex I*: 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*

Category 1A *Category 1B*	*distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).* *Known human reproductive toxicant* *The classification of a substance in this Category 1A is largely based on evidence from humans.* *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*

Annex I: ***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.*

Annex I: ***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.*

Annex I: ***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.

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

l. 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.

m. 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.

n. 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. Classification of substances containing CMR constituents

From a compositional and a toxicological point of view the situation for substances containing CMR constituents, additives or impurities is the same as for mixtures containing components classified for these endpoints. For this reason the classification procedure for CMR endpoints that is foreseen by CLP for mixtures containing CMR components, is considered applicable also to substances containing CMR constituents, additives or impurities (see Section 1.1.6.1). As discussed in Section 3.7.3 below, mixtures containing components classified as germ cell mutagens shall be normally classified using only the relevant available information for the individual substances in the mixture. Further, in cases where the available test data on the mixture itself demonstrate CMR effects which have not been identified from the information on the individual substances, those data shall also be taken into account. For CMR endpoints the lowest incidence possible to detect in the tests is by far unacceptable in humans. Thus a dose as high as possible (such as maximal tolerated dose, MTD dose) is needed to be able to detect CMR hazards. Dilution, as would be the case if mixtures or substances containing CMR constituents were tested, would increase the risk that CMR hazards would not be detected.

According to article 10 (1) substances in other substances and substances in mixtures are treated in the same way regarding the use of GCLs and SCLs.

3.7.2.6. Setting of specific concentration limits

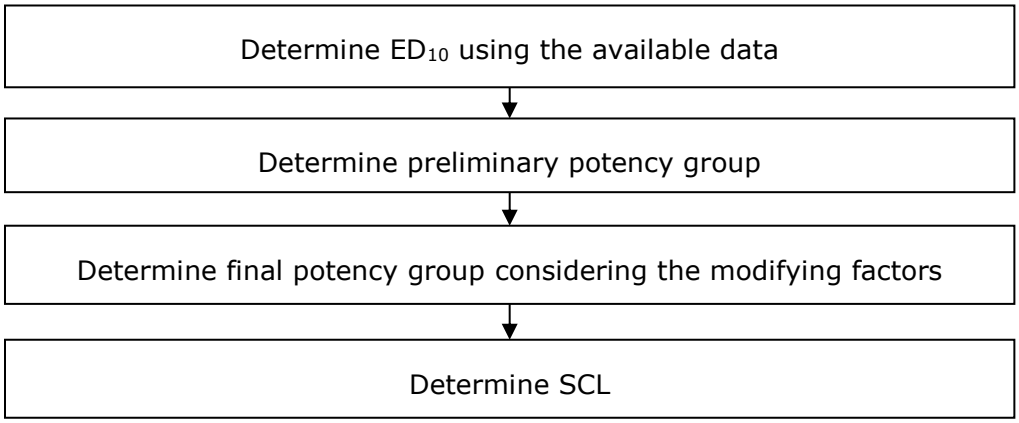
> ***Article 10(1)*** *Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to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

3.7.2.6.1. 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 3.7.2.6.5. 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 3.6. 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 3.6 Procedure for setting SCL for reproductive toxicity



3.7.2.6.2. 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.

3.7.2.6.3. 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 Section 3.7.2.6.3.2).

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.

3.7.2.6.3.1. 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.

3.7.2.6.3.2. 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 3.10, 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 3.10 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.

3.7.2.6.3.3. 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 3.11, 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 3.11 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	

3.7.2.6.3.4. 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 3.12 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 3.12, 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.

3.7.2.6.3.5. 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 G*uidance 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

3.7.2.6.4. 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 3.13 are used.

Table 3.13 Boundaries of the potency groups[69].

Potency group	Boundaries
High potency group	ED_{10} value ≤ 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 ≥ 400 mg/kg bw/day.

3.7.2.6.5. 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 3.13 above).

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

[69] See Annex VI of this guidance document for more details.

of the modifying factor exceeds the distance of the preliminary ED_{10} 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.

3.7.2.6.5.1. 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.

3.7.2.6.5.2. 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}.

3.7.2.6.5.3. 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.

3.7.2.6.5.4. 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.

3.7.2.6.5.5. 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.

3.7.2.6.5.6. 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.

3.7.2.6.6. 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 3.14 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.

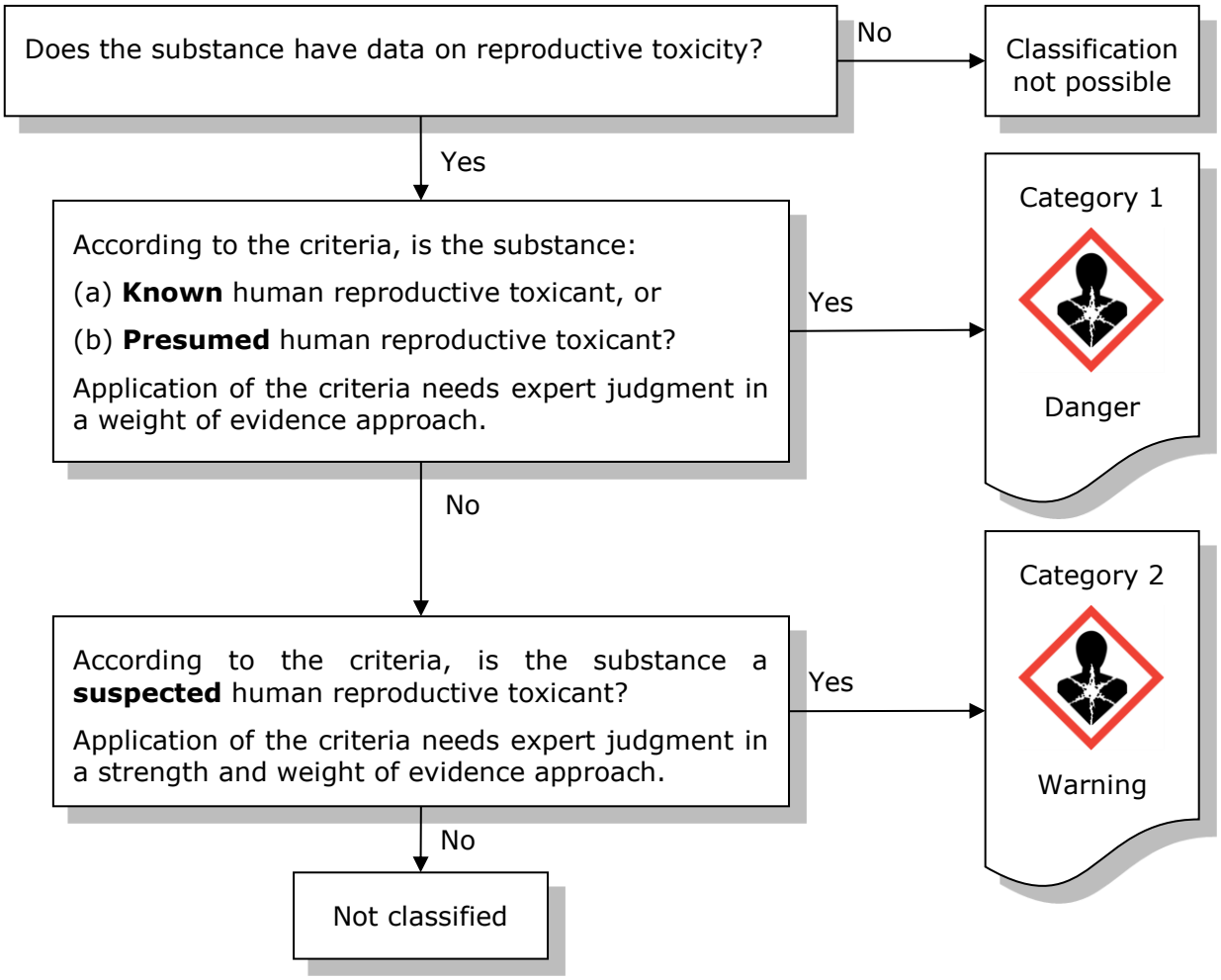
3.7.2.6.6.1. Assigning two SCLs to a substance

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)

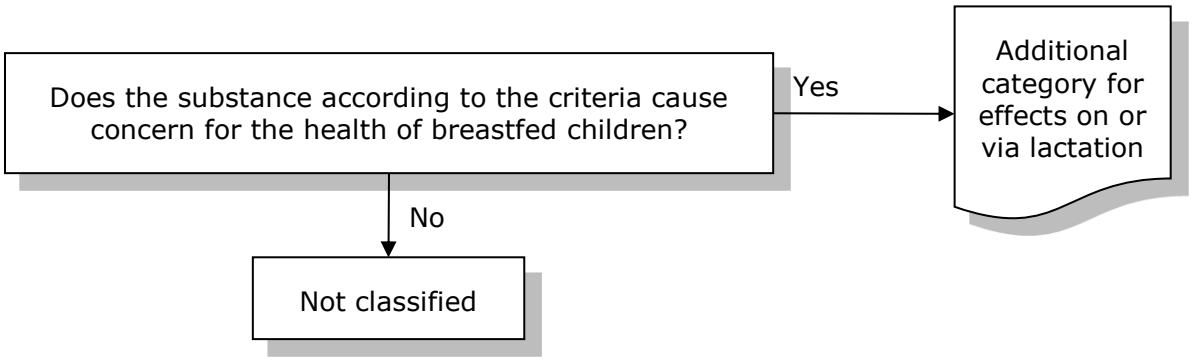
3.7.2.7. Decision logic for classification of substances

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



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).

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.*

Annex I: *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

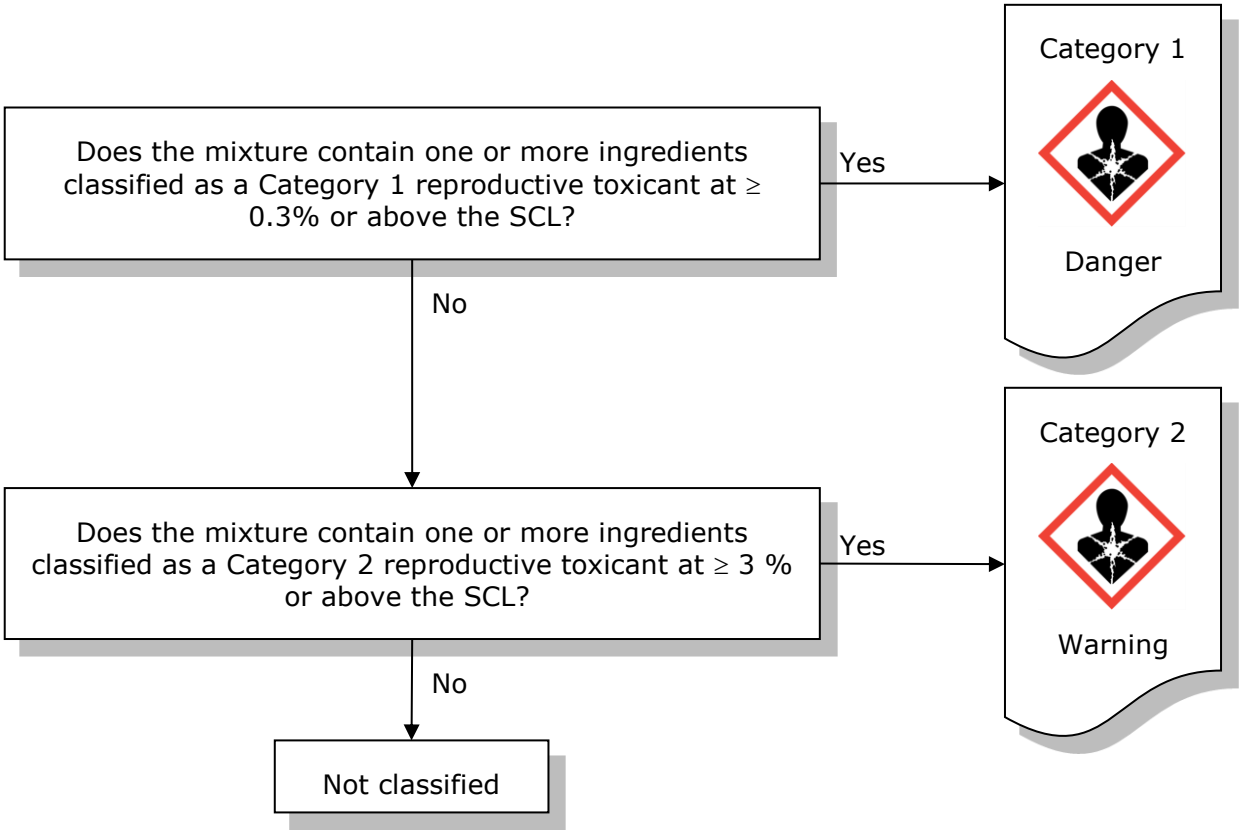
(see CLP Annex 1, 1.1.3.3 and 1.1.3.4)

3.7.3.2. Decision logic for classification of mixtures

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.

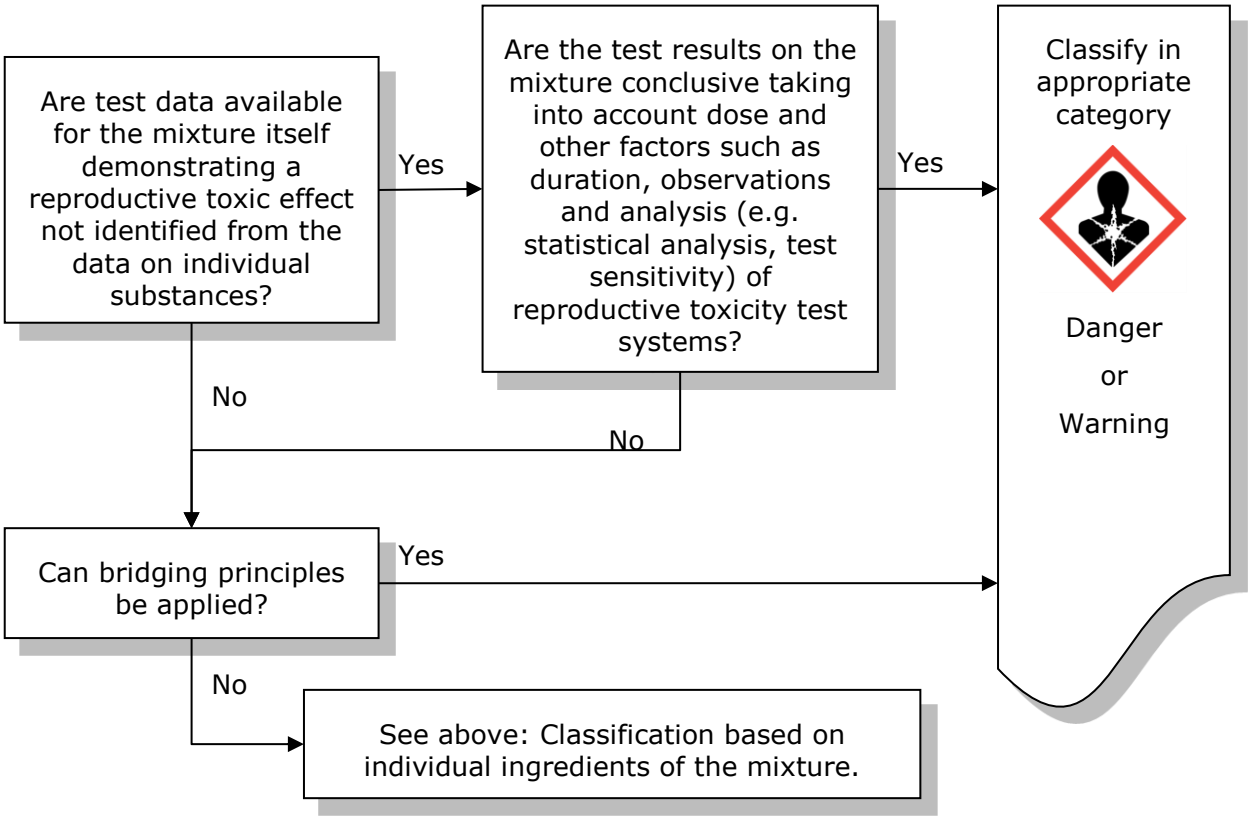
Classification of mixtures for fertility or developmental effects

Classification based on individual ingredients of the mixture



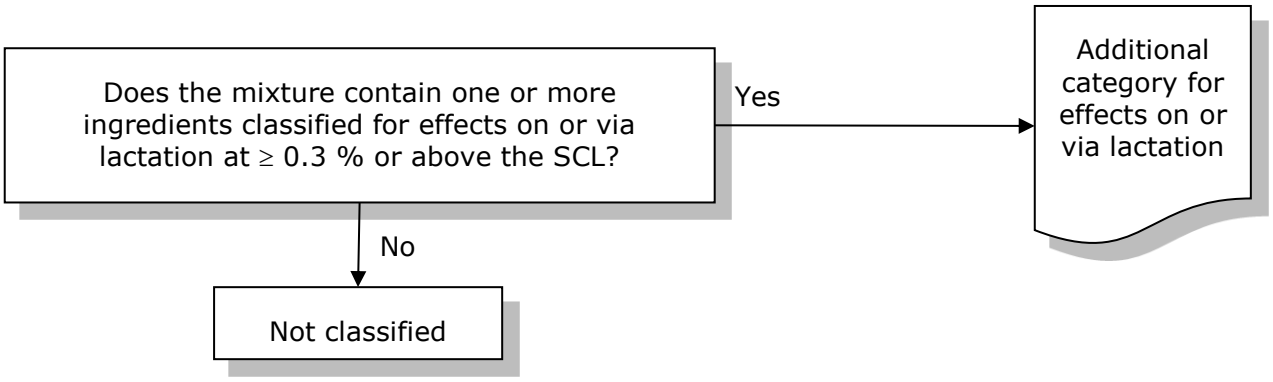
Modified classification on a case-by-case basis

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)).



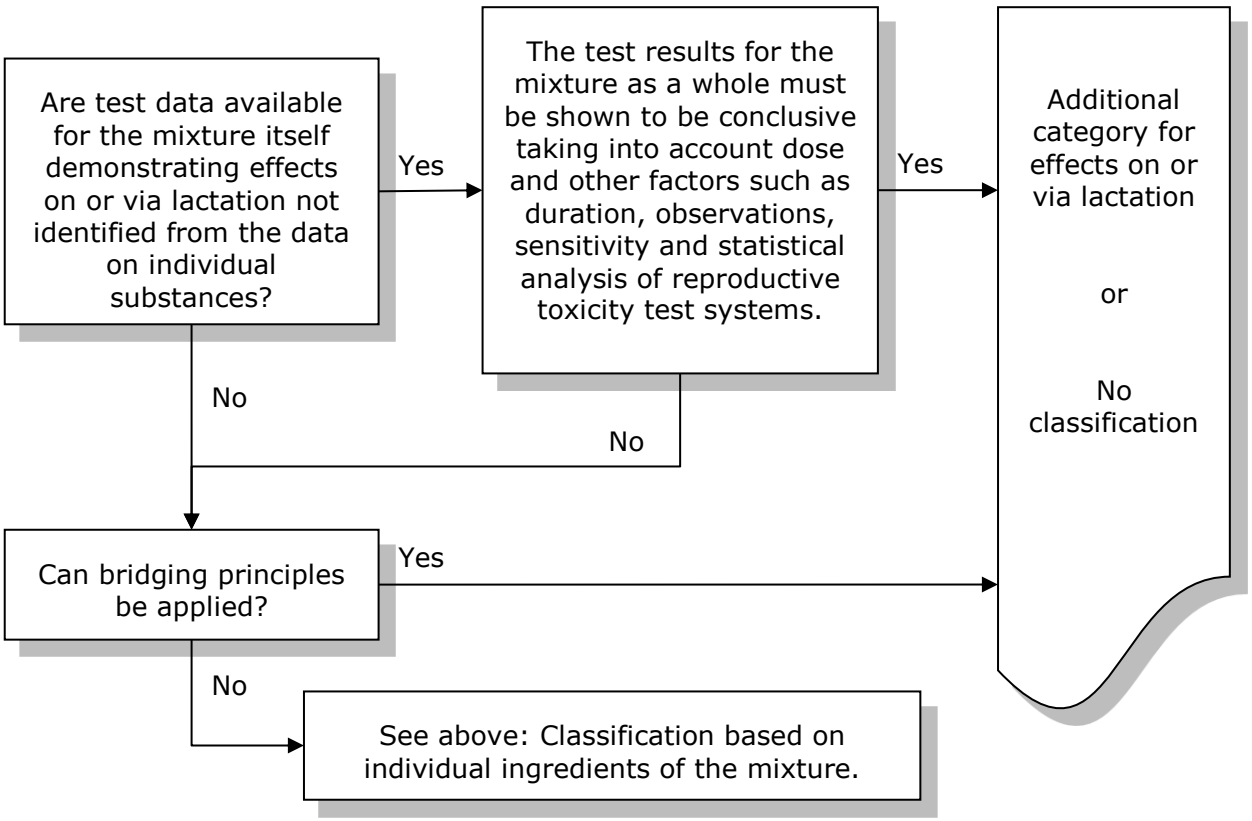
Classification of mixtures for effects via lactation

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 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 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* *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;effects on fertility and/or development: "May damage/Suspected of damaging fertility or the unborn child". 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. 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 3.15 Hazard statements for reproductive toxicity: H360 and H361, and their specifications

H No.	Hazard statement
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.*

H No.	Hazard statement
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. Examples

3.7.5.1. Examples of the determination of SCLs

Four examples are given below:

3.7.5.1.1. Example 1

1. Identification

Substance Name:	XXXXXX

2. EU CLP classification

Repro	1B
H	360D

3. ED_{10} in animals

3.1. 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.

3.2. 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

3.3. 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.

3.4. 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

3.7.5.1.2. Example 2 (developmental part only)

1. Identification

Substance Name:	XXXXXX

2. EU CLP classification

Repro	1B
H	360 FD

3. ED_{10} in animals

3.1. 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.

3.2. 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

3.3. Determination of the ED_{10} value

ED10 was determined as 33 mg/kg.

Control skeletal malformations is 9%. ED10 rate would be 19%. Interpolation between NOAEL (classification) (12% at 25 mg/kg) and LOAEL (classification) (33% at 50 mg/kg) leads to an ED10 of 33.3 mg/kg bw/day.

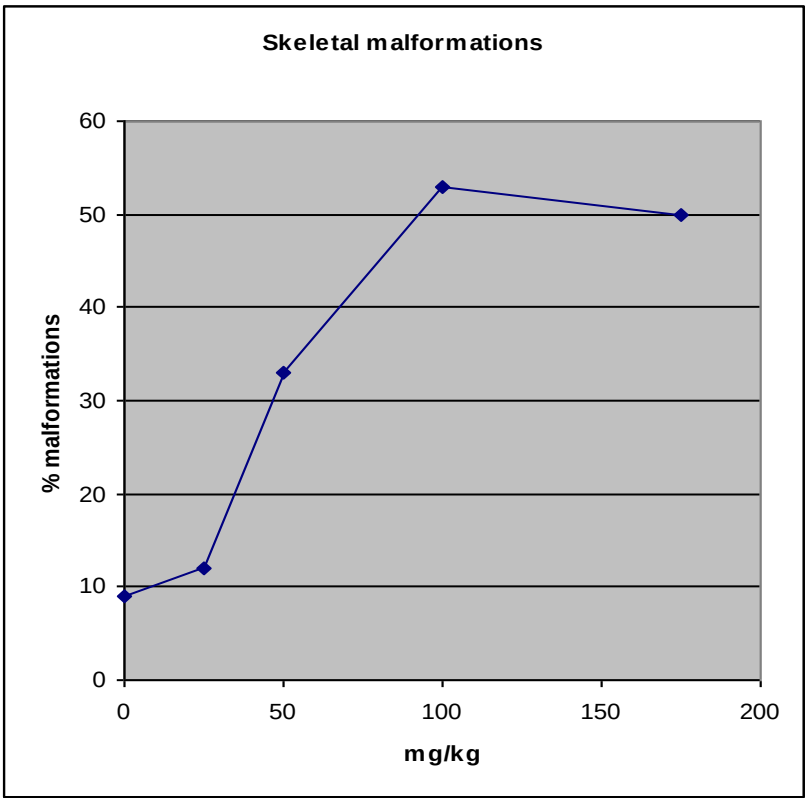
Calculation:

(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.

3.4. Preliminary potency group	Medium potency group.

4. Elements that may modify the preliminary potency evaluation

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

3.7.5.1.3. Example 3 (limited to developmental toxicity)

1. Identification

Substance Name:	XXXXXX

2. EU CLP classification

Repro	1B
H	360 fD

3. ED_{10} in animals

3.1. 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.

3.2. Remarks on the study used for the determination of the ED_{10}

Species, strain, sex:	CD(Sprague-Dawley) rats male and female
Study type:	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

3.3. 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.

3.4. 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.
4.4. 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

3.7.5.1.4. Example 4

1. Identification

Substance Name:	XXXXXX

2. EU CLP classification

Repro	2
H	361f

3. ED_{10} in animals

3.1. 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.

3.2. 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

3.3. 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.

3.4. 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 ED_{10} 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.*

Annex I*: 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.*

Annex I*: 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 Sections 3.8.2.4.3 and 3.8.2.4.2 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 1.4 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).

Annex I: *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 I: *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 I: *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 I: *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 I: *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 I: *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 I*: 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: H2S).

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 I*: 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 I: *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 I: *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).

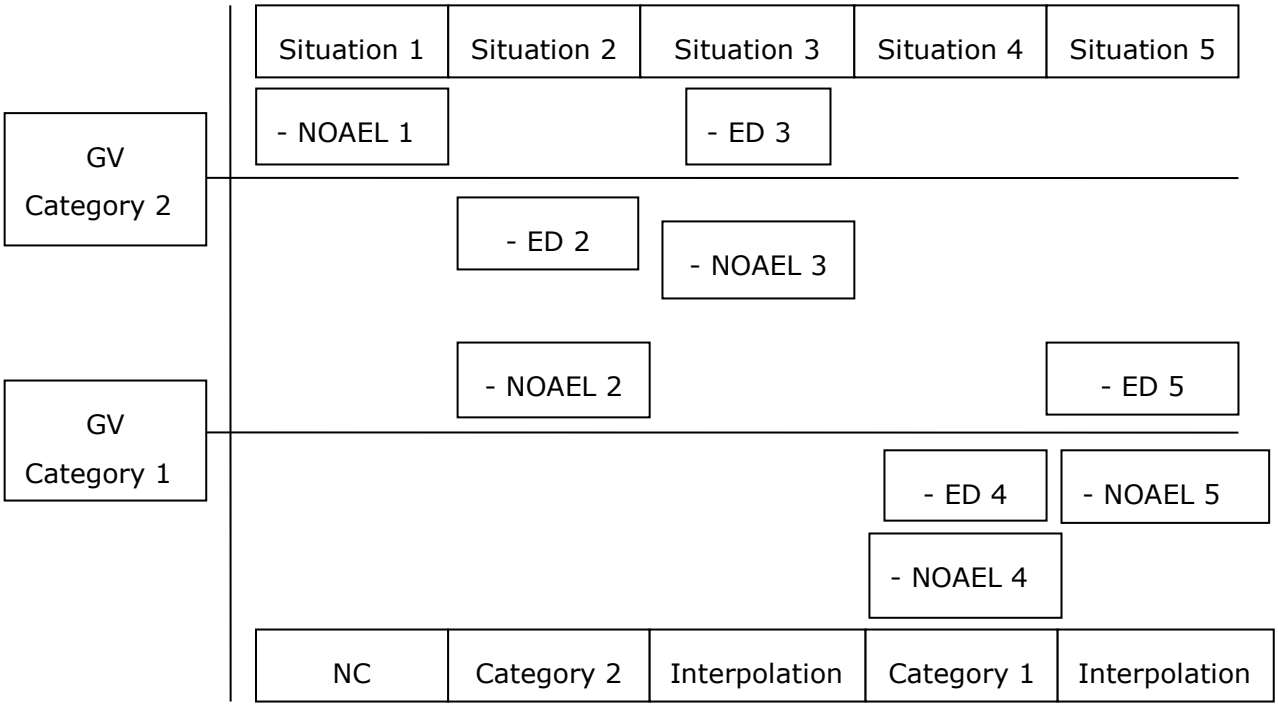
Categories 1 and 2

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 3.7). 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 3.7).

In a case where the ED is above a GV but the NOAEL is below the GV (situations 3 and 5 in Figure 3.7) 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 3.7 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 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:*

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 Section 3.8.2.4.5.

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:

Equation 3.8.2.6.1

$$SCLCat.1 = \frac{ED}{GV1} \times 100\%$$

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[70] (1, 2 or 5).

Example of determining STOT-SE SCL for a Category 1 substance:

$$= \frac{0.7mg/kgbw}{300mg/kgbw} \times 100\%$$ = 0.23% --> 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:

Equation 3.8.2.6.2

$$SCLCat.2 = \frac{ED}{GV2} \times 100\%$$

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.

Example for a substance in SCL Category 2:

$$= \frac{0.7mg/kgbw}{2000mg/kgbw} \times 100\%$$ = 0.035 --> 0.02% (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 ≥ 0.2% ; Cat 2: 0.02% ≤ 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

[70] 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.

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.

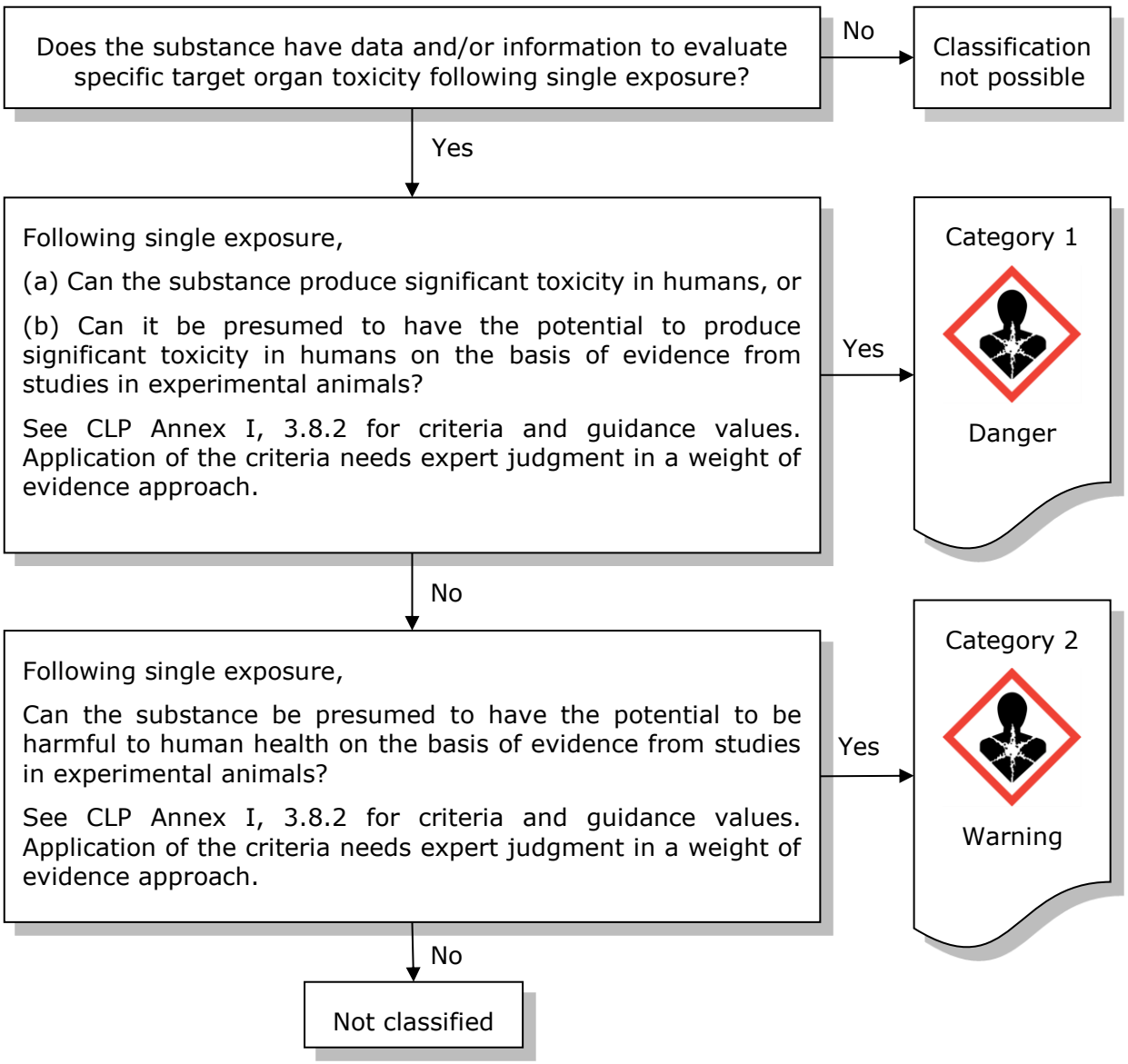
For narcotic effects, the factors to be taken into consideration in order to set lower or higher SCLs are the effective dose/concentration, and in addition for liquids, the volatility (saturated vapour concentration) of the substance.

3.8.2.7. Decision logic for classification of substances

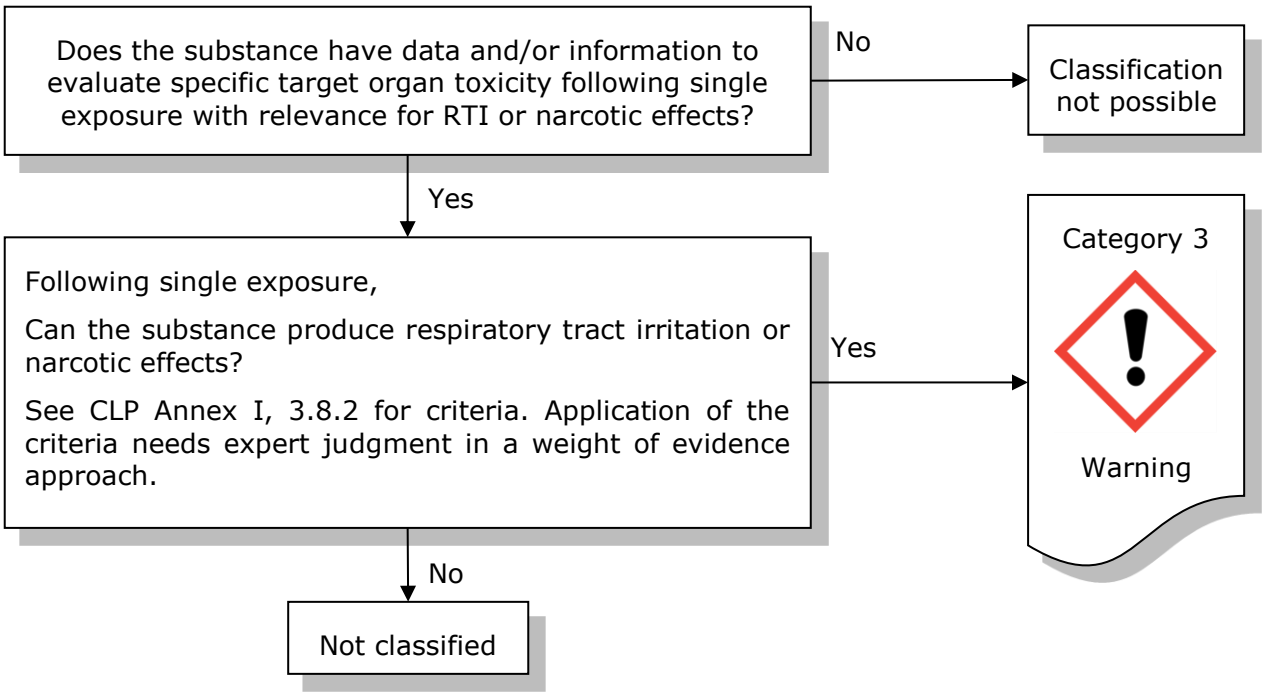
The decision logic is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

This decision logic deviates slightly from the original 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



Classification in Category 3



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 I: ***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 I: ***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 I: *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 I: *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 I: *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.* **Annex I*: 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.* **Annex I*: 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 classification of mixtures

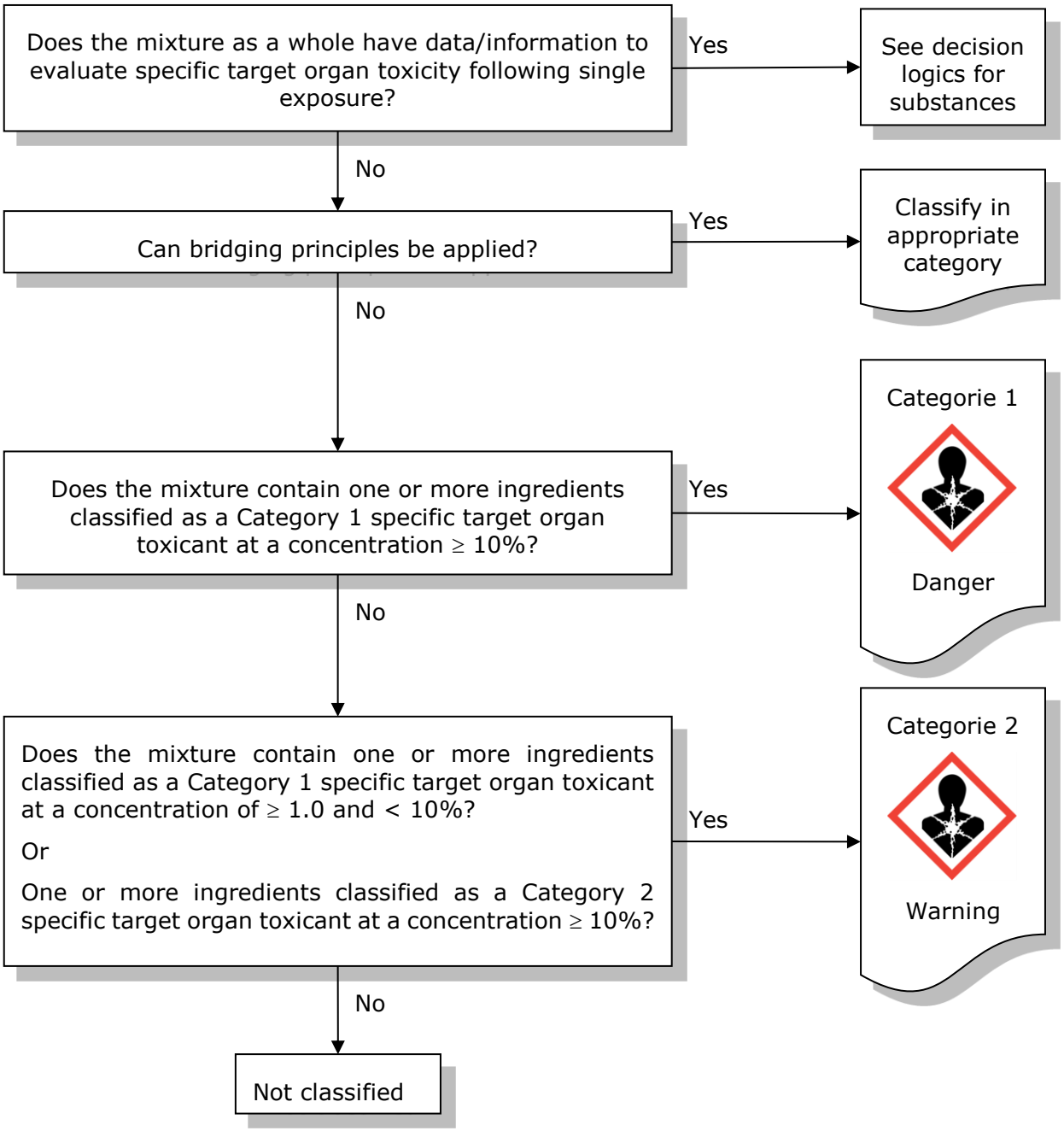
A mixture should be classified either in Category 1 or in Category 2, according to the criteria described above. The corresponding hazard statement (H370 for Category 1 or H371 for Category 2) should be used without specifying the target organs, except if the classification of the mixture is based on 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.

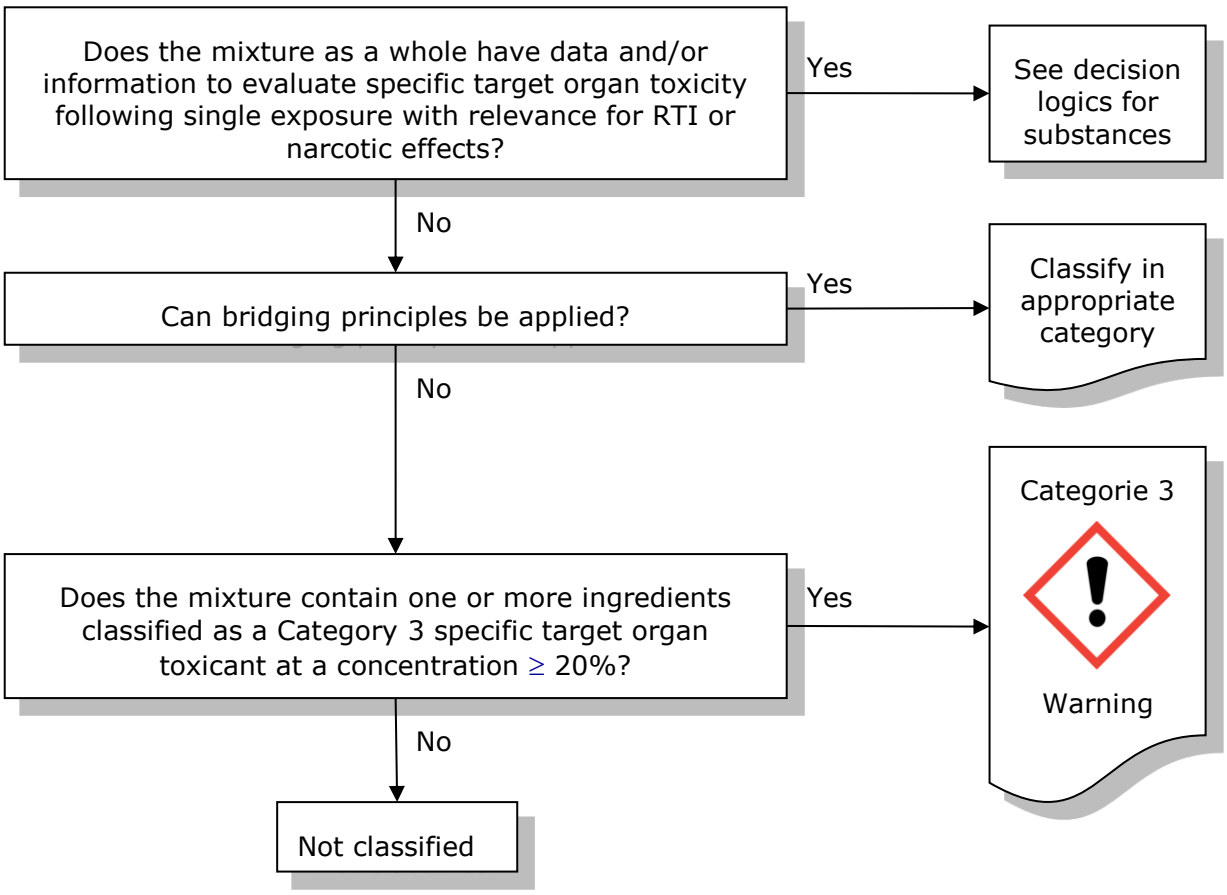
The decision logic is provided as additional guidance. It is strongly recommended that the person responsible for classification study the criteria for classification before and during use of the decision logic.

This decision logic deviates slightly from the original 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 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

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	*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. Examples of classification for STOT-SE

3.8.5.1. Examples of substances fulfilling the criteria for classification

3.8.5.1.1. 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	STOT-SE Category 1	The classification criteria for Category 1 are fulfilled: clear human evidence of a specific target organ toxicity effect

	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)		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.		

3.8.5.1.2. 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.		

3.8.5.1.3. 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		

3.8.5.1.4. 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		

3.8.5.2. Examples of substances not fulfilling the criteria for classification

3.8.5.2.1. 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

3.8.5.2.2. 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. 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 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.
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.*

Annex I*: 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.*

Annex I*: 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).*	

⚠ *NOTE: In the Note above (in green box) '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 I: *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 I*: 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 I*: 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 I: *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 I: *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 3.16 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

Annex I: *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 I: *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 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.*

Annex I: ***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

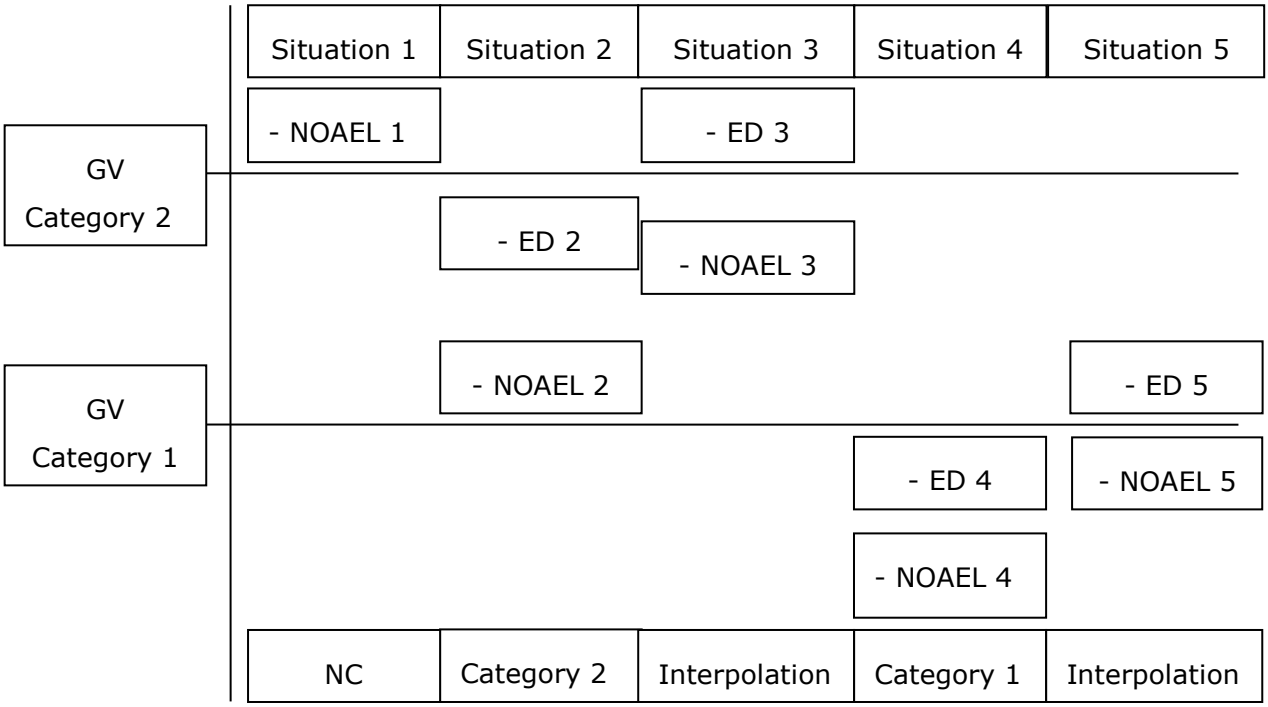
Annex I: ***3.9.2.7.3.*** *Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening 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 3.8 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 3.8).

In a case where the ED is above a GV but the NOAEL is below the GV (situations 3 and 5 Figure 3.8) 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 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 3.17 and Table 3.18 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 3.17 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 3.18 Conversion drinking water

Animal	Weight (kg)	Drinking water 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:

$$mg/l = ppm \times MW \times 1/24{,}450$$

3.9.2.3.4. Weight of evidence

Annex I: *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.*

Annex I*: 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 I: *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.*

Annex I: *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 I: ***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 I: ***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 I: ***3.9.2.8.*** *Effects considered not to support classification for specific target organ toxicity following repeated exposure*

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

(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 3.19 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 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, (2000) in EU Commission Summary Record of Meeting of the Commission Working Group on C&L of Dangerous Substances ECBI/44/01).

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[71] 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, 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: *3.9.2.7.3.*

(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:

[71] 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.

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.

α-2-μ globulin nephropathy in male rats

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.

Specific thyroid toxicity via liver enzyme induction

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 (see ECBI/22/98 Add1, EU Commission Meeting of the Commission Working Group on C&L of Dangerous Substances ECBI/27/98 Rev.2).

Peroxisome induction/proliferation

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).

Lung Overload

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:

Equation 3.9.2.6.1

$$SCLCat.1 = \frac{ED}{GV1} \times 100\%$$

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[72] (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:

Equation 3.9.2.6.2

$$SCLCat.2 = \frac{ED}{GV2} \times 100\%$$

SCL Cat 2: 0.12 mg/kg bw**/**100 mg/kg bw x 100%=0.12% --> 0.1%

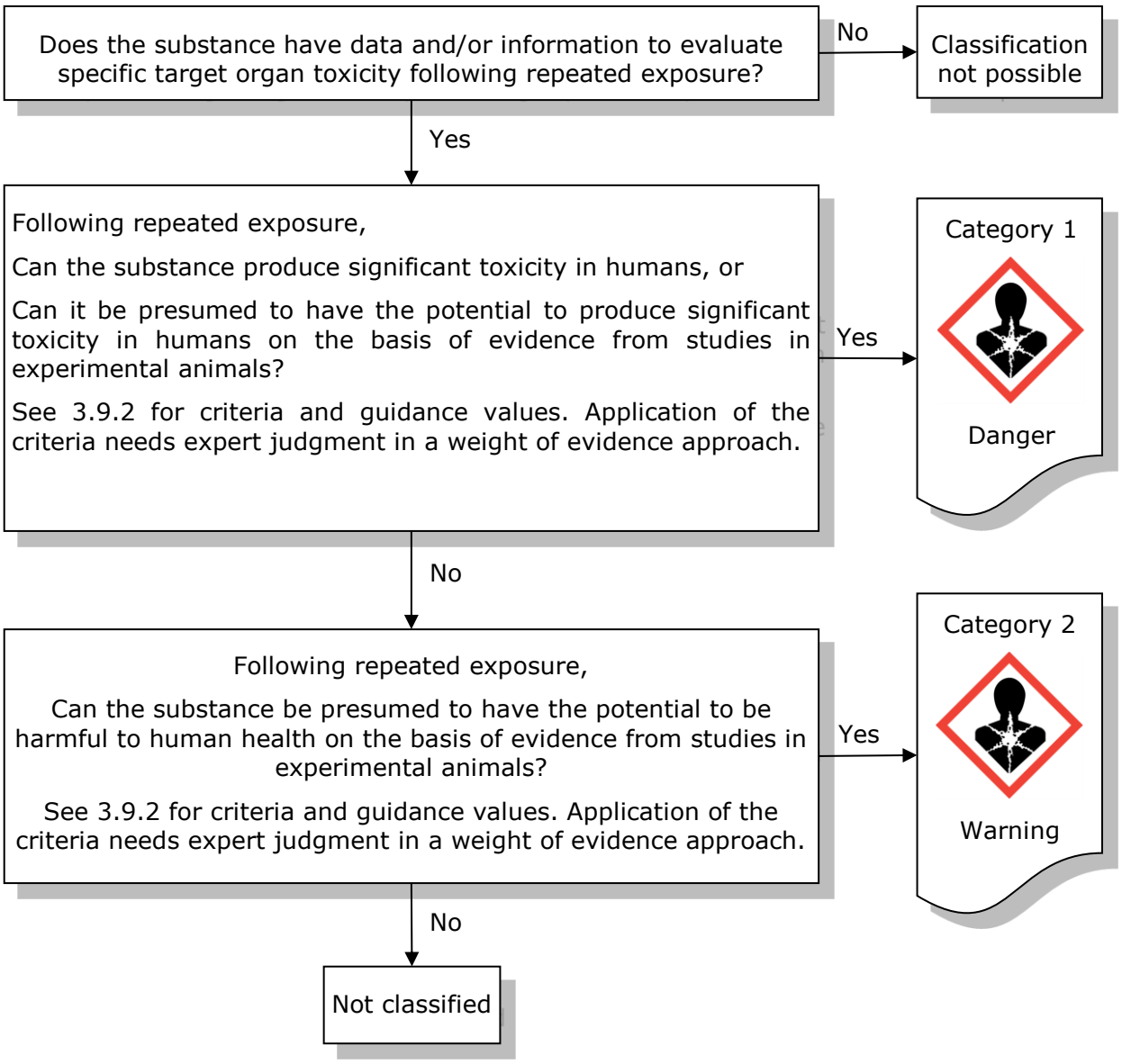
In this formula the ED (effective dose) is the dose inducing specific target organ toxicity and GV2 is the upper guidance value for Category 2 according to CLP Annex I, Table 3.9.3 corrected for the exposure duration. The resulting SCL is rounded down to the nearest preferred values (1, 2 or 5).

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%.

[72] 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.

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 I: ***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 I: ***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 I: 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 I: ***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 I: *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 I: ***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.*

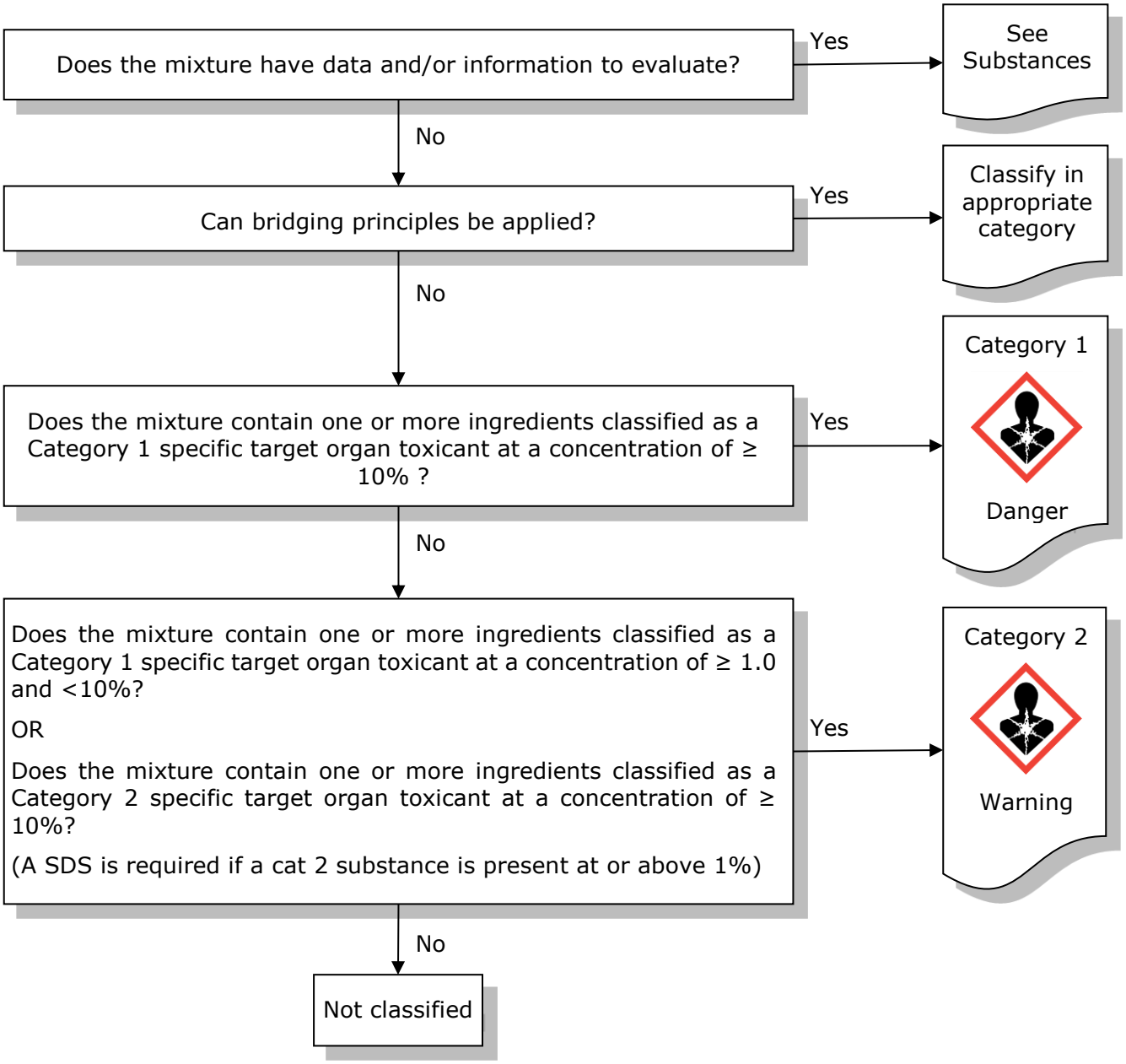
In the case a specific concentration limit has been established for one or more ingredients these SCLs have precedence over the respective generic concentration limit.

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 classification of 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 I: ***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. Examples of classification for STOT-RE

NOTE: 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).

3.9.5.1. Examples of substances fulfilling the criteria for classification

3.9.5.1.1. 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:

6. Human experience: No information available
7. 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 < 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:

- 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 & Labelling:

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

(See also ECBI/ 14/3/ Add 3 (2003) and ECBI/56/04 Rev 1 in EU Commission Meeting of the Commission Working Group on C&L of Dangerous Substances ECBI/139/04 Rev.2)

3.9.5.1.2. Example 2: But-2-yn-1,4-diol (EC No 203-788-6; CAS No 110-65-6)

Application of criteria for evaluation/classification and allocation of hazard statements with respect to specific target organs and route of exposure

Available information:

8. Human experience: no information available
9. 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
- 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 & Labelling:

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.

To note: 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)

3.9.5.1.3. 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:

- Human experience: No information available
- 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)

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)**	
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

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, 97/98 weeks 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	15 (M: 5.2, F: 3.1)		Category 2 based on the effects on blood. Category 2 based on the effects on the retina

Classification & Labelling:

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.

3.9.5.2. Examples of substances not fulfilling the criteria for classification

3.9.5.2.1. 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:

- Human experience: No information available
- 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.

KEY CHRONIC TOXICITY DATA: SUMMARY OF DATA FOR REPEATED EXPOSURE

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 & Labelling:

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 give an idea what are the actual effects around the cut-off values (10-100 mg/kg bw) but probably we could come to the same conclusion, i.e. the effect is not enough to justify the classification in any category.

3.9.5.3. Examples of mixtures fulfilling the criteria for classification

3.9.5.3.1. 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 & Labelling:

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.

3.9.5.3.2. 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 & Labelling:

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%.

3.9.5.3.3. 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 & Labelling:

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.

3.9.5.3.4. 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 & Labelling:

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.

3.9.5.4. Example of mixtures not fulfilling the criteria for classification

3.9.5.4.1. 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 & Labelling:

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.

3.9.6. References

Muller, A. *et al* (2006) Regulatory Toxicology and Pharmacology 45, 229-241

4. PART 4: ENVIRONMENTAL HAZARDS

4.1. HAZARDOUS TO THE AQUATIC ENVIRONMENT

4.1.1. Introduction

Guidance for the application of the criteria covering effects on the aquatic compartment was developed by OECD and incorporated as Annexes 9 and 10 in the 'Globally Harmonised System of classification and labelling of chemicals (UN GHS)' (United Nations GHS (Rev. 3) 2009)).

The text in this chapter, and even more so in some of the Annexes to this chapter, is largely based on the text in UN GHS (Rev. 3, 2009). The guidance given in Annexes 9 and 10 of UN GHS relates to substances, but not mixtures. Some parts have therefore been slightly revised to take into account recent developments and additional guidance documents provided by ECHA. Furthermore guidance on the classification of mixtures has been brought into this chapter as well as classification examples for both substances and mixtures.

4.1.2. Scope

Annex I: ***4.1.1.3.1*** *Classification of substances and mixtures for environmental hazards requires the identification of the hazards they present to the aquatic environment. The aquatic environment is considered in terms of the aquatic organisms that live in the water, and the aquatic ecosystem of which they are part. The basis, therefore, of the identification of short-term (acute) and long-term (chronic) hazards is the aquatic toxicity of the substance or mixture, although this shall be modified by taking account of further information on the degradation and bioaccumulation behaviour, if appropriate.*

The classification scheme has been developed with the objective of identifying those chemicals that present, through their intrinsic properties, a hazard to the aquatic environment covering the aquatic freshwater and marine ecosystems. For most substances, the majority of data available addresses this environmental compartment. The classification scheme is limited in scope in that it does not, as yet, include aquatic sediments, nor higher organisms at the top end of the aquatic food-chain, although these may to some extent be covered by the criteria selected.

Although limited in scope, it is widely accepted that this compartment is vulnerable, in that it is the receiving environment for many harmful substances, and the organisms that live there can be very sensitive. It is also complex since any system that seeks to identify hazards to the environment must seek to define those effects in terms of wider effects on ecosystems rather than on individuals within a species or population. However, for practical reasons a limited set of specific properties has been selected through which the short-term (acute) and long-term (chronic) hazards, can be best described: acute aquatic toxicity; chronic aquatic toxicity; lack of rapid degradability; and potential or actual bioaccumulation. Relevant definitions for aquatic hazard classification of substances i.e. acute and/or chronic aquatic toxicity, availability and bioavailability to the aquatic environment are outlined in the CLP Regulation, Annex I, Section 4.1.1.1. Some further guidance can be viewed in the IR&CSA[73], Chapter B.6.3. The rationale for the selection of these properties as the means to define the aquatic hazard will be described in more detail in the following sections of this guidance.

[73] *Guidance on Information Requirements and Chemical Safety Assessment*.

4.1.3. Classification of substances hazardous to the aquatic environment

4.1.3.1. Information applicable for classification of substances hazardous to the aquatic environment

4.1.3.1.1. Substance properties used for classification

Generally speaking, in deciding whether a substance should be classified, a search of appropriate databases and other sources of data should be made for at least the following substance properties: water solubility, octanol/water partition coefficient (log K_{ow}), acute aquatic toxicity (L(E)C_{50}), chronic aquatic toxicity (NOEC or equivalent EC_x[74]), degradation (evidence of rapid degradability, hydrolysis) and bioaccumulation (preferably bioconcentration factor in fish (BCF)). Other information might be considered on a case-by-case basis.

Although not used directly in the criteria, the water solubility and stability data are important since they are a valuable help in the data interpretation of the other properties. However, water solubility may be difficult to determine and is frequently recorded as simply being low, insoluble or less than the detection limit. This may create problems in interpreting aquatic toxicity and bioaccumulation studies (see also Annex III). Hydrolysis data (Test Methods Regulation (EC) No 440/2008; OECD Test guideline 111) and information on the hydrolysis products as well as their behaviour in water might be helpful as well. As an example, for substances where the degradation half-life (DT_{50}) is less than 12 hours, environmental effects are likely to be attributed to the hydrolysis products rather than to the parent substance itself (IR&CSA, Chapter R7.8).

4.1.3.1.2. Information and data availability

Annex I: ***4.1.1.2.2*** *Preferably data shall be derived using the standardised test methods referred to in Article 8(3). In practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in section 1 of Annex XI to Regulation (EC) No 1907/2006. In general, both freshwater and marine species toxicity data are considered suitable for use in classification provided the test methods used are equivalent. Where such data are not available classification shall be based on the best available data. See also part 1 of Annex I to Regulation (EC) No 1272/2008.*

The data used to classify a substance can be drawn from data required for other regulatory purposes as well as the relevant literature. A number of internationally recognised databases exist which can act as a good starting point. Such databases vary widely in quality and comprehensiveness and it is unlikely that any one database will hold all the information necessary for classification to be made. Some databases specialise in aquatic toxicity and others in environmental fate. Information can also be gathered from data submitted under plant protection products and/or biocidal products legislation.

Non-testing information

Information derived from (Q)SAR and read-across, grouping and categorisation can also be used, see also IR&CSA, Chapter R.6.

Information sources

IR&CSA Chapter R.3.4.1 specifies a selection of freely available databases and databanks which might be consulted for classification purposes. All ECHA guidance documents are available on

[74] If available, preference is given to EC_{10}, see OECD 2006.

the Agency's website (http://echa.europa.eu/web/guest/support/guidance-on-reach-and-clp-implementation).

Data can also be found through the eChemPortal, which is a global portal to information on chemical substances. The eChemPortal provides access to a number of databases, including the OECD HPV (Existing Chemicals Database) and the SIDS UNEP (Screening Information Dataset for High Volume Chemicals). The eChemPortal is currently hosted by the OECD: (http://www.echemportal.org/)

Further guidance is given in Annex IV to this document.

4.1.3.2. Evaluation of available information

4.1.3.2.1. General considerations

The term substance covers a wide range of chemicals (consult the *Guidance for identification and naming of substacnes under REACH and CLP*, Chapter 3) many of which pose challenges to a classification system based on rigid criteria. This section will thus provide some guidance on how these challenges can be dealt with based both on experience in use and clear scientific rationale.

The range of interpretational problems can be extensive and as a result such interpretation will always rely on the ability and expertise of the individuals responsible for classification. However, it is possible to identify some commonly occurring difficulties and provide guidance. Such difficulties can fall into a number of overlapping issues:

a. The difficulty in applying the current test procedures to some types of substances;

b. The difficulty in interpreting the data derived both from these 'difficult to test' substances and from other substances;

c. The difficulty in interpretation of diverse datasets derived from a wide variety of sources (e.g. Weight of Evidence).

d. The difficulty of interpreting 'other' information

Regarding the use of test data, in general, only reliable information (i.e. with a Klimisch reliability score of 1 (reliable without restrictions) or 2 (reliable with restrictions)) should be used for classification purposes. However, good quality data may not always be available for all trophic levels. It will be necessary to consider data of lower quality for those trophic levels for which good quality data are not available. Consideration of such data, however, will also need to take into account the difficulties that may have affected the likelihood of achieving a valid result. For larger data sets, preference should be given to information with Klimisch score 1, while information with Klimisch score 2 can be used as supporting information. For more information on the Klimisch reliability scoring system, see IR&CSA, Chapter R.4.2.

4.1.3.2.2. Substances difficult to test

For many organic substances, the testing and interpretation of data present no problems when applying both the relevant Test Methods Regulation (EC) No 440/2008 and/or OECD Test Guidelines and the classification criteria. There are a number of typical interpretational problems, however, that can be characterised by the properties of the substance being studied. These are commonly called 'difficult substances':

a. poorly soluble substances: these substances are difficult to test because they present problems in the preparation of a test solution, maintenance of test concentrations and verification of exposure during aquatic toxicity testing. In addition, many available data for such substances have been produced using 'solutions' in excess of the water solubility resulting in major interpretational problems in defining the true L(E)C_{50} or NOEC/EC_x for the purposes of classification. Interpretation of the partitioning behaviour can also be problematic where the poor solubility in water and octanol may be

compounded by insufficient sensitivity in the analytical method. Water solubility may be difficult to determine and is frequently recorded as simply being less than the detection limit, creating problems in interpreting both aquatic toxicity and bioaccumulation studies. In biodegradation studies, poor solubility may result in low bioavailability and thus lower than expected biodegradation rates. The specific test method or the choice of procedures used can thus be of key importance;

b. unstable substances: such substances that degrade (or react) rapidly in the test system present both testing and interpretational problems. It will be necessary to determine whether the correct methodology in line with the guidance provided in Section 4.1.3.3 has been used, whether it is the substance or the degradation/reaction product that has been tested, and whether the data produced is relevant to the classification of the parent substance;

c. volatile substances: such substances that can clearly present testing problems when used in open systems should be evaluated to ensure adequate maintenance of exposure concentrations. Loss of test material during biodegradation testing is inevitable in certain methods and will lead to misinterpretation of the results;

d. complex or multi-constituent[75] substances: such substances, for example, complex hydrocarbons, or other UVCB[76] substances, frequently cannot be dissolved into a homogeneous solution, and the multiple components make monitoring impossible. For organics, consideration therefore needs to be given to using the data derived from the testing of water-accommodated fractions (WAFs) for aquatic toxicity, and the use of such data in the classification scheme[77]. Biodegradation, bioaccumulation, partitioning behaviour and water solubility all present problems of interpretation, where each component of these complex or multi-constituent substances may behave differently;

e. polymers: such substances frequently comprise a wide range of molecular masses, which individually might have different water solubilities. Special methods are available to determine the water soluble fraction and these data will need to be used in interpreting the test data against the classification criteria;

f. inorganic compounds and metals: such substances, which can interact with the media, can produce a range of aquatic toxicities dependent on factors such as pH, water hardness etc. Difficult interpretational problems also arise from the testing of essential elements that are beneficial at certain levels. For metals and inorganic metal compounds, the concept of degradability as applied to organic compounds has limited or no meaning. Equally the use of bioaccumulation data should be treated with care (see also Annex IV);

g. surface active substances: such substances can form emulsions in which the bioavailability is difficult to ascertain, even with careful preparation of solutions. Micelle formation can result in an overestimation of the bioavailable fraction even when 'solutions' are apparently formed. This presents significant problems of interpretation in each of the water solubility, partition coefficient, bioaccumulation and aquatic toxicity studies;

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[75] Further definitions are provided in the *Guidance for identification and naming of substances under REACH and CLP* (ECHA).

[76] UVCB means Substances of Unknown or Variable composition, Complex reaction products or Biological materials, see Chapter 4.3 of the *Guidance for identification and naming of substances under REACH and CLP*.

[77] Note that the toxicity is sometimes expressed as LL_{50}, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria (see also Annex I.4.5 of this guidance document).

h. ionisable substances: such substances can change the extent of ionisation according to the level of counter ions in the media. Acids and bases, for example, will show radically different partitioning behaviour depending on the pH;

i. coloured substances: such substances can cause problems in the algal/aquatic plant testing because of the blocking of incident light;

j. impurities: some substances can contain impurities that can change in percentage and in chemical nature between production batches. Interpretational problems can arise where either or both the toxicity and water solubility of the impurities are greater than the parent substance, thus potentially influencing the toxicity data in a significant way. In general, the substance as manufactured including impurities should be tested and the classification should be based on these test results. To assess the sameness of two substances containing the same impurity in different amount see *Guidance for identification and naming of substances under REACH and CLP*, Chapter 5;

k. essential substances: some substances are essential to life, even though, like any substance, excessive concentrations can be harmful. This can lead to complex concentration/dose-response curves;

l. substances which can chelate or sequester essential elements, leading to the same problems of interpretation as in (k).

For further details see the OECD Guidance Document on aquatic toxicity testing of difficult substances and mixtures (OECD 2000) and also the IR&CSA Guidance, Chapter R.7b, Appendix 7.8.1 and Annex I to this guidance.

4.1.3.2.3. Interpretation of data for aquatic toxicity, degradation and bioaccumulation

4.1.3.2.3.1. Aquatic toxicity

Annex I: ***4.1.2.7.1*** *Acute aquatic toxicity is normally determined using a fish 96 hour LC_{50}, a crustacea species 48 hour EC_{50} and/or an algal species 72 or 96 hour EC_{50}. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species (e.g. Lemna spp.) shall also be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC_{50}s are treated as acute values for classification purposes (see note 2).*

Annex I: ***4.1.2.7.2*** *For determining chronic aquatic toxicity for classification purposes data generated according to the standardised test methods referred to in Article 8(3) shall be accepted, as well as results obtained from other validated and internationally accepted test methods. The NOECs or other equivalent EC_x (e.g. EC_{10}) shall be used.*

Fish, crustacea and algae or other aquatic plants are tested as surrogate species representing a range of trophic levels and taxa, and the test methods are highly standardised (see Annex I for further details). Valid data for short- and long-term tests on other species at the same trophic level shall also be considered, provided they are equivalent in terms of species relevance, testing conditions and test endpoints.

The purpose of classification is to characterise both the acute and long-term hazards in the aquatic environment. The acute and long-term hazards represent distinct types of hazard and should be applied independently.

The lowest available toxicity value(s) between and within the different trophic levels (fish, crustacea, algae/aquatic plants) will normally be used to define the appropriate hazard category(ies), although there may be circumstances where a weight of evidence approach is required (see Section 4.1.3.2.4).

Care should be taken when classifying substances like ionisable organic chemicals or organo-metallic substances as the observed results may express different toxicities in freshwater and marine environments and/or poorly soluble substances (water solubility < 1 mg/l), where there is evidence that the acute test does not provide a true measure of the intrinsic toxicity.

Relevant descriptions of the type of acute and/or chronic aquatic toxicity tests have been outlined in detail in Annex I to this guidance and in IR&CSA, Sections R.7.8.3-R.7.8.4. For classification and labelling purposes, tests using organisms outside the specified size (generally smaller) and/or tests with a differing test duration could be used if no other acceptable data are available.

Currently *in vitro* studies are only validated for some human health endpoints and according to IR&CSA, Chapters R.7.8.3-R.7.8.4, there are currently no validated fish cell systems available for use as alternative data to determine acute and long-term hazards within the scope of classification and labelling.

4.1.3.2.3.2. Degradation

Annex I: ***4.1.2.9.1*** *Substances that rapidly degrade can be quickly removed from the environment. While effects of such substances can occur, particularly in the event of a spillage or accident, they are localised and of short duration. In the absence of rapid degradation in the environment a substance in the water has the potential to exert toxicity over a wide temporal and spatial scale.*

Annex I: ***4.1.2.9.2*** *One way of demonstrating rapid degradation utilises the biodegradation screening tests designed to determine whether an organic substance is "readily biodegradable". Where such data are not available, a BOD(5 days)/COD ratio ≥ 0,5 is considered as indicative of rapid degradation. Thus, a substance which passes this screening test is considered likely to biodegrade "rapidly" in the aquatic environment, and is thus unlikely to be persistent. However, a fail in the screening test does not necessarily mean that the substance will not degrade rapidly in the environment. Other evidence of rapid degradation in the environment may therefore also be considered and are of particular importance where the substances are inhibitory to microbial activity at the concentration levels used in standard testing. Thus, a further classification criterion is included which allows the use of data to show that the substance did actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. Thus, if degradation is demonstrated under environmentally realistic conditions, then the criterion of "rapid degradability" is met.*

The definition of degradation covers both biotic (biodegradation) and abiotic degradation processes. Data on degradation properties of a substance may be available from standardised tests, from other types of investigations, or they may be estimated from the structure of the molecules (see Section 1.4). In Section II.2 of Annex II to this guidance a general overview of relevant definitions on how to use different (bio)degradability tests and guidance for the interpretation of test data in the context of classification and labelling is given. Additional information on (bio)degradation testing methods can be found in IR&CSA, Chapter R.7.9. The OECD test methods 301A-F (C.4-A to F of the Test Methods Regulation 440/2008), OECD 310, or equivalent tests, are commonly used to determine 'ready biodegradability'. Some guidance on the use of QSAR methods for degradability is presented in IR&CSA, Chapter R.7.9.3.1.

The paragraphs below will focus on the guidance for using degradability data for classification & labelling under CLP. It should be noted that the guidance on degradability pertains primarily to individual substances. In the case of complex or multi-constituent substances, the proposed test approaches do not normally allow an unequivocal interpretation of the degradability of the individual components of the substances. Thus, results of biodegradability tests on complex or multi-constituent substances should be carefully evaluated before use for classification purposes is considered.

Annex I: ***4.1.2.9.3*** *Many degradation data are available in the form of degradation half-lives and these can be used in defining rapid degradation provided that ultimate biodegradation of the substance, i.e. full mineralisation, is achieved. Primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.*

Annex I: ***4.1.2.9.4*** *The criteria used reflect the fact that environmental degradation may be biotic or abiotic. Hydrolysis can be considered if the hydrolysis products do not fulfil the criteria for classification as hazardous to the aquatic environment.*

Annex I: ***4.1.2.9.5*** *Substances are considered rapidly degradable in the environment if one of the following criteria holds true:*

(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:

(i) tests based on dissolved organic carbon: 70 %;

(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.

These levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10 % of the substance has been degraded; unless the substance is identified as an UVCB or as a complex, multi-constituent substance with structurally similar constituents. In this case, and where there is sufficient justification, the 10-day window condition may be waived and the pass level applied at 28 days, or

(b) if, in those cases where only BOD and COD data are available, when the ratio of BOD_5/COD is ≥ 0,5; or

(c) if other convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70 % within a 28-day period.

The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment.

A substance is considered to be **not** rapidly degradable **unless** at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the ten-day window condition may be waived for complex multi-component substances and the pass level applied at 28 days, as discussed in point II.2.3 of Annex II to this document). If this is not possible, then the pass level should be evaluated within a 14 days time window if possible, or after the end of the test; or

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days); or

c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydroysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70 % within 28 days), and it can be demonstrated that the degradation

products do not fulfill the criteria for classification as hazardous to the aquatic environment.

When these preferred data types are not available rapid degradation may be demonstrated if one of the following criteria is justified:

a. The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

b. In those cases where only BOD5 and COD data are available, the ratio of BOD5/COD is greater than or equal to 0.5. The same criterion applies to ready biodegradability tests of a shorter duration than 28 days, if the half-life furthermore is < 7 days; or

c. A weight of evidence approach based on read-across provides convincing evidence that a given substance is rapidly degradable.

If none of the above types of data are available then the substance is considered as **not** rapidly degradable. This decision may be supported by fulfilment of at least one of the following criteria:

i. the substance is not inherently degradable in an inherent biodegradability test; or

ii. the substance is predicted to be slowly biodegradable by scientifically valid QSARs, e.g. for the Biodegradation Probability Program, the score for rapid degradation (linear or non-linear model) < 0.5; or

iii. the substance is considered to be not rapidly degradable based on indirect evidence, such as knowledge from structurally similar substances; or

iv. no other data regarding degradability are available.

The percentage degradation reached after 28 days in ready biodegradability tests may be used directly for the assessment of 'rapid degradability' if no specific information on the time window is available or if the data were derived with the MITI 1 test (OECD 301C, 2006 or C.4-E of the Test Methods Regulation 440/2008). In the Closed Bottle test (OECD 301D, or C.4-F of the Test Methods Regulation 440/2008) a 14-day window may be used when measurements have not been made after 10 days. For some industrial chemicals that in terms of composition can be seen as multi-component substances testing for 'ready biodegradability' can lead to interpretational problems (see Annex II to this guidance).

Selection of test systems

As regards paragraph 4.1.2.9.5 point c in Annex I to CLP, the evaluation of the fulfilment of this criterion should be conducted on a case-by-case basis by expert judgement. Test systems that can be used to demonstrate the occurrence of rapid degradability are listed in Annex II. This includes e.g. simulation tests under realistic conditions, mesocosms and field monitoring.

Inherent- (OECD 302A and B, or C.9 and C.12 of the Test Methods Regulation 440/2008) and sewage treatment simulation (OECD 303, or C.10 of the Test Methods Regulation 440/2008) tests are not normally used in this context, due to the high levels of adapted biomass. Anaerobic degradation tests (OECD 311/ISO 11734 and analogous tests) do not qualify because of the specificity of the anaerobic compartments. Also the newly defined category of 'Enhanced Ready Biodegradation (Screening) Tests' in IR&CSA, Chapter R.7.9 do not qualify for use in classification and labelling, as they are presently not reviewed and internationally standardised.

Use of SARs and QSARs

The estimation of degradation via SARs and/or QSARs for hydrolysis and biodegradation is a rapidly developing field. The predictions from QSAR models may be considered as contributing to a decision on ready or rapid degradation for classification purposes. QSAR models should be used with great care, taking into account the applicability domain and validation of the models.

Current practice is to use the outcome of these biodegradation models to predict that a substance is not readily degradable, rather than *vice versa*. This is because models such as BIOWIN tend to predict non-biodegradability more accurately than biodegradability. However, QSAR information can be used as a part of expert judgement and Weight of Evidence practices, for example where very consistent measured and predicted data are available for a structurally analogous compound.

General interpretation problems and substances difficult to test

Both the UN GHS Annex 9 and the INS discuss substances that are inherently difficult to test for biodegradability, and possible adjustments to overcome testing problems. Testing or interpretational problems may occur with e.g. complex multi-constituent substances, surface active agents, highly volatile or insoluble substances, substances that are toxic to micro-organisms at normal test concentrations, and unstable molecules.

4.1.3.2.3.3. Bioaccumulation

> **Annex I:** ***4.1.2.8.1*** *Bioaccumulation of substances within aquatic organisms can give rise to toxic effects over longer time scales even when actual water concentrations are low. For organic substances the potential for bioaccumulation shall normally be determined by using the octanol/water partition coefficient, usually reported as a log K_{ow}. The relationship between the log K_{ow} of an organic substance and its bioconcentration as measured by the bioconcentration factor (BCF) in fish has considerable scientific literature support. Using a cut-off value of log $K_{ow} \geq 4$ is intended to identify only those substances with a real potential to bioconcentrate. While this represents a potential to bioaccumulate, an experimentally determined BCF provides a better measure and shall be used in preference if available. A BCF in fish of* **≥** *500 is indicative of the potential to bioconcentrate for classification purposes. Some relationships can be observed between chronic toxicity and bioaccumulation potential, as toxicity is related to the body burden.*

The potential for bioaccumulation is an important criterion to determine whether a chemical substance is a potential hazard to the environment. Bioaccumulation of a substance into an organism is not a hazard in itself, but should be considered in relation to potential long-term effects. Chemical concentration and accumulation may result in internal concentrations of a substance in an organism (body burden), which may or may not lead to toxic effects over long-term exposures. Further guidance on bioaccumulation is given in Annex III to this guidance. Bioaccumulation of metals is discussed in Annex IV.

Information on actual bioaccumulation of a substance may be available from standardised tests (e.g. Test Methods Regulation (EC) No 440/2008, OECD 305: Bioconcentration – Flow through fish test) or information on the bioaccumulation potential, for organic substances, may be estimated from the structure of the molecule.

In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A surrogate measure of lipophilicity is the n-octanol/water partition coefficient (K_{ow}) which, for lipophilic non-ionised organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, K_{ow} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{ow}. For those organic substances, estimation methods are available for calculating the K_{ow}. Data on the bioconcentration properties of non-ionised organic substances may thus be:

1. Experimentally determined;
2. Estimated from experimentally determined K_{ow}; or
3. Estimated from Kow values derived by use of Quantitative Structure Activity Relationships (QSARs).

Experimentally derived BCF values of high quality are ultimately preferred for classification purposes. BCF results from poor or questionable quality studies should not be used for classification purposes if high quality data on log K_{ow} are available. If no BCF is available for fish species, high quality data on the BCF for some invertebrates (e.g. blue mussel, oyster and/or scallop) may be used as a worst case surrogate.

For non-ionised organic substances, experimentally derived high quality K_{ow} values are preferred. If no experimental data of high quality are available validated Quantitative Structure Activity Relationships (QSARs) for log K_{ow} may be used in the classification process. If data are available but not validated, expert judgement should be used. For ionised organic substances problems may occur with e.g. changes in pH which may significantly affect the water solubility and partition coefficient of the substance. Further guidance on how to deal with such difficulties is provided in the OECD Guidance Document on aquatic toxicity testing of difficult substances and mixtures (OECD 2000).

4.1.3.2.4. Using weight of evidence in evaluations in the context of C&L

4.1.3.2.4.1. General aspects of weight of evidence

The weight of evidence approach is described in IR&CSA, Chapter B.4.4 as follows: 'The weight of evidence (WoE) approach is not a scientifically well-defined term or an agreed formalised concept. It involves assessing the relevance, reliability and adequacy of each piece of available information, holding the various pieces of information up against each other and reaching a conclusion on the hazard. This process always involves expert judgement. It is important to document and communicate how the evidence-based approach was used in a reliable, robust and transparent manner'.

Where there is only one experimental data entry per endpoint, classification and labelling decisions are relatively straightforward. However this is often not the case when dealing with data deficient substances or substances for which more than one valid piece of data is available for a given data element. In both situations, available information needs to be evaluated carefully. Data deficiency may occur for substances for which there are no, or limited experimental data with relevance for classification and labelling. This might be the case for substances exempted from REACH such as polymers or substances manufactured in quantities < 1 tonne/annum.

The taxa chosen, fish, crustacea and aquatic plants that represent the 'base-set' in most hazard profiles, represent a minimum dataset for a fully valid description of hazard. The lowest of the available toxicity values will normally be used to define the hazard category. Given the wide range of species in the environment, the three taxa tested can only be a poor surrogate and the lowest value is therefore taken for precautionary reasons to define the hazard category. In doing so, it is recognised that the distribution of species sensitivity can be several orders of magnitude wide, and that there will thus be both more and less sensitive species in the environment. Therefore, when data are limited, the use of the most sensitive species tested gives a cautious but acceptable definition of the hazard. There are some circumstances where it may not be appropriate to use the lowest toxicity value as the basis for classification. This will usually only arise where it is possible to define the sensitivity distribution with more accuracy than would normally be possible, such as when large datasets are available. Such large datasets should be evaluated with due caution.

Conversely, as CLP allows the use of expert judgment in employing non-testing information such as QSARs, the classification of data deficient substances could potentially be conducted in the absence of any experimental data.

In applying the WoE approach, the reliability of the experimental information under evaluation needs to be taken into due account. Typically, this information originates from studies which have been ranked according to the Klimisch criteria. The scores assigned to the studies may serve as an indication of the 'weight' that the corresponding information could have in 'weighing the evidence'.

4.1.3.2.4.2. Guidance on WoE for data deficient substances

Either for those substances for which the standard data set of acute aquatic testing in fish, crustacea and algae/aquatic plants is not available or where there are data gaps, REACH introduces the concept of an 'Integrated Testing Strategy' (for further guidance see IR&CSA, Chapter R.7B, Figure R.7.8-2). This outlines a stepwise approach on the use of test data and non-testing information, such as reliable QSARs and *in vitro* testing. It outlines how the relevant information is collected and evaluated and in the final step, expert judgement is used to reach an overall assessment of the aquatic toxicity of the substance under evaluation, taking into consideration also metabolites, reaction products, analogues.

For classification purposes, representative species should be chosen which cover a range of trophic levels and taxonomic groups, namely fish, crustacea and primary producers. Annex I to this document also provides guidance on the following where no experimental data are available:

> 'QSARs can be relied upon to provide predictions of acute toxicity to fish, crustacea (Daphnia and Mysid) and algae for non-electrolytes, non-electrophilic, and otherwise non-reactive substances. Care should be taken when evaluating the toxicity of poorly water soluble substances, where the quoted toxicity may be greater than the water solubility'.

4.1.3.2.4.3. Guidance on WoE for substances for which more than one valid piece of data is available for a given data element

The best quality data should be used as the fundamental basis for classification. Classification should preferably be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Where multiple studies for a taxonomic group are available, all studies that are assessed to have sufficient quality should be taken into consideration. The study showing the highest toxicity (e.g. the one with the lowest L(E)C_{50} or NOEC or EC_x) should normally be chosen as key study for aquatic hazard classification for that taxonomic group. However, in a WoE approach, a different weight may be given to studies irrespective the test results. For example: a judgement has to be made on a case-by-case basis whether Klimish 1 studies in a dataset are given more weight than Klimish 2 studies or valid QSAR data available for the same taxonomic group.

Lower quality information showing no or low toxicity should specifically be treated with care, especially where the quality assessment has revealed points of concern regarding methodology and reporting (e.g. maintenance of test concentrations). In addition it should be noted that substances which are difficult to test may yield apparent results that are not indicating the true toxicity. Expert judgement would also be needed for classification in these cases.

Assessment of data quality includes assessment of adequacy of the information for classification purposes and an assessment of both relevance and reliability. Details on the assessment of quality can be found in IR&CSA, Chapter R.4.

Where more than one acceptable test is available for the same taxonomic group, the most sensitive (the one with the lowest L(E)C_{50} or NOEC/EC_{10}) is generally used for classification. However, this must be dealt with on a case-by-case basis. When larger data sets (four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. In estimating a mean value, it is not advisable to combine tests of different species within a taxonomic group or in different life stages or tested under different conditions or duration. This implies that for substances, where four or more ecotoxicity data on the same species and endpoint are available, the data should be grouped, and the geometric mean used as a representative toxicity value for that species.

In case of very large data sets meeting the criteria for applying the Species Sensitivity Distribution (SSD) approach (see IR&CSA, Chapter R.10), statistical techniques (e.g. HC_5

derivation) can be considered to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC_{50} or NOEC), in a weight of evidence approach.

4.1.3.2.4.4. Outliers

The WoE approach would also address potential outliers, since as a starting point, all data points for a specific trophic level/taxonomic group would be considered to come from the same sensitivity distribution. Only if a sufficiently large number of data were available, appropriate statistical tests would be performed to confirm or disprove a particular value as an outlier.

The issue of possible 'outliers', which may exist, particularly in large data sets can be tackled according to a proposal in IR&CSA, Chapter R.7.8.4.1.

4.1.3.2.4.5. Weight of evidence in degradation

Where multiple or conflicting datasets exist for a single chemical, the most reliable data should be selected first, and subsequently a 'weight of evidence' approach followed based on these data. This implies that if both positive (i.e. above the pass level) and negative results (below pass level) have been obtained for a substance in rapid degradability tests, then the data of the highest quality and the best documentation should be used for determining the rapid degradability of the substance. Thus, given the conservative nature of ready biodegradability tests positive results could be used irrespective of negative results when the scientific quality is good and the test conditions are well documented, i.e. the guideline criteria are fulfilled. See Annex II for further guidance.

4.1.3.2.4.6. Weight of evidence in bioaccumulation

When conflicting bioaccumulation data is available, see Annex III for guidance.

4.1.3.3. Classification categories and criteria

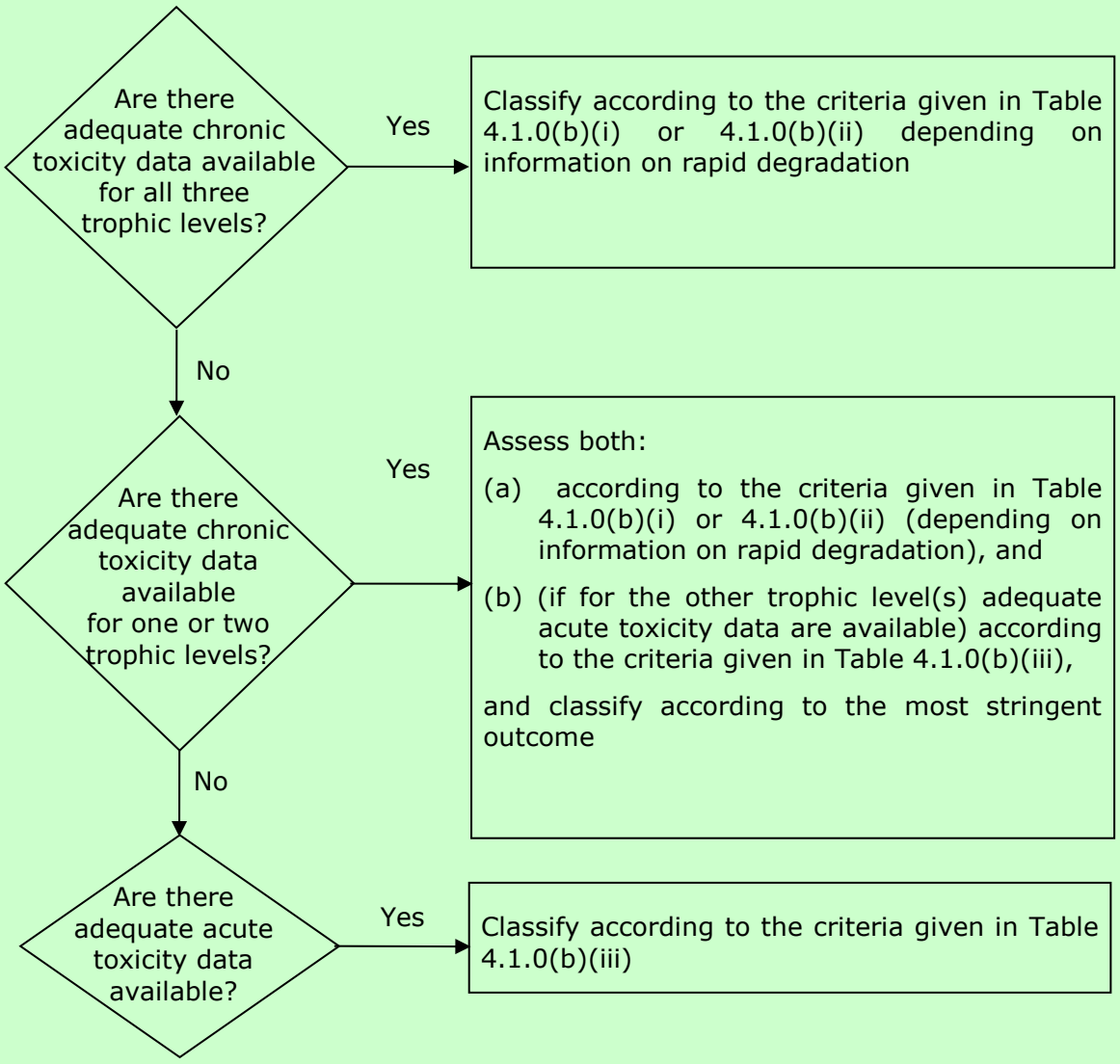
4.1.3.3.1. Outline of the core classification system

Annex I: ***4.1.2.2.*** *The core classification system for substances consists of one short-term (acute) hazard classification category and three long-term (chronic) hazard classification categories. The short-term (acute) and the long-term (chronic) hazard classification categories are applied independently.*

Annex I: ***4.1.2.3.*** *The criteria for classification of a substance in category Acute 1 are defined on the basis of acute aquatic toxicity data only (EC_{50} or LC_{50}). The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if available information on chronic toxicity merits long-term (chronic) hazard classification. In absence of adequate chronic toxicity data, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data) (see Figure 4.1.1).*

Figure 4.1.1

Categories for substances long-term (chronic) hazardous to the aquatic environment



4.1.2.1. *The system for classification recognises that the intrinsic hazard to aquatic organisms is represented by both the acute and chronic toxicity of a substance. For the long-term (chronic) hazard separate hazard categories are defined representing a gradation in the level of hazard identified. The lowest of the available toxicity values between and within the different trophic levels (fish, crustacean, algae/aquatic plants) shall normally be used to define the appropriate hazard category(ies). There are circumstances, however, when a weight of evidence approach is appropriate.*

Where adequate chronic toxicity data exist for the three trophic levels and the lowest chronic toxicity value (that normally would define the appropriate hazard category) is below or equal to 1 mg/l, a long-term hazard classification is warranted. The actual category is also depending on the information on rapid degradation.

While recognising that for packaged goods the long-term hazard represents the principal concern, it must also be recognised that chronic toxicity data are expensive to generate and generally not readily available for most substances. On the other hand, acute toxicity data are more often readily available than chronic toxicity data, or can be generated according to highly standardised test protocols. It is this acute toxicity which has therefore been used as the core property in defining both the acute and the long-term hazard if no adequate chronic test data are available. Nevertheless, it has been recognised that chronic toxicity data, if available, should be preferred in defining the long-term hazard category.

Chronic toxicity data (EC_x or NOEC) would normally override acute data for long-term hazard classification. However, when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor.

The combination of chronic toxicity and degradation properties reflects the potential hazard of a substance. Substances that do not rapidly degrade have a higher potential for longer term exposures and therefore should be classified in a more severe category than substances which are rapidly degradable.

A review of the existing adequate appropriate acute toxicity data and environmental fate data (degradability and bioaccumulation) is required for those trophic levels where adequate chronic toxicity data may be absent; to decide if a long-term hazard classification may be warranted.

While recognising that acute toxicity itself is not a sufficiently accurate predictor of chronic toxicity to be used solely and directly for establishing hazard, it is considered that, in combination with either a potential to bioaccumulate (i.e. experimentally determined BCF $\geq$ 500 or, if absent, the log $K_{ow} \geq 4$) or potential longer term exposure (i.e. lack of rapid degradation) it can be used as a suitable surrogate for classification purposes. Substances rapidly degrading that show acute toxicity with a significant degree of bioaccumulation will normally show chronic toxicity at a significantly lower concentration. Equally, substances that do not rapidly degrade have a higher potential for giving rise to longer term exposures which again may result in long-term toxicity being realised.

The hazard categories for acute and chronic aquatic toxicity and their related criteria are set out in CLP, Annex I, Section 4.1, Table 4.1.0.

Annex I: *Table 4.1.0* ***Classification categories for hazardous to the aquatic environment***	
(a) Short-term (acute) aquatic hazard	
Category Acute 1: *(Note 1)*	
96 hr LC_{50} (for fish)	*≤ 1 mg/l and/or*
48 hr EC_{50} (for crustacea)	*≤ 1 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*≤ 1 mg/l. (Note 2)*
(b) Long-term (chronic) aquatic hazard	
(i) Non-rapidly degradable substances (Note 3) for which there are adequate chronic toxicity data available	
Category Chronic 1: *(Note 1)*	
Chronic NOEC or EC_x (for fish)	*≤0,1 mg/l and/or*

Chronic NOEC or EC_x (for crustacea)	*≤0,1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤0,1 mg/l.*
Category Chronic 2:	
Chronic NOEC or EC_x (for fish)	*≤1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤1 mg/l.*

(ii) Rapidly degradable substances (Note 3) for which there are adequate chronic toxicity data available

***Category Chronic 1**: (Note 1)*	
Chronic NOEC or EC_x (for fish)	*≤0,01 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤0,01 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤0,01 mg/l*
Category Chronic 2:	
Chronic NOEC or EC_x (for fish)	*≤0,1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤0,1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤0,1 mg/l*
Category Chronic 3:	
Chronic NOEC or EC_x (for fish)	*≤1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤1 mg/l.*

(iii) Substances for which adequate chronic toxicity data are not available

***Category Chronic 1**: (Note 1)*	
96 hr LC_{50} (for fish)	*≤1 mg/l and/or*
48 hr EC_{50} (for crustacea)	*≤1 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*≤1 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

***Category Chronic 2**:*	
96 hr LC50 (for fish)	*>1 to ≤10 mg/l and/or*
48 hr EC50 (for crustacea)	*>1 to ≤10 mg/l and/or*
72 or 96 hr ErC50 (for algae or other aquatic plants)	*>1 to ≤10 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

***Category Chronic 3**:*	
96 hr LC_{50} (for fish)	*> 10 to ≤ 100 mg/l and/or*
48 hr EC_{50} (for crustacea)	*> 10 to ≤ 100 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*> 10 to ≤ 100 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

Note 1: When classifying substances as Acute Category 1 and/or Chronic Category 1 it is necessary at the same time to indicate then appropriate M-factor(s) (see table 4.1.3).

Note 2: Classification shall be based on the ErC_{50} [= EC_{50} (growth rate)]. In circumstances where the basis of the EC_{50} is not specified or no ErC_{50} is recorded, classification shall be based on the lowest EC_{50} available.

Note 3: When no useful data on degradability are available, either experimentally determined or estimated data, the substance should be regarded as not rapidly degradable.

Classifications may also be made in cases where data are not available on all three trophic levels. In these cases, the classification may be subject to further information becoming available. In general, all the data available will need to be considered prior to assigning a classification. Where good quality data are not available, lower quality data will need to be considered. In these circumstances, a judgement will need to be made regarding the true level of hazard. For example, where good quality data are available for a particular species or taxa, this should be used in preference to any lower quality data which might also be available for that species or taxa. However, good quality data may not always be available for all trophic levels. It will be necessary to consider data of lower quality for those trophic levels for which good quality data are not available. Consideration of such data, however, will also need to consider the difficulties that may have affected the likelihood of achieving a valid result. For example, the test details and experimental design may be critical to the assessment of the usability of some data, such as that from hydrolytically unstable chemicals, while less so for other chemicals. Such difficulties are described further in Annex I to this guidance.

Normally, the identification of hazard, and hence the classification will be based on information directly obtained from testing of the substance being considered. There are occasions, however, where this can create difficulties or the outcomes do not conform to common sense. For example, some chemicals, although stable in the bottle, will react rapidly (or slowly) in water giving rise to degradation products that may have different properties. Where such degradation is rapid, the available test data will frequently define the hazard of the degradation products since it will be these that have been tested. These data may be used to classify the parent substance in the normal way. However, where degradation is slower, it may be possible to test the parent substance and thus generate hazard data in the normal manner. The subsequent degradation may then be considered in determining whether an acute or long-term hazard category should apply. There may be occasions, however, when a substance so tested may degrade to give rise to a more hazardous product. In these circumstances, the classification of the parent compound should take due account of the hazard of the degradation product, and the rate at which it can be formed under normal environmental conditions (for detailed information please check also the Annexes to this guidance).

4.1.3.3.2. The 'safety net'

Annex I: ***4.1.2.4*** *The system also introduces a "safety net" classification (referred to as Chronic 4) for use when the data available do not allow classification under the formal criteria for Acute 1 or Chronic 1 to 3 but there are nevertheless some grounds for concern (see example in Table 4.1.0).*

Annex I: *4.1.2.6. Table 4.1.0. continued*

'Safety net' classification

Chronic Category 4

Cases when data do not allow classification under the above criteria but there are nevertheless some grounds for concern. This includes, for example, poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility (note 4), and which are not rapidly degradable in accordance with Section 4.1.2.9.5 and have an experimentally determined BCF ≥ 500 (or, if absent, a log K_{ow} ≥ 4), indicating a potential to bioaccumulate, which will be classified in this category unless other scientific evidence exists showing classification to be unnecessary. Such evidence includes chronic toxicity NOECs > water solubility or > 1 mg/l, or other evidence of rapid degradation in the environment than the ones provided by any of the methods listed in Section 4.1.2.9.5.

Note 4: 'No acute toxicity' is taken to mean that the L(E)C_{50}(s) is/are above the water solubility. Also for poorly soluble substances, (water solubility < 1 mg/l), where there is evidence that the acute test does not provide a true measure of the intrinsic toxicity.

Category Chronic 4 is for example triggered in the following cases. For some poorly soluble substances, which are normally considered as those having a water solubility < 1 mg/l, no acute toxicity is expressed in toxicity tests performed at the solubility limit. If for such a substance, however, the BCF ≥ 500, or if absent, the log K_{ow} ≥ 4 (indicating a bio-accumulating potential) and the substance is also not rapidly degradable, a safety net classification, Chronic 4 is assigned. For these types of substances the exposure duration in short-term tests may well be too short for a steady-state concentration of the substance to be reached in the test organisms. Thus, even though no acute toxicity has been measured in a short-term (acute) test, it remains a real possibility that such non-rapidly degradable and bioaccumulative substances may exert chronic effects, particularly since such low degradability may lead to an extended exposure period in the aquatic environment.

The precise definitions of the core elements of this system are described in detail in Annexes I-III to this guidance document.

4.1.3.3.3. Setting an M-factor for highly toxic substances

4.1.2.5 *Substances with acute toxicities below 1 mg/l or chronic toxicities below 0,1 mg/l (if non-rapidly degradable) and 0,01 mg/l (if rapidly degradable) contribute as components of a mixture to the toxicity of the mixture even at a low concentration and shall normally be given increased weight in applying the summation of classification approach (see Note 1 of Table 4.1.0 and 4.1.3.5.5).*

When a substance is classified as category Acute 1 and/or category Chronic 1, (a) multiplying factor(s) (M-factor) has/have to be assigned (as described Article 10 of CLP). Where appropriate, M-factors shall be set for acute and long-term hazards separately. This means that there can be two different M-factors (one for acute and one for long-term hazard) for one substance. It is important to also include the M-factor(s) in the SDS as other users in the supply chain might need it, e.g. for classification of mixtures containing that substance.

The M-factor itself can be taken from the table below and is dependent on the toxicity band of the substances. For a substance with an acute toxicity of 0.005 mg/l for example an M-factor of 100 needs to be assigned. Whereas e.g. with a chronic toxicity of 0.005 mg/l an M-factor of 10 needs to be assigned for non-rapidly degrable substance and an M-factor of 1 to rapidly degradable substances.

Annex I: *Table 4.1.3*

Multiplying factors for highly toxic components of mixtures

Acute toxicity	*M factor*	*Chronic toxicity*	*M factor*	
L(E)C_{50} value		*NOEC value*	*NRD[a] components*	*RD[b] components*
0,1 < L(E)C_{50} ≤ 1	*1*	*0,01 < NOEC ≤ 0,1*	*1*	-
0,01 < L(E)C_{50} ≤ 0,1	*10*	*0,001 < NOEC ≤ 0,01*	*10*	*1*
0,001 < L(E)C_{50} ≤ 0,01	*100*	*0,0001 < NOEC ≤ 0,001*	*100*	*10*
0,0001 < L(E)C_{50} ≤ 0,001	*1000*	*0,00001 < NOEC ≤ 0,0001*	*1000*	*100*
0,00001 < L(E)C_{50} ≤ 0,0001	*10000*	*0,000001 < NOEC ≤ 0,00001*	*10000*	*1000*
(continue in factor 10 intervals)		*(continue in factor 10 intervals)*		

[a] *Non-rapidly degradable.*

[b] *Rapidly degradable.*

The NOEC value in Table 4.1.3 (Annex I to CLP) refers to both NOEC and EC_x (toxicity values are in mg/l). The first two columns in Table 4.1.3 refer to the classification system in Table 4.1.0 (a)(b, point iii), the last three columns refer to the respective classification system in Table 4.1.0 (b, points i & ii). In cases where chronic data are not available and Table 4.1.0 (a)(b, point iii) is used for defining long-term aquatic hazard, the resulting M-factor derived for acute aquatic hazard classification is also applied to the long-term aquatic hazard classification.

4.1.3.4. Decision on classification: examples for substances

If the evaluation shows that the criteria are fulfilled, one category for acute aquatic hazard and/or one for long-term aquatic hazard should be assigned, as well as (an) M-factor(s) where applicable. For the labelling elements, such as hazard pictograms, signal words, hazard statements and precautionary statements, see Section 4.1.6 of this guidance.

Further classification examples specific to metals and metal compounds are given in Annex IV to this guidance document.

The examples in this section are focussed on self-classification based on relevant data available. Mandatory use of harmonised classification for substances included in Table 3.1 of Annex VI, the use of information from the classification and labelling inventory and the use of the translation Table in Annex VII are not taken into account in these examples.

After data collection self-classification starts with evaluation of the adequateness of the data collected and assessment of the results and concluding on endpoints relevant for environmental hazard classification. Where the assessment shows that criteria for environmental classification are fulfilled, one category for acute aquatic hazard and/or one category for long-term aquatic hazards should be assigned and M-factor(s) should be deducted where applicable.

List of the examples on substance classification included in this section:

- Example A: Hydrophilic substance, straightforward classification based on acute and chronic toxicity data;
- Example B: Hydrophilic substance, straightforward classification based on acute data, no chronic toxicity data available;
- Example C: Moderately water soluble substance, straightforward classification based on acute data, chronic toxicity data available for two trophic levels; combined set of QSAR data and experimental data;
- Example D: Substance with several toxicity data for one trophic level;
- Example E: "Safety net" classification category Chronic 4;
- Example F: Substance difficult to test, toxicity above level of water solubility.

Further classification examples specific to metals and metal compounds are given in Annex IV to this guidance.

The examples are presented using a logical format starting with a table listing for all relevant data elements the information available, followed by an aquatic hazard assessment for each data element, a section showing the aquatic hazard classification, a section with the reasoning behind the conclusions and finally a table presenting the applicable labelling elements.

Explanation of data elements used in the examples:

- Physico-chemical properties important for evaluation of aquatic hazards for the purpose of classification: Generally this consists of water solubility (mg/l) and log octanol/water partition coefficient (log K_{ow});
- Acute aquatic toxicity: Generally expressed in terms of LC_{50} or EC_{50} (mg/l);
- Long-term aquatic toxicity: Generally expressed in terms of NOEC or EC_x(mg/l);
- Degradation (evidence of rapid degradation): Generally expressed in terms of biotic or abiotic degradation of organic substances (or transformation of inorganic substances). In case of rapid primary degradation, information shall be given whether the degradation products can be classified as hazardous to the aquatic environment or not;
- Bioaccumulation: Generally expressed in terms of bioconcentration factor in fish.

Information on reliability is not taken into account in the exemplification. For the purpose of the examples the reliability score is assumed to be high (e.g. for experimental tests, Klimisch score 1 or 2) unless otherwise stated. Note that assigning a reliability score to studies is important - if a study is assessed as poorly reliable it is normally not usable for classification purposes.

Besides the conclusion from studies on relevant endpoints for classification the following information is presented for each example in a separate column:

- Referral to applicable test method according to the EU Test Methods Regulation (EC) No 440/2008 or OECD test guideline or QSAR model used;
- Some basic information on the test design (pH of the test media, renewal regime of test media (static, semi-static, flow-through);
- Use of measured or nominal test concentrations;
- Compliance of the experiment and reporting with OECD Good Laboratory Practice (GLP) rules;
- Specific information related to the relevant endpoints, as appropriate.

This information plays a crucial role when the adequacy of the data and the assessment of the study results are being evaluated for their applicability in the classification and labelling scheme. However, in these examples this information is included mainly to make the data more realistic.

4.1.3.4.1. Example A: Hydrophilic substance, straightforward classification based on acute and chronic toxicity data

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		1200 mg/l	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		2.75	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	12 mg/l (96 h LC_{50})	C.1. / static, non-GLP
	Lepomis macrochirus:	2.7 mg/l (96 h LC_{50})	C.1. / static, GLP
Crustacea	*Daphnia magna*:	18 mg/l (48 h EC_{50})	C.2. / static, non-GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.056 mg/l (96 h ErC_{50})	C.3. / static, GLP
	Lemna gibba:	0.031 mg/l (7 d ErC_{50})	C.26. / semi-static, GLP
Chronic aquatic toxicity			
Fish	*Danio rerio*:	1.2 mg/l (21 d NOEC)	OECD 210 / Early Life Stage toxicity test, flow-through, GLP
Crustacea	*Daphnia magna*:	1.1 mgl (21 d NOEC)	C.20. / semi-static, GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.01 mg/l (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation:		86 % in 28 days (10 day-window fulfilled)	C.4-C / pH:7.5, GLP
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is readily soluble. Log K_{ow} < 4, indicating low potential for bioaccumulation, which can be used in absence of BCF data.

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values is between 0.01 and 0.1 mg/l.

Long-term aquatic toxicity:

- The long-term aquatic toxicity based on the lowest of the available toxicity values is between 0.001 and 0.01 mg/l.

Degradation (evidence of rapid degradation):

- 70 % degradation in 28 days based on dissolved organic carbon (DOC) fulfils the criteria for rapid degradation.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 10.

Long-term aquatic hazard: category Chronic 1, M-factor: 1.

Reasoning:

Acute aquatic hazard: acute toxicity L(E)C_{50} ≤ 1 mg/l. M-factor based on L(E)C_{50} between 0.01 and 0.1 mg/l.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate long-term toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see Section 4.1.3.3 and Table 4.1.0.

- Adequate long-term toxicity data for all three trophic levels, long-term toxicity NOEC ≤ 0.01 mg/l, rapidly degradable. M-factor based on NOEC between 0.001 and 0.01 mg/l (rapidly degradable).

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[78]
Precautionary statement(s)	P273, P391, P501

[78] Note that in accordance with CLP Article 27 the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

4.1.3.4.2. Example B: Hydrophilic substance, straightforward classification based on acute data, no chronic data available

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		1200 mg/l	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		2.75	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	12 mg/l (96 h LC_{50})	C.1. / static, non-GLP
	Lepomis macrochirus:	2.7 mg/l (96 h LC_{50})	C.1. / static, GLP
Crustacea	*Daphnia magna*:	18 mg/l (48 h EC_{50})	C.2. / static, non-GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.056 mg/l (96 h ErC_{50})	C.3. / static, GLP
	Lemna gibba:	0.031 mg/l (7 d ErC_{50})	C.26. / semi-static, GLP
Chronic aquatic toxicity			
Fish:		No data	
Crustacea:		No data	
Algae/aquatic plants:		NOEC not reported	
Degradation (evidence of rapid degradation)			
Biotic degradation:		86 % in 28days (10 day-window fulfilled)	C.4-C / pH:7.5, GLP
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		560 l/kg	C.13. / pH: 7.8, GLP, BCF (related to total radioactive residues because data for parent compound not available)

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is readily soluble. Log K_{ow} < 4, indicating low potential for bioaccumulation, which can be used in absence of BCF data (see bioaccumulation assessment).

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values is between 0.01 and 0.1 mg/l.

Long-term aquatic toxicity:

- No adequate chronic toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- 70 % degradation based on dissolved organic carbon (DOC) fulfils the criteria for rapid degradation.

Bioaccumulation:

- BCF > 500, hence high potential for bioaccumulation. BCF value overrules the use of logKow value which in this case is lower than the cut-off value of 4.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M-factor: 10.

Long–term aquatic hazard: category Chronic 1, M-factor: 10.

Reasoning:

Acute (short-term) aquatic hazard: acute toxicity L(E)C_{50} ≤ 1 mg/l. M-factor based on L(E)C_{50} between 0.01 and 0.1 mg/l.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate long-term toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see Section 4.1.3.3 and Table 4.1.0.

- No adequate long-term toxicity data available (for all three trophic levels);
- Lowest acute toxicity L(E)C_{50} ≤ 1 mg/l;
- Substance is rapidly degradable but the experimentally determined BCF > 500;
- Since the conclusion is based on Table 4.1.0 (b) (iii), therefore the M-factor is based on the acute toxicity between 0.01 and 0.1 mg/l. In this case, the same factor M applies for both acute and long-term hazard.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[79]
Precautionary statement(s)	P273, P391, P501

[79] Note that in accordance with CLP Article 27 the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

4.1.3.4.3. Example C: Moderately water soluble substance, straightforward classification based on acute data, chronic data available for two trophic levels only; combined set of QSAR data and experimental data

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		25 mg/l	A.6. / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		5.75 3.9	A.8. / pH: 7.5, GLP QSAR KOWINN, valid, non-GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*: *Lepomis macrochirus*:	12.3 mg/l (96 h LC_{50}) 22.5 mg/l (96 h LC_{50})	C.1. / static, non-GLP C.1. / static, GLP
Crustacea	*Daphnia magna*: *Daphnia magna*:	0.79 mg/l (48 h EC_{50}) 1.06 mg/l (48 h EC_{50})	C.2. / static, non-GLP QSAR, ECOSAR, valid, non-GLP
Algae/aquatic plants *Scenedesmus subspicatus*:		1.53 mg/l (96 h ErC_{50})	C.3. / static, GLP
Chronic aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	0.56 mg/l (21 d NOEC)	OECD 210 / Early Life Stage toxicity test, flow-through, GLP
Crustacea:		No data	
Algae/aquatic plants *Scenedesmus subspicatus*:		0.23 mg/l (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation:		45 % in 28 days	C.4-C / pH: 7.5, GLP
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is moderately soluble. Log K_{ow} 5.75. Based on weight of evidence, valid K_{ow} estimated with QSAR is overruled by valid GLP experimental data.

Note that use of experimental data and QSAR data for estimation log K_{ow} should be carefully considered on a case by case basis. The validity of data may be dependant on the structure of the chemical. See Annex III, Section III.2.2 for more details on the use of log K_{ow} data and Annex III, Section **Error! Reference source not found.** for details on chemical classes that eed special attention in this respect.

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values is between 0.1 and 1 mg/l;
- For *Daphnia magna* two valid values are presented. A weight of evidence approach is applied in which the QSAR data are outweighed by the valid experimental data. Hence, the lowest acute toxicity value of 0.79 mg/l is used for crustaceans.

Long-term aquatic toxicity:

- Adequate chronic toxicity data available only for fish and algae/aquatic plants, not for crustaceans;
- The chronic aquatic toxicity based on the lowest of the available toxicity values for fish and algae/aquatic plants is between 0.1 and 1 mg/l.

Since there is adequate chronic toxicity data available for two trophic levels, assess both:

a. according to the criteria given in Table 4.1.0(b)(i) or 4.1.0(b)(ii) (depending on information on rapid degradation), and
b. (if for the other trophic level(s) adequate acute toxicity data are available) according to the criteria given in Table 4.1.0(b)(iii),

and classify according to the most stringent outcome.

Degradation (evidence of rapid degradation):

- < 70 % degradation in 28 days based on dissolved organic carbon (DOC), does not fulfil the criteria for rapid degradation.

Bioaccumulation:

- Log K_{ow} 5.75, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M factor: 1.

Long-term aquatic hazard: category Chronic 1, M factor: 1.

Reasoning:

Acute (short-term) aquatic hazard: lowest acute aquatic toxicity L(E)C_{50} ≤ 1 mg/l. M-factor based on L(E)C_{50} between 0.1 and 1 mg/l.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate long-term toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). In this example the absence of long-term study for the species/trophic level (i.e. Daphnia/Crustacea) with the lowest acute toxicity value supports using the surrogate system. For details see Section 4.1.3.3 and Table 4.1.0.

- NOEC-based system (Table 4.1.0 (b)(i): lowest long-term aquatic toxicity NOEC ≤ 1 mg/l, not rapidly degradable, hence category Chronic 2;
- Surrogate system (Table 4.1.0 (b)(iii): lowest acute aquatic toxicity L(E)C_{50} < 1 mg/l, not rapidly degradable (and Log K_{ow}>4), hence category Chronic 1;
- Conclusion: category Chronic 1 applies following the most stringent outcome;
- Since the conclusion is based on the surrogate system (Table 4.1.0 (b) (iii)) the M-factor is based on the acute aquatic toxicity between 0.1 and 1 mg/l.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[80]
Precautionary statement(s)	P273, P391, P501

[80] Note that in accordance with CLP Article 27 the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

4.1.3.4.4. Example D: Substance with several toxicity data for a trophic level

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		120 mg/l	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		4.9	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Lepomis macrochirus*:	108 mg/l (96 h LC_{50})	C.1. / static, GLP
Crustacea[81]	*Daphnia magna*:	40 mg/l (48 h EC_{50})	C.2. / static, GLP
	Procambarus clarkii:	0.12 mg/l (48 h EC_{50})	Method na. / static, GLP
	Asellus aquaticus:	0.4 mg/l (48 h EC_{50})	Method na. / static, non-GLP
	Mysidopsis bahia:	0.5 mg/l (48 h EC_{50})	Method na. / static, GLP
	Chironomus tentans:	0.8 mg/l (48 h EC_{50})	Method na. / static, GLP
Algae/aquatic plants *Pseudokirchneriella subcapitata:*		22 mg/l (96 h ErC_{50})	C.3. / static, GLP
Chronic aquatic toxicity			
Fish	*Pimephales promelas:*	1.1 mg/l (21 d NOEC)	OECD 210 / Early Life Stage toxicity test, flow-through, GLP, endpoint: growth
Crustacea	*Daphnia magna:*	1.2 mg/l (21 d NOEC)	C.20. / semi-static, GLP, endpoint: reproduction
Algae/aquatic plants *Pseudokirchneriella subcapitata:*		8.5 mg/l (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation		No data	
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

[81] Some species in this trophic level may be representatives of other taxonomic groups than crustecea e.g. the non-biting midge *Chironomus tentans* is a representative of the subphylum Hexapoda (class Insecta).

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is water soluble. Log K_{ow} 4.9.

Acute aquatic toxicity:

- The acute aquatic toxicity (based on the lowest of the available toxicity values) is between 0.1 and 1 mg/l. The classification in this example should be based on the most sensitive species which is the crustacean *Procambarus clarkii*;

Note that in general for substances for which multiple toxicity data is available for a taxonomic group (in this case crustaceans) on a case-by-case basis the toxicity data may be evaluated by weighting the evidence. If for example four or more acute LC50 values were available for the same fish species, then a geometric mean may be calculated (see Section 4.1.3.2.4.3). In this specific example, acute toxicity data on five separate crustacean species is available and all – except one – are from GLP studies that are weighed equally in a weight of evidence approach. Accordingly, the lowest value is used for classification purposes.

Chronic aquatic toxicity:

- Adequate long-term toxicity data available only for fish and algae/aquatic plants. The chronic aquatic toxicity (based on the lowest of the two available toxicity values) is above 1 mg/l;
- For crustaceans chronic data is available for *Daphnia magna* which based upon the relatively large acute dataset is clearly the least sensitive of the species for which data is available. Hence, the chronic aquatic toxicity data on *Daphnia magna* in this case should be considered not in conformity with the definition of 'adequate chronic data'.

Degradation (evidence of rapid degradation):

- No data available for this substance. In this case the substance is considered as not rapidly degradable (see Table 4.1.0, Note 3).

Bioaccumulation:

- Log K_{ow} 4.9, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M factor: 1.

Long-term aquatic hazard: category Chronic 1, M factor 1.

Reasoning:

Acute aquatic hazard: Acute aquatic toxicity L(E)C_{50} > 0.001 and < 0.01 mg/l;

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate long-term toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see Section 4.1.3.3 and Table 4.1.0.

- Adequate Chronic toxicity data available for two out of three trophic levels (fish and algae/aquatic plants), lowest NOEC above 1 mg/l. Conclusion for these two trophic

levels: NOEC-based system (Table 4.1.0 (b)(i): lowest long-term aquatic toxicity NOEC > 1 mg/l, hence not classified;

- Surrogate system (Table 4.1.0 (b)(iii): lowest acute aquatic toxicity L(E)C_{50} < 1 mg/l (0.12 mg/l Procambarus clarkii), not rapidly degradable (and log Kow > 4), hence category Chronic 1;
- Conclusion: category Chronic 1 applies following the most stringent outcome;
- Since the conclusion is based on the surrogate system (Table 4.1.0 (b) (iii)) the M-factor is based on the acute aquatic toxicity between 0.1 and 1 mg/l.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[82]
Precautionary statement(s)	P273, P391, P501

[82] Note that in accordance with CLP Article 27 the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

4.1.3.4.5. Example E: 'Safety net' classification category Chronic 4

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties		
Water solubility:	0.009 mg/l	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):	5.4	A.8. / pH:7.5, GLP
Acute aquatic toxicity		
Fish:	No data	
Crustacea *Daphnia magna*:	> 1 mg/l (48 h EC_{50})	C.2. / static, nominal concentration, non-GLP
Algae/aquatic plants:	No data	
Chronic aquatic toxicity		
Fish:	No data	
Crustacea:	No data	
Algae/aquatic plants:	No data	
Degradation (evidence of rapid degradation)		
Biotic degradation:	No data	
Abiotic degradation, hydrolysis (half-life (d)):	No data	
Bioaccumulation		
Bioconcentration factor in fish (BCF):	No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is poorly soluble. Log K_{ow} > 4, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Acute aquatic toxicity:

- Data poor substance. No acute toxicity recorded at levels up to the limit of water solubility.

Long-term aquatic toxicity:

- No adequate chronic toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- The substance is considered not rapidly degradable by default in absence of measured data.

Bioaccumulation:

- Log K_{ow} 5.4, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute hazard: Not classified.

Long-term hazard: 'Safety net' classification category Chronic 4.

Reasoning:

Acute hazard: No acute aquatic toxicity recorded at levels up to the limit of water solubility;

Long-term hazard: No adequate chronic toxicity data available for all three trophic levels. Substance nevertheless of concern based on the following findings:

- Poorly soluble substance;
- No acute aquatic toxicity recorded at levels up to the limit of water solubility;
- Not rapidly degradable (by default in absence of measured data);
- High potential for bioaccumulation (in absence of BCF data, log K_{ow} > 4);
- No evidence on NOEC being > water solubility for all three trophic levels;
- No other evidence of rapid degradation in the environment.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	-
Hazard Statement	H413
Precautionary statement(s)	P273, P501

4.1.3.4.6. Example F: Substance difficult to test, toxicity above level of water solubility

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties		
Water solubility:	< 0.2 mg/l	A.6. / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):	No data	Not determined due to instability of the substance in water
Acute aquatic toxicity		
Fish *Oncorhynchus mykiss*:	12 mg/l (96 h LC_{50})	C.1. / static, nominal concentration, non-GLP
Crustacea *Daphnia magna:*	18 mg/l (48 h EC_{50})	C.2. / static, nominal concentration, non-GLP
Algae/aquatic plants *Pseudokirchneriella subcapitata:*	3.56 mg/l (96 h ErC_{50})	C.3. / static, nominal concentration, non-GLP
Chronic aquatic toxicity		
Fish:	No data	
Crustacea:	No data	
Algae/aquatic plants:	No data	
Degradation (evidence of rapid degradation)		
Biotic degradation:	No data	
Abiotic degradation, hydrolysis (half-life (d)):	< 0.5 days (longest half-life within pH 4-9)	C.7. / pH: 7.0, non-GLP
Bioaccumulation		
Bioconcentration factor in fish (BCF):	No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The water solubility test is not considered to be valid (Klimisch 3) as the substance is known to rapidly hydrolyse and this was not considered in this study. Log K_{ow} not determined.

Acute aquatic toxicity:

- This data is based on initial measured concentrations in the suspension and the reported EC_{50} values are far above the water solubility (Klimisch score 3). Tests undertaken in a static regime which is inappropriate for a substance which rapidly hydrolyses (see also IR&CSA R.7b for guidance on how to test difficult substances);
- It is not clear whether the reported effects in the acute toxicity studies are due to physical effects of the undissolved substance particles in the test media on the test species or inherent toxicity of the substance.

Long-term aquatic toxicity:

- No adequate long-term toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- In the assessment of rapid degradability hydrolysis can be considered if the hydrolysis products do not fulfil the criteria for classification as hazardous to the aquatic environment. In this example hydrolysis is sufficient to show a rapid degradability of the parent substance in the environment but no information is available about the breakdown product(s). More data on degradation of this/these compound(s) would be necessary;
- In absence of data to show a rapid degradation of the breakdown product(s) the parent substance is considered not rapidly degradable.

Bioaccumulation:

- Log K_{ow} could not be determined experimentally. The parent substance has a low potential for bioaccumulation due to hydrolytical instability.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: Not classified in absence of adequate data (data of poor quality).

Long-term aquatic hazard: category Chronic 4.

Reasoning:

Acute hazard (Table 4.1.0 (a)): No acute aquatic toxicity as no adequate acute data available;

Long-term hazard: No adequate long-term toxicity data available for all three trophic levels. Substance nevertheless of concern based on the following findings:

- Poorly soluble substance (< 0.2 mg/l);
- No acute aquatic toxicity recorded at levels up to the limit of water solubility;
- Not rapidly degradable (see Section 4.1.3.2.3.2 of this guidance (CLP legal text: point 4.1.2.9.3);
- No evidence of NOEC being > water solubility for all three trophic levels.
- No information available on the hydrolysis products and hence dataset not decisive whether these fulfil the criteria for classification as hazardous to the aquatic environment based upon:
    - Toxicity;
    - Rapid degradability;
    - Bioaccumulation.

- In this case the safety net classification should be applied because of the large uncertainty on the fate and effects of the hydrolysis products.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	-
Hazard Statement	H413
Precautionary statement(s)	P273, P501

4.1.4. Classification of mixtures hazardous to the aquatic environment

4.1.4.1. General considerations for classification of mixtures hazardous to the aquatic environment

Note that general principles for classification of mixtures under CLP are given in Section 1.1.6.2 and Section 1.6 of part 1 of this guidance document.

The basic principle of mixture classification under CLP is shown in the green box below and in Figure 4.1.2 which is also explained in the text below the box.

Annex I: *4.1.3.2* *The approach for classification of aquatic environmental hazards is tiered, and is dependent upon the type of information available for the mixture itself and for its components. Figure 4.1.2 outlines the process to be followed.*

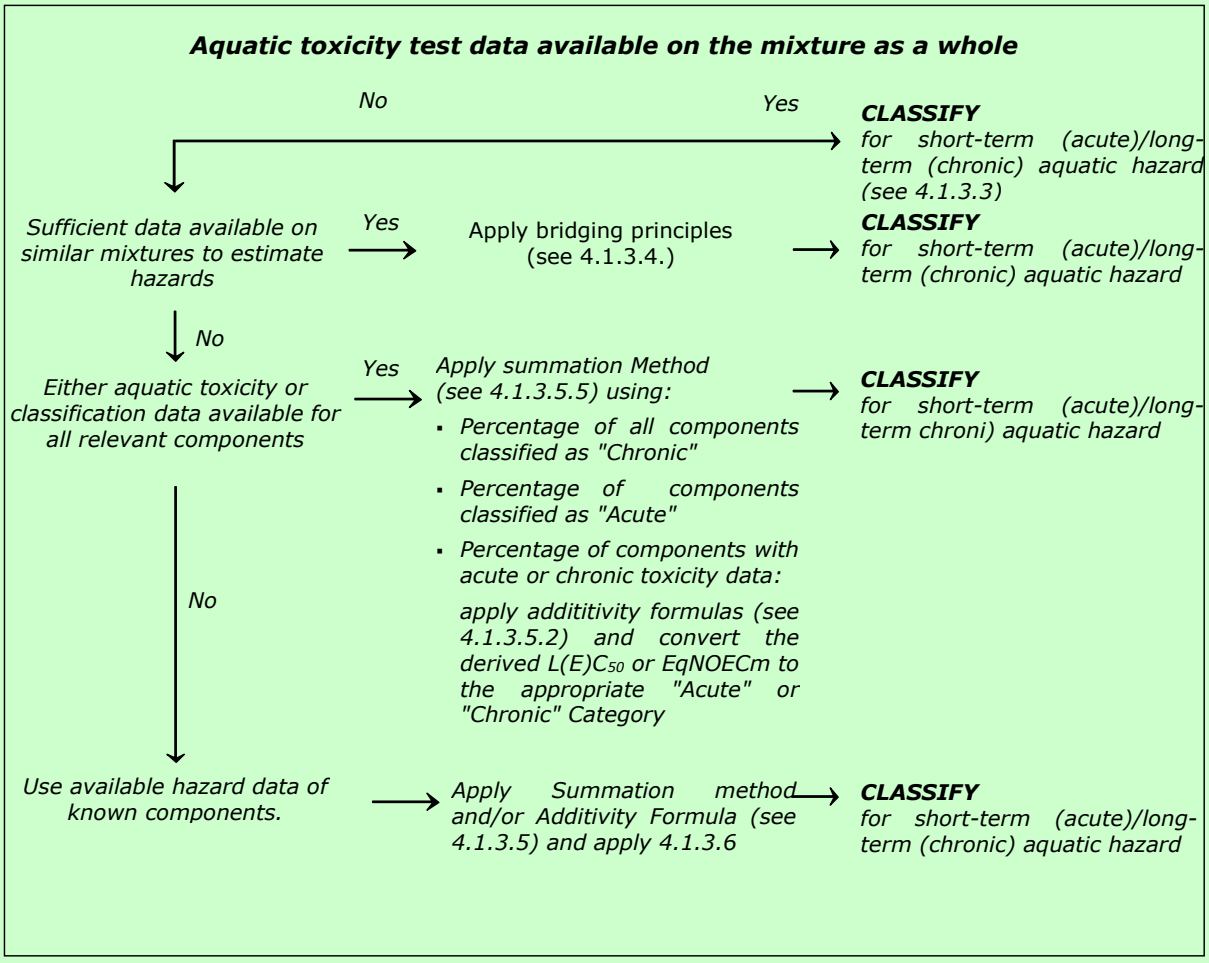
Elements of the tiered approach include:

classification based on tested mixtures;

classification based on bridging principles;

the use of "summation of classified components" and/or an "additivity formula".

Figure 4.1.2
Tiered approach to classification of mixtures for short-term (acute) and long-term (chronic) aquatic environmental hazards



Explanation of Figure 4.1.2:

- Horizontal arrow in first row: In some cases, particularly where specific and valid test data are already available on the mixture, there is a general obligation to use these data on the mixture itself for classification purposes. Valid data must normally then be available on each of fish, crustacea and algae or other aquatic plants, unless a decision to classify in the most stringent category(ies) (Acute 1 and/or Chronic 1) can be made without a full dataset (see Section 4.1.4.3 of this document).
- Horizontal arrows in second row: In other cases, sufficient data may be available on similar tested mixtures to estimate hazards using the bridging principles (see Section 4.1.4.4 of this document).
- Horizontal arrows in third row: In general, however, where either aquatic toxicity or classification data are available for all relevant components of a mixture the aquatic hazard classification shall be made through the identification of the hazards of the respective components in a first step, and then in a second step through the summation of the quantities of these hazardous components, applying the summation method (see Section 4.1.4.5 of this document). When doing so:
    - The percentage of all components classified as Acute 1 and/or Chronic 1, 2, 3 & 4 is fed straight into the summation method (for relevant components see point 4.1.3.1 of Annex I to CLP);
    - For the percentage of the other components with acute or long-term toxicity data, the additivity formulas (see point 4.1.3.5.2 of Annex I to CLP) may be applied. The derived L(E)C_{50} or EqNOECm is converted to the appropriate "Acute" or "Chronic" Category and then, in a second step, fed into the summation method.[83]
- Horizontal arrows in fourth (last) row: Use available hazard data of known components.
    - This applies to mixtures containing unknown components and/or known components, for which neither toxicity data nor classifications are known. In these cases, apply the additional statement on the label and in the safety data sheet: "*Contains x % of components with unknown hazards to the aquatic environment*" (see the green box below). For classification based on the known part of the mixture, use the Summation Method and/or the Additivity Formula (see Section 4.1.4.5 of this document).

Annex I: 4*.1.3.6.1* *In the event that no useable information on short-term (acute) and/or long-term (chronic) aquatic hazard is available for one or more relevant components, it is concluded that the mixture cannot be attributed to one or more definitive hazard category(ies). 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: "Contains x % of components with unknown hazards to the aquatic environment".*

4.1.4.2. Information requirements

Before a classification can be made, available information on toxicity of the mixture as a whole as well as all the available information on the composition of the mixture and the hazard category of relevant components (substances) should be gathered. Note that manufacturers, importers or downstream users are not requested by the CLP Regulation to generate new data for determining the aquatic hazard classification of the mixture. Rather the supplier should be

[83] As manufacturers and importers are obliged to classify all substances placed on the market within the EU, the summation method can usually be directly applied and the additivity formula will be of limited application.

contacted if it is considered that the information on the substance or mixture supplied is not sufficient for classification purposes.

Generally, therefore, the constituent substance classifications should be used as the basis for derivation of the correct hazard classification of the final mixture (see also Section 1.6.4 of this guidance document).

Article 11 of the CLP-Regulation refers to cut-off values. These values are the minimum concentrations for a substance to be taken into account for classification purposes. The substances meeting these criteria are relevant ingredients or relevant components. When a classified substance is present in a concentration above the generic cut-off value it contributes to the mixture classification even if it may not trigger classification of the mixture directly.

Annex I: ***4.1.3.1.*** *The classification system for mixtures covers all classification categories which are used for substances, i.e. categories Acute 1 and Chronic 1 to 4. In order to make use of all available data for purposes of classifying the aquatic environmental hazards of the mixture, the following is applied where appropriate:*

The "relevant components" of a mixture are those which are classified "Acute 1"or "Chronic 1" and present in a concentration of 0.1 % (w/w) or greater, and those which are classified "Chronic 2", "Chronic 3" or "Chronic 4" and present in a concentration of 1 % (w/w) or greater, unless there is a presumption (such as in the case of highly toxic components (see 4.1.3.5.5.5)) that a component present in a lower concentration can still be relevant for classifying the mixture for aquatic environmental hazards. Generally, for substances classified as "Acute 1" or "Chronic 1" the concentration to be taken into account is (0.1/M) %.
(For explanation M-factor see 4.1.3.5.5.5).

For aquatic hazards the cut-off values are further addressed under point 1.1.2.2.2 (b) of Annex I to CLP. The calculation referred to in point (b)(i) of that point, is found in point 4.1.3.1 of Annex I to CLP (see the green box above).

This signals that highly toxic components will need to be considered at lower levels than the generic cut-off values, and this applies to any substance to which an M-factor greater than 1 has been assigned (see Section 4.1.4.5 of this document).

Note that generic concentration limits (GCLs) should be given in weight percentages except for certain gaseous mixtures where they may be best described in volume percentage, e.g. a single hazardous component in an inert diluent, e.g. nitrogen or helium.

When the information on the mixture has been gathered and validated, the following guidance should be followed depending on the type and level of information available.

4.1.4.3. Classification criteria for mixtures hazardous to the aquatic environment based on test data on the mixture as a whole

The testing of a mixture for aquatic toxicity is highly complex, both in terms of the conduct of the test, and in the interpretation of data from such testing. The different physico-chemical properties, such as water solubility, vapour pressure, and adsorption, make it almost impossible to prepare an exposure concentration that is characteristic of the mixture, while the multi-component analysis needed to verify such an exposure concentration is both complex and expensive.

Therefore, before any such new testing is conducted, alternative approaches such as the summation method, should be considered, particularly where testing would involve the use of vertebrate animals such as fish (see also Section 1.1.6.2 of this document). Nevertheless, there are circumstances where test data may already be available, and should then be examined to assess its relevance for the purposes of classification. Data which has been prepared for Regulatory use in compliance with standard guidelines, such as test data on plant protection or

biocidal products, may be considered as acceptable for classification. Where such valid test data, both acute and chronic, are available, they may be used in accordance with the general guidance below.

Annex I: ***4.1.3.3.1*** *When the mixture as a whole has been tested to determine its aquatic toxicity, this information can be used for classifying the mixture according to the criteria that have been agreed for substances. The classification is normally based on the data for fish, crustacea and algae/plants (see sections 4.1.2.7.1 and 4.1.2.7.2). When adequate acute or chronic toxicity data for the mixture as a whole are lacking, "bridging principles" or "summation method" should be applied (see sections 4.1.3.4 and 4.1.3.5).*

4.1.3.3.2 *The long-term (chronic) hazard classification of mixtures requires additional information on degradability and in certain cases bioaccumulation. Degradability and bioaccumulation tests for mixtures are not used as they are usually difficult to interpret, and such tests may be meaningful only for single substances.*

4.1.3.3.3 *Classification for category Acute 1*

(a) When there are adequate acute toxicity test data (LC_{50} or EC_{50}) available for the mixture as a whole showing $L(E)C_{50} \leq 1$ mg/l:

Classify mixture as Acute 1 in accordance with point (a) of Table 4.1.0.

(b) When there are acute toxicity test data (LC_{50}(s) or EC_{50}(s)) available for the mixture as a whole showing $L(E)C_{50}$(s) >1 mg/l for normally all trophic levels:

No need to classify for short-term (acute) hazard.

4.1.3.3.4 *Classification for categories Chronic 1, 2 and 3*

(a) When there are adequate chronic toxicity data (EC_x or NOEC) available for the mixture as a whole showing EC_x or NOEC of the tested mixture ≤ 1mg/l:

(i) Classify the mixture as Chronic 1, 2 or 3 in accordance with point (b)(ii) of Table 4.1.0. as rapidly degradable if the available information allows the conclusion that all relevant components of the mixture are rapidly degradable;

(ii) Classify the mixture as Chronic 1 or 2 in all other cases in accordance with point (b)(i) of Table 4.1.0. as non-rapidly degradable;

(b) When there are adequate chronic toxicity data (ECx or NOEC) available for the mixture as a whole showing ECx(s) or NOEC(s) of the tested mixture > 1 mg/l for normally all trophic levels:

No need to classify for long-term (chronic) hazard in categories Chronic 1, 2 or 3.

4.1.3.3.5 *Classification for category Chronic 4*

If there are nevertheless reasons for concern:

Classify the mixture as Chronic 4 (safety net classification) in accordance with Table 4.1.0.

Where a classification is made based on test data, valid data should normally be available on each of fish, crustacea and algae or other aquatic plants, unless a decision to classify in the most stringent category(ies) (Acute 1 and/or Chronic 1) can be made without a full dataset. To be valid, it would normally be necessary to show that the tested organism has been exposed to the toxic components of the mixture in proportion to the composition of the mixture, and that this exposure has been maintained for the duration of the test. If this cannot be accomplished the classification should be based on information on the individual components. It is insufficient to simply prepare a water-accommodated fraction (WAF) for testing.

When there is adequate toxicity test data available for the mixture as a whole, this may be simplified to two basic rules for each of acute and long-term hazard classification:

Classification for acute (short-term) aquatic hazard:

i. If the lowest valid acute/short-term L(E)C_{50} is ≤ 1 mg/l, classify as Acute 1.

ii. If valid acute/short-term test data are available on fish, crustacea and algae/aquatic plants (i.e. all three trophic levels), and all showing L(E)C_{50} > 1 mg/l, there is no need to classify for acute aquatic hazard.

Classification for long-term aquatic hazard:

i. If the lowest valid chronic toxicity test data (NOEC or EC_x) is ≤ 1 mg/l, classify as Chronic 1, 2 or 3, depending on the information on components degradability, e.g. if all components are known to be rapidly degradable.

ii. If valid chronic toxicity test data are available on fish, crustacea and algae/aquatic plants (i.e. all three trophic levels), and all showing NOEC or EC_x >1 mg/l, there is no need to classify for long-term aquatic hazard in Chronic 1, 2 or 3.

4.1.4.4. When experimental aquatic toxicity data are not available for the complete mixture: bridging principles

Annex I: ***4.1.3.4.1*** *Where the mixture itself has not been tested to determine its aquatic environmental hazard, but there are sufficient data on the individual components and similar tested mixtures to adequately characterise the hazards of the mixture, this data shall be used in accordance with the bridging rules set out in Section 1.1.3. However, in relation to application of the bridging rule for dilution, sections 4.1.3.4.2 and 4.1.3.4.3 shall be used.*

4.1.3.4.2 *Dilution: if a mixture is formed by diluting another tested mixture or a substance classified for its aquatic environmental hazard with a diluent which has an equivalent or lower aquatic hazard classification than the least toxic original component and which is not expected to affect the aquatic hazards of other components, then the resulting mixture may be classified as equivalent to the original tested mixture or substance. Alternatively, the method explained in section 4.1.3.5 may be applied.*

4.1.3.4.3 *If a mixture is formed by diluting another classified mixture or substance with water or other totally non-toxic material, the toxicity of the mixture can be calculated from the original mixture or substance.*

For circumstances where no or inadequate test data are available on the mixture itself, the classification of a mixture may be determined based on sufficient data for individual components of the mixture and on another similar tested mixture by an appropriate application of any of the specified 'bridging principles'. The identified relevant information needs to be evaluated for the purpose of classification, by comparing it with the criteria in point 1.1.3 of Annex I to CLP. Those rules allow characterisation of the hazards of the mixture without performing tests on it, but rather by building on the available information on similar tested mixtures (see also Part 1, Section 1.6.3.2 of this guidance document).

4.1.4.5. When hazard data (information on toxicity or classification) are available for all the components of the mixture

Annex I: ***4.1.3.5.1*** *The classification of a mixture is based on summation of the classification of its components. The percentage of components classified as "Acute" or "Chronic" is fed straight in to the summation method. Details of the summation method are described in 4.1.3.5.5.*

Where no or inadequate test data on the mixture itself is available and the bridging principles are not applicable, the classification of the mixture is based on information on the components. The information that will most usually be available to aid classification of a mixture will be the

classification applied to the individual components (substances). These data and any associated M-factor(s) are included in the safety data sheets (SDS) and also in the Classification and Labelling Inventory (C&L Inventory) established and maintained by the Agency in the form of a database [http://echa.europa.eu/information-on-chemicals/cl-inventory-database]. In cases the aquatic hazard classification of a mixture will be made based on data on the components, it is therefore generally the summation of the quantities of the hazardous components that should be used to determine a specific hazard classification of the mixture.

Provided the classification data, in part or in total, and the % of these components in the mixture are known, a classification of the mixture can be made according to the summation method. The following text from CLP describes the application of this method.

Annex I*: 4.1.3.5.5* *Summation method*

4.1.3.5.5.1 *Rationale*

4.1.3.5.5.1.1 *In case of the substance classification categories Chronic 1 to Chronic 3, the underlying toxicity criteria differ by a factor of 10 in moving from one category to another. Substances with a classification in a high toxicity band therefore contribute to the classification of a mixture in a lower band. The calculation of these classification categories therefore needs to consider the contribution of any substance classified as Chronic 1, 2 or 3.*

4.1.3.5.5.2. *Classification procedure*

4.1.3.5.5.2.1 *In general a more severe classification for mixtures overrides a less severe classification, e.g. a classification with Chronic 1 overrides a classification with Chronic 2. As a consequence, in this example, the classification procedure is already completed if the result of the classification is Chronic 1. A more severe classification than Chronic 1 is not possible. Therefore it is not necessary to undergo the further classification procedure.*

4.1.3.5.5.3 *Classification for category Acute 1*

4.1.3.5.5.3.1 *First all components classified as Acute 1 are considered. If the sum of the concentrations (in %) of these components multiplied by their corresponding M-factors is greater than 25 % the whole mixture is classified as Acute 1.*

4.1.3.5.5.3.2 *The classification of mixtures for short-term (acute) hazards based on this summation of classified components is summarised in Table 4.1.1.*

Table 4.1.1
Classification of a mixture for short-term (acute) hazards, based on summation of classified components

Sum of components classified as:	*Mixture is classified as:*
Acute 1 × M ([a]) ≥ 25 %	*Acute 1*

(a) For explanation of the M-factor see 4.1.3.5.5.5

4.1.3.5.5.4 *Classification for the categories Chronic 1, 2, 3 and 4*

4.1.3.5.5.4.1 *First all components classified as Chronic 1 are considered. If the sum of the concentrations (in %) of these components multiplied by their corresponding M-factors is equal to or greater than 25 %, the mixture is classified as Chronic 1. If the result of the calculation is a classification of the mixture as Chronic 1, the classification procedure is completed.*

4.1.3.5.5.4.2 *In cases where the mixture is not classified as Chronic 1, classification of the mixture as Chronic 2 is considered. A mixture is classified as Chronic 2 if 10 times the sum of the concentrations (in %) of all components classified as Chronic 1 multiplied by their corresponding M-factors plus the sum of the concentrations (in %) of all components classified as Chronic 2 is equal to or greater than 25 %. If the result of the calculation is classification of the mixture as Chronic 2, the classification process is completed.*

4.1.3.5.5.4.3 *In cases where the mixture is not classified either as Chronic 1 or Chronic 2, classification of the mixture as Chronic 3 is considered. A mixture is classified as Chronic 3 if 100 times the sum of the concentrations (in %) of all components classified as Chronic 1 multiplied by their corresponding M-factors plus 10 times the sum of the concentrations (in %) of all components classified with Chronic 2 plus the sum of the concentrations (in %) of all components classified as Chronic 3 is ≥ 25 %.*

4.1.3.5.5.4.4 *If the mixture is still not classified in Chronic 1, 2 or 3, classification of the mixture as Chronic 4 shall be considered. A mixture is classified as Chronic 4 if the sum of the concentrations (in %) of components classified as Chronic 1, 2, 3 and 4 is equal to or greater than 25 %.*

4.1.3.5.5.4.5 *The classification of mixtures for long-term (chronic) hazards, based on this summation of the concentrations of classified components, is summarised in Table 4.1.2.*

Table 4.1.2
Classification of a mixture for long-term (chronic) hazards, based on summation of the concentrations of classified components

Sum of components classified as:	*Mixture is classified as:*
Chronic 1 × M (a) ≥ 25 %	*Chronic 1*
(M × 10 × Chronic 1) + Chronic 2 ≥ 25 %	*Chronic 2*
(M × 100 × Chronic 1) + (10 × Chronic 2) + Chronic 3 ≥ 25 %	*Chronic 3*
Chronic 1 + Chronic 2 + Chronic 3 + Chronic 4 ≥ 25 %	*Chronic 4*

(a) For explanation of the M-factor, see 4.1.3.5.5.5

4.1.3.5.5.1.2 *When a mixture contains components classified as Acute 1 or Chronic 1, attention must be paid to the fact that such components, when their acute toxicity is below 1 mg/l and/or chronic toxicity is below 0,1 mg/l (if non-rapidly degradable) and 0.01 mg/l (if rapidly degradable) contribute to the toxicity of the mixture even at a low concentration. Active ingredients in pesticides often possess such high aquatic toxicity but also some other substances like organometallic compounds. Under these circumstances the application of the normal generic concentration limits leads to an "under-classification" of the mixture. Therefore, multiplying factors shall be applied to account for highly toxic components, as described in section 4.1.3.5.5.5.*

For those components for which only toxicity data are available the additivity formulas offer a way for estimating what the toxicity of a mixture would be if the individual substance toxicities could be 'added' to each other in a straightforward way. Thus it assumes a similar 'mode of action' for each component.

To make full use of this approach access to the whole aquatic toxicity dataset and the necessary knowledge to select the best and most appropriate data is required. Clearly, the best use would be to add up separately each of the fish toxicity data, the crustacean toxicity data and the algae/aquatic plants toxicity data to derive a specific toxicity value for each trophic level. The lowest of the toxicity values would normally be used to define the appropriate hazard category for the mixture. Indeed, if it is only possible to characterise part of the mixture in this way, that part can be assigned a hazard category (and an M-factor for categories Acute 1 and/or Chronic 1) and then, in a second step, be used in the summation method.

The use of the additivity formulae is limited to those circumstances where the substance hazard category is not known. The following text from CLP describes the application of the additivity formulae.

Annex I: ***4.1.3.5.2*** *Mixtures can be made of a combination of both components that are classified (as Acute 1 and/or Chronic 1, 2, 3, 4) and others for which adequate toxicity test data is available. When adequate toxicity data are available for more than one component in the mixture, the combined toxicity of those components is calculated using the following additivity formulas (a) or (b), depending on the nature of the toxicity data:*

(a) Based on acute aquatic toxicity:

$$\frac{\sum \mathrm{Ci}}{\mathrm{L(E)C_{50m}}} = \sum_{\eta} \frac{\mathrm{Ci}}{\mathrm{L(E)C_{50i}}}$$

where:

C_i = concentration of component i (weight percentage);

$L(E)C_{50\,i}$ = (mg/l) LC_{50} or EC_{50} for component i;

η = number of components, and i is running from 1 to n;

$L(E)C_{50\,m}$ = L(E) C_{50} of the part of the mixture with test data;

The calculated toxicity may be used to assign to that portion of the mixture a short-term (acute) hazard category which is then subsequently used in applying the summation method;

(b) Based on chronic aquatic toxicity:

$$\frac{\sum Ci + \sum Cj}{EqNOEC_m} = \sum_{n} \frac{Ci}{NOECi} + \sum_{n} \frac{Cj}{0{,}1 \times NOECj}$$

where:

C_i = concentration of component i (weight percentage) covering the rapidly degradable components;

Cj = concentration of component j (weight percentage) covering the non- rapidly degradable components;

$NOEC_i$ = NOEC (or other recognized measures for chronic toxicity) for component i covering the rapidly degradable components, in mg/l;

NOECj = NOEC (or other recognized measures for chronic toxicity) for component j covering the non-rapidly degradable components, in mg/l;

n = number of components, and i and j are running from 1 to n;

$EqNOEC_m$ = Equivalent NOEC of the part of the mixture with test data;

The equivalent toxicity thus reflects the fact that non-rapidly degrading substances are classified one hazard category level more "severe" than rapidly degrading substances.

The calculated equivalent toxicity may be used to assign that portion of the mixture a long-term (chronic) hazard category, in accordance with the criteria for rapidly degradable

substances (point (b)(ii) of Table 4.1.0.), which is then subsequently used in applying the summation method.

4.1.3.5.3. *When applying the additivity formula for part of the mixture, it is preferable to calculate the toxicity of this part of the mixture using for each substance toxicity values that relate to the same taxonomic group (i.e. fish, crustacean, algae or equivalent) and then to use the highest toxicity (lowest value) obtained (i.e. use the most sensitive of the three taxonomic groups). However, when toxicity data for each component are not available in the same taxonomic group, the toxicity value of each component is selected in the same manner that toxicity values are selected for the classification of substances, i.e. the higher toxicity (from the most sensitive test organism) is used. The calculated acute and chronic toxicity is then used to assess whether this part of the mixture shall be classified as Acute 1 and/or Chronic 1, 2 or 3 using the same criteria described for substances.*

Note that generic concentration limits (GCLs) should be given in weight percentages except for certain gaseous mixtures where they may be best described in volume percentage, e.g. a single hazardous component in an inert diluent, e.g. nitrogen or helium.

NOTICE: With the aquatic toxicity data at hand the ingredient substance classification and M-factor(s) could easily be gained by a direct comparison with the substance criteria, which then could be fed straight into the summation method. It will therefore usually not be necessary to use the additivity formulae.

4.1.4.6. When hazard data (information on toxicity or classification) are available for only some components of the mixture

This section is related to Figure 4.1.1 where one can decide to apply the summation method and/or the additivity formulae (see point 4.1.3.5 of Annex I to CLP) and apply point 4.1.3.6 of Annex I to CLP.

Use available hazard data of known components.

- This applies to mixtures containing unknown components and/or known components, for which neither toxicity data nor classifications are known. In these cases, for labelling purposes consider the provisions of point 4.1.3.6 in Annex I to CLP. For classification based on the known part of the mixture, use the summation method and/or the additivity formula (see point 4.1.3.5 of Annex I to CLP).
- NOTE: If a mixture is classified in more than one way, the method yielding the most stringent result should be used.

4.1.4.7. Decision on classification: examples for mixtures

If the evaluation shows that the criteria are fulfilled, one category for acute aquatic hazard and/or one category for long-term aquatic hazards should be assigned. For the labelling elements, such as: hazard pictograms, signal words, hazard statements and precautionary statements, see Section 4.1.6.

List of the examples on mixtures classification included in this section:

The classification system for mixtures is complex as different methods are available. Which method to use is dependent upon the type of information available.

- Example A: When classification data are available for some or all components of a mixture: straightforward application of the summation method.
- Example B1: When toxicity test data on the mixture as a whole are available for all three trophic levels: classification based on test data on the mixture.
- Example B2: When information on the classification of the components and test data on the mixture as a whole are available for some, but not all three trophic levels: classification based on the summation method.
- Example C: When no data are available on the mixture as a whole and its components, but test data are available on a similar tested mixture: use of the bridging principles – dilution with water.
- Example D: When only test data are available for some, but not all components of the mixture: use of the additivity formulae and of the summation method.

4.1.4.7.1. Example A: When classification data are available for some or all components of a mixture: straightforward application of the summation method

INFORMATION ON INGREDIENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Astralamid	Acute 1	10	Chronic 1	10	1
Bastralamid	Acute 1	1	Chronic 2	-	3
Castralamid	Not classified	-	Chronic 2	-	10
Dastralamid	Not classified	-	Chronic 3	-	10
Estralamid	Not classified	-	Not classified	-	10
Festralamid	Not classified	-	Not classified	-	66

M = M-factor; C = Concentration

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Category Chronic 2

Reasoning:

- Valid test data on the mixture as a whole (for all three trophic levels) are not available.
- Valid test data on similar tested mixtures are not available, either, meaning that any bridging principle cannot be used.

Therefore, classification should be considered based on individual components using the summation method.

Acute aquatic hazard: Information on classification including associated M-factors and the % of the components in the mixture are available.

Classify for acute hazard if: $\sum$ (Acute 1 $\times$ M) $\geq$ 25%

Using the classification of the components of the mixture: (1 $\times$ 10) + (3 $\times$ 1) = 13 (which is < 25%). Hence, no classification for acute aquatic hazard.

Long-term aquatic hazard:

Step 1: Classify as Chronic 1 if: $\sum$ (Chronic 1 $\times$ M) $\geq$ 25% (if not, then go to Step 2).

Step 2: Classify as Chronic 2 if: $\sum$ (10 $\times$ Chronic 1 $\times$ M) + $\sum$ (Chronic 2) $\geq$ 25% (if not, then go to Step 3).

Step 3: Classify as Chronic 3 if: $\sum$ (100 $\times$ Chronic 1 $\times$ M) + $\sum$ (10 $\times$ Chronic 2) + $\sum$ (Chronic 3) $\geq$ 25% (if not, then go to Step 4).

Step 4: Classify as Chronic 4 if: $\sum$ (Chronic 1) + $\sum$ (Chronic 2) + $\sum$ (Chronic 3) + $\sum$ (Chronic 4) $\geq$ 25%

Using the classification of the components of the mixture:

Step 1: (1 × 10) = 10 (which is < 25% → Step 2).

Step 2: (10 × 1 × 10) + 3+10 = 113 (which is > 25%). Hence, classify as Category Chronic 2.

Labelling elements based on the classification:

Element	Aquatic hazard information that could appear on the label
GHS Pictogram	GHS09
Signal Word	-
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

4.1.4.7.2. Example B1: When toxicity data on the mixture as a whole is available for all three trophic levels: classification based on test data for the mixture

INFORMATION ON INGREDIENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Frusthrin	Acute 1	1	Chronic 1	1	40
Gladobrin	Acute 1	1	Chronic 3	-	60

M = M-factor; C = Concentration

Acute (short-term) aquatic toxicity	**Value**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Fish: Mixture (*Cyprinus carpio*)	19 mg/l (96 hr LC_{50})	C.1 / static, GLP
Crustacea: Mixture (*Daphnia magna*)	3.5 mg/l (48 hr EC_{50})	C.2 / static, GLP
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	15 mg/l (72 or 96 hr ErC_{50})	C.3 / static, GLP
Chronic (long-term) aquatic toxicity		
Fish: Mixture (*Cyprinus carpio*)	0.09 mg/l (12 d NOEC)	OECD 210 / Early Life Stage, flow through, GLP
Crustacea: Mixture (*Daphnia magna*)	0.05 mg/l (21 d NOEC)	C.20 / semi-static, GLP
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	1.5 mg/l (96 h NOEC)	C.3 / static, GLP

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Chronic 1.

Reasoning:

Acute aquatic hazard:

Valid test data for all the three trophic levels are available for the mixture as a whole, therefore no need to consider bridging principles or classification of individual components for acute hazard classification of the mixture. Test data showed that $L(E)C_{50}$ > 1 mg/L. Consequently - no classification for acute aquatic hazard.

Long-term aquatic hazard:

Valid test data for all the three trophic levels are available for the mixture as a whole, therefore no need to consider classification of individual components for long-term hazard classification of the mixture. Test data showed that NOEC < 0.1 mg/l. No information on rapid degradation. Hence, the mixture is considered being not rapidly degradable. The mixture is classified as category Chronic 1.

Labelling elements based on the classification:

Element	Aquatic hazard information that could appear on the label
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410
Precautionary statement(s)	P273, P391, P501

4.1.4.7.3. Example B2: When information on the classification of the components is available and toxicity data on the mixture as a whole is available for some, but not all three trophic levels: use of the summation method

INFORMATION ON COMPONENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Frusthrin	Acute 1	1	Chronic 1	1	40
Gladobrin	Acute 1	1	Chronic 3	-	60

M = M-factor; C = Concentration

Acute (short-term) aquatic toxicity	**Value**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	15 mg/l (72 or 96 hr ErC_{50})	C.3 / static, GLP
Chronic (long-term) aquatic toxicity		
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	1.5 mg/l (96 h NOEC)	C.3 / static, GLP

Aquatic hazard classification:

Acute aquatic hazard: Acute 1.

Long-term aquatic hazard: Chronic 1.

Reasoning:

- Valid test data on the mixture as a whole are available for one, but not for all the three trophic levels and we don't know if algae is clearly the most sensitve trophic level for the mixture.
- Neither is valid test data on similar tested mixtures available, meaning that the bridging principles could not be used.

Therefore, classification should for both acute hazard and long-term hazard be considered based on individual components using the summation method. Testing should not be conducted for the mixture for the remaining trophic levels.

Acute aquatic hazard:

Information on classification including associated M-factors and the % of the components in the mixture are available.

Classify for acute hazard if: Σ (Acute 1 $\times$ M) $\geq$ 25%

Using the classification of the components of the mixture: (40 × 1) + (60 × 1) = 100 (which is ≥ 25%). Hence - category Acute 1.

Long-term aquatic hazard:

Information on classification including associated M-factors and the % of the components in the mixture are available.

Step 1: Classify as Chronic 1 if: ∑ (Chronic 1 × M) ≥ 25% (if not, then go to Step 2).

Using the classification of the components of the mixture:

Step 1: (40 × 1) = 40 (which is ≥ 25%). Hence - Category Chronic 1.

Labelling elements based on the classification:

Element	Aquatic hazard information that could appear on the label
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[84]
Precautionary statement(s)	P273, P391, P501

[84] Note that in accordance with article 27 hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6.

4.1.4.7.4. Example C: When no data are available on the mixture as a whole and its components, but test data are available on a similar tested mixture: use of the bridging principles – dilution with water

Test Species	Information / Data
Fish	No data available
Crustacea	No data available
Algae	No data available

A reference mixture has shown a LC_{50} of 0.5 mg/l and adequate NOECs in the range 0.07 to < 0.1 mg/L. Based on this data it has been classified as Category Acute 1 and Category Chronic 1.

Subsequently, this mixture has been diluted in water by factor of 10 and the newly diluted mixture shall now be classified.

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Category Chronic 2.

Reasoning:

The mixture is formed by diluting another classified mixture with water, the toxicity of the mixture can therefore be calculated from the original mixture. (see Section 4.1.4.4 of this document and CLP Annex I, point 4.1.3.4.3.)

Acute aquatic hazard: LC_{50} = 5 mg/l (0.5x10). Hence - not classified.

Long-term aquatic hazard: Adequate NOECs in the range 0.7 to < 1 mg/l (0.07x10 and 0.1x10). Hence - category Chronic 2.

Labelling elements based on the classification:

Element	Aquatic hazard information that could appear on the label
GHS Pictogram	GHS09
Signal Word	-
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

4.1.4.7.5. Example D: When test data are available for some, but not all components of the mixture: use of the additivity formula and of the summation method

INFORMATION ON COMPONENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Component 1	-	-	-	-	50
Component 2	-	-	-	-	10
Component 3	-	-	-	-	10
Component 4	Not classified	-	Chronic 1	-	30

COMPONENT TOXICITY DATA			
Data elements	**Component 1 (50% of the mixture)**	**Component 2 (10% of the mixture)**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Physico-chemical properties			
Water solubility (S_w):	200 mg/l	1000 mg/l	A.6 / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):	No data	No data	
Acute (short-term) aquatic toxicity			
Fish: *Oncorhynchus mykiss*	No data	0.3 mg/l (96 hr LC_{50})	C.1 / static, GLP
Crustacea: *Daphnia magna*	0.55 mg/l (48 hr EC_{50})	No data	C.2 / static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus*	0.37 mg/l (72 hr E_rC_{50})	1.37 mg/l (72 hr E_rC_{50})	C.3 / static, GLP
Long-term aquatic toxicity			
Fish: *Oncorhynchus mykiss*	0.07 mg/l (28 d NOEC)	1.3 mg/l (28 d NOEC)	OECD 210 / semi-static
Crustacea: *Daphnia magna*	0.09 mg/l (21 d NOEC)	1.4 mg/l (21 d NOEC)	C.20 / semi-static, GLP

Algae/aquatic plants: *Scenedesmus subspicatus*	0.13 mg/L (72 hr NOEC)	0.53 mg/L (72 hr NOEC)	C.3 / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation (% degradation in 28 days (or, if absent, half-life in water (d)):	No data	No data	
Abiotic degradation (Hydrolysis) (half-life (d)):	No data	No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):	No data	No data	

Chronic classification is known for 30% of the mixture.

Test data is available for 60% of the mixture.

For 10% of the mixture no information is available.

Aquatic hazard classification:

Acute aquatic hazard: Category Acute 1.

Long-term aquatic hazard: Category Chronic 1.

Reasoning:

- Valid test data on the mixture as a whole (for all three trophic levels) are not available.
- Valid test data on similar tested mixtures are not available, either, meaning that any bridging principle cannot be used.

Therefore, classification should be considered based on individual components using the summation method.

NOTICE! In the case of the downstream user or importer not having the classification of all the components, further dialogue with the supplier may be necessary to obtain additional information. The suppliers in a supply chain shall cooperate to meet the requirements for classification, labelling and packaging (see CLP Article 4(9)). This particular example, however, shows what could be done if the classification of some components in any case is not available (which, for example, could be the case when importing certain mixtures).

Acute aquatic hazard:

For component 1 the most sensitive species showed a L(E)C_{50} 0.37mg/l. Thus, component 1, comprising 50% of the mixture, is classified as Acute 1; M factor 1.

Subsequently used in the summation method, more than 25% of the mixture is classified as category Acute 1. Hence, the mixture is classified as Acute 1.

Alternatively: You can calculate the combined toxicity for components 1 and 2 applying the *Additivity Formula*[85]:

$L(E)C_{50m}$ = 60 / (50/0.37 mg/L + 10/0.3mg/L) = 0.36 mg/L

Assign category Acute 1. This means that 60% of this mixture is classified as category Acute 1 and hence, subsequently used in the summation method, the whole mixture is classified as Acute 1.

Long-term aquatic hazard:

Assign hazard categories for each component for which there are adequate chronic toxicity data available:

	Relevant information	Category	C (%)
Component 1	0.07 mg/L (28 d NOEC Fish); No information on degradation. Hence, the substance is considered not rapidly degradable.	Assign Chronic 1, M factor 1	50 %
Component 2	0.53 mg/L (72 hr NOEC Algae); No information on degradation	Assign Chronic 2	10%
Component 3	No data	-	10%
Component 4	Not classified	Chronic 1	30 %

More than 25% of the mixture is classified as category Chronic 1 and thus, the mixture is classified as category Chronic 1.

Alternatively: You can apply the *Additivity Formula*[86] to calculate the combined toxicity for components 1 and 2 (60% of the mixture)

$EqNOEC_m$ = 60 / (50/(0.1 x 0.07) + 10/(0.1 x 1.3)) = 0.008 mg/l for fish

$EqNOEC_m$ = 60 / (50/(0.1 x 0.09)) + 10/(0.1 x 1.4)) = 0.011 mg/l for crustaceans

$EqNOEC_m$ = 60 / (50/(0.1 x 0.13) + 10/(0.1 x 0.53)) = 0.015 mg/l for algae

The lowest calculated $EqNOEC_m$ is 0.008 mg/l.

Apply CLP, Annex I, point (b) (ii) of Table 4.1.0. Assign category Chronic 1, M factor 10 to that part of the mixture.

In addition component 4 of the mixture is classified as category Chronic 1 and comprises 10% of the mixture.

The long-term hazard category assigned to that part of the mixture the mixture is then subsequently used in applying the summation method:

Classify as Chronic 1 if: Σ (Chronic 1 $\times$ M) $\geq$ 25%

[85] In many cases it is possible to use the summation method straight away by assigning hazard categories to single components of a mixture when data is available.

[86] See also Section 4.1.4.6 of this guidance.

$\Sigma (60 \times 10) + 10 = 70$

Thus, the mixture is classified as category Chronic 1.

Labelling elements based on the classification:

Element	Aquatic hazard information that could appear on the label
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410[87]
Precautionary statement(s)	P273, P391, P501

In the SDS and on the label it has to be stated: 'Contains 10% of components with unknown hazards to the aquatic environment'.

[87] Note that in accordance with CLP Article 27, the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

4.1.5. Metal and metal compounds

4.1.2.10. *Inorganic compounds and metals*

4.1.2.10.1. *For inorganic compounds and metals, the concept of degradability as applied to organic compounds has limited or no meaning. Rather, such substances may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally the use of bioaccumulation data shall be treated with care([1]).*

4.1.2.10.1. *Poorly soluble inorganic compounds and metals may be acutely or chronically toxic in the aquatic environment depending on the intrinsic toxicity of the bioavailable inorganic species and the rate and amount of this species which enter solution. All evidence must be weighed in a classification decision. This would be especially true for metals showing borderline results in the Transformation/Dissolution Protocol.*

_____

([1]) Specific guidance has been issued by the European Chemicals Agency on how these data for such substances may be used in meeting the requirements of the classification criteria.

Annex IV provides the detailed guidance on the classification of metals and metal compounds.

The guidance on classification of alloys and complex metal containing materials is limited so far. More guidance is needed (see also Annex IV.5.5).

4.1.6. Hazard communication for hazards to the aquatic environment

A substance or mixture classified as hazardous and contained in packaging shall bear a label in accordance with the rules in Title III of CLP. The elements to be included in labels should be specified in accordance with the hazard pictograms, signal words, hazard statements and precautionary statements which form the core information of the CLP system. For general guidance on labelling see the *Introductory Guidance on the CLP Regulation (ECHA, 2009)* and also the *Guidance on Labelling and Packaging in accordance with Regulation (EC) No 1272/2008 (ECHA, 2011).*

Label elements shall be used for substances or mixtures meeting the criteria for classification in the hazard class *Hazardous to the Aquatic Environment* in accordance with Table 4.1.4 of Annex I to CLP.

Pictogram

The hazard pictogram shall satisfy the provisions of Annex V and Annex I, part 1.2 to the Regulation.

Symbol: *Environment*; Pictogram Code: *GHS09*

The pictogram GHS09 is required only for substances or mixtures classified as:

- Acute hazard category 1 and/or
- Long-term hazard categories 1 or 2

Signal word

The label shall include the relevant signal word in accordance with the classification of the hazardous substance or mixture. The signal word relevant for the hazard class *Hazardous to the Aquatic Environment* is:

WARNING

Signal Word Code: *Wng*

The signal word 'Warning' is required only for substances or mixtures classified as:

- Acute 1 and/or
- Chronic 1

Where the signal word 'Danger' is used on the label due to classification into another hazard class(es), the signal word 'Warning' shall not appear on the label.

Hazard statements

The label shall include the relevant hazard statements in accordance with the classification of the hazardous substance or mixture and shall be worded in accordance with Annex III to CLP.

The hazard statements (and the Hazard statement Codes) relevant for the hazard class *Hazardous to the Aquatic Environment* are:

- Very toxic to aquatic life (H400)
- Very toxic to aquatic life with long lasting effects (H410)
- Toxic to aquatic life with long lasting effects (H411)
- Harmful to aquatic life with long lasting effects (H412)
- May cause long lasting harmful effects to aquatic life (H413)

The hazard statement H400 is required only for substances or mixtures classified as:

- Acute 1

The hazard statements H410 to H413 are respectively required for substances or mixtures classified as:

- Chronic 1, 2, 3 or 4

Article 27 of CLP states that if a substance or mixture is classified within several hazard classes or differentiations of a hazard class, all hazard statements resulting from the classification shall appear on the label, unless there is evident duplication or redundancy.

This means that in line with Part 1 of Annex III to CLP, where the hazard statement H410 is used on the label due to classification in the long-term hazard category Chronic 1, the hazard statement H400 does not need to appear on the label. Furthermore, where a substance or a mixture is classified both in acute and long-term hazard categories, it is possible to use only hazard statement H410 on the label (see Table 4.1).

Table 4.1 Hazard statement Codes relevant for the hazard class Hazardous to the Aquatic Environment

Aquatic hazard classification	Associated hazard statement	Associated hazard statement that could appear on the label
Acute 1	H400	H400
Acute 1 and Chronic 1	H400; H410	H410
Acute 1 and Chronic 2	H400; H411	H410
Acute 1 and Chronic 3	H400; H412	H410
Acute 1 and Chronic 4[88]	H400; H413	H410
Chronic 1	H410	H410
Chronic 2	H411	H411
Chronic 3	H412	H412
Chronic 4	H413	H413

Precautionary statements

In accordance with CLP Articles 17 and 22 the label shall include the relevant precautionary statements. The precautionary statements that can in principle be used for the hazard class *Hazardous to the Aquatic Environment* are:

- Avoid release to the environment (P273)
- Collect spillage (P391)
- Dispose of contents/container to … (P501)

4.1.7. Re-classification of substances and mixtures classified as hazardous to the aquatic environment according to DSD/DPD

For the re-classification of substances and mixtures with regard to their hazards to the aquatic environment, a supplier has to apply the classification criteria specified in Annex I, part 4 of CLP. For this reason, all available information shall be re-evaluated in order to apply the criteria, as stated in CLP, accordingly. It is not suggested that new testing should be performed, but instead, available information should be evaluated for its relevance and reliability.

Besides the fact that M-factors need to be established for Acute 1 and Chronic 1 classifications, a direct translation of classification from the DSD/DPD to CLP can only be done in absence of chronic toxicity data. But also then, the translation for substances is not straightforward in all cases, for example:

[88] Please note that this combined classification only applies for mixtures.

Differences between the CLP classification and the DSD classification of substances to which R53 - alone or in combination with R50, R51 or R52 - is applied. This is based on the slightly different criteria for classification, in particular higher cut-off values for logKow (i.e. 4 in CLP compared to 3 in DSD) and BCF (i.e. 500 in CLP compared to 100 in DSD). That means that only for those substances for which adequate chronic toxicity data is not available, for which the long-term aquatic hazard classification is based on a combination of acute toxicity data and bioaccumulation data (without data on rapid biodegradability affecting classification) and to which the currently applied R53 is based exclusively on a BCF between 100 and 500 or a $\log K_{ow}$ between 3 and 4 the classification would be subject to re-consideration.

4.1.8. References

European Communities, 2003: *Technical guidance Document on Risk Assessment*. Part II. European Commission, Joint Research Centre

OECD 2000: *Series on Testing and Assessment Number 23, Guidance Document on aquatic toxicity Testing of difficult substances and mixtures*. ENV/JM/MONO(2000)6

OECD 2006: *Series on Testing and Assessment Number 54, Current approaches in the statistical analysis of ecotoxicity data: a guidance to application*. ENV/JM/MONO(2006)18

5. PART 5: ADDITIONAL HAZARDS

5.1. HAZARDOUS TO THE OZONE LAYER

The criteria chapter for classification and labelling of substances and mixtures hazardous to the ozone layer are short and the need for guidance is limited to the actual ODP-value that would trigger classification for a substance.

Annex I:

5.1.2 Classification criteria for substances

5.1.2.1. A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

5.1.3 Classification criteria for mixtures

5.1.3.1. Mixtures shall be classified as Hazardous to the Ozone Layer (Category 1) on the basis of the individual concentration of the substance(s) contained therein that are also classified as Hazardous to the Ozone Layer (Category 1), in accordance with Table 5.1.

Any substances having an Ozone Depleting Potential (ODP) greater or equal to the lowest ODP (i.e. 0.005) of the substances currently listed in Annex I to Regulation (EC) No 1005/2009[89] should be classified as hazardous to the ozone layer (category 1).

[89] Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

ANNEXES

I ANNEX I: AQUATIC TOXICITY

I.1 Introduction

The basis for the identification of a hazard to the aquatic environment for a substance is the aquatic toxicity of that substance. Classification is predicated on having toxicity data for fish, crustacea, and algae/aquatic plant available. These taxa are generally accepted as representative of aquatic fauna and flora for hazard identification. Data on these particular taxa are more likely to be found because of this general acceptance by regulatory authorities and the chemical industry. Other information on the degradation and bioaccumulation behaviour is used to better delineate the aquatic hazard. This section describes the appropriate tests for ecotoxicity, provides some basic concepts in evaluating the data and using combinations of testing results for classification. Further detailed guidance is given in the Integrated Testing Strategy (ITS) for aquatic toxicity for the substance (IR&CSA (R.7a) Chapters 7.8.3 – 7.8.5).

I.2 Description of tests

For classifying substances in the harmonised system, freshwater and marine species toxicity data can be considered as equivalent data. It should be noted that some types of substances, e.g. ionisable organic chemicals or organometallic substances may express different toxicities in freshwater and marine environments. Since the purpose of classification is to characterise hazard in the aquatic environment, the result showing the highest toxicity should normally be chosen. However, there are circumstances where a weight of evidence approach is appropriate.

The criteria for determining aquatic hazards should be test method neutral, allowing different approaches as long as they are scientifically sound and validated according to international procedures and criteria already referred to in existing systems for the hazard of concern and produce mutually acceptable data. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in Section 1 of Annex XI to the REACH Regulation (EC) No 1907/2006.

According to the proposed system (OECD 1998):

"Acute toxicity would normally be determined using a fish 96 hour LC_{50} (OECD Test Guideline 203 or equivalent), a crustacea species 48 hour EC_{50} (OECD Test Guideline 202 or equivalent) and/or an algal species 72 or 96 hour EC_{50} (OECD Test Guideline 201 or equivalent). These species are considered as surrogate for all aquatic organisms and data on other species such as the duckweed Lemna may also be considered if the test methodology is suitable."

Chronic testing involves an exposure that covers a significant period of time when compared to the organism´s life cycle. The term can signify periods from days to a year, or more depending on the reproductive cycle of the aquatic organism. Chronic tests can be done to assess certain information relating to growth, survival, reproduction and development.

"*Chronic toxicity data are less available than acute data and the range of testing procedures less standardised. Data generated according to the OECD Test Guidelines 210 (Fish Early Life Stage), 202 Part 2 or 211 (Daphnia Reproduction) and 201 (Algal Growth Inhibition) or equivalent can be accepted. Other validated and internationally accepted tests could also be used. The NOECs or other equivalent EC_x should be used."*

It should be noted that several of the test guidelines cited as examples for classification are being revised or are being planned for updating. Such revisions may lead to minor modifications of test conditions. Therefore, the expert group that developed the harmonised criteria for

classification intended some flexibility in test duration and/or species and number of animals used.

Guidelines for conducting acceptable tests with fish, crustacea, and algae can be found in many sources (Test Methods Regulation 440/2008; OECD e.g. the OECD monograph No.11, Detailed Review Paper on Aquatic Toxicity Testing for Industrial Chemicals and Pesticides, 1999; EPA, 1996; ASTM, 1999; ISO EU).

I.2.1 Fish tests

I.2.1.1 Acute testing

Acute tests are generally performed with young juveniles 0.1 – 5 g in size for a period of 96 hours. The observational endpoint in these tests is mortality. Fish larger than this range and/or durations shorter than 96 hours are generally less sensitive. However, for classification, they could be used if no acceptable data with the smaller fish for 96 hours are available or the results of these tests with different size fish or test durations would influence classification in a more hazardous category. Tests consistent with OECD Test Guideline 203 (Fish 96 hour LC_{50}) or equivalent should be used for classification.

I.2.1.2 Chronic testing

Chronic or long-term tests with fish can be initiated with fertilized eggs, embryos, juveniles, or reproductively active adults. Tests consistent with OECD Test Guideline 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used in the classification scheme. Durations can vary widely depending on the test purpose (anywhere from 7 days to over 200 days). Observational endpoints can include hatching success, growth (length and weight changes), spawning success, and survival. Technically, the OECD 210 Guideline (Fish Early Life Stage) is not a 'chronic' test, but a sub-chronic test on sensitive life stages. It is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system. Fish early life stage toxicity data are much more available than fish life cycle or reproduction studies.

I.2.2 Tests with Crustaceae

I.2.2.1 Acute testing

Acute tests with crustacea generally begin with first instar juveniles. For daphnids, test duration of 48 hours is used. For other crustacea, such as mysids or others, duration of 96 hours is typical. The observational endpoint is mortality or immobilisation as a surrogate to mortality. Immobilisation is defined as unresponsive to gentle prodding. Tests consistent with OECD Test Guideline 202 Part 1 (Daphnia acute) or USA-EPA OPPTS 850.1035 (Mysid acute toxicity) or their equivalents should be used for classification.

I.2.2.2 Chronic testing

Chronic tests with crustacea also generally begin with first instar juveniles and continue through maturation and reproduction. For daphnids, in particular *Daphnia magna*, 21 days is sufficient for maturation and the production of 3 broods. For mysids, 28 days is necessary. Observational endpoints include time to first brood, number of offspring produced per female, growth, and survival. It is recommended that tests consistent with OECD test guidelines 211 and/or 202 Part 2 (Daphnia reproduction) or US-EPA 850.1350 (Mysid chronic) or their equivalents be used in the classification scheme.

I.2.3 Algae / other aquatic plant tests

I.2.3.1 Tests with algae

Algae are cultured and exposed to the test substance in a nutrient-enriched medium. Tests consistent with OECD Test Guideline 201 (Algal growth inhibition) should be used. Standard test

methods employ a cell density in the inoculum in order to ensure exponential growth through the test, usually 3 to 4 days duration.

The algal growth inhibition test is a short-term test that provides both acute and chronic endpoints. However, the EC_{50} is treated as an acute value for classification purposes. Classification shall be based on both, the algal growth rate reduction endpoint, ErC_{50} [= EC_{50} (growth rate)] and NOErC [= NOEC (growth rate)] provided that the control growth is exponential (greater than a factor of 16). This endpoint is preferred because it is not dependent on the test design, whereas the endpoint, biomass (growth) inhibition (EbC_{50}) depends on both, growth rate of the test species as well as test duration and other elements of test design. Thus in circumstances where the basis of the EC_{50} is not specified and no ErC_{50} is recorded, classification shall be based on the lowest EC_{50} available. Where the algal toxicity ErC_{50} [= EC_{50} (growth rate)] falls more than 100 times below the next most sensitive species and results in a classification based solely on this effect, consideration should be given to whether this toxicity is representative of the toxicity to aquatic plants. Where it can be shown that this is not the case, professional judgment should be used in deciding if classification should be applied.

I.2.3.2 Tests with aquatic macrophytes

The most commonly used vascular plants for aquatic toxicity tests are duckweeds (*Lemna gibba* and *L. minor*). The tests last for up to 14 days and are performed in nutrient enriched media similar to that used for algae, but may be increased in strength. The observational endpoint is based on change in the number of fronds produced. Tests consistent with OECD Test Guideline on Lemna (2006) and US-EPA 850.4400 (aquatic plant toxicity, Lemna) should be used.

Under the REACH Regulation growth inhibition study on aquatic plants, algae are the preferred species.

I.3 Aquatic toxicity concepts

This section addresses the use of acute and chronic toxicity data in classification, and special considerations for exposure regimes, algal toxicity testing, and use of QSARs.

I.3.1 Acute toxicity

Acute toxicity for purposes of classification refers to the intrinsic property of a substance to be injurious to an organism in a short-term exposure to that substance. Acute toxicity is generally expressed in terms of a concentration which is lethal to 50 % of the test organisms (lethal concentration, LC_{50}), causes a measurable adverse effect to 50 % of the test organisms (e.g. immobilisation of daphnids, EC_{50}), or leads to a 50 % reduction in test (treated) organism responses from control (untreated) organism responses (e.g. growth rate in algae, ErC_{50}).

Acute aquatic toxicity is normally determined using a fish 96 hour LC_{50}, a crustacea species 48 hour EC_{50}, an algal species 72 or 96 hour EC_{50} and/or aquatic plants 7 days EC50. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species shall also be considered if the test methodology is suitable. Since the purpose of classification is to characterise hazard in the aquatic environment, the result showing the highest toxicity should be chosen. However, there are circumstances, when a weight of evidence approach is appropriate.

Substances with an acute toxicity determined to be less than one part per million (1 mg/l) are generally recognised as being very toxic. The handling, use, or discharge into the environment of these substances poses a high degree of hazard and they are classified in category Acute 1. When classifying substances as Acute 1, it is necessary at the same time to indicate an appropriate Multiplying factor, M-factor. The multiplying factors are defined using a toxicity value (see Section 4.1.3.3.2).

I.3.2 Chronic toxicity

Chronic toxicity, for purposes of classification, refers to the intrinsic property of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life-cycle of the organism. Such chronic effects usually include a range of sublethal endpoints and are generally expressed in terms of a No Observed Effect Concentration (NOEC), or an equivalent EC_x. Observable endpoints typically include survival, growth and/or reproduction. Chronic toxicity exposure durations can vary widely depending on the test endpoint measured and test species used.

For the classification based on chronic toxicity a differentiation is made between rapidly degradable and non-rapidly degradable substances. Substances that do rapidly degrade are classified in category Chronic 1 when the chronic toxicity NOEC or EC_x is determined to be ≤ 0.01 mg/l. Decimal bands are accepted for categorising chronic toxicity above this category. Substances with a chronic toxicity NOEC or EC_x between 0.01 and 0.1 mg/l are classified in category Chronic 2 for chronic toxicity. Substances with a chronic toxicity NOEC or EC_x between 0.1 and 1.0 mg/l are classified in category Chronic 3 for chronic toxicity. Finally, those substances with chronic toxicity NOECs or EC_xs over 1.0 mg/l are not classifiable for long-term hazard in any of the categories Chronic 1, 2 or 3. For substances that do not rapidly degrade or for which such has to be assumed by worst case (i.e. this applies in case where no information on rapid degradation is available) two chronic categories are used: category Chronic 1 if the chronic toxicity NOEC or EC_x is determined to be ≤ 0.1 mg/l and category Chronic 2 if the chronic toxicity NOEC or EC_x is determined to be between 0.1 and 1.0 mg/l.

When classifying substances as Chronic 1, it is necessary at the same time to indicate an appropriate M-factor. The multiplying factors are defined using a toxicity value (see Section 4.1.3.3.2).

Since chronic toxicity data are less common in certain sectors than acute data, for classification schemes, the potential for long-term hazard is in absence of chronic toxicity data, is identified by appropriate combinations of acute toxicity, lack of degradability, and/or the potential or actual bioaccumulation. However, where adequate chronic toxicity data exist, this shall be used in preference over the classification based on the combination of acute toxicity with degradability and/or bioaccumulation. In this context, the following general approach should be used.

a. If adequate chronic toxicity data are available for all three trophic levels this can be used directly to determine an appropriate long-term hazard category;

b. If adequate chronic toxicity data are available for one or two trophic levels, it should be examined if acute toxicity data are available for the other trophic level(s). A potential classification is made for the trophic level(s) with chronic data and compared with that made using the acute toxicity data for the other trophic level(s). The final classification shall be made according to the most stringent outcome;

c. In order to remove or lower a long-term aquatic classification, using chronic toxicity data, it must be demonstrated that the NOEC(s) (or equivalent EC_x) used would be suitable to remove or lower the concern for all taxa which resulted in classification based on acute data in combination with degradability, and/or bioaccumulation. This can often be achieved by using a long-term NOEC for the most sensitive species identified by the acute toxicity. Thus, if a classification has been based on a fish acute LC_{50}, it would generally not be possible to remove or lower this classification using a long-term NOEC from an invertebrate toxicity test. In this case, the NOEC would normally need to be derived from a long-term fish test of the same species or one of equivalent or greater sensitivity. Equally, if classification has resulted from the acute toxicity of more than one taxonomic group, it is likely that NOECs from each taxonomic group will be needed. In case of classification of a substance as Chronic 4, sufficient evidence should be provided

that the NOEC or equivalent EC_x for each taxonomic group is greater than 1 mg/l or greater than the water solubility of the substances under consideration.

I.3.3 Exposure regimes

Four types of exposure conditions are employed in both acute and chronic tests and in both freshwater and saltwater media: static, static-renewal (semi-static), recirculation, and flow-through. The choice for which test type to use usually depends on test substance characteristics, test duration, test species, and regulatory requirements.

I.3.4 Test media for algae and Lemna

Algal and Lemna tests are performed in nutrient-enriched media and use of one common constituent, EDTA, or other chelators, should be considered carefully. When testing the toxicity of organic chemicals, trace amounts of a chelator like EDTA are needed to complex micronutrients in the culture medium; if omitted, growth can be significantly reduced and compromise test utility. However, chelators can reduce the observed toxicity of metal test substances. Therefore, for metal compounds, it is desirable that data from tests with high concentration of chelators and/or tests with stoichiometrical excess of chelator relative to iron be critically evaluated. Free chelator may mask heavy metal toxicity considerably, in particular with strong chelators like EDTA (see Annex IV to this guidance on Metals and inorganic metal compounds). However, in the absence of available iron in the medium the growth of algae and Lemna can become iron limited, and consequently data from tests with no or with reduced iron and EDTA should be treated with caution.

I.3.5 Use of substance categorisation (read-across and grouping) and (Q)SARs for classification and labelling

See Section 1.4 of this guidance.

I.4 Substances which are difficult to test

For classification of organic compounds, it is desirable to have stabilised and analytically measured test concentrations. Although measured concentrations are preferred, classification may, under certain circumstances, be based on studies where nominal concentrations are the only valid data available. If the material is likely to substantially degrade or otherwise be lost from the water column, care must be taken in data interpretation and classification should be done taking into account the loss of the toxicant during the test, if relevant and possible. Additionally, metals present their own set of difficulties and are discussed separately (see Annex IV on metals).

In most cases where test conditions are hard to define, the actual test concentration is likely to be less than the nominal or expected test concentration. Where acute toxicities ($L(E)C_{50}$) are estimated to be less than 1 mg/l for a difficult to test substance, one can be fairly confident the classification as Acute 1 (and Chronic 1, if appropriate) is warranted. However, if the estimated toxicity is greater than 1 mg/l, the estimated toxicity is likely to under-represent the toxicity. In these circumstances, expert judgement is needed to determine the acceptability of a test with a difficult substance for use in classification. In addition, caution is also needed when deriving appropriate M-factors, in particular when the nominal effect concentrations are close to the thresholds for diverging M-factors. Where the nature of the testing difficulty is believed to have a significant influence on the actual test concentration when toxicity is estimated to be greater than 1 mg/l and the test concentration is not measured, then the test should be used with due caution in classification.

The following paragraphs provide some detailed guidance on some of these problems of interpretation. In doing so it should be remembered that this is guidance and hard and fast

rules cannot be applied. The nature of many of the difficulties mean that expert judgement must always be applied both in determining whether there is sufficient information in a test for a judgement to be made on its validity, and also whether a toxicity level can be determined suitable for use in applying the classification criteria.

I.4.1 Unstable substances

While testing procedures should ideally have been adopted which minimise the impacts of instability in the test media, in practice, in certain tests, it can be almost impossible to maintain a concentration throughout the test. Common causes of lack of constant exposure concentration during the test are oxidation, hydrolysis, photodegradation and biodegradation. While the latter forms of degradation can more readily be controlled, such controls are frequently absent in much existing testing. Nevertheless, for some testing, particularly acute and chronic fish toxicity testing, a choice of exposure regimes is available to help minimise losses due to instability, and this should be taken into account in deciding on the test data validity.

Where instability is a factor in determining the level of exposure during the test, an essential prerequisite for data interpretation is the existence of measured exposure concentrations at suitable time points throughout the test. In the absence of analytically measured concentrations at least at the start and end of test, no valid interpretation can be made and the test should be considered as invalid for classification purposes. Where measured data are available, a number of practical rules can be considered by way of guidance in interpretation:

a. where measured data are available for the start and end of test (as is normal for the acute Daphnia and algal tests), the L(E)C_{50}, for classification purposes, may be calculated based on the geometric mean concentration of the start and end of test. Where concentrations at the end of test are below the analytical detection limit, such concentrations shall be considered to be half that detection limit;

b. where measured data are available at the start and end of media renewal periods (as may be available for the semi-static tests), the geometric mean for each renewal period should be calculated, and the mean exposure over the whole exposure period calculated from these data;

c. where the toxicity can be attributed to a degradation breakdown product, and the concentrations of this are known, the L(E)C50 for classification purposes may be calculated based on the geometric mean of the degradation product concentration, back calculated to the parent substance;

d. similar principles may be applied to measured data in chronic toxicity testing.

I.4.2 Poorly soluble substances

These substances, usually taken to be those with a solubility in water of < 1 mg/l, are frequently difficult to dissolve in the test media, and the dissolved concentrations will often prove difficult to measure at the low concentrations anticipated. For many substances, the true solubility in the test media will be unknown, and will often be recorded as < detection limit in purified water. Nevertheless such substances can show toxicity, and where no toxicity is found, judgement must be applied to whether the result can be considered valid for classification. Judgement should err on the side of caution and should not underestimate the hazard.

Ideally, tests using appropriate dissolution techniques and with accurately measured concentrations within the range of water solubility should be used. Where such test data are available, they should be used in preference to other data. It is normal, however, particularly when considering older data, to find such substances with toxicity levels recorded in excess of the water solubility, or where the dissolved levels are below the detection limit of the analytical method. Thus, in both circumstances, it is not possible to verify the actual exposure concentrations using measured data. Where these are the only data available on which to classify, some practical rules can be considered by way of general guidance:

a. where the acute toxicity is recorded at levels in excess of the water solubility, the L(E)C_{50} for classification purposes may be considered to be equal to or below the measured water solubility. In such circumstances it is likely that category Chronic 1 and/or category Acute 1 should be applied. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. Where this is considered the likely cause of the effects observed, the test should be considered as invalid for classification purposes;

b. where no acute toxicity is recorded at levels in excess of the water solubility, the L(E)C_{50} for classification purposes may be considered to be greater than the measured water solubility. In such circumstances, consideration should be given to whether the category Chronic 4 should apply. In making a decision that the substance shows no acute toxicity, due account should be taken of the techniques used to achieve the maximum dissolved concentrations. Where these are not considered as adequate, the test should be considered as invalid for classification purposes;

c. where the water solubility is below the detection limit of the analytical method for a substance, and acute toxicity is recorded, the L(E)C_{50} for classification purposes may be considered to be less than the analytical detection limit. Where no toxicity is observed, the L(E)C_{50} for classification purposes, may be considered to be greater than the water solubility. Due consideration should also be given to the quality criteria mentioned above;

d. where chronic toxicity data are available, the same general rules should apply. In principle, only data showing no observed effect concentrations at levels above the water solubility limit, or greater than 1 mg/l need be considered. Again, where these data cannot be validated by measuring the concentrations, the techniques used to achieve the maximum dissolved concentrations must be considered as appropriate.

I.4.3 Other factors contributing to concentration loss

A number of other factors can also contribute to losses of test material from solution and, while some can be avoided by correct study design, interpretation of data where these factors have contributed may, from time to time, be necessary.

a. sedimentation: this can occur during a test for a number of reasons. A common explanation is that the substance has not truly dissolved despite the apparent absence of particulates, and agglomeration occurs during the test leading to precipitation. In these circumstances, the L(E)C_{50} for classification purposes, may be considered to be based on the end of test concentrations. Equally, precipitation can occur through reaction with the media. This is considered under instability above;

b. adsorption: this can occur for substances of high adsorption characteristics such as high log Kow substances. Where this occurs, the loss of concentration is usually rapid and exposure may best be characterised by the end of test concentrations;

c. bioaccumulation: losses may occur through the bioaccumulation of a substance into the test organisms. This may be particularly important where the water solubility is low and log K_{ow} correspondingly high. The L(E)C_{50} for classification purposes, may be calculated based on the geometric mean of the start and end of test concentrations.

I.4.4 Perturbation of the test media

Strong acids and bases may exert their toxicity through extreme pH. Generally however changes of the pH in aquatic systems are normally prevented by buffer systems in the test medium. If no data are available on a salt, the salt should generally be classified in the same way as the anion or cation, i.e. as the ion that receives the most stringent classification. If the effect concentration is related to only one of the ions, the classification of the salt should take

the molecular weight difference into consideration by correcting the effect concentration by multiplying with the ratio: $MW_{salt}/MW_{ion.}$

Polymers are typically not available in aquatic systems. Dispersible polymers and other high molecular mass materials can perturb the test system and interfere with uptake of oxygen, and give rise to mechanical or secondary effects. These factors need to be taken into account when considering data from these substances. Many polymers behave like complex substances, however, having a significant low molecular mass fraction which can leach from the bulk polymer. This is considered further below.

I.4.5 Complex substances

Complex substances are characterised by a range of chemical structures, frequently in a homologous series, but covering a wide range of water solubilities and other physico-chemical characteristics. On addition to water, equilibrium will be reached between the dissolved and undissolved fractions which will be characteristic of the loading of the substance. For this reason, such complex substances are usually tested as a WSF or WAF, and the $L(E)C_{50}$ recorded based on the loading or nominal concentrations. Analytical support data are not normally available since the dissolved fraction will itself be a complex mixture of components. The toxicity parameter is sometimes referred to as LL_{50}, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria.

Polymers represent a special kind of complex substance, requiring consideration of the polymer type and their dissolution/dispersal behaviour. Polymers may dissolve as such without change, (true solubility related to particle size), be dispersible, or portions consisting of low molecular weight fractions may go into solution. In the latter case, in effect, the testing of a polymer is a test of the ability of low molecular mass material to leach from the bulk polymer, and whether this leachate is toxic. It can thus be considered in the same way as a complex mixture in that a loading of polymer can best characterise the resultant leachate, and hence the toxicity can be related to this loading.

I.5 References

US EPA 1996. *Ecological Effects Test Guidelines - OPPTS Harmonized Test Guidelines Series 850.1000* -- Public Drafts, EPA 712-C-96-113. http://www.epa.gov/ocspp/pubs/frs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1000.pdf

ASTM 1999. *Annual book of ASTM standards*, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA

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Test Methods Regulation (EC) No 440/2008. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0739:en:PDF

II ANNEX II: RAPID DEGRADATION

II.1 Introduction

Degradability is one of the important properties of substances that have impact on the potential for substances to exert an aquatic hazard. Non-degradable substances will persist in the environment and may consequently have a potential for causing long-term adverse effects on biota. In contrast, degradable substances may be removed in the sewers, in sewage treatment plants or in the environment. It should be noted that data from degradability tests on mixtures are difficult or impossible to interpret, and are therefore not used in classification and labelling.

Classification of substances is primarily based on their intrinsic properties. However, the degree of degradation depends not only on the intrinsic degradability or recalcitrance of the molecule, but also on the actual conditions in the receiving environmental compartment such as redox potential, pH, temperature, presence of suitable micro-organisms, concentration of the substance and occurrence and concentration of other substrates. The interpretation of the degradation properties in an aquatic hazard classification context therefore requires detailed criteria that balance the intrinsic properties of the substance and the prevailing environmental conditions into a concluding statement on the potential for long-term adverse effects.

The term degradation is defined in Section 4.1 of Annex I to CLP as 'the decomposition of organic molecules to smaller molecules and eventually to carbon dioxide, water and salts'. For inorganic compounds and metals, the concept of degradability has no meaning. Rather the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Therefore, the present section applies only to organic and organo-metal compounds. A separate section on the classification & labelling (C&L) of metals is provided in Part 4, section 4.1.5 and Annex IV to the CLP guidance.

Data on degradation properties of a substance may be available from standardised tests, or from other types of investigations, or they may be estimated from the structure of the molecules i.e. via SAR or QSAR approaches. The interpretation of such degradation data for classification purposes often requires detailed evaluation of the (test) data. The use of biodegradation data for classification purposes is only applicable to substances. Biodegradation data on mixtures cannot be used as it does not provide a reliable indication of environmental fate (CLP Annex I, point 4.1.3.3.1).

II.2 Interpretation of degradability data

Often a diverse range of test data is available that does not necessarily fit directly with the classification criteria. Consequently, guidance is needed on interpretation of existing test data in the context of the aquatic hazard classification. Based on the harmonised criteria, guidance for interpretation of degradation data is prepared below for several types of data comprised by the expression 'rapid degradation' in the aquatic environment.

II.2.1 Ready biodegradability

Ready biodegradability is defined in the OECD Test Guidelines No. 301 methods A-F (OECD 1992), OECD 306 (marine water) and OECD 310 (OECD 2006). All organic substances that degrade to a level higher than the pass level in a standard OECD ready biodegradability test or in a similar test should be considered readily biodegradable, and consequently also rapidly degradable. Many test data found in the open literature, however, do not specify all of the conditions that should be evaluated to demonstrate whether or not the test fulfils the requirements of a ready biodegradability test. Expert judgement is therefore needed as regards the validity of the data before use for classification purposes. Before concluding on the ready biodegradability of a test substance, however, at least the following parameters should be considered.

II.2.1.1 Concentration of test substance

Relatively high concentrations of test substance are used in the OECD ready biodegradability tests (2-100 mg/l). Many substances may however be toxic to the inocula at such high concentrations, resulting in a low degradation of the substances in these tests, although the substances might be rapidly degradable at lower non-toxic concentrations. A toxicity test with micro-organisms, or inhibition of the inoculum observed with a positive control substance may demonstrate the toxicity of the test substance. Guidance on the selection of suitable microbial inhibition test methods is provided in IR&CSA Parts R7.8.14. When it is likely that inhibition is the reason for a substance being not readily degradable, results from a test employing lower non-toxic concentrations of the test substance should be used when available.

II.2.1.2 Time window

The harmonised criteria include a general requirement for all of the ready biodegradability tests on achievement of the pass level within ten days of the onset of biodegradation. This is not in line with the OECD Test Guideline 301 in which the ten-day time window applies to the OECD ready biodegradability tests except to the MITI I test (OECD Test Guideline 301C). In the Closed Bottle test (OECD Test Guideline 301D), a 14-days window may be used instead when measurements have not been made after ten days. Moreover, often only limited information is available in references of biodegradation tests. Thus, as a pragmatic approach the percentage of degradation reached after 28 days may be used directly for assessment of ready biodegradability when no information on the ten days time window is available. This should, however, only be accepted for existing test data and data from tests where the ten-day window does not apply.

Where there is sufficient justification, the ten-day window condition may be waived for certain complex substances and the pass level is applied at 28 days. This applies to multi-constituent and certain UVCB substances (such as oils and surfactants) consisting of structural similar constituents with different chain-lengths, degree and/or site of branching or stereo-isomers, even in their most purified commercial forms. Testing of each individual constituent may be costly and impractical. If a test on such a complex substance is performed and it is anticipated that a sequential biodegradation of the individual constituents is taking place, then the ten-day window should not be applied to interpret the results of the test. A case by case evaluation should however take place on whether a biodegradability test on such a substance would give valuable information regarding its biodegradability as such i.e. regarding the degradability of all the constituents, or whether instead an investigation of the degradability of carefully selected individual constituents of the complex substance is required (OECD 2006).

II.2.2 BOD_5/COD

Information on the 5-day biochemical oxygen demand (BOD_5) will be used for classification purposes only when no other measured degradability data are available. Thus, priority is given to data from ready biodegradability tests and from simulation studies regarding degradability in the aquatic environment. Therefore, this test should not be performed anymore for assessment of the ready biodegradability of substances. Older test data may however be used when no other degradability data are available. For substances where the chemical structure is known, the theoretical oxygen demand (ThOD) can be calculated and this value should be used instead of the chemical oxygen demand (COD).

II.2.3 Other convincing scientific evidence

Rapid degradation in the aquatic environment may be demonstrated by other data than a ready biodegradability test, or a BOD_5/COD ratio. These may be data on biotic and/or abiotic degradation. Data on primary degradation can only be used where it is demonstrated that the degradation products shall not be classified as hazardous to the aquatic environment, i.e. that they do not fulfil the classification criteria.

The fulfilment of criterion (c) of paragraph 4.1.2.9.5 of CLP requires that the substance is degraded in the aquatic environment to a level of > 70 % within a 28-day period. If first-order kinetics are assumed, which is reasonable at the low substance concentrations prevailing in most aquatic environments, the degradation rate will be relatively constant for the 28-day period. Thus, the degradation requirement will be fulfilled with an average degradation rate constant, $k > -(\ln 0.3 - \ln 1)/28 = 0.043$ day^{-1}. This corresponds to a degradation half-life, $t_{½} < \ln 2/0.043 = 16$ days.

Moreover, as degradation processes are temperature dependent, this parameter should also be taken into account when assessing degradation in the environment. Data from studies employing environmentally realistic temperatures e.g. 5 – 25 °C should be used for the evaluation. When data from studies performed at different temperatures need to be compared, the traditional Q10 approach could be used, i.e. that the degradation rate is halved when the temperature decreases by 10°C.

The evaluation of data on fulfilment of this criterion should be conducted on a case-by-case basis by expert judgement. However, guidance on the interpretation of various types of data that may be used for demonstrating a rapid degradation in the aquatic environment is given below. In general, only data from aquatic biodegradation simulation tests are considered directly applicable. However simulation test data from other environmental compartments could be considered as well, but such data require in general more scientific judgement before use.

II.2.3.1 Aquatic simulation tests

Aquatic simulation tests (e.g. OECD 309, 2004) are tests conducted in the laboratory, but simulating environmental conditions and employing natural samples as inoculum. Results of aquatic simulation tests may be used directly for classification purposes, when realistic environmental conditions in surface waters are simulated, i.e.:

a. substance concentration that is realistic for the general aquatic environment (often in the low µg/l range);

b. inoculum from a relevant aquatic environment;

c. realistic concentration of inoculum (10^3-10^6 cells/ml);

d. realistic temperature e.g. 5 °C to 25 °C; and

e. ultimate degradation is determined i.e. determination of the mineralisation rate or the individual degradation rates of the total biodegradation pathway.

II.2.3.2 Field investigations

Parallel to laboratory simulation tests are field investigations or mesocosm experiments. In such studies, fate and/or effects of chemicals in the environment or in environmental enclosures may be investigated. Fate data from such experiments can in principle be used for assessing the potential for a rapid degradation. This may, however, often be difficult, as it requires that ultimate degradation can be demonstrated. This may be documented by preparing mass balances showing that no non-degradable intermediates are formed, and which take the fractions into account that are removed from the aqueous system due to other processes such as sorption to sediment or volatilisation from the aquatic environment.

II.2.3.3 Monitoring data

Monitoring data may demonstrate the removal of contaminants from the aquatic environment. Such data are, however, very difficult to use for classification purposes. The following aspects should be considered before use:

a. Is the removal a result of degradation, or is it a result of other processes such as dilution or distribution between compartments (sorption, volatilisation)?

b. Is formation of non-degradable intermediates excluded?

Only when it can be demonstrated that removal as a result of ultimate degradation fulfils the criteria for rapid degradability, can such data be considered for use for classification purposes. In general, monitoring data should only be used as supporting evidence for demonstration of either persistence in the aquatic environment, or of rapid degradation.

II.2.3.4 Inherent and Enhanced Ready Biodegradability tests

Substances that are degraded more than 70% in tests for inherent biodegradability (OECD Test Guidelines 302) have the potential for ultimate biodegradation. However, because of the optimised conditions in these tests, the rapid biodegradability of inherently biodegradable substances in the environment cannot be assumed. The optimised conditions in inherent biodegradability tests stimulate adaptation of the micro-organisms thus increasing the biodegradation potential, compared to natural environments. Therefore, positive results in general should not be interpreted as evidence for rapid degradation in the environment.

IR&CSA Chapters R.7b and R.11 refer in the context of persistence testing to a new category of tests, i.e. the 'enhanced ready (screening) biodegradability tests'. These are in essence ready biodegradability tests to which more flexibility is given to demonstrate the occurrence of degradation e.g. via prolonged testing times, larger test volumes, adaptation, etc. These methods are not yet validated and/or standardised for C&L.

II.2.3.5 Sewage treatment plant simulation tests

Results from tests simulating the conditions in a sewage treatment plant (STP) e.g. the OECD Test Guideline 303 cannot be used for assessing the degradation in the aquatic environment. The main reasons for this are that the microbial biomass in a STP is significantly different from the biomass in the environment, that there is a considerably different composition of substrates, and that the presence of rapidly mineralised organic matter in waste water may facilitate degradation of the test substance by co-metabolism.

II.2.3.6 Soil and sediment degradation data

It has been argued that for many non-sorptive substances more or less the same degradation rates are found in soil and in surface water. For sorptive substances, a lower degradation rate may generally be expected in soil than in water due to a lower bioavailability caused by sorption. Thus, when a substance has been shown to be degraded rapidly in a soil simulation study, it is most likely also rapidly degradable in the aquatic environment. It is therefore proposed that an experimentally determined rapid degradation in soil is sufficient documentation for a rapid degradation in surface waters when:

a. no pre-exposure (pre-adaptation) of the soil micro-organisms has taken place; and
b. an environmentally realistic concentration of substance is tested; and
c. the substance is ultimately degraded within 28 days with a half-life < 16 days corresponding to a degradation rate > 0.043 day^{-1} .

The same argumentation is considered valid for data on degradation in sediment under aerobic conditions.

II.2.3.7 Anaerobic degradation data

Data regarding anaerobic degradation cannot be used in relation to deciding whether a substance should be regarded as rapidly degradable, because the aquatic environment is generally regarded as the aerobic compartment where the aquatic organisms, such as those employed for aquatic hazard classification, live.

II.2.3.8 Hydrolysis

Data on hydrolysis e.g. OECD Test Guideline 111 might be considered for classification purposes only when the longest half-life $t_{½}$ determined within the pH range 4-9 is shorter than 16 days. However, hydrolysis is not an ultimate degradation and various intermediate degradation

products may be formed, some of which may be only slowly degradable. Only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment, data from hydrolysis studies could be considered.

When a substance is quickly hydrolysed e.g. with $t_{½}$ < a few days, this process is a part of the degradation determined in biodegradation tests. Hydrolysis may be the initial transformation process in biodegradation.

II.2.3.9 Photochemical degradation

Information on photochemical degradation e.g. OECD 1997 is difficult to use for classification purposes. The actual degree of photochemical degradation in the aquatic environment depends on local conditions e.g. water depth, suspended solids, turbidity as well as seasonal influences, and the hazard of the degradation products is usually not known. Probably only seldom will enough information be available for a thorough evaluation based on photochemical degradation.

II.2.3.10 Estimation of degradation

Hydrolysis: Certain QSARs have been developed for prediction of an approximate hydrolysis half-life, which should only be considered when no experimental data are available, or in a Weight of Evidence approach. However, a hydrolysis half-life can only be used with great care in relation to classification, because hydrolysis does not concern ultimate degradability (see 'Hydrolysis' of this Section). Furthermore the QSARs developed until now have a rather limited applicability and are only able to predict the potential for hydrolysis on a limited number of chemical classes (see also IR&CSA Chapter R.7.9.3.1).

Biodegradation: In general, no quantitative estimation method (QSAR) for estimating the degree of biodegradability of organic substances is yet sufficiently accurate to unequivocally predict rapid degradation. However, results from such methods may be used to predict that a substance is not rapidly degradable, or be used in a Weight of Evidence approach. For example, when in the Biodegradation Probability Program e.g. BIOWIN version 3.67, Syracuse Research Corporation the probability is < 0.5 estimated by the linear or non-linear methods, the substances should be regarded as not rapidly degradable (OECD, 1994; Pedersen *et al.*, 1995 & Langenberg *et al.*, 1996). Also other (Q)SAR methods may be used as well as expert judgement, for example, when degradation data for structurally analogue compounds are available, but such judgement should be conducted with great care. See also IR&CSA Chapter R.7.9.3.1.

In general, a QSAR prediction that the substance is not rapidly degradable is considered a better documentation for classification than application of a default classification, when no useful degradation data are available.

Degradation data from structurally related substances may provide evidence that a given substance displays very similar degradation properties. Such information may be employed in a read-across or weight of evidence approach for C&L.

II.2.3.11 Volatilisation

Chemicals may be removed from some aquatic environments by volatilisation. The intrinsic potential for volatilisation is determined by the Henry's Law constant (H) of the substance. Volatilisation from the aquatic environment is highly dependent on the environmental conditions of the specific water body in question, such as the water depth, the gas exchange coefficients (depending on wind speed and water flow) and stratification of the water body. Because volatilisation only represents removal of a chemical from the water phase, and not degradation, the Henry's Law constant cannot be used for assessment of degradation in relation to aquatic hazard classification of substances (see also Pedersen *et al.*, 1995).

II.2.4 No degradation data available

When no useful data on degradability are available - either experimentally determined or estimated data - the substance should be regarded by default as not rapidly degradable.

II.3 General interpretation problems

II.3.1 Complex substances

The harmonised criteria for classification of chemicals as hazardous for the aquatic environment focus on single substances. Some intrinsically complex substances are multi-constituent substances. They are typically of natural origin and need occasionally to be considered. This may be the case for chemicals that are produced or extracted from mineral oil or plant material. Such complex chemicals are normally considered as single substances in a regulatory context. In most cases they are defined as a homologous series of substances within a certain range of carbon chain length and/or degree of substitution. When this is the case, no major difference in degradability is foreseen and the degree of degradability can be established from tests of the complex chemical. One exception would be when a borderline degradation is found because in this case some of the individual substances may be rapidly degradable and others may not be rapidly degradable. This requires a more detailed assessment of the degradability of the individual constituents in the complex substance. When the constituents that are not-rapidly-degradable constitute a significant part of the complex substance e.g. more than 20 %, or for a hazardous constituent, an even lower content, the substance should be regarded as not rapidly degradable.

II.3.2 Availability of the substance

The present standard methods for investigating degradability of substances are developed for readily soluble test compounds. However, many organic substances are only slightly soluble in water. As the standard tests require 2-100 mg/l of the test substance, sufficient availability may not be reached for substances with low water solubility. In general, the DOC Die-Away test (OECD Test Guideline 301A) and the Modified OECD Screening test (OECD Test Guideline 301E) are less suitable for testing the biodegradability of poorly soluble substances since adsorption may be confused with degradation. In such cases, test adaptations may be considered with e.g. continuous mixing and/or an increased exposure time. Also tests with a special design, where concentrations of the test substance lower than the water solubility have been employed e.g. with radiolabelled test chemicals, could be relevant.

II.3.3 Test duration less than 28 days

Sometimes degradation is reported for tests terminated before the 28 day period specified in the standards e.g. the MITI, 1992. These data are of course directly applicable when a degradation greater than or equal to the pass level is obtained. When a lower degradation level is reached, the results need to be interpreted with caution. One possibility is that the duration of the test was too short and that the chemical structure would probably have been degraded in a 28-day biodegradability test. If substantial degradation occurs within a short time period, the situation may be compared with the criterion $BOD_5/COD \geq 0.5$ or with the requirements on degradation within the 10-days time window. In these cases, a substance may be considered readily degradable (and hence rapidly degradable), if:

a. the ultimate biodegradability exceeds 50 % within 5 days; or

b. the ultimate degradation rate constant in this period is greater than 0.1 day^{-1} corresponding to a half-life of 7 days.

These criteria are proposed in order to ensure that rapid mineralisation did occur, although the test was ended before 28 days and before the pass level was attained. Interpretation of test data that do not comply with the prescribed pass levels must be made with great caution. It is mandatory to consider whether a biodegradability result below the pass level was due to a partial degradation of the substance and not a complete mineralisation. If partial degradation is the probable explanation for the observed biodegradability, the substance should be considered not readily biodegradable.

II.3.4 Primary biodegradation

In some tests, only the disappearance of the parent compound i.e. primary degradation is determined for example by following the degradation by specific or group specific chemical analyses of the test substance. Data on primary biodegradability may be used for demonstrating rapid degradability only when it can be satisfactorily demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

II.3.5 Conflicting results from screening tests

The situation where more degradation data are available for the same substance introduces the possibility of conflicting results. In general, conflicting results for a substance which has been tested several times with an appropriate biodegradability test could be interpreted by a 'weight of evidence approach'. This implies that if both positive i.e. higher degradation than the pass level and negative results have been obtained for a substance in ready biodegradability tests, then the data of the highest quality and the best documentation should be used for determining the ready biodegradability of the substance. However, positive results in ready biodegradability tests could be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, i.e. guideline criteria are fulfilled, including the use of non-pre-exposed (non-adapted) inoculum.

The suitability of the inoculum for degrading the test substance depends on the presence and amount of competent degraders. When the inoculum is obtained from an environment that has previously been exposed to the test substance, the inoculum may be adapted as demonstrated by a degradation capacity greater than that of an inoculum from a non-exposed environment. As far as possible the inoculum must be sampled from an unexposed environment, but for substances that are used ubiquitously in high volumes and released widespread or more or less continuously, this may be difficult or impossible. When conflicting results are obtained, the origin and density of the inoculum should be checked in order to clarify whether or not differences in the adaptation of the microbial community may be the reason.

As mentioned above, many substances may be toxic or inhibitory to the inoculum at the relatively high concentrations tested in ready biodegradability tests. Especially in the Modified MITI (I) test (OECD Test Guideline 301C) and the Manometric Respirometry test (OECD Test Guideline 301F) high concentrations (100 mg/l) are prescribed. The lowest test substance concentrations are prescribed in the Closed Bottle test (OECD Test Guideline 301D) where 2-10 mg/l is used. The possibility of toxic effects may be evaluated by including a toxicity control in the ready biodegradability test or by comparing the test concentration with toxicity test data on micro-organisms (for test methods see IR&CSA Chapter R.7.8.14).

Volatile substances should only be tested in closed systems as the Closed Bottle test (OECD Test Guideline 301D), the MITI I test (OECD Test Guideline 301C) the Manometric Respirometry test (OECD Test Guideline 301F), or OECD 310 (CO2 in sealed vessels – Headspace Test). Results from other tests should be evaluated carefully and only considered if it can be demonstrated, e.g. by mass balance estimates, that the removal of the test substance is not a result of volatilisation.

II.3.6 Variation in simulation test data

A number of simulation test data may be available for certain high priority chemicals. Often such data provide a range of half-lives in environmental media such as soil, sediment and/or surface water. The observed differences in half-lives from simulation tests performed on the same substance may reflect differences in test conditions, all of which may be environmentally relevant. A suitable half-life in the higher end i.e. a realistic worst case of the observed range of half-lives from such investigations should be selected for classification by employing a weight of evidence approach and taking the realism and relevance of the employed tests into account in relation to environmental conditions. In general, simulation test data of surface water are preferred relative to aquatic sediment or soil simulation test data in relation to the evaluation of rapid degradability in the aquatic environment.

II.4 Decision scheme

The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment.

A substance is considered to be **not** rapidly degradable **unless** at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the ten-day window condition may be waived for complex multi-component substances and the pass level applied at 28 days, as discussed in II.2.3). If this is not possible, then the pass level should be evaluated within a 14 days time window if possible, or after the end of the test; or

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days); or

c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydroysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of > 70 % within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment.

When these preferred data types are not available rapid degradation may be demonstrated if one of the following criteria is justified:

a. The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

b. In those cases where only BOD5 and COD data are available, the ratio of BOD5/COD is greater than or equal to 0.5. The same criterion applies to ready biodegradability tests of a shorter duration than 28 days, if the half-life furthermore is < 7 days; or

c. A weight of evidence approach based on read-across provides convincing evidence that a given substance is rapidly degradable.

If none of the above types of data are available then the substance is considered as **not** rapidly degradable. This decision may be supported by fulfilment of at least one of the following criteria:

i. the substance is not inherently degradable in an inherent biodegradability test; or

ii. the substance is predicted to be slowly biodegradable by scientifically valid QSARs, e.g. for the Biodegradation Probability Program, the score for rapid degradation (linear or non-linear model) < 0.5; or

iii. the substance is considered to be not rapidly degradable based on indirect evidence, such as knowledge from structurally similar substances; or

iv. no other data regarding degradability are available.

II.5 References

OECD (2006). *Revised introduction to the OECD guidelines for testing of chemicals*, section 3. OECD, 23 March 2006.

OECD (1992), *OECD Test Guidelines No. 301 methods A-F*

Pedersen, F., H. Tyle, J. R. Niemeldi, B. Guttmann, L. Lander, and A. Wedebrand 1995. *Environmental Hazard Classification – data collection and interpretation guide*. TemaNord 1995:581

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III ANNEX III: BIOACCUMULATION

III.1 Introduction

Bioaccumulation of a substance by an organism is not in itself a hazard. However, the bioaccumulation of a substance should be considered in relation to the potential for that substance to exert long-term effects. Chemical concentration and accumulation may result in internal concentrations of a substance in an organism (body burden), which may or may not lead to toxic effects over long-term exposures. For most organic chemicals uptake from water (bioconcentration) is believed to be the predominant route of uptake. Only for very hydrophobic substances does uptake from food become important. The classification criteria use the bioconcentration factor (BCF) or in the absence of it the octanol/water partition coefficient (log K_{ow}) as the measure of the potential for bioaccumulation. For these reasons, the present guidance document mainly considers bioconcentration and does not discuss in detail uptake via food or other routes. However, the possibility to use information on the biomagnification factor (BMF) as supportive evidence for bioaccumulation of highly lipophilic substances may be taken into account on a case by case basis.

Classification of a substance is primarily based on its intrinsic properties. However, the degree of bioconcentration also depends on factors such as the degree of bioavailability, the physiology of test organism, maintenance of constant exposure concentration, exposure duration, metabolism inside the body of the target organism and excretion from the body. The interpretation of the bioconcentration potential in a chemical classification context therefore requires an evaluation of the intrinsic properties of the substance, as well as of the experimental conditions under which bioconcentration factor (BCF) has been determined. IR&CSA (R.7c) Chapter 7.10.5.1 discusses the suitability of bioconcentration data, log K_{ow} data and other information (e.g. evidence for limited bioaccumulation potential) for classification purposes. Use of measured biomagnification data is discussed in relation to the screening approach in IR&CSA (R.7c) Chapter 7.10.4.5. Bioaccumulation of metals is discussed in Annex IV.

Information on the bioaccumulation potential of a substance may be available from standardised tests or may be estimated from the structure of the molecule. The interpretation of such bioconcentration data for classification purposes often requires detailed evaluation of test data. Guidance has been developed in IR&CSA in order to facilitate this evaluation. Chapter 7.1.8 (R.7a) gives guidance on n-octanol/water partition coefficient and Chapter 7.10.4 (R.7c) gives guidance on how to evaluate laboratory data on aquatic bioaccumulation. The use of bioaccumulation data for classification purposes is only applicable to substances. Bioaccumulation data on mixtures cannot be used as it does not provide a reliable indication of environmental fate (CLP Annex I, point 4.1.3.3.1).

III.2 Interpretation of bioconcentration data

Aquatic hazard classification of a substance is normally based on existing data on its environmental properties. Test data will only seldom be produced with the main purpose of facilitating a classification. Often a diverse range of test data is available which does not necessarily match the classification criteria. Further guidance on how to use this data is given in Chapter 7.10.5 of IR&CSA (R.7c).

Bioconcentration of an organic substance can be experimentally determined in bioconcentration experiments, during which BCF is measured as the concentration in the organism relative to the concentration in water under steady-state conditions and/or estimated from the uptake rate constant and the elimination rate constant. In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A measure of lipophilicity is the n-octanol/water partition coefficient (K_{ow}) which, for lipophilic non-ionised organic

substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, K_{ow} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{ow}. For those organic substances, estimation methods are available for calculating the K_{ow}. Data on the bioconcentration properties of non-ionised organic substances may thus be (i) experimentally determined, (ii) estimated from experimentally determined K_{ow}, or (iii) estimated from K_{ow} values derived by use of Quantitative Structure Activity Relationships (QSARs). Guidance for interpretation of such data is given in Chapters 7.10.4 and 7.10.5 of IR&CSA (R.7c). Guidance is also given on ionised chemicals and other classes that need special attention (see Section III.3.1).

III.2.1 Bioconcentration factor (BCF)

The bioconcentration factor is defined as the ratio on a weight basis between the concentration of the chemical in biota and the concentration in the surrounding medium, here water, at steady state. BCF can thus be experimentally derived under steady-state conditions, on the basis of measured concentrations. In addition BCF can also be calculated as the ratio between the first-order uptake and elimination rate constants; a method which does not require steady state (equilibrium conditions).

Different test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted, the most generally applied being the OECD test guideline 305 [90] (OECD, 1996; C.13 in Test Methods Regulation 440/2008 is a corresponding test).

Experimentally derived BCF values of high quality studies are ultimately preferred for classification purposes as such data override surrogate data, e.g. K_{ow}.

High quality data are defined as data where the validity criteria for the test method applied are fulfilled and described. Further guidance is provided in Chapter 7.10.4 of IR&CSA (R.7c).

BCF results from poor or questionable quality may give an erroneous BCF value. Therefore, such data should be carefully evaluated before use and consideration should be given to using K_{ow} instead.

If there is no BCF value for fish species, high-quality data on the BCF value for invertebrate species may be used. An invertebrate (mussel, oyster or scallop) BCF can be used as a worst case (conservative) value for fish. BCF for algae should not be used.

Experimental BCF data on highly lipophilic substances (e.g. with log K_{ow} above 6) will have a higher level of uncertainty than BCF values determined for less lipophilic substances. For highly lipophilic substances, e.g. with log K_{ow} above 6, experimentally derived BCF values tend to decrease with increasing log K_{ow}. Conceptual explanations of this non-linearity mainly refer to either reduced membrane permeation kinetics or reduced biotic lipid solubility for large molecules. A low bioavailability and uptake of these substances in the organism will thus occur. Other factors comprise experimental artifacts, such as equilibrium not being reached, reduced bioavailability due to sorption to organic matter in the aqueous phase, and analytical errors. Special care should thus be taken when evaluating experimental data on BCF for highly lipophilic substances as these data will have a much higher level of uncertainty than BCF values determined for less lipophilic substances.

III.2.1.1 BCF in different test species

BCF values used for classification are based on whole body measurements. As stated previously, the optimal data for classification are BCF values derived using the OECD test guideline 305 or corresponding EU test guideline C.13 or internationally equivalent methods, which uses small

[90] Note that OECD 305 is currently under revision. All adopted OECD guidelines can be freely accessed via the OECD iLibrary.

fish. Due to the higher gill surface-to-weight ratio in smaller organisms than in larger ones, steady-state conditions will be reached sooner in smaller organisms than in larger ones. The size of the organisms (fish) used in bioconcentration studies is thus of considerable importance in relation to the time used in the uptake phase, when the reported BCF value is based solely on measured concentrations in fish and water at steady-state. Thus, if large fish, e.g. adult salmon, have been used in bioconcentration studies, it should be evaluated whether the uptake period was sufficiently long for steady state to be reached or to allow for a kinetic uptake rate constant to be determined precisely. Also possible growth dilution should be taken into account when calculating the BCF values for smaller fish that grow during the bioconcentration studies.

Furthermore, when using existing data for classification, it is possible that the BCF values could be derived from several different fish or other aquatic species (e.g. clams) and for different organs in the fish. Thus, to compare diverse measured BCF data from different species to each other and to the criteria, normalisation to common basis lipid content will be required to reduce variability. Detailed guidance can be found in IR&CSA (R.7c) Chapter 7.10.4.1 for 'correction factors'.

Generally, the highest valid BCF value expressed on this common lipid basis is used to determine the wet weight based BCF-value in relation to the cut off value for BCF of 500 of the classification criteria.

III.2.1.2 Use of radio-labelled substances

The use of radio-labelled test substances can facilitate the analytical measurents in water and fish samples. However, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of the parent substance as well as possible metabolite(s) and possible metabolised carbon, which have been incorporated in the fish tissue in organic molecules. BCF values determined by use of radio-labelled test substances are therefore normally overestimated.

When using radio-labelled substances, the labelling is most often placed in the stable part of the molecule, for which reason the measured BCF value includes the BCF of the metabolites as well as the BCF from the parent substance. For some substances it is the metabolite which is the most toxic or which has the highest bioconcentration potential. Selective measurements of the parent substance as well as the metabolites may thus be important for the interpretation of the aquatic hazard (including the bioconcentration potential) of such substances.

In experiments where radio-labelled substances have been used, high radio-label concentrations are often found in the gall bladder of fish. This is interpreted to be caused by biotransformation in the liver and subsequently by excretion of metabolites in the gall bladder (Comotto *et al.*, 1979; Wakabayashi *et al.*, 1987; Goodrich *et al.*, 1991; Toshima *et al.*, 1992).

The BCF from radio-labelled studies should, preferentially, be based on the parent compound. If these are unavailable, for classification purposes, the BCF based on total radio-labelled residues can be used. If the BCF, in terms of radio-labelled residues, is ≥ 1000, the identification and quantification of degradation products documented to be ≥ 10 % of total residues in fish tissues at steady state, are strongly recommended.

When fish do not eat, the content of the gall bladder is not emptied into the gut, and high concentrations of metabolites may build up in the gall bladder. The feeding regime may thus have a pronounced effect on the measured BCF. In the literature many studies are found where radio-labelled compounds are used, and where the fish are not fed. In these studies the bioconcentration may in most cases have been overestimated.

III.2.2 Octanol-water-partitioning coefficient (K_{ow})

For organic substances experimentally derived high-quality K_{ow} values are preferred over other determinations of K_{ow}. When no experimental data of high quality are available, validated Quantitative Structure Activity Relationships (QSARs) for log K_{ow} may be used in the

classification process. Such validated QSARs may be used without modification to the agreed criteria if they are restricted to chemicals for which their applicability domain is well characterised. For substances like strong acids and bases, substances which react with the eluent, or surface-active substances, a QSAR estimated value of K_{ow} or an estimate based on individual *n*-octanol and water solubilities should be provided instead of an analytical determination of K_{ow}. Measurements should be taken on ionisable substances in their non-ionised form (free acid or free base) only by using an appropriate buffer with pH below pK for free acid or above the pK for free base. If multiple log K_{ow} data are available for the same substance, the reasons for any differences should be assessed before selecting a value. Generally, the highest valid value should take precedence.

III.2.2.1 Experimental determination of Kow

For experimental determination of K_{ow} values, several different methods are described in standard guidelines. Chapter 7.1.8.3 in IR&CSA (R.7a) gives guidance on direct measurement methods (Shake Flask Method, Generator Column Method, and Slow Stirring Method), and on one indirect measurement method (Reverse Phase HPLC Method).

III.2.2.2 Use of QSARs for determination of log Kow

When an estimated K_{ow} value is found, the estimation method has to be taken into account. Numerous QSARs have been and continue to be developed for the estimation of K_{ow}. The performances of top six programs, as evaluated in 2007, are given in the table below. It is recommended that at least one of the below software programs be used for the prediction of log K_{ow}. If possible, the average of several predictions should be taken. More guidance is provided is Chapter 7.1.8.4 in IR&CSA (R.7a).

Table III. 1 Examples of software programs for the estimation of log K_{ow}

Software	Website	Availability	Batch Operation	% Predicted within 0.5 Log unit	Standard Error
ADMET	www.simulationsplus.com	Purchase	Yes	94.2	0.27
ACDLabs	www.acdlabs.com	Purchase	Yes	93.5	0.27
ChemSilico	www.logp.com	Free on line	No	93.5	0.30
KOWWIN	http://www.epa.gov/opptintr/exposure/pubs/episuite.htm	Free to download	Yes	89.1	0.34
SPARC	ibmlc2.chem.uga.edu/sparc	Free on line	No	88.5	0.33
ClogP	www.daylight.com	Purchase	Yes	88.4	0.29

III.3 Chemical classes that need special attention with respect to BCF and K_{ow} values

There are certain physico-chemical properties of substances, which can make the determination of BCF or its measurement difficult. These may be substances, which do not bioconcentrate in a manner consistent with their other physico-chemical properties, e.g. steric hindrance or substances which make the use of descriptors inappropriate, e.g. surface activity, which makes both the measurement and use of log K_{ow} inappropriate.

III.3.1 Substances difficult to test

The methods presented above are generally designed for non-ionised organic substances. They are therefore of limited usefulness for a large number of other substances, collectively termed difficult substances, which include complex mixtures and chemicals that are charged at environmental pH (such as inorganic compounds). Substances difficult to test may be poorly soluble substances, complex mixtures, high molecular weight substances, surface active substances, inorganic substances, ionisable substances, or organic substances that do not partition to lipid. Some guidance is given in this Chapter. More detailed guidance is provided in IR&CSA (R.7c), mainly in Chapter 7.10.7.

In order to bioconcentrate in aquatic organisms, an organic substance needs to be present in the water, available for transfer across the fish gills and soluble in lipids. Factors that may alter this availability will thus change the actual bioconcentration of a substance, when compared with the prediction. For example, readily biodegradable substances may only be present in the aquatic compartment for short periods of time. Similarly, volatility, and hydrolysis will reduce the concentration and the time during which a substance is available for bioconcentration. A further important parameter, which may reduce the actual exposure concentration of a substance, is adsorption, either to particulate matter or to surfaces in general. There are a number of substances, which have shown to be rapidly transformed in the organism, thus leading to a lower BCF value than expected. Substances that form micelles or aggregates may bioconcentrate to a lower extent than would be predicted from simple physico-chemical properties. This is also the case for hydrophobic substances that are contained in micelles formed as a consequence of the use of dispersants. Therefore, the use of dispersants in bioaccumulation tests is discouraged. Further guidance is given in IR&CSA (R.7c) Chapter 7.10.3.4 on how to consider the factors that affect the bioaccumulation potential of many substances and that are important especially in the absence of a fully valid BCF test result.

In general, for substances difficult to test, measured BCF and K_{ow} values – based on the parent substance – are a prerequisite for the determination of the bioconcentration potential. Furthermore, proper documentation of the test concentration is a prerequisite for the validation of the given BCF value.

III.3.2 Poorly soluble and complex substances

Special attention should be paid to poorly soluble substances. Frequently the solubility of these substances is recorded as less than the detection limit, which creates problems in interpreting the bioconcentration potential. Where the test data indicate that the concentrations in the study are below the limit of detection, then the test is invalid and cannot be used. For such substances the bioconcentration potential should be based on experimental determination of log K_{ow} or QSAR estimations of log K_{ow} (see Section III.2.2). Complex substances contain a range of individual substances which can have a great variation in their physico-chemical and toxicological properties. It is generally not recommended to estimate an average or weighted BCF value. It is preferable to identify one or more representative constituents for further consideration. Further guidance is given in Chapter 7.10.7.2 in IR&CSA (R.7c).

III.3.3 High molecular weight substances

A number of regulatory systems use molecular weight as an indicator for reduced or minimal bioconcentration. It is, however, concluded in IR&CSA (R.7c), Chapter 7.10.3.4 that molecular mass and size should not be used in isolation as confirmatory evidence of lack of bioaccumulation (ECETOC 2005). However, supported by other data and by employing expert judgement, it may be concluded by a weight of evidence argument that such substances are unlikely to have a high bioconcentration factor (regardless of the log K_{ow} value). More details can be found in PBT assessment guidance (IR&CSA (R.11)).

III.3.4 Surface-active substances (surfactants)

Surfactants consist of an apolar, lipophilic part (most often an alkyl chain) (the hydrophobic tail) and a polar part (the hydrophilic headgroup). According to the charge of the headgroup, surfactants are subdivided into classes of anionic, cationic, non-ionic, or amphoteric surfactants. Due to the variety of different headgroups, surfactants are a structurally diverse class of compounds, which is defined by surface activity rather than by chemical structure. The bioaccumulation potential of surfactants should thus be considered in relation to the different subclasses (anionic, cationic, non-ionic, or amphoteric) instead of to the group as a whole. Surface-active substances may form emulsions, in which the bioavailability is difficult to ascertain. Micelle formation can result in a change of the bioavailable fraction even when the solutions are apparently formed, thus giving problems in interpretation of the bioaccumulation potential. See Chapter 7.10.7.4 in IR&CSA (R.7c) for further guidance.

Measured (experimentally derived) BCF values on surfactants show that BCF tends to increase with increasing alkyl chain length and be dependent on the site of attachment of the head group, other structural features and whether the alkyl part is subject to biotransformation.

III.3.4.1 Octanol-water-partition coefficient (K_{ow})

The octanol-water partition coefficient for surfactants cannot be determined using the shakeflask or slow stirring method because of the formation of emulsions. In addition, the surfactant molecules will exist in the water phase almost exclusively as ions, whereas they will have to pair with a counter-ion in order to be dissolved in octanol. Therefore, experimental determination of K_{ow} does not characterise the partition of ionic surfactants (Tolls, 1998). On the other hand, it has been shown that the bioconcentration of anionic and non-ionic surfactants increases with increasing lipophilicity (Tolls, 1998). Tolls (1998) showed that for some surfactants, an estimated log K_{ow} value using LOGKOW could represent the bioaccumulation potential; however, for other surfactants some 'correction' to the estimated log K_{ow} value using the method of Roberts (1989) was required. These results illustrate that the quality of the relationship between log K_{ow} estimates and bioconcentration depends on the class and specific type of surfactants involved. Therefore, the classification of the bioconcentration potential based on log K_{ow} values should be used with caution. Further guidance is provided in Chapter 7.10.7.4 in IR&CSA (R.7c).

III.4 Conflicting data and lack of data

III.4.1 Conflicting BCF data

When multiple BCF data are available for the same substance, the possibility of conflicting results may arise. In general, conflicting results for a substance, which has been tested several times with an appropriate bioconcentration test, should be interpreted by a 'weight of evidence approach'. This implies that if experimentally determined BCF data, both ≥ and < 500, have been obtained for a substance the data of the highest quality and with the best documentation should be used for determining the bioconcentration potential of the substance. If differences still remain, if for example high-quality BCF values for different fish species are available, generally the highest valid value should be used as the basis for classification. When larger data sets (4 or more values) are available for the same species and life stage, the geometric mean of the BCF values may be used as the representative BCF value for that species.

III.4.2 Conflicting log K_{ow} data

When multiple log K_{ow} data are available for the same substance, the possibility of conflicting results might arise. If log K_{ow} data both ≥ and < 4 have been obtained for a substance, then the data of the highest quality and the best documentation should be used for determining the bioconcentration potential of the substance. If differences still exist, generally the highest valid value should take precedence. In such situation, QSAR estimated log K_{ow} could be used as guidance.

III.4.3 Expert judgement

If no experimental BCF or log K_{ow} data or no predicted log K_{ow} data are available, the potential for bioconcentration in the aquatic environment may be assessed by expert judgement. This may be based on a comparison of the structure of the molecule with the structure of other substances for which experimental bioconcentration or log K_{ow} data or predicted K_{ow} are available. IR&CSA (R.7c) gives guidance on read-across and categories in Chapter 7.10.3.2.

III.5 Decision scheme

Based on the above discussions and conclusions, a decision scheme has been elaborated which may facilitate decisions as to whether or not a substance has the potential for bioconcentration in aquatic species.

Experimentally derived BCF values of high quality are ultimately preferred for classification purposes. BCF results from poor or questionable quality studies should not be used for classification purposes. If no BCF is available for fish species, high quality data on the BCF for some invertebrates (e.g. blue mussel, oyster and/or scallop) may be used as a worst case surrogate.

For non-ionised organic substances, experimentally derived high quality K_{ow} values, or values which are evaluated in reviews and assigned as the "recommended values", are preferred. If no experimental data of high quality are available, validated Quantitative Structure Activity Relationships (QSARs) for log K_{ow} may be used in the classification process. Such validated QSARs may be used without modification in relation to the classification criteria, if restricted to chemicals for which their applicability is well characterised. For difficult substances like strong acids and bases, metal complexes, and surface-active substances a QSAR estimated value of K_{ow} or an estimate based on individual *n*-octanol and water solubilities should be provided instead of an analytical determination of K_{ow}.

If data are available but not validated, expert judgement should be used.

Whether or not a substance has a potential for bioconcentration in aquatic organisms could thus be decided in accordance with the following scheme:

Valid/high quality experimentally determined BCF value → YES:

→ BCF ≥ 500: *The substance meets the criterion*

→ BCF < 500: *The substance does not meet the criterion*

Valid/high quality experimentally determined BCF value → NO:

→ Valid/high quality experimentally determined log K_{ow} value →YES:

→ log K_{ow} ≥ 4: *The substance meets the criterion*

→l og K_{ow} < 4: *The substance does not meet the criterion*

Valid/high quality experimentally determined BCF value → NO:

Valid/high quality experimentally determined log K_{ow} value → NO:

Use of validated QSAR for estimating a log K_{ow} value → YES:

→ log K_{ow} ≥ 4: *The substance meets the criterion*

→ log K_{ow} < 4: *The substance does not meet the criterion*

III.6 References

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IV ANNEX IV: METALS AND INORGANIC METAL COMPOUNDS

IV.1 Introduction

The harmonised system for classifying chemical substances is a hazard-based system, and the basis of the identification of hazard is the aquatic toxicity of the substances, and information on the degradation and bioaccumulation behaviour (OECD 2001). Since this document deals only with the hazards associated with a given substance when the substance is dissolved in the water column, exposure from this source is limited by the solubility of the substance in water and bioavailability of the substance to organisms in the aquatic environment. Thus, the hazard classification schemes for metals and metal compounds are limited to the acute and long-term hazards posed by metals and metal compounds when they are available (i.e. exist as dissolved metal ions, for example, as M+ when present as M-NO_3), and do not take into account exposures to metals and metal compounds that are not dissolved in the water column but may still be bioavailable, such as metals in foods. This section does not take into account the non-metallic ion (e.g. CN^-) of metal compounds which may be toxic. For such metal compounds the hazards of the non-metallic ions must also be considered.

Also organometal compounds may be of concern given they may pose bioaccumulation or persistence hazards. Organometals do not dissociate or dissolve in water as the metal ion, as metals and inorganic metal compounds do. Organometals (e.g. methyl mercury or tributyltin) that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in Section 4. Metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and classified according to this annex (e.g. zinc acetate).

The level of the metal ion which may be present in solution following the addition of the metal and/or its compounds, will largely be determined by two processes: the extent to which it can be dissolved, i.e. its water solubility, and the extent to which it can react with the media to transform to water soluble forms. The rate and extent at which this latter process, known as 'transformation' for the purposes of this guidance, takes place can vary extensively between different compounds and the metal itself, and is an important factor in determining the appropriate hazard class. Where data on transformation are available, they should be taken into account in determining the classification. The Protocol for determining this rate is available as Annex 10 to the UN GHS.

Generally speaking, the rate at which a substance dissolves is not considered relevant to the determination of its intrinsic toxicity. However, for metals and many poorly soluble inorganic metal compounds, the difficulties in achieving dissolution through normal solubilisation techniques are so severe that the two processes of solubilisation and transformation become indistinguishable. Thus, where the compound is sufficiently poorly soluble that the levels dissolved following normal attempts at solubilisation do not exceed the available L(E)C_{50}, it is the rate and extent of transformation, which must be considered. The transformation will be affected by a number of factors, not least of which will be the properties of the media with respect to pH, water hardness, alkalinity, temperature etc. In addition to these properties, other factors such as the size and, in particular, the specific surface area of the particles which have been tested, the length of time over which exposure to the media takes place and, of course the mass or surface area loading of the substance in the media will all play a part in determining the level of dissolved metal ions in the water. Transformation data can generally, therefore, only be considered as reliable for the purposes of classification if conducted according to the standard protocol in Annex 10 to UN GHS. This protocol aims at standardising the principal variables such that the level of dissolved ion can be directly related to the loading of the substance added. It is this loading level which yields the level of metal ion equivalent to the available L(E)C_{50} or NOEC/EC_{10} that can then be used to determine the acute or long-term

hazard category appropriate for classification. The testing methodology is detailed in Annex 10 to the UN GHS. The strategy to be adopted in using the data from the testing protocol, and the data requirements needed to make that strategy work, are described in Annex IV.2, IV.3 and in more detail in Annex IV.5 of this document.

In considering the classification of metals and metal compounds, both readily and poorly soluble, recognition has to be paid to a number of factors. As defined in Annex II, Section II.1, the term 'degradation' refers to the decomposition of organic molecules. For inorganic compounds and metals, clearly the concept of degradability, as it has been considered and used for organic substances, has limited or no meaning. Rather, the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally, the log K_{ow} cannot be considered as a measure of the potential to accumulate. Nevertheless, the concept that a substance, or a toxic metabolite/reaction product may not be rapidly lost from the environment and/or may bioaccumulate, are as applicable to metals and metal compounds as they are to organic substances.

Speciation of the soluble form can be affected by pH, water hardness and other variables, and may yield particular forms of the metal ion which are more or less toxic. In addition, metal ions could be made non-available from the water column by a number of processes (e.g. mineralisation and partitioning). Sometimes these processes can be sufficiently rapid to be analogous to degradation in assessing chronic (long-term) aquatic hazard. However, partitioning of the metal ion from the water column to other environmental media does not necessarily mean that it is no longer bioavailable, nor does it necessarily mean that the metal has been made permanently unavailable.

Information pertaining to the extent of the partitioning of a metal ion from the water column, or the extent to which a metal has been or can be converted to a form that is less toxic or non-toxic is frequently not available over a sufficiently wide range of environmentally relevant conditions, and thus, a number of assumptions will need to be made as an aid in classification. These assumptions may be modified if available data show otherwise. In the first instance it should be assumed that the metal ions, once in the water, are 'not rapidly partitioned' from the water column. Underlying this is the assumption that, although speciation can occur, the species will remain available under environmentally relevant conditions. This may not always be the case, as described above, and any evidence available that would suggest changes to the bioavailability over the course of 28 days, should be carefully examined.

The bioaccumulation of metals and inorganic metal compounds is a complex process and bioaccumulation data should be used with care. The application of bioaccumulation criteria will need to be considered on a case-by-case basis taking due account of all the available data.

A further assumption that can be made, which represents a cautious approach, is that, in the absence of any solubility data for a particular metal compound, either measured or calculated, the metal compound will be assumed to be sufficiently soluble to cause toxicity at the level of the ecotoxicity reference value (ERV), being the acute ERV (expressed as $L(E)C_{50}$), and/or the chronic ERV (expressed as the NOEC/ECx or an HC5 for extensive data sets) and thus may be classified in the same way as other soluble salts of the metal. Again, this is clearly not always the case, and it may be wise to generate appropriate solubility data. Absence of solubility data on the metallic form for a metal for which the soluble salts are classified for the environment, will therefore lead to a default classification due to potential hazard concerns.

This Annex IV deals with metals and inorganic metal compounds. Within the context of this guidance document, metals and metal compounds are characterised as follows:

a. metals (M^0) in their elemental state are not soluble in water but may transform to yield the available form (e.g. Fe^0 will not dissolve as such but the Fe^0 molecules present at the surface of a massive/powder will be first transformed into Fe^{2+} or Fe^{3+} compounds prior to their solubilisation). This means that a metal in the elemental state may react with water or a dilute aqueous electrolyte to form soluble cationic or anionic products, and in

the process the metal will oxidise, or transform, from the neutral or zero oxidation state to a higher one;

b. in a simple metal compound, such as an oxide or sulphide, the metal already exists in the oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium.

Organo-metals are outside the scope of this section.

While oxidisation may not change, interaction with the media may yield more soluble forms. A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield a small amount of the available form by dissolution. However, it should be recognised that the final solution concentration may be influenced by a number of factors, including the solubility product of some metal compounds precipitated during the transformation/dissolution test, e.g. aluminium hydroxide.

IV.2 Application of aquatic toxicity data and solubility data for classification

IV.2.1 Interpretation of aquatic toxicity data

Ecotoxicity data of soluble inorganic compounds are used and combined to define the toxicity of the metal ion under consideration. The ecotoxicity of soluble inorganic metal compounds is dependent on the physico-chemistry of the medium, irrespective of the original metal species released in the environment. Reading across metal compounds can therefore be conducted by comparing the soluble metal ion concentration (µg Me/L) causing the ecotoxicity effect and translating this towards the compound under investigation. A molecular weight correction of the ecotoxicity reference value may be required to classify soluble metal compounds (MW soluble substance/MW metal ion[91]). Poorly soluble metal compounds and metals do not require Molecular weight correction given the amount used for Transformation Dissolution already recognises this into the loading calculation. The comparison is therefore directly done by comparing the soluble fraction measured after Transformation Dissolution with the ecotoxicity reference values of the soluble metal ion (based on the UN GHS, 2009).

When evaluating ecotoxicity data, the general guidance on the weight of evidence (see Section 4.1.3.2.4 of this document) is also applicable to metals.

The term adequacy covers here both the **reliability** *(*inherent quality of a test relating to test methodology and the way that the performance and results of a test are described) and the **relevance** *(*extent to which a test is appropriate to be used for the derivation of an ecotoxicity reference value) of the available ecotoxicity data.

Under the reliability criteria, metal specific considerations include the description of some abiotic parameters in the test conditions for enabling the consideration of the bioavailable metal concentration and free metal ion concentration:

- **Description of the physical test conditions**: further to the general parameters (O_2, T°, pH, …) abiotic parameters such as dissolved organic carbon (DOC), hardness, alkalinity of the water that govern the speciation and hence the metal bioavailability is required. A proper description of culture conditions related to the level of essential metals is required to avoid artefacts due to acclimatisation/adaptation (see also below);
- **Description of test materials and methods**: to calculate the free metal ion concentration with speciation models the concentrations of dissolved major ions and cations like Al, Fe, Mg, Ca… are required;

[91] Note that this calculation needs to be adjusted to reflect the stoichiometry of the compound, for example for $Zn_3(PO_4)_2$ the MW metal would be multiplied by three.

- **Concentration-effect relationship; hormesis**: sometimes an increased performance in growth or reproduction is seen at low metal doses that exceed the control values, referred to as hormesis. Such effects can be important especially for major trace nutrients such as Fe, Zn and Cu but can also occur with a wide variety of non-essential substances. In such cases, positive effects should not be considered in the derivation of acute ERV's and especially chronic ERV's, likely other models than the conventional log-logistic dose-response model should be used to fit the dose-response curve and consideration should be given to the adequacy of the control diet/exposure. Due to the essential nutritional needs, caution is needed with regards to extrapolation of the dose-response curve (e.g. to derive an acute ERV) below the lowest tested concentration.

Under the relevancy criteria, certain considerations need to be made, related to the relevancy of the test substance and to acclimatisation/adaptation:

- **Relevance of the test substance**: soluble metal salts should be used for the purpose of classification of inorganic metals/metal compounds. The ecotoxicity adapted from organic metal compounds exposure should not be used.
- **Acclimatisation/adaptation**: for essential metals, the culture medium should contain a minimal concentration not causing deficiency for the test species used. This is especially relevant for organisms used for long-term toxicity tests where the margin between essentiality and toxicity may become small. As an example, for algae, depletion of the strong complexing agent EDTA from the medium may result in iron deficiency.

Aquatic toxicity studies carried out according to a recognised protocol should normally be acceptable as valid for the purposes of classification. Annex I should also be consulted for generic issues that are common to assessing any aquatic toxicity data point for the purposes of classification.

IV.2.1.1 Metal complexation and speciation

The toxicity of a particular metal in solution, appears to depend primarily on (but is not strictly limited to) the level of dissolved free metal ions and the physico-chemistry of the environment. Abiotic factors including alkalinity, ionic strength and pH can influence the toxicity of metals in two ways: (i) by influencing the chemical speciation of the metal in water (and hence affecting the availability) and (ii) by influencing the uptake and binding of available metal by biological tissues. For the classification of metals, Transformation/Dissolution is carried out over a pH range. Ideally both T/D and ecotoxicity data are compared at a similar pH since both parameters will vary with pH. However, the majority of ecotoxicity tests are performed at the higher pH range (i.e. > pH 7.5) and ecotoxicity data obtained at lower pH are often scarce. Bioavailability and speciation models (e.g. respectively Biotic Ligand Models and WHAM (Tipping, 1994), as discussed below) may allow to normalise ecotoxicity data obtained at a given pH to other pH values, relevant to the T/D data. The applicability of the bioavailability models to the biological species for which data are available must be evaluated. Guidance on the Bioavailability correction for metals can be found in IR&CSA Annex R.7.13.2).

Where chemical speciation is important, it may be possible to model the concentrations of the different chemical forms of the metal, including those that are likely to cause toxicity. Analysis methods for quantifying exposure concentrations, which are capable of distinguishing between the complexed and uncomplexed fractions of a test substance, may not always be available or economic.

Complexation of metals to organic and inorganic ligands in test media and natural environments can be estimated from metal speciation models. Speciation models for metals, including pH, hardness, DOC, and inorganic substances such as MINTEQ (Brown and Allison, 1987), WHAM (Tipping, 1994) and CHESS (Santore and Driscoll, 1995) can be used to calculate the uncomplexed and complexed fractions of the metal ions.

Alternatively, and when available for the metal, the Biotic Ligand Model (BLM), allows, for the calculation of the acute and/or chronic ERV's of the metal ion, for different pH values, through integration of metal speciation and its interaction with the organism. The BLM model has at present been validated for a number of metals, organisms, and end-points (Santore and Di Toro, 1999). The models and formula used for the characterisation of metal complexation in the media should always be clearly reported, allowing for their translation back to natural environments (OECD, 2000). In case a metal-specific BLM is available covering an appropriate pH range, a normalised comparison of aquatic toxicity data can be made using the entire effects database for different reference pH values.

IV.2.2 Interpretation of solubility data

When considering the available data on solubility, their validity and applicability to the identification of the hazard of metal compounds should be assessed. In particular, the pH and the medium in which the data were generated should be known.

IV.2.2.1 Assessment of existing data

Existing data will be in one of the three forms: **for soluble, insoluble metal compounds and the metallic form**. For some well-studied metals, there will be solubility products and/or solubility data for the various inorganic metal compounds. It is also possible that the pH relationship of the solubility will be known. However, for many metals or metal compounds, it is probable that the available information will be descriptive only, e.g. poorly soluble or resulting from the water solubility test form the OECD 105 physico-chemical water dissolution test. Unfortunately there appears to be very little (consistent) guidance about the solubility ranges for such descriptive terms. Where these are the only information available it is most probable that solubility data will need to be generated using the Transformation/Dissolution Protocol (Annex 10 to the UN GHS).

IV.2.2.2 Screening T/D test for assessing solubility of metal compounds

In the absence of solubility data, a simple 'Screening Test' for assessing solubility, based on the high rate of loading (100 mg/l) for 24 h and rigid stirring conditions, should be used for metal compounds as described in the Transformation/Dissolution Protocol (Annex 10 to the UN GHS). The function of the screening test is to identify those metal compounds which undergo either dissolution or rapid transformation such that they are indistinguishable from soluble forms and hence may be classified based on the dissolved ion concentration and those who dissolves slowly and can be assessed in the same way as the metallic form. Where data are available from the screening test detailed in the Transformation/Dissolution Protocol, the maximum solubility obtained over the tested pH range should be used. Where data are not available over the full pH range, a check should be made that this maximum solubility has been achieved by reference to suitable thermodynamic speciation models or other suitable methods (see Section IV.2.1 of this document). It should be noted that this test is only intended to be used for inorganic metal compounds. Metals should immediately be assessed at the level of the full T/D test.

IV.2.2.3 Full T/D test for assessing solubility of metals and metal compounds

The Full Transformation Dissolution test should be carried out at the pH[92] that maximises the concentration of dissolved metal ions in solution and that expresses the highest toxicity.

Based on the data from the Full Test, it is possible to generate a concentration of the metal ions in solution after 7 days (short-term test) for each of the three loadings (i.e. 1 mg/l as 'low', 10 mg/l as 'medium' and 100 mg/l as 'high loading') used in the test. If the purpose of the test is to assess the long-term hazard of the substance, then the loadings[93] should be 0.01 mg/l, 0.1 mg/l or 1 mg/l depending on the transformation rate and the duration of the test being extended to 28 days (long-term test).

[92] The UN GHS transformation/dissolution protocol specifies a pH range of 6-8.5 for the 7days test and 5.5 to 8.5 for the 28 days test. Considering the difficulty in carrying out transformation/dissolution tests at pH 5.5, the OECD only validated the test in the pH range of 6-to 8.5.

[93] The standard protocol in Annex 10 to UN GHS presently only foresees a long-term loading rate of 1 mg/l and lower loading rates may not even be practically feasible for each case. While TDp testing at lower loading rates is in principle the best way forward it is technically often not feasible for the lower chronic loading rates. Extensive experience with the T/D protocol demonstrated that reliable predictions can be made for other loading rates. In order to make maximal use of existing Transformation Dissolution data, the 28 days results for the lower chronic loading rates (0,1 and 0,01 mg/l) can therefore be derived by extrapolation from TDp evidence from other loading rates. Such read-across should be justified on a case by case basis and supported by reliable information on the T/D at different loading rates, e.g. over 7 and/or 28 days. It should be noted that the relationship between loading rate and dissolved metal concentration may well not be linear. Therefore extrapolation of T/D data to lower loadings should preferably be made by using the equations of section A10.6.1 of the UN-Annex 10 transformation dissolution protocol or alternatively by extrapolating in a precautionary way.

The UN announced to change/update Annex 10 in the near future to bring it better in line with the chronic classification strategy an aim that is already anticipated in this guidance note for the CLP.

IV.2.3 Comparison of aquatic toxicity data and solubility data

A decision on whether or not the substance is classified will be made by comparing aquatic toxicity data and solubility data. Depending on the available data two approaches can be followed.

1. When only a **limited dataset** is available existing data should be taken together irrespective of whether the toxicity and dissolution data are at the same pH and the lowest data point should give the basis for classification (this should be used as the default approach). This default approach may lead to the lowest toxicity data point compared with the highest Transformation Dissolution result each derived at different pH levels used for the purpose of classification.
2. When a more **extensive toxicity/dissolution dataset** is available, a split of the acute and chronic ecotoxicity reference values can be performed according to their pH used during T/D test. The worst case classification entry across pHs should be used based on comparing TDp data with relevant ecotox data across the pH range. Meaning that toxicity data and transformation data are in this case always compared at the same pH.

This split of the effects data into pH classes would apply in an equal way to the acute and the long-term effects data sets.

IV.3 Assessment of environmental transformation

Environmental transformation of one species of a metal to another species of the same metal does not constitute 'degradation' as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. In addition naturally occurring geochemical processes can partition metal ions from the water column while also other processes may remove metal ions from the water column (e.g. by precipitation and speciation). Data on water column residence time, the processes involved at the water – sediment interface (i.e. deposition and re-mobilisation) are fairly extensive for some metals. Using the principles and assumptions discussed above in Section IV.1 of this document, it may therefore be possible to incorporate this approach into the classification.

Such assessments are difficult to give guidance for and will normally be addressed on a case-by-case approach. However, the following may be taken into account:

a. Changes in speciation if they are to non-available forms, however, the potential for the reverse change to occur must also be considered;

b. Changes to a metal compound which is considerably less soluble than that of the metal compound being considered.

Some caution is recommended; see Section IV.1 of this document, the 5th and 6th paragraph.

⚠ Comment by ECHA: Please note that in the light of a lack of scientific consensus and continuing discussions on the interpretation of rapid removal from the water column in the context of classification, it has been decided to remove certain parts from the Annex IV for the time being until agreement on the validity of use of the concept of rapid removal for classification purposes has been reached.

IV.4 Bioaccumulation

While log Kow is a good predictor of BCF for certain types of organic compounds e.g. nonpolar organic substances, it is irrelevant for inorganic substances such as inorganic metal compounds because metals, in contrast to organic substances, are not lipophilic and are not passively transported through cellular membranes. Uptake of metal ions occurs through active processes.

The mechanisms for uptake and depuration rates of metals are very complex and variable and there is at present no general model to describe this. Instead the bioaccumulation of metals according to the classification criteria should be evaluated on a case-by-case basis using expert judgement.

While BCFs are indicative of the potential for bioaccumulation there may be a number of complications in interpreting measured BCF values for metals and inorganic metal compounds. For most metals and inorganic metal compounds the relationship between water concentration and BCF in aquatic organisms is inverse, and bioconcentration data should therefore be used with care. This is particularly relevant for metals that are biologically essential. Metals that are biologically essential are actively regulated in organisms in which the metal is essential (homeostasis). Removal and sequestration processes that minimise toxicity are complemented by an ability to up-regulate concentrations for essentiality. Since nutritional requirement of the organisms can be higher than the environmental concentration, this active regulation can result in high BCFs and an inverse relationship between BCFs and the concentration of the metal in water. When environmental concentrations are low, high BCFs may be expected as a natural consequence of metal uptake to meet nutritional requirements and can in these instances be viewed as a normal phenomenon. Also, while a metal may be essential in a particular organism, it may not be essential in other organisms. Therefore, where the metal is not essential or when the bioconcentration of an essential metal is above nutritional levels, special consideration should be given to the potential for bioconcentration and environmental concern.

Non- essential metals are also actively regulated to some extent and therefore also for non-essential metals, an inverse relationship between the metal concentration and the external concentration may be observed (McGeer *et al*., 2003).

Consequently for both essential and non-essential elements, measured BCFs decline as external concentration increases. When external concentrations are so high that they exceed a threshold level, or overwhelm the regulatory mechanism, this can cause harm to the organism

BCF and BAF may be used to estimate metal accumulation by:

a. Considering information on essentiality and homeostasis of metals/ metal compounds. As a result, of such regulation, the 'bioaccumulative' criterion is not applicable to these metals.

b. Assessing bioconcentration factors for non-essential metals, should preferably be done from BCF studies using environmentally relevant concentrations in the test media.

IV.5 Classification strategies for metals and metal compounds

IV.5.1 Introduction

⚠ Notice! *Acute and long-term hazards* are assessed individually.

For determination of long-term hazards preference should be given in applying the approach based on chronic toxicity data. Such evidence is often frequently available for the bioavailable forms of metals.

The schemes for the determination of acute and long-term aquatic hazards of metals and metal compounds are described below and summarised diagrammatically in the figures:

IV.5.2.1 (acute hazard classification of metals);

IV.5.2.2 (a and b) (long-term hazard of metals);

IV.5.3.1 (acute hazard classification of metal compounds);

IV.5.3.2 (a and b) (long-term hazard of metal compounds).

There are several stages in these schemes where data are used for decision purposes. It is not the intention of the classification schemes to generate new ecotoxicity data. In the absence of valid data, it will be necessary to use all available data and expert judgement.

In the following sections, the reference to the acute and chronic ERV's refer to the data point(s) that will be used to select the hazard category(ies) for the metal or metal compound.

When considering acute and chronic ERV's data for metal compounds, it is important to ensure that the data point to be used as the justification for the classification is expressed in the weight of the molecule of the metal compound to be classified. This is known as correcting for molecular weight. Thus while most metal data is expressed in, for example, mg/l of the metal (ion), this value will need to be adjusted to the corresponding weight of the metal compound. Thus:

Acute $ERV_{compound}$ = acute ERV of the metal compound = acute ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

Chronic $ERV_{compound}$ = chronic ERV of the metal compound = chronic ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

IV.5.2 Classification strategies for metals

⚠ Notice! *Acute and long-term hazards* are assessed individually.

IV.5.2.1 Classification strategy for determining acute aquatic hazard for metals

The scheme for the determination of *acute* aquatic hazard for metals are described in this section and summarised diagrammatically in Figure IV. 1.

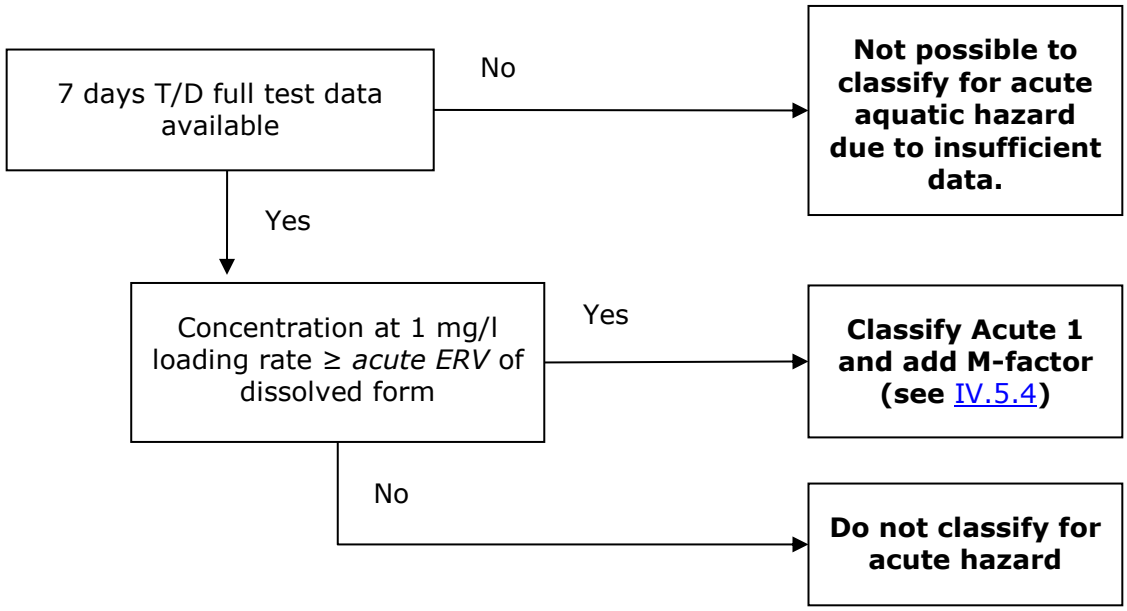
Where *the acute ERV* for the metal ions of concern is greater than 1 mg/l the metals need not be considered further in the classification scheme for acute hazard.

Where the acute ERV for the metal ions of concern is less than or equal to 1 mg/l consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where 7d data from the Transformation/Dissolution protocol are available, then the results should be used to classify, according to the following rule:

Classify the metal as ***Category Acute 1*** if the dissolved metal ion concentration after a period of 7 days (or earlier for a significant time period) at a loading rate of 1 mg/l exceeds that of the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4).

Figure IV. 1 Classification strategy for determining acute aquatic hazard for metals



IV.5.2.2 Classification strategy for determining long-term aquatic hazard for metals

The scheme for the determination of *long-term* aquatic hazard for metals are described in this section and summarised diagrammatically in Figure IV. 2 and IV. 3.

Metals can be classified for long-term aquatic hazards:

1. using chronic reference data when available; or
2. using the surrogate approach in absence of appropriate chronic toxicity reference data.

In case relevant chronic ecotoxicity data (chronic ERV) are available the approach comparing chronic ERV with 28 days transformation/dissolution reference should be applied as described under IV.5.2.2.1 while otherwise the surrogate approach (see IV.5.2.2.2) should be followed.

IV.5.2.2.1 Approach based on available chronic toxicity reference data

Where *the chronic ERV* for the metal ions of concern is greater than 1 mg/l, the metals need not be considered further in the classification scheme.

Where the chronic ERV for the metal ions of concern is less than or equal to 1 mg/l consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 28 d period.

Where such T/Dp data are unavailable the surrogate approach should be applied (see Section IV.5.2.2.2). Where 28d data from the Transformation/Dissolution protocol are available, then, the results should be used to aid classification according to the following rules:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained at a loading rate of 0.1 mg/l is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained at a loading rate of 1 mg/l is greater than or equal to the chronic ERV.

If there is evidence of rapid environmental transformation:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained at a loading rate of 0.01 mg/l is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained at a loading rate of 0.1 mg/l is greater than or equal to the chronic ERV; or

c. **Classify** the metal as ***Category Chronic 3*** if the dissolved metal ion concentration obtained at a loading rate of 1 mg/l is greater than or equal to the chronic ERV.

Do not classify for long-term hazard if the dissolved metal ion concentration obtained from the 28 day Transformation/Dissolution test at ***a loading rate of 1 mg/l*** is less than the chronic ERV of the metal ion.

IV.5.2.2.2 The surrogate approach

Where the acute ERV for the metal ions of concern is less than or equal to 100 mg/l consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where such T/Dp data are unavailable, i.e. there is no clear data of sufficient validity to show that the transformation to metal ions will not occur; the safety net classification (Category Chronic 4) should be applied since the known classifiable toxicity of these soluble forms is considered to give rise to sufficient concern.

Where T/Dp data are available classification should be according to the following rules:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the low loading rate (1 mg/l) is greater than or equal to the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4);

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the medium loading rate (10 mg/l) is greater than or equal to the acute ERV;

c. **Classify** the metal as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/l) is greater than or equal to the acute ERV.

d. **Classify** the metal as ***Category Chronic 4*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/l) is lower than the acute ERV.

Figure IV. 2 Classification strategy for determining long-term aquatic hazard for metals

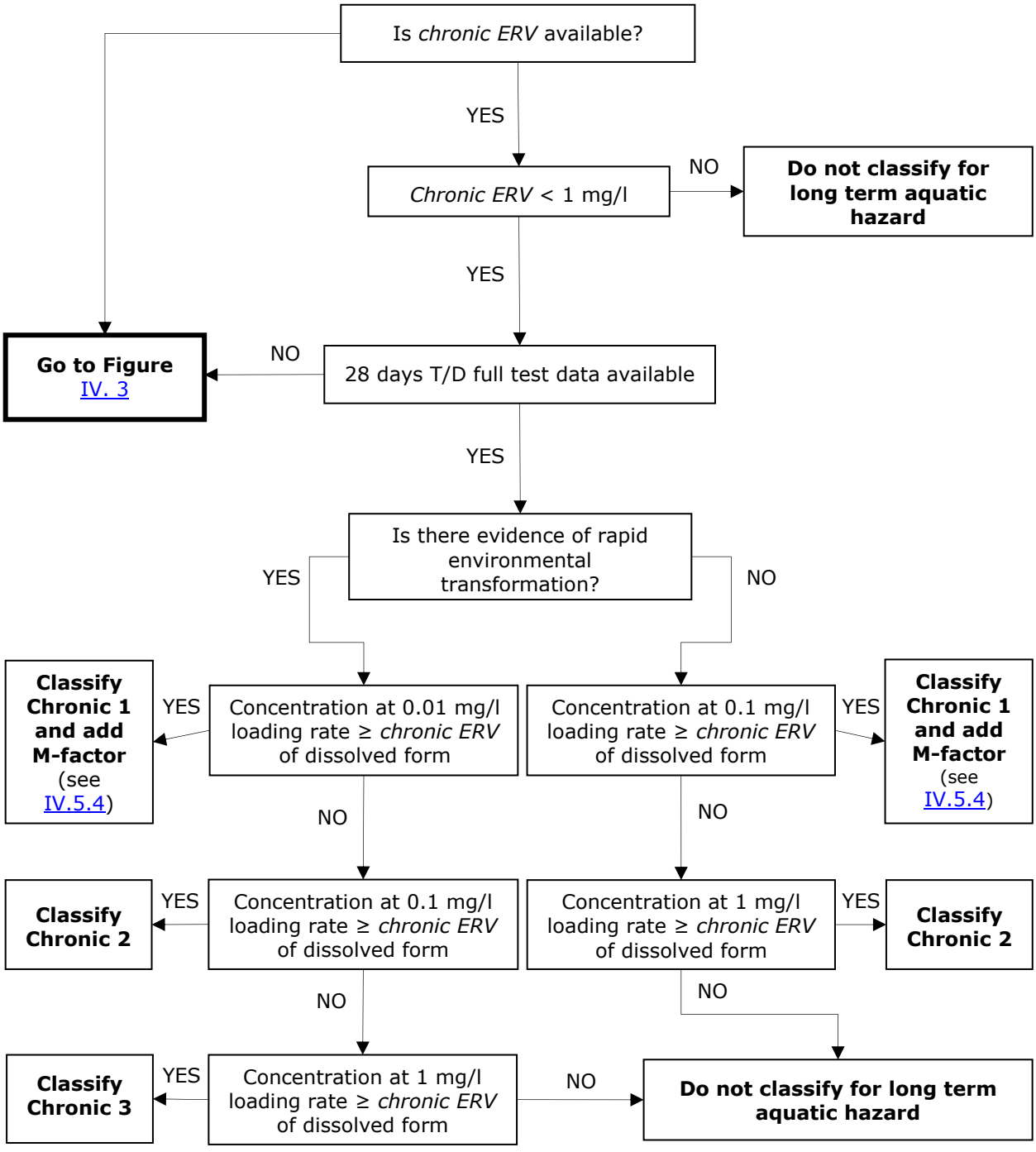
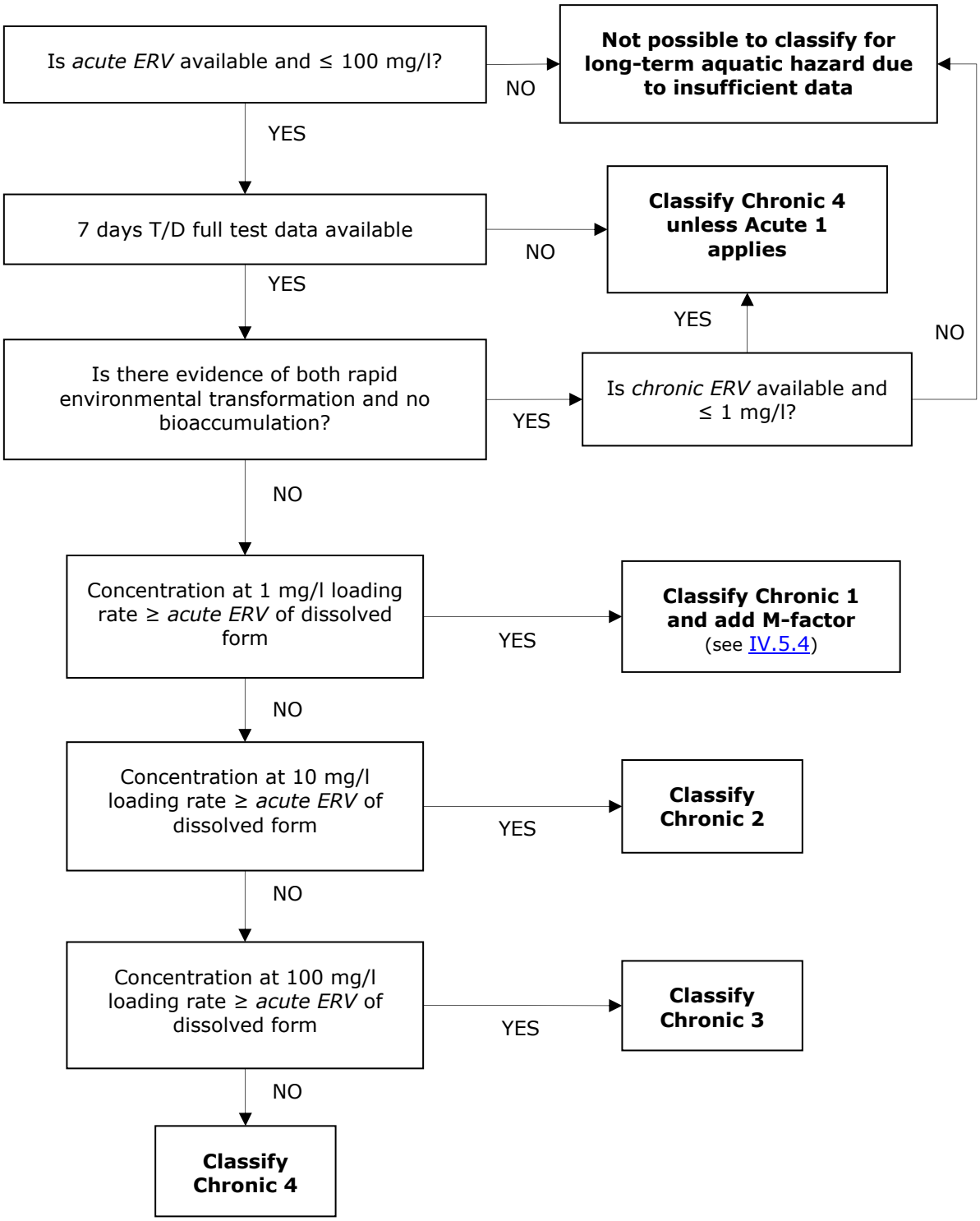


Figure IV. 3 Classification strategy for determining long-term aquatic hazard for metals in absence of appropriate chronic toxicity reference and/or T/Dp data



IV.5.3 Classification strategies for metal compounds

Notice! Acute and long-term hazards are assessed individually

A metal compound will be considered as ***readily soluble*** if:

- the water solubility (measured through a 24-hour Dissolution Screening test or estimated e.g. from the solubility product) is greater or equal to the acute ERV of the dissolved metal ion concentration; or
- if such data are unavailable, i.e. there are no clear data of sufficient validity to show that the transformation to metal ions will not occur.

Care should be exercised for metal compounds whose solubility is close to the acute toxicity reference value as the conditions under which solubility is measured could differ significantly from those of the acute toxicity test. In these cases the results of the Dissolution Screening Test are preferred.

Metal compounds that have lower water solubility than the acute ERV through a 24-hour Dissolution Screening test or estimated from the solubility product, are considered as ***poorly soluble metal compound.***

IV.5.3.1 Classification strategies for determining acute aquatic hazard for metal compounds

The scheme for the determination of *acute* aquatic hazard for metal compounds are described in this section and summarised diagrammatically in Figure IV. 4.

Where the acute ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as *acute* $ERV_{compound}$) is greater than 1 mg/l, the metal compounds need not to be considered further in the classification scheme for acute hazard.

Where the acute $ERV_{compound}$ is less than or equal to 1 mg/l, consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal compound. Such data, to be valid and useable should have been generated using the T/D (Annex 10 to UN GHS).

Readily soluble metal compounds

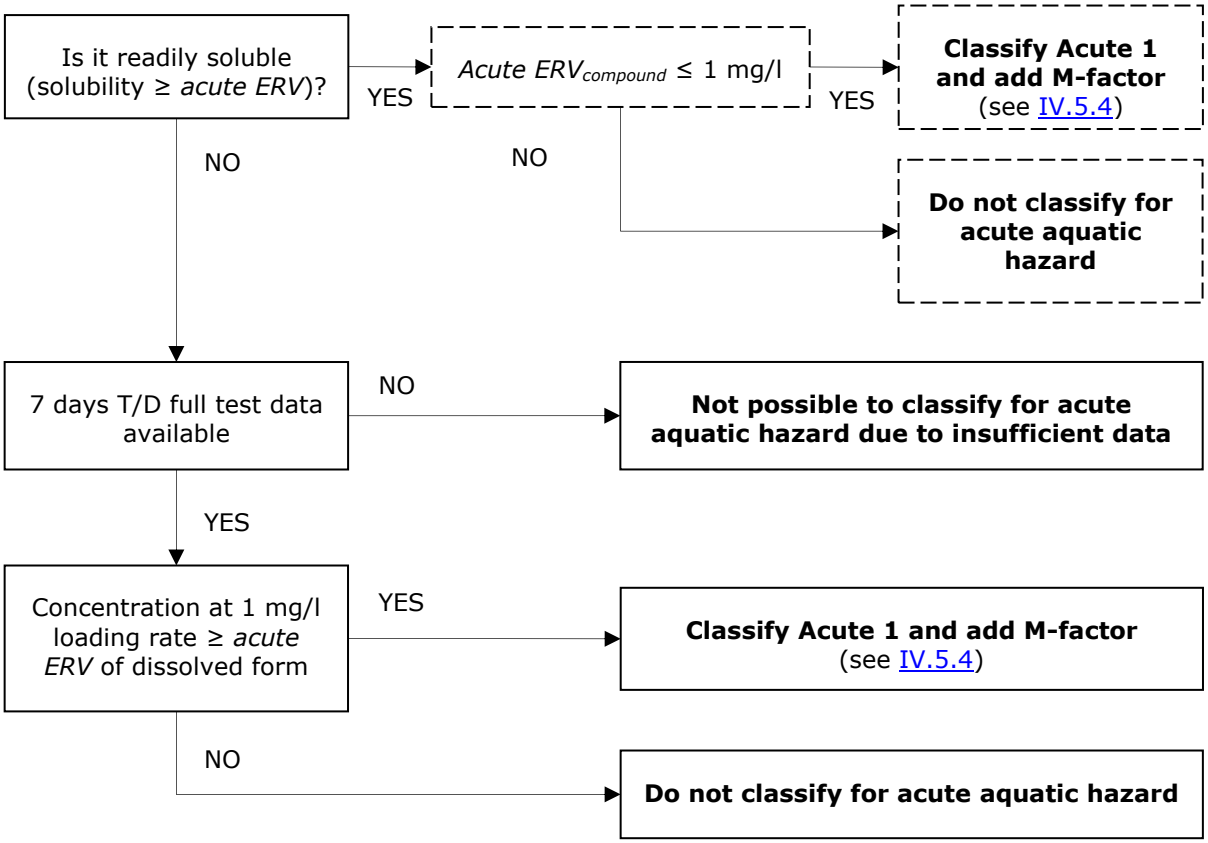
Classify the metal compound as ***Category Acute 1*** if the acute $ERV_{compound} \leq 1$ mg/l, an M-factor must also be established as part of this classification (see IV.5.4).

Poorly soluble metal compounds

Where 7d data from the Transformation/Dissolution protocol are available, then the results should be used to classify sparingly soluble metal compounds, according to the following rule:

> Classify the metal compound as *Category Acute 1* if the dissolved metal ion concentration after a period of 7 days (or earlier for a significant time period) at a loading rate of 1 mg/l exceeds that of the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4).

Figure IV. 4 Classification strategy for determining acute aquatic hazard for metal compounds



IV.5.3.2 Classification strategy for determining long-term aquatic hazard for metal compounds

The scheme for the determination of *long-term* aquatic hazard for metal compounds are described in this section and summarised diagrammatically in Figure IV. 5 and IV. 6.

Metal compounds can be classified for long-term aquatic hazards:

1. using chronic reference data when available; or
2. using the surrogate approach in absence of appropriate chronic toxicity reference data.

In case relevant chronic ecotoxicity data (chronic ERV) are available the approach comparing chronic ERV of the dissolved metal ion with release data of 28 days transformation/dissolution, should be applied as described under IV.5.3.2.1 while otherwise the surrogate approach (see IV.5.3.2.2) should be followed.

IV.5.3.2.1 Approach based on available chronic toxicity reference data

Where the chronic ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as *chronic* $ERV_{compound}$) is greater than 1 mg/l, the metal compounds need not to be considered further in the classification scheme for long-term hazard.

Readily soluble metal compounds

Readily soluble metal compounds are classified on the basis of chronic ERV of the dissolved metal ion, corrected for the molecular weight of the compound (further called as chronic $ERV_{compound}$) .

If there is *no evidence* of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the chronic $ERV_{compound} \leq 0.1$ mg/l, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the chronic $ERV_{compound} >$ 0.1mg/l and ≤ 1 mg/l.

If there is *evidence* of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the chronic $ERV_{compound} \leq 0.01$ mg/l,an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the chronic $ERV_{compound} >$ 0.01mg/l and ≤ 0.1 mg/l; or

c. **Classify** the metal compound as ***Category Chronic 3*** if the chronic $ERV_{compound} >$ 0.1mg/l and ≤ 1 mg/l.

Poorly soluble metal compounds

Where *the chronic ERV* for the metal ions of concern is greater than 1 mg/l, the metals need not be considered further in the classification scheme.

Where the chronic $ERV_{\mathbf{compound}}$ is less than or equal to 1 mg/l consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal compound. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 28d period.

Where 28d T/Dp data are unavailable, the surrogate approach should be applied (see Section IV.5.3.2.2).

Where 28d data from the Transformation/Dissolution protocol are available, then classify according to the following rules:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.1 mg/l is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 1 mg/l is greater than or equal to the chronic ERV.

If there is evidence of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.01 mg/l is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.1 mg/l is greater than or equal to the chronic ERV; or

c. **Classify** the metal compound as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 1 mg/l is greater than or equal to the chronic ERV.

Do not classify for long-term hazard if the dissolved metal ion concentration obtained from the 28 day Transformation/Dissolution test at a loading rate of 1 mg/l is less than the chronic ERV of the dissolved metal ion.

IV.5.3.2.2 The surrogate approach

Readily soluble metal compounds

In absence of relevant chronic toxicity data, and unless there is evidence of both rapid environmental transformation and evidence of no bioaccumulation (see Sections IV.3 and IV.4), *readily soluble metal compounds* are classified as:

a. **Category Chronic 1** if the acute $ERV_{compound} \leq 1$ mg/l, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Category Chronic 2** if the acute $ERV_{compound} > 1$mg/l and ≤ 10 mg/l; or

c. **Category Chronic 3** if the acute $ERV_{compound} > 10$mg/l and ≤ 100 mg/l.

Poorly soluble metal compounds

Where the acute $ERV_{compound}$ is less than or equal to 100 mg/l consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where such 7d T/Dp data are unavailable, i.e. there is no clear data of sufficient validity to show that the transformation to metal ions will not occur; the safety net classification (Category Chronic 4) has to be applied.

Where T/Dp data are available but relevant chronic ERVs are absent, the results should be used to aid classification according to the following rules:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the low loading rate (1 mg/l) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation, an M-factor must also be established as part of this classification (see IV.5.4);

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the medium loading rate (10 mg/l) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation;

c. **Classify** the metal compound as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/l) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation;

d. **Classify** the metal compound as ***Category Chronic 4*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/l) is lower than the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation.

Figure IV. 5 Classification strategy for determining long-term aquatic hazard for metal compounds

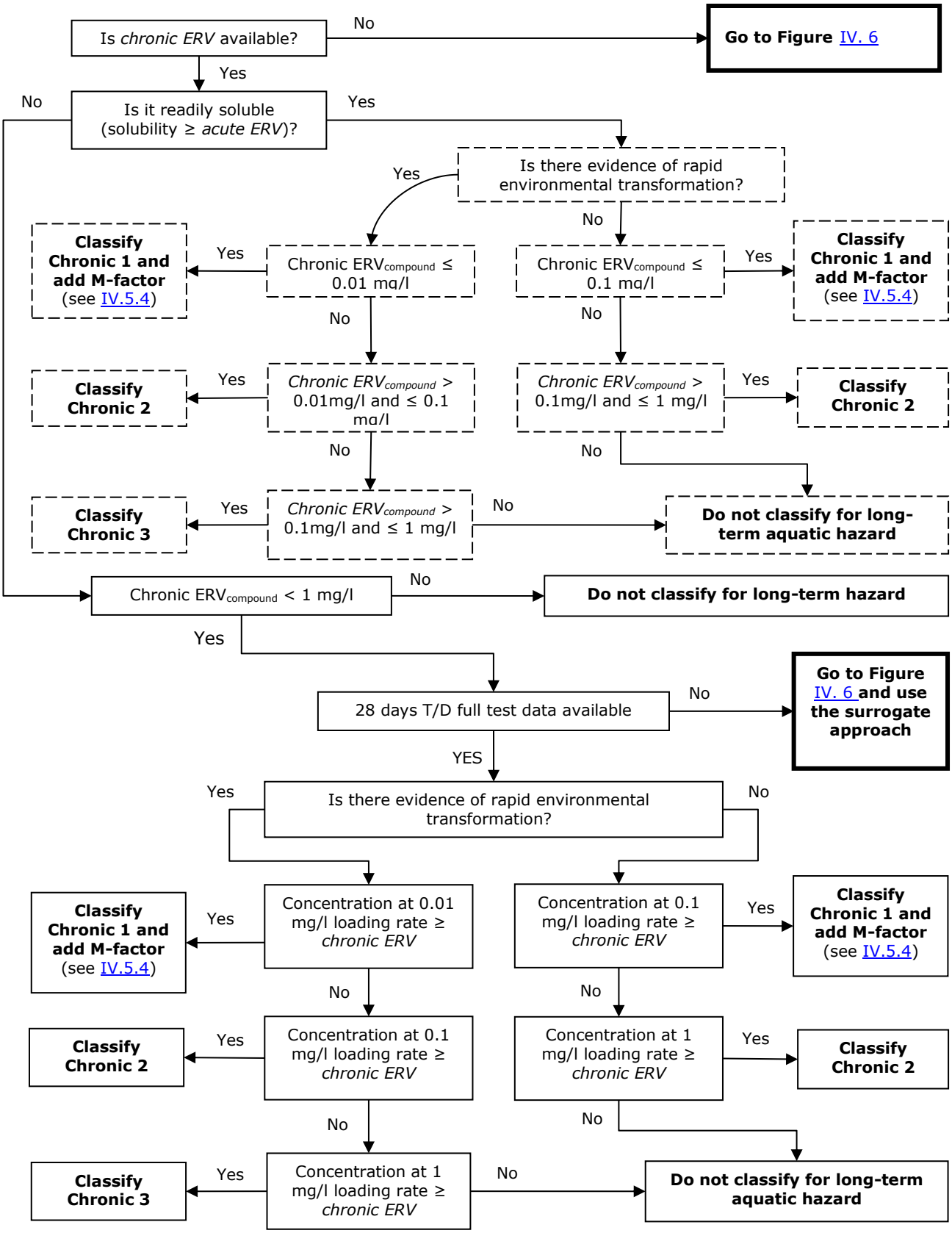
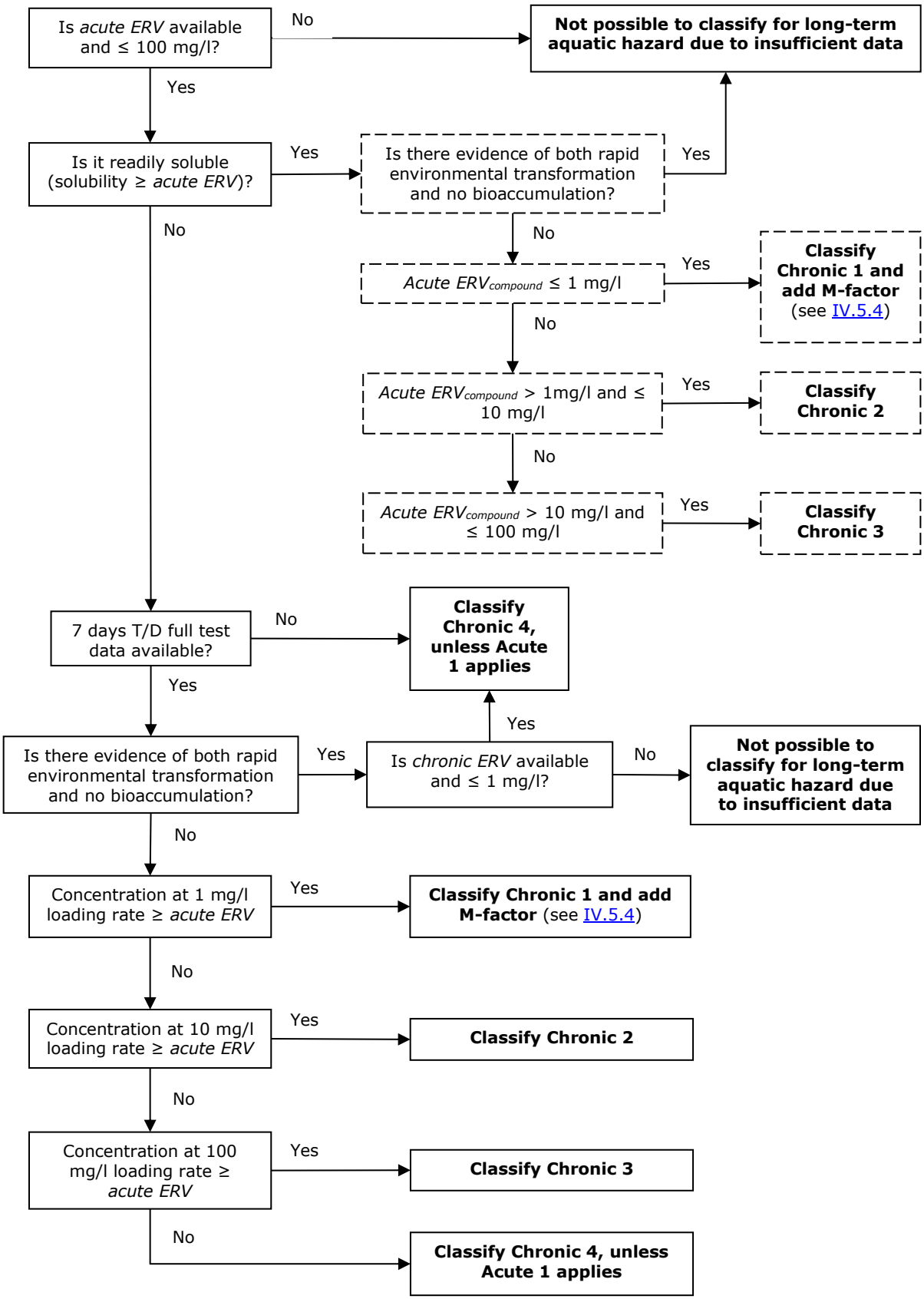


Figure IV. 6 Classification strategy for determining long-term aquatic hazard for metal compounds in absence of appropriate chronic toxicity reference and/or T/Dp data



IV.5.4 Setting M-factors for metals and inorganic metal compounds

For the hazard class "Hazardous to the Aquatic Environment", SCLs are not applicable. Instead the M-factors concept is used.

The M-factors are used in application of summation method for classification of mixtures containing substances that are classified as very toxic. The concept of M-factors has been established to give an increased weight to very toxic substances when classifying mixtures. M-factors are only applicable to the concentration of a substance classified as hazardous to the aquatic environment (categories Acute 1 and Chronic 1) and are used to derive by the summation method the classification of a mixture in which the substance is present. They are, however, substance-specific and it is important that they are being established already when classifying substances.

M-factors should have been established in accordance with Article 10 of CLP and be available in the C&L Inventory.

For the harmonised classifications in Annex VI to CLP, M-factors shall be set by the manufacturer, importer or downstream user in case there is no M-factor provided, in accordance with CLP Article 10(4).

For *soluble metal compounds* M-factors are applied as for organic substances (see Table IV. 1).

For poorly soluble metal compounds and metals M-factors can be estimated from the ratio of the soluble metal ions concentrations obtained from Transformation Dissolution (at respectively 7 d or 28 d's for a loading of 1 mg/l) and the ERV of the dissolved metal ion taking the considerations mentioned in I.V.2.3 into account. If this ratio is:

- below 10 then an M-factor of 1 should be applied;
- 10 and < 100 then the M-factor would be 10;
- 100 and < 1000 then the M-factor would be 100.

Continue in factor 10 intervals

Table IV. 1 M-factors for inorganic substances

Acute ERV (mg/L)	Multiplying factors (M)
0,1 < Acute ERV < 1	1
0,01 < Acute ERV < 0,1	10
0,001 < Acute ERV < 0,01	100
0,0001 < Acute ERV < 0,001	1000
Continue in factor 10 intervals	10000

Chronic ERV (mg/L)	Multiplying factors (M)	
	No rapid environmental transformation	Rapid environmental transformation
0,01 < Chronic ERV < 0,1	1	1
0,001 < Chronic ERV < 0,01	10	1
0,0001 < Chronic ERV < 0,001	100	10
0,00001 < Chronic ERV < 0,0001	1000	100
Continue in factor 10 intervals		

IV.5.5 Particle size and surface area

Surface area is a crucial parameter in that any variation in surface area tested may cause a significant change in the levels of metals ions released in a given time-window. Thus, particle size or surface area is fixed for the purposes of the transformation test, allowing the comparative classifications to be based solely on the loading level. Normally, the classification data generated would have used the smallest particle size marketed to determine the extent of transformation. There may be cases where data generated for a particular metal powder are not considered as suitable for classification of the massive forms. For example, where it can be shown that the tested powder is structurally a different material (e.g. different crystallographic structure) and/or it has been produced by a special process and is not generally generated from the massive metal, classification of the massive can be based on testing of a more representative particle size or surface area, if such data are available. The powder may be classified separately based on the data generated on the powder. However, in normal circumstances it is not anticipated that more than two classification proposals would be made for the same metal.

Metals with a particle size smaller than the default diameter value of 1 mm can be tested on a case-by-case basis. One example of this is where metal powders are produced by a different production technique or where the powders give rise to a higher dissolution (or reaction) rate than the massive form leading to a more stringent classification.

The particle sizes tested and/or used for classification and labelling depend on the substance being assessed and are shown in the table below:

Type	Particle size	Comments
Metal compounds	Smallest representative size sold	Never larger than 1 mm
Metals – powders	Smallest representative size sold	May need to consider different sources if yielding different crystallographic/ morphologic properties
Metals – massive	1 mm	Default value may be altered if sufficient justification

Massives will usually be tested as 1 mm particles. Alternatively, the T/D testing of materials with different surface area's may result in highly reliable dissolution kinetic equations that allows to define the 'Critical Particle Diameter' (CPD) for appropriate loadings for the acute and long-term hazard assessment.

For most metals and some metal compounds, it is possible, using the Transformation/ Dissolution Protocol (Annex 10 to UN GHS), to obtain a correlation between the concentration of the metal ion after a specified time interval as a function of the surface area loadings of the forms tested. Such correlations should be established for the relevant pH ranges as specified in the protocol. In such cases, it could then be possible to estimate the level of dissolved metal ion concentration at a given pH of the metal with different particles, using the critical surface area approach [Skeaff *et. al.* (2000)]. From this correlation and a linkage to the appropriate toxicity data at corresponding pH level, it is possible to determine a "Critical Surface Area" (CSA) of the substance that delivers the $L(E)C_{50}$ to the dissolution medium and then to convert the CSA to a Critical Particle Diameter (CPD) (see example). This CPD at appropriate mass loadings for acute and long-term hazard assessment can then be used to:

- determine the classification category of powders based on the finest representative powder on the market; and
- determine an accurate classification of the massive metal by applying a 1 mm (default) diameter.

Within the CSA Approach an equation is developed to predict metal ion release (based on previously measured metal ion release from different loadings of the metal), which is correlated to measured surface area, and a corresponding calculated equivalent particle diameter. The basis of the CSA Approach is that ***the release of metal ions is dependent on the surface area of the substance***, with this release being predictable once the relationship has been established. The CSA is the surface area loading (mm^2/l) to a medium that delivers a selected ecotoxicity reference value to that medium. The term *SA* is the measured specific surface area (m^2/g) of the metal sample. The measured specific critical surface area (SA_{crit}) (m^2/g) is the measured specific surface areas for the corresponding low, medium and high loadings which are associated with the respective acute and long-term aquatic toxicity classification categoriess in the classification scheme for metals and metal compounds. A typical equation for this relationship for a given substance, aquatic medium, pH and retention time is:

$$\log (C_{Me(aq)}, \text{mg/l}) = a + b \log(A_{meas})$$

$C_{Me(aq)}$ = total dissolved concentration of metal ion (mg/l) at a particular length of test time (*i.e.* 168 hours for acute toxicity transformation testing) under certain conditions (*i.e.* pH, specified medium, etc.), as determined by transformation/dissolution testing of different surface area loadings

a, *b* = regression coefficients

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) X (substance mass loading in g/l) X 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

IV.5.6 Classification of mixtures of metals and metal compounds

Simple composed metal or metal compound mixtures should be handled as mixtures and classified according to the mixtures rules described in Section 4.1.4 given they normally express toxicity as a function of their composing ingredients. Ores and concentrates and UVCB inorganics are considered as substances in respect to CLP, but follow in general the mixture ruling to determine their classification unless specific ecotoxicity data are available for the mineral(s) under consideration.

Ores and concentrates and inorganic UVCBs are considered substances under CLP. In the absence of substance specific ecotoxicity data, their classification can be assessed by applying the mixtures rule. The metals industry has developed classification tools that allow for the hazard ID and environmental classification of these complex materials, by integrating all aspects of this guidance with a knowledge of their mineralogical and other typical metal properties.

Metal alloys are defined by the CLP as 'special preparations' because their (eco)toxicity profile differs from that of their constituents. Further information on how to assess the environmental hazard classification of alloys and other complex metal containing materials is provided hereunder.

IV.5.6.1 Classification of alloys and complex metal containing materials

Metal alloys, or alloy manufacturing products are not simple mixtures of metals or metal compounds, since the alloy has clearly distinctive properties compared to a classical mixture of its metal components. Justified by their intrinsic properties, the solubility properties can differ substantially from what is observed for each individual constituent in that alloy (eg the rate and extend of metals release from pure metals are different from the ones from alloys). The rate and extend to which the ingredient of the alloy react with the media to transform to water soluble forms can be measured in the same way as with metals (by using the OECD Transformation/Dissolution test (Annex 10 to UN GHS)). However, alloys often react slowly and to a very limited extent, making the application of the T/D protocol more complex. Special care should be taken in this respect to the detection limit and the accurate determination of the measured surface. Initial testing of alloys, using the T/D protocol, shows that this can be useful but **further additional guidance on this aspect is recommended**.

More complex metals or metal compounds containing inorganic substances like e.g. ores and concentrates are not simple mixtures of metals or metal compounds. Justified by their intrinsic properties, the solubility properties can differ substantially from what is observed for each individual constituent of that complex substance (e.g. the rate and extent of metals release from e.g. ores/concentrates are different from the ones from simple metals). All these materials are typically not readily soluble in any aqueous medium. In addition, these materials are often heterogeneous in size and composition on a microscopic/macroscopic scale. Therefore, adequate amounts of the material could be used to evaluate the extent to which the substances can be dissolved, i.e. its water solubility and/or the extent to which the metals can react with the media to transform to water soluble forms e.g. through Transformation/Dissolution tests. Additional guidance on this aspect is needed for complex metal mixtures.

An **ecotoxicity validation step** may be important for alloys and complex metal containing materials (e.g. ores, concentrates, slags), where binding of the metal to abiotic and biological binding sites will in many cases be competitive. Therefore the 'additivity mode' is not necessarily valid and additional information may be relevant.

Therefore, information from ecotoxicity validation steps could be useful in cases where a significant uncertainty is associated with the existing toxicity data. This ecotoxicity validation should have been derived from tests using most sensitive species at dissolved ion concentrations equivalent to those measured in the T/D medium. However, information from ecotoxicity testing directly in the T/D medium is not recommended because the composition of this medium is unlikely to meet the requirements for standard test media to ensure proper

survival and/or reproduction. Therefore, ecotoxicity tests should have been conducted in standard media dosed at metal concentration equivalent to the concentration level actually measured in the T/D medium.

IV.6 References

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IV.7 Decision on classification: examples for metals and metal compounds

List of examples:

- **Example A**: Soluble metal compound with acute and chronic toxicity data and no evidence of rapid environmental transformation (Me_2 $(SO4)_2$).
- **Example B**: Poorly soluble metal compound with acute and chronic toxicity data, Transformation/Dissolution data at 7 days (low loading rate) and at 28 days (only low and medium loading rates) and no evidence of rapid environmental transformation.
- **Example C**: Metal in powder and massive form with acute and chronic toxicity data and Transformation/Dissolution data at 7 days (low, medium and high loading rates) and at 28 days (only the high loading rate) and no evidence of rapid environmental transformation.
    - *Explanatory note to Example D* - Critical Surface Area (CSA) Approach.
- **Example D**: Hazard classification of a soluble metal salt: the case of rapid environmental transformation through speciation in the water column.

IV.7.1 Example A: Soluble metal compound with acute and chronic toxicity data and no evidence of rapid environmental transformation ($Me_2(SO4)_2$).

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence		
Screening test (24 h) at 100 mg/l loading	pH 6 : 6240 µg/l pH 8 : 840 µg/l	Metals TDp, non-GLP
7 d TDp test	Not applicable	
28 d TDp test	Not applicable	
MWT of the metal ion versus compound		
	60 / 312	
Acute aquatic toxicity of metal ion[94]		
Fish: *Oncorhynchus mykiss*	120 µg/l (96 h LC_{50}) at pH 7,8 106 µg/l (96 h LC_{50}) at pH 7,8 104 µg/l (96 h LC_{50}) at pH 7,8 78 µg/l (96 h LC_{50}) at pH7,8 (species mean: 102 µg/l at pH 7,8)	C.1. / static, GLP C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP
Crustacea: *Daphnia magna*	180 µg/l (48 h EC_{50}) at pH 8	C.2. / static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus* *Lemna gibba*	 154 µg/l (72 h ErC_{50}) at pH 8 670 µg/l (7 d ErC_{50}) at pH 8	 C.3. / static, GLP C.26. / semi-static, GLP
Chronic aquatic toxicity[95]		
Fish: *Danio rerio* *Marine Fish*	24 µg/l (28 d NOEC) at pH 6 87 µg/l (28 d NOEC) at pH 8 1414 µg/l (28 d EC10)	OECD 210 / 28 d flow-through, non-GLP OECD 210 /28 d flow through, GLP) OECD 210 /28 d flow through, GLP)

[94] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

[95] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Crustacea:	*Daphnia magna*	37 µg/l (21 d EC_{10}) at pH 7.8 8.6 µg/l (21 d NOEC) at pH 6.4	C.20. / semi-static, GLP C.20./semi-static non-GLP
	Marine decapoda	1612 µg/l (21 d NOEC)	Non standard test
Algae/aquatic plants:	*Scenedesmus subspicatus*	21.6 µg/l (72 h NOEC) at pH 8 8.7 µg/l (72 h NOEC) at pH 6.2	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)			
Rapid environmental transformation		No evidence.	
Bioaccumulation			
Bioconcentration factor in fish		+/- 200 at NOEC level	

Aquatic hazard assessment, conclusions and comments:

Transformation Dissolution:

- The substance passes the 24 h screening TDp test at pH 6 given the dissolution at a loading of 100 mg/l is 6240 µg/l > acute ERV of the soluble ion being 102 µg/l at pH 7.8.

Acute aquatic toxicity:

- The acute ecotoxicity reference value is driven by the Fish data. No data are available for the low pH end.
- The acute ERV for the metal compound is 102 * (312/(2*60)) = **265 µg/l**.

Evidence of rapid environmental transformation:

- No information available, so substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity:

- The chronic aquatic ecotoxicity reference toxicity value based on the lowest of the available toxicity values is slightly below 10 µg/l for Daphnia magna at pH 6,4 for the metal ion.
- The chronic ERV for the metal compound is 8.6 * (312/(2*60)) = **22.4 µg/l**.

Aquatic hazard classification and, where applicable, established M-factor(s):

- Acute (short-term) aquatic hazard: category Acute 1, M-factor: 1
- Long-term aquatic hazard: category Chronic 1, M-factor: 1

Reasoning:

Acute aquatic hazard

- The acute ecotoxicity reference value is driven by the Fish data. A species mean of 102 µg/l for the metal ion, is calculated for *Oncorhynchus mykiss* given 4 or more toxicity data for the same species under comparable conditions are available.
- Acute aquatic hazard expressed as the ERV for the metal compound after molecular weight correction ≤ 1 mg/l. M-factor is 1 given the acute ERV is between 1 and 0.1 mg/l.

- The molecular weight correction recognises that 2 metal ions are included.
- The substance passes the 24 h screening dissolution test by comparing acute toxicity data at pH 7.8 with TDp data at pH6 given an acute toxicity data set at pH 6 is lacking and the chronic data indicate more toxic behaviour of the metal at the lower pH end.

Long-term aquatic hazard:

- Adequate information on chronic toxicity (all 3 trophic levels) is available allowing long-term hazard classification (no use of the surrogate approach).[96]
- Marine toxicity data are not included in the chronic ERV assessment given far less sensitive as fresh water toxicity references and data for 3 trophic levels for the freshwater are available.
- The Daphnia magna reference at pH6 is the lowest and determines the chronic ERV.
- A molecular weight correction is applied to the substance recognising that 2 metal ions are included.
- Rapid environmental transformation cannot be demonstrated given the lack of sufficient information.
- The M-factor of 1 is based on the chronic ERV of 22 µg/l (so between 0.01 and 0.1 mg/l.) without rapid environmental transformation.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H400, H410 → H410[97]
Precautionary statement(s)	P273, P391, P501

[96] In absence of adequate chronic toxicity data for all trophic levels, the subsequent step is to combine two types of information, i.e. chronic info for the trophic level with such data and acute aquatic toxicity data and environmental fate information for lacking info on trophic levels. For details see Section 4.1.3.3 and Table 4.1.0.

[97] In accordance with CLP Article 27, the hazard statement H400 may be considered redundant on the label and therefore not included on the label because hazard statement H410 also applies, see Section 4.1.6 of this document.

IV.7.2 Example B: Poorly soluble metal compound with acute and chronic toxicity data, transformation/dissolution data at 7 days (low loading rate) and at 28 days (only low and medium loading rates) and no evidence of rapid environmental transformation

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence		
Screening test (24 h) at 100 mg/l loading	pH 6: 74 µg/l pH 8: 34 µg/l	Metals TDp, non-GLP
7 d TDp test at 1 mg/l loading	pH 6: 50 µg/l pH 8: 16 µg/l	Metals TDp, non-GLP Metals TDp, non-GLP
28 d TDp test at 0.1 mg/l loading at 0.01 mg/l loading	pH 6: no data available pH 8: no data available pH 6: 9 µg/l pH 8: <1 (DL)	Metals TDp, non-GLP Metals TDp, non-GLP Metals TDp, non-GLP Metals TDp, non-GLP
MWT of the metal ion versus compound		
MWT of the metal ion versus compound	60 / 91	
Acute aquatic toxicity of metal ion[98]		
Fish: *Oncorhynchus mykiss*	186µg/l (48 h LC_{50}) at pH 7 120 µg/l (96 h LC_{50}) at pH 7.8 106 µg/l (96 h LC_{50}) at pH 7.8 104 µg/l (96 h LC_{50}) at pH 7.8 78 µg/l (96 h LC_{50}) at pH 7.8 (species mean for four values : 102 µg/l at pH 7.8) *78 µg/l* (96 h LC_{50}) at pH 6.4	C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP
Crustacea: *Daphnia magna*	180 µg/l (48 h EC_{50}) at pH 8 106 µg/l (48 h EC_{50}) at pH 8	C.2. / static, non-GLP
Algae/aquatic plants *Scenedesmus subspicatus*	154 µg/l (72 h ErC_{50}) at pH 8 78 µg/l (72 h ErC_{50}) at pH 6	C.3. / static, GLP
Lemna gibba	670 µg/l (7 d ErC_{50}) at pH 8	C.26. / semi-static, GLP

[98] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Chronic aquatic toxicity[99]		
Fish: *Danio rerio*	24 µg/l (28 d NOEC) at pH 6 87 µg/l (28 d NOEC) at pH 8	OECD 210 / 28 d flow-through, non-GLP OECD 210 /28 d flow through, GLP)
Crustacea: *Daphnia magna*	37 µg/l (21 d EC_{10}) at pH 7.8 8.6 µgl (21 d NOEC) at pH 6.4	C.20. / semi-static, GLP C.20. / semi-static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus*:	21.6 µg/l (96 h NOEC) at pH 8 8.7 µg/l (72 h EC_{10}) at pH 6.2	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)		
Rapid environmental transformation	No data available therefore considered as not rapidly transformed.	
Bioaccumulation		
Bioconcentration factor in fish	+/- 200 at NOEC level	

Aquatic hazard assessment, conclusions and comments:

Transformation Dissolution screening outcome:

- The substance fail the 24 h screening Transformation Dissolution test given the dissolution at a loading of 100 mg/l :
  - at pH 6 is 74 µg/l < acute ERV of the soluble ion being 78 µg/l (borderline case)
  - at pH 8 is 34 µg/l < acute ERV of the soluble ion being 102 µg/l

Acute aquatic toxicity:

- Adequate data on pH 6 and 8 are available allowing to derive an acute ERV for the (soluble) metal ion :
  - at the lower pH end (around pH 6) : 78 µg/l
  - at the higher pH end (around pH 8) : 102 µg/l

7 days Transformation/Dissolution outcome :

- The acute release after 7 d is the highest at pH 6 (50 µg/l) being lower than the acute toxicity level (78 µg/l) at this corresponding pH
- The acute release is lower at or around pH 8 (16 µg/l), which is significantly lower than the acute toxicity level (102 µg/l) at this corresponding pH

Evidence of rapid environmental transformation:

- No information available and therefore substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity for a substance not rapidly transformed:

[99] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

- The chronic ERV for the (soluble) metal ion is ***8.6 µg/l*** around pH 6 and ***21.6 µg/l*** around pH 8.

28 days Transformation dissolution outcome for a substance not rapidly transformed:

- The release after 28 d at pH 6 at a loading of 0.1 mg/l is not available and needs to be extrapolated from the 0.01 loading rate assuming a 10 times higher dissolution level (10x9=90 µg/l), which is significantly larger than the chronic ERV at pH 6 (8.6 µg/l).
- The release for the 0.1 mg/l loading is also extrapolated in the same way and is much lower at pH 8. The calculated release rate of < 10 µg/l is still lower than the chronic toxicity level 21.6 µg/l at this pH level. The calculated release rates at 1 mg/l loading would be < 100 µg/l which is significantly larger than the chronic ERV at pH 8.

Aquatic hazard classification and, where applicable, established M-factor(s):

- Acute (short-term) aquatic hazard: no acute classification
- Long-term aquatic hazard: category Chronic 1, M-factor 10

Reasoning:

The metal compound is considered as poorly soluble since it fails the OECD transformation dissolution screening test at a 100 mg/l loading. The test confirmed pH 6 as the pH of the highest release rate.

Acute aquatic hazards:

- The acute ecotoxicity reference value is driven by the Fish data for the high pH and by algae data for the low pH level. For the high pH end (around pH 8) a species mean of 102 µg/l for the metal ion is calculated for *Oncorhynchus mykiss* and a single reference of 78 µg/l for *Scenedesmus subspicatus* at around pH 6.
- A poorly soluble substance is evaluated for classification by comparing the dissolved metal ion level resulting from the TDp at 7d, at a loading rate of 1 mg/l with the acute ERV as determined for the (soluble) metal ion. A molecular weight correction for the poorly soluble metal compound is consequently not required given this factor has already been included for the loading rate of the TDp test.
- The dissolution level of the poorly soluble metal compound from the 7d TDp at 1 mg loading is lower than the acute ERVs of the soluble metal ion for both pH levels, thereby not resulting in an acute classification.

Long-term aquatic hazard:

- Adequate information on chronic toxicity (all 3 trophic levels) for the higher and lower pH levels are available allowing direct long-term hazard classification (no use of the surrogate approach).
- No valid info is available on rapid transformation by normal environmental processes so the poorly soluble metal compound is considered to be not rapidly transformed.
- No Molecular Weight Correction is applied for the poorly soluble metal compound given the classification scheme is based on the comparison of the dissolved fraction of the poorly metal compound with the chronic ERV of the soluble metal ion at both pH 6 and pH 8.
- No TDp data are available for the 0.1 mg/l and 1 mg/l loading. The calculated dissolution level from the 28d TDp at pH 6 at 0.1mg/l loading (+/- 90 µg/l) for the poorly soluble metal compound is much higher than the chronic ERV's of the soluble metal ion for pH 6 (8.6 µg/l) warranting a chronic 1 classification. The classification is much less sensitive at pH 8 given a less toxic and a lower dissolution rate.

- The M-factor associated with the long-term hazard classification is derived by using the solubility level derived from the 28d TDp test at the 0,1 mg/l loading (90 µg/l at pH 6) divided by the ERV of the dissolved metal ion (8.6 µg/l at pH 6): 90/8.6=10.45. Accordingly to Section IV.5.4 the substance will get an M-factor 10, given this factor was between 10 and 100.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	WARNING
Hazard Statement	H410
Precautionary statement(s)	P273, P391, P501

IV.7.3 Example C: Metal in powder and massive form with acute and chronic toxicity data and Transformation/Dissolution data at 7 days (low, medium and high loading rates) and at 28 days (only the high loading rate) and no evidence of rapid environmental transformation

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence **For metal in POWDER form**		
Screening test (24 h) at 100 mg/l loading	Not applicable for metals	Metals TDp, non-GLP
7 d TDp test at 1 mg/l loading at 10 mg/l loading at 100 mg/l loading	pH 6 : 1.7 µg/l (.) pH 8 : 3 µg/l pH 6 : 24 µg/l pH 8 : 29 µg/l pH 6 : 340 µg/l pH 8 : 280 µg/l	Metals TDp, non-GLP
28 d TDp test at 1 mg/l loading at 0.1 mg/l loading at 0.01 mg/l loading	pH 6: 2.3 µg/l pH 8: 3.5 µg/l no measured data available no measured data available	Metals TDp, non-GLP
MWT of the metal		
MWT of the metal	59	
Acute aquatic toxicity of metal ion[100]		
Fish:	Large data sets available for the 2 pH ends but less sensitive than crustacean at high pH end and Algae at low pH end	C.1. / static, non-GLP C.1. / static, GLP
Crustacea: *Ceriodaphnia dubia*	Most sensitive species at high ph end (pH 8.3-8.7) : Geometric mean for 6 values under comparable test conditions (EC_{50} 48h): 68 µg metal ion/l	C.2. / static, non-GLP

[100] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Algae/aquatic plants: *Pseudokirchneriella subcapitata*	Data sets available for the 2 pH ends but less sensitive than crustacean at high pH end and most sensitive endpoint at low end. Most sensitive value (96 h EC_{10}) at the low pH range: 120 µg metal ion/l	C.3. / static, GLP And non-GLP C.26. / static, non GLP
Chronic aquatic toxicity[101]		
Fish	Large data sets available for different pHs but less sensitive than crustacean at high and low pH	
Crustacea: *Ceriodaphnia dubia*	Most sensitive species at high and low pH end: - At low pH (NOEC 21d): 20 µg/l - At high pH: (EC10 21d): 2.4 µg /l	C.20. / semi-static, non-GLP
Algae/aquatic plants:	Large data sets available for different pH's but less sensitive than crustacean at high and low pH	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)		
Rapid environmental transformation	No information.	.
Bioaccumulation		
Bioconcentration factor in fish	<< 500 at NOEC or EC50 level	

Transformation Dissolution screening outcome: not applicable for metals

Acute aquatic toxicity:

- Adequate data at high and low pH are available allowing deriving an acute ERV for the (soluble) metal ion
  - at the lower pH end (around pH 6) : ***120 µg/l***
  - at the higher pH end (above pH 8) : ***68 µg/l***

[101] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

7 days Transformation/Dissolution outcome for the powder form:

- The release after 7 d's is the highest at pH 8 while lower at pH 6. The table below compares the TDp results with the acute ERV values at the corresponding pH ranges

Loading (mg metal ion/l)	pH*	Highest dissolution (mg metal/l)	Reference toxicity value (mg metal/l)	Dissolution > toxicity reference value?
1	low	0.0017	0.12	No
10	low	0.024	0.12	No
100	low	0.35	0.12	Yes
1	high	0.003	0.068	No
10	high	0.029	0.068	No
100	high	0.28	0.068	Yes

* pH value at which dissolution testing was conducted and similar to the pH for the acute toxicity reference value

- The release from the metal powder[102] at a loading of 100 mg/l is for both pH ranges higher than the acute ERV.

7 days Transformation/Dissolution outcome for the massive form :

- The CSA Approach can be used to calculate a Critical Particle Diameter (CPD) for the dissolution rates from the metal powder. The metal in massive form will be classified as hazardous to the aquatic environment if the CPD is above or equal to 1 mm. The measured critical surface area (SA_{crit}) that releases sufficient ions to reach the acute ERV for the most critical pH (6) is ***SA_{crit}* 0.101 m^2/g** corresponding to an equivalent critical spherical particle diameter (CD_{spec}) of 6.67 μm at a 100 mg/l loading rate. This is far less than 1 mm.

Evidence of rapid environmental transformation:

- No information available and therefore substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity:

- The chronic ERV for the (soluble) metal ion is ***2.4 µg/l*** at around pH 8 and ***20 µg/l*** around pH 6 which is an inverse relationship with pH as for the acute level.

28 days Transformation/Dissolution outcome for a substance not rapidly transformed:

- The release after 28 d at a loading of 1 mg/l is slightly higher at ***pH 8*** (3.5 µg/l) than at pH 6 (2.3 µg/l).
- TDp data for lower loadings are not available and were calculated given that the rate of metal ion release from the metal in the OECD 203 medium at high pH at the 28 days can be predicted by the equation: log (CMe(aq)) = -5.144 + 1.0229log(Ameas), whereby

$C_{me(aq)}$ = total dissolved concentration of metal (mg/l)

[102] The finest representative metal powder should be used for TDp testing.

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) X 10], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

An equal approach can be followed for the lower pH level.

- Measured and estimated transformation dissolution data for the *metal powder* are listed in the table below

Loading (mg metal ion/l)	Measured or *calculated*	pH*	Highest dissolution (mg metal/l)	Reference toxicity value (mg metal/l)	Dissolution > toxicity reference value?
1	Measured	low	0.0023	0.020	No
1	Measured	high	0.0035	0.0024	Yes
0.1	Estimated	Low	0.00023	0.020	No
0.1	Estimated	High	0.00035	0.0024	No

* pH value at which dissolution testing was conducted and similar to the pH for the acute toxicity reference value

- The release after 28 days at the 1 mg/l loading for the higher pH level slightly exceeds the chronic ERV, while no such effect is noted at pH 6 mainly due to the lower sensitivity of the species.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute (short-term) aquatic hazard:

- for the powder form: no acute hazard classification
- for the massive form: no acute hazard classification

Long-term aquatic hazard:

- for the powder form: category Chronic 2
- for the massive form: no long-term hazard classification

Reasoning:

The single environmental classification for all ***metal powders*** (spherical diameter ≤ 1 mm) of the considered metal can be derived by comparing the transformation/dissolution data for the smallest commercially representative metal powder with the acute and chronic toxicity reference values (for the soluble metal compounds).

Acute hazard classification:

- The ***dissolution rate for the finest powder*** on the market does not reach the concentration corresponding with the ERV, within 7 days at a loading of 1 mg/l. This is only reached at a loading of 100 mg/l. Therefore, **no acute hazard classification is required.**
- The ***dissolution rate for the massive forms*** (spherical diameter > 1 mm) is lower than those for powders given the lower available surface area. The Critical surface area approach confirms that above a diameter of 6.7 µm the acute ERV cannot be reached within 7 days at a loading of 1 mg/l. (Not even at a 100 mg/l loading.) Thereby confirming no need for an acute hazard classification. More explanation on the CSA

assessment of the powder form for this metal is included in the explanatory note to example D (see below).

Long-term hazard classification:

- The metal does not fulfil the criterion for rapid environmental transformation.
- T/D data are only available for 1 mg/l loading rate. The medium loading rate of 0,1 mg/l required for the long-term hazard assessment could be safely extrapolated from existing evidence given clear relationships between concentration and dissolution were established for both pH levels.
- The comparison of chronic ERV's with the 28 days TDp results concludes that the chronic ERV for the metal ion is only reached at a loading rate of 1 mg/l at pH 8. Therefore, ***chronic 2 hazard classification for the metal in the powder form is warranted.***
- Given the surface of the particle reference ***for massive metal*** is > 100 larger than for the smallest commercially representative form this corresponds to a Critical Particle Diameter > 1 mm at the high loading rate. Therefore there is no need to classify the massive form for long-term hazard.

Labelling elements based on the classification for the powder form:

Element	Code
GHS Pictogram	none
Signal Word	none
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

Labelling elements based on the classification for the massive form: none

Element	Code
GHS Pictogram	none
Signal Word	none
Hazard Statement	none
Precautionary statement(s)	none

IV.7.3.1 Explanatory note to Example C - Critical Surface Area (CSA) approach

Acute hazard:

For the metal powder in this example, the data showed that the concentration of metal released in the OECD 203 medium at pH 8 at the 168 hr can be predicted by the equation:

$\log (C_{Me(aq)}) = -5.122 + 0.9875 \log (A_{meas})$

$C_{Mel(aq)}$ = total dissolved concentration of Metal ion (mg/l) at 168 hr and pH 8;

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) × 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

The CSA approach can subsequently determine what surface areas and particle diameters would result in different levels of aquatic toxicity classification using the regression coefficients from the above equation, *a* (-5.122) and *b* (0.9875), and the proposed acute toxicity reference value (0.068 mg Me/l) as the $C_{Me(aq)}$. The critical surface area (*CSA*) would be the A_{meas} at which the metal ion is released at the concentration of the acute toxicity reference value. The following equations can be used to derive these values for this case:

$\log L(E)C_{50} = -5.122 + 0.9875 \log CSA$

$L(E)C_{50}$ = acute ecotoxicity reference value for classification (mg/l)

CSA = critical surface area (mm^2/l) that releases metal ion in the concentration of the acute ecotoxicity reference value to the aquatic medium

The *CSA* can be derived as follows:

$$\log CSA = \left(\frac{\log L(E)C_{50} + 5.122}{0.9875} \right)$$

For an acute toxicity reference value of 0.068 mg Me/l, the *CSA* is thus 10,100 mm^2/l. This is the surface area loading of metal that will deliver the reference value amount of metal ion to the OECD 203 medium at pH 8 and at a time of 168 hr.

The critical specific surface areas, SA_{crit}s for a loading of 1 mg/l will deliver the acute toxicity reference value to the OECD 203 medium at pH 8 and a time of 168 hr can be calculated by:

SA_{crit} = critical specific surface area (m^2/g) corresponding to the acute ecotoxicity reference value

CP = classification cut-off loading of 1 mg/l that yield a classification as acute 1)

Thus, for the metal powder under consideration a *CSA* of 10.100 mm^2/l and the *CP* of 1 mg/l, the SA_{crit} is 10,1 m^2/g.

The equivalent critical spherical particle diameter (CD_{spec}) associated with the acute ecotoxicity reference value is determined by:

$$CDspec = \left(\frac{6}{SA_{crit} \times \rho Me} \right)$$

ρ_{Me} = density of the metal (g/cm^3)

CD_{spec} = critical diameter of the sphere (μm) corresponding to the acute ecotoxicity reference value

For the above SA_{crit} of 10,1 m^2/g, corresponding to the 1 mg/l loading, the critical diameter would be 0,067 μm. The EU-CLP system defines that the finest representative metal powder should be used for TDp testing and classification of the metal powder form.

An acute toxicity classification can therefore be assigned to all metal powders (diameter ≤ 1 mm) by **measuring the real surface area** using the BET nitrogen adsorption-desorption technique and comparing it to SA_{crit}. If the surface area of the reference material is greater than the SA_{crit} for the associated acute toxicity classification then the representative metal sample would classify for that acute hazard category ***and classify all powder types of that metal in the same way***. If the measured surface area is less than the SA_{crit}s of all of the classification categories then all powders of this metal would not classify for aquatic toxicity.

The CSA Approach can consequently be used to assign an acute hazard classification to the metal powders based on measured surface area using the **measured surface area of0.43 m^2/g** for the smallest representative size powder on the EU market. Since this surface area is greater than 0.1 m^2/g but less than 1 m^2/g, there is according to this approach no need for an ***acute hazard classification of the metal powders in this example***.

The CSA Approach can also be used to calculate a Critical Particle Diameter (CPD) to be used to determine an accurate classification of the **metal massive** (diameter > 1 mm), where the measured surface area of the tested granules is 0.086 m^2/g. This surface area is far less than all of the SA_{crit} so there is ***no need for an acute classification for the metal massive***.

Long-term hazard: For this example it has been shown that rate of metal ion release from the metal in the OECD 203 medium at high pH at the 672 hr can be predicted by the equation:

$$\log (C_{Me(aq)}) = -5.144 + 1.0229 \log(A_{meas})$$

$C_{me(aq)}$ = total dissolved concentration of metal (mg/l)

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) X 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

The CSA Approach can determine what surface areas and particle diameter would result in chronic (long-term) hazard classification by using the regression coefficients from the above equation, *a* (-5.144) and *b* (1.0229), and the proposed chronic toxicity reference value (0.0024 mg Me/l) as the $C_{Me(aq)}$. The critical surface area (*CSA*) would be the A_{meas} at which metal ion is released at the concentration of the chronic toxicity reference value. The following equations can be used to derive these values.

$$\log \textit{chronic toxicity} = -5.144 + 1.0229 \log \textit{CSA}$$

chronic toxicity = chronic ecotoxicity reference value for classification (mg/l), using calculated EC_{10}s or measured NOECs (if the EC_{10} is less than the NOEC)

CSA = critical surface area (mm^2/l) that releases metal in the concentration of the chronic toxicity reference value to the aquatic medium

The *CSA* can be derived as follows:

$$\log CSA = \left(\frac{\log chronictoxicity + 5.144}{1.0229} \right)$$

For the chronic hazard classification derivation exactly the same approach as for the acute hazard assessment can be followed to define SA_{crit} and CD_{spec}. For this metal powder example this results in a CSA of 3,420 mm^2/l and the CP of 1 mg/l, the SA_{crit} is 0.342 m2/g.

For a SA_{crit} of 0.342 m^2/g, corresponding to the 1 mg/l loading, the critical diameter would be 2 μm.

Equivalent as for the assessment of the acute hazard the CSA Approach can be used to assign a long-term hazard classification to all powders based on measured surface area of the reference powder, using the measured surface area at 100 mg/l loading (0.43 m^2/g) for the smallest representative size powder on the EU market. Since this surface area is greater than 0.342 m^2/g, ***all metal powders would be classified as Chronic 3***.

The CSA Approach can also be used to **classify the massive metal (diameter > 1 mm)**, where the measured surface area of the massive at 100 mg/l loading) is 0.086 m^2/g. This surface area is less than the chronic SA_{crit} so the massive metal form would ***not be classified for long-term environmental hazard***.

IV.7.4 Example D: Hazard classification of a soluble metal salt: the case of rapid environmental transformation through speciation in the water column

General approach

This example was selected to:

i. illustrate the use of information on the metal oxidation and resulting transformation of metal ions in the water column for classification decisions;

ii. provide further information related to testing of sparingly soluble metal salts.

The metal ion selected for this example, Me(II), is unstable when its solutions are exposed to air, and it oxidises to the Me(III), which then forms the familiar insoluble, hydrated, amorphous, gelatinous precipitate, $Me(OH)_3$ (metal hydroxide). The question then arises as to whether the metal hydroxide precipitate forms rapidly enough to decrease the concentration of Me(II) and Me(III) ions to levels below which there is no cause for concern over the aquatic environment. Consideration of the rates at which Me(II) oxidises to Me(III) is relevant to this question to proof rapid environmental transformation.

Additionally, the classification of substances of concern for the aquatic environment requires evaluation of aquatic toxicity. Results for this case were evaluated against standard acceptability criteria for use in this classification assessment.

Results

Assessment of the rapid environmental transformation:

A review of the scientific literature on the oxidation of metal sulphate reveals the following: Metal sulphate reacts with oxygen in water to form metal hydroxide ($MeOH_2$), moderately insoluble, Ksp = 1.6×10^{-14}) this in turn undergoes further oxidation to form metal hydroxide ($MeOH_3$) which is highly insoluble (Ksp = 1×10^{-36}). Formation of metal hydroxide at pH levels above 5.0 limits the presence of metal ions in aqueous systems. In sediments the metal hydroxide is expected to result in enriched concentrations of insoluble metal sulphide.

The rates at which dissolved metal sulphate (Me^{++}) oxidises to (Me^{+++}) and forms the metal hydroxide [$Me(OH)_3$] precipitate:

- Is highly dependent on pH (100 fold from pH 6 to 8);
- decreases with increase in ionic strength of the aqueous medium (pristine waters contain less metal ions);
- dependent to some extent on the anions present in solution such as sulphate and chloride;
- increases 10-fold for a 15 °C increase in temperature;
- exhibits a linear dependence on the partial pressure of oxygen; and
- dependent on the initial concentration of metal sulphate and exhibits linear reaction kinetics at Me(II) loadings less than ~50 micromolar (~3 mg/l). At concentrations

greater than 50 micromolar, rates of reaction increase with increasing concentration of metal sulfate (about 4× for each order of magnitude).

Based on literature data and empirical reaction kinetics, it can be calculated that, at low pH (reasonable worst case scenario) in the OECD 203 medium (diluted by 10 as per the Transformation/Dissolution Protocol), the half-times for the oxidation of Me(II) are 11, 9 and 3.6 hr, for 1, 10 and 100 mg/l loadings of $MeSO_4$, respectively. At high pH, the reaction is estimated to be as short as 8 seconds. The rapid precipitation of metal ions from aqueous systems accounts for low 'metal' concentrations found in most natural aquatic systems (all except natural waters at very low pH values (i.e. < pH 5.5)). Under the reasonable worst case scenario of low pH and a low initial concentration of 1 mg/l $MeSO_4$, the 70 % removal from solution is calculated to be achieved in 19hr and 90 % removal would be achieved by 36hr. Since the removal of the metal sulphate are due to reaction with oxygen in water to form highly insoluble and non classifiable metal hydroxide and the half life for the removal of the soluble species are less than 16 days this can be considered as rapidly transformed in the water column and the substance considered for classification purposes as rapidly degradable.

To support this, evidence of rapid loss of 'Metal ions' (and other metals) from the water column has been reported in mesocosm lake experiments (Perch Lake). The data are presented as half lives as a function of time, partition coefficient and first stability constant. Half lives for metal ions in the mesocosms are calculated to be approximately 11 days under the given conditions. The data support that half lives are short and loss from the water column can be related to both formation of the metal hydroxide but also to sorption to suspended particles that are settling.

Aquatic Toxicity

Acute ERV values lie in the range of 1-37 mg/l (see Table). Two values for *Daphnia magna* were less than 10 mg/l. Four *Daphnia magna* studies were performed and the geometric mean value for this species is 5.77 mg/l. The values for fish were all greater than 10 mg/l. No algal studies were deemed reliable. All these values are expressed as mg/l Me. If the classification relates specifically to metal sulphate of which the most common form is the heptahydrate $MeSO_4.7H_2O$. The numerical ERV values detailed should be adjusted according to the table below and the species under consideration to calculate the toxicity on a metal sulfate basis.

Chemical Species	Molecular Weight	Ratio
$MeSO_47H_2O$	278.0	4.978
$MeSO_4H_2O$	169.91	3.043
$MeSO_4$	151.90	2.720
Me	55.84	1.0

The data cover all the reliable results available for aquatic toxicity of binary 'metal' and any observed toxicity effects could relate to the Me ion which could be in Me(II) or metal Me(III) oxidation states.

Conversion of the acute ERV values for the metal ion to those appropriate for $MeSO_4.7H_2O$ implies an acute toxicity range of 6.4 to 199 mg/l.

Table IV. 2 Acute toxicity data deemed reliable for 'Metal' are presented as mg/l Me

Test substance	Test organism	Duration	Endpoints	L(E)C_{50} (mg Me L^{-1})
$MeCl_3.6H_2O$	*Pimephales promelas* *Lepomis macrochirus*	96h 96h	Survival Survival	21.8 20.3
$MeSO_4.7H_2O$	*Oncorhynchus mykiss*	96h	Survival	16.6
$Me_2(SO_4)_3$	*Oncorhynchus mykiss*	96h	Survival	>27.9
$MeSO_4$	*Daphnia pulex*	24h	Immobility	36.9
$MeSO_4$	*Daphnia magna*	24h	Immobility	17
$MeCl_3.6H_2O$	*Daphnia pulex*	48h	Immobility	12.9
$Me_2(SO_4)_3$	*Daphnia longispina*	48h	Immobility	11.5
$MeCl_3.6H_2O$	*Daphnia magna*	48 h	Immobility	9.6
$MeSO_4$	*Daphnia magna*	24h	Immobility	5.25
$MeSO_4.7H_2O$	*Daphnia magna*	48h	Immobility	1.29

Table IV. 3 Chronic toxicity data deemed reliable for 'Metal' are presented as mg/l Me

Test substance	Test organism	Duration	Endpoints	NOEC/LOEC (mg Me L^{-1})
$Fe(OH)_3$	*Salvelinus fontinalis*	30 days	Hatching Growth Survival	>10.3
$Fe(OH)_3$	*Oncorhynchus kisuth*	30 days	Hatching Growth Survival	>10.3 2.81/>10.3 >10.3
$FeCl_3.6H_2O$	*Pimephales promelas*	33 days	Survival Length Weight	1.0/1.6 1.61/2.81
$FeCl_3.6H_2O$	*Daphnia pulex*	21 days	Immobility Total offspring Brood size	2.51/5.01 0.63/1.26 1.26/2.51
$FeCl_3.6H_2O$	*Daphnia magna*	21 days	Immobility Reproduction	5.9 EC50 4.4 EC16

Aquatic hazard classification*:*

Acute hazard: Not classified.

Long-term hazard: Not classified.

Reasoning:

Acute aquatic toxicity > 1 mg/l.

Since all chronic aquatic toxicity values are higher than 1 mg/l and rapid transformation to a metal hydroxide takes place by normal environmental processes, no classification is warranted.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	none
Signal Word	none
Hazard Statement	none
Precautionary statement(s)	none

V ANNEX V: COLLECTION OF INTERNET LINKS FOR THE USERS OF THE GUIDANCE

Reference/Site name	Host	URL
ECHA website	ECHA	http://echa.europa.eu/web/guest
UN GHS	UN	http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html
eChemPortal	OECD	http://www.echemportal.org/
REACH guidance	ECHA	http://echa.europa.eu/guidance-documents/guidance-on-reach
OECD Series on Testing and Assessment	OECD	http://www.oecd.org/document/30/0,3746,en_2649_34377_1916638_1_1_1_1,00.html
EU Test Method Regulation 440/2008	EC	http://eur-lex.europa.eu/lexuriserv/lexuriserv.do?uri=celex:32008r0440:en:not
OECD test guidelines	OECD	http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm l
Public C&L Inventory	ECHA	http://www.echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

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

VI.1 Executive summary

Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (the 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 the 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.

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 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 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 following SCLs 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.

VI.2 Introduction

VI.2.1 General description of the classification system for reprotoxic substances and mixtures

The CLP Regulation contains rules for the classification of substances and mixtures. In CLP Annex I, 3.7.2.1.1 Table 3.7.1 (a), the criteria are given for the classification of substances as reprotoxicants in one of the following categories:

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.*

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. The 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 in 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 must 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.

VI.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.

VI.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 an 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, potency is defined as the dose at which a 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 three 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.

VI.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[103], LOAEL[104], 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 VI. 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 Muller et al., 2012. As the data for these two main types of reproductive toxicity were analysed separately, the results are provided separately.

VI.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

[103] NOAEL means No Observed Adverse Effect Level.

[104] LOAEL means Lowest Observed Adverse Effect Level.

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 VI. 2). 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 VI. 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 VI. 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 VI. 2**Error! Reference source not ound.**). 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 VI. 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 Table VI. 1 and Table VI. 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 VI. 1 is mainly due to the absence of an ED_{10} for some very potent substances.

VI.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 six parameters for potency is less than a factor of four. This is small compared to the difference in potency between substances for each parameter of up to 30,000 (Table VI. 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 VI. 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 VI. 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 VI. 4).

Table VI. 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 Table VI. 3 and Table VI. 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 VI. 3 is due to the absence of an ED_{10} for some very potent substances.

VI.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 can 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 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.

VI.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 below). It should be noted that several of the elements may be interrelated.

VI.3.1 Boundaries of the potency groups

Table VI. 5 Boundaries of the potency groups

Potency group	Boundaries
High potency group	ED_{10} value ≤ 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 ≥ 400 mg/kg bw/day.

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 IV.4 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 3.7.2.6.5).

VI.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:

VI.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.

VI.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.

VI.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.

VI.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 (CLP Annex I 3.8.2.1.10.4 and CLP Annex I 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, (CLP Annex I Table 3.1.1: see section 3.1.2.2 of this Guidance) or guidance values (CLP Annex I Table 3.8.2 – see section 3.8.2.2.1 of this Guidance and Annex I Table 3.9.2 and 3.9.3- see section 3.9.2.2 of this Guidance) 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.

VI.5 Potency groups and specific concentration limits

VI.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.

VI.5.1.1 General considerations on potency groups

VI.5.1.1.1 Legal requirements

According to the second subparagraph of CLP Article 10(1):

> **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):

> **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.

VI.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.

VI.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 VI. 5) 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 VI. 6 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.

VI.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.

VI.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 VI. 6 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 VI. 6 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 VI. 6 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 agreed that volatility was not considered a modifying factor and thus, the ED_{10} distribution changes as shown in Table VI. 7. 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 VI. 7 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 Table VI.

6 and Table VI. 7 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.

VI.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 VI. 8.

Table VI. 8 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 potent substances[B])	ED_{10} (classification) 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 ≤ 400 mg/kg bw/day	0.3% (GCL)	$ED_{10} \geq 4$ mg/kg bw/day, and ≤ 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).

VI.5.2 Assigning SCLs

The SCL or GCL for each substance can be determined using the final potency group of the substance using Table VI. 6.

VI.6 References

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VII ANNEX VII: RELATION BETWEEN TRANSPORT AND CLP CLASSIFICATION REGARDING PHYSICAL HAZARDS

Table VII. 1 on physical hazards only, provided in this annex, contains additional information on transport classifications in relation to CLP classifications that could be of added value. However, these comparisons have certain restrictions with regard to their applicability. In particular, the area of applicability of the transport regulation is different from the CLP Regulation (ADR 49 countries, IMDG-Code, ICAO-TI international regulations). Therefore, the table should be used as reference for deriving CLP classifications and not vice versa.

The transport classification of named substances or mixtures in the transport regulations reflects the transport conditions and therefore were not adapted to take into account the GHS criteria. The transport classifications may be based on experience or certain events that are specific to transport. The transport classification of named substances or mixtures is legally binding for transport and should not be used to derive a CLP classification without an expert review.

The transport regulations include the concept of precedence of hazards which guarantees that information on the most dangerous hazards is communicated with precedence. CLP does not apply a precedence of hazards and therefore substances or mixtures might need to be classified in additional hazard classes under CLP, which in the transport classification are allocated and noted under the respective UN-Number (giving information on subsidiary risks, appropriate packaging and transport conditions).

It needs to be noted that a substance may have more than one entry in the Dangerous Goods List. These are usually within the same class, but transport conditions are different because of different severity of the hazard for different concentrations of this substance.

The following table refers only to physical hazards, as health hazards are not harmonised regarding cut-off values, and/or allowed methods.

Tabel VII. 1 Relation between transport and CLP classifications regarding physical hazards

⚠ (NOTE that within transport, the term 'substances' covers also mixtures in CLP terms.)

Transport classification		Physical state	CLP-classification		Remarks
Transport class and (sub)division (if applicable)	Packing group, division, type, group or code		Hazard class	Hazard category, division, type or group	
Class 1	Division 1.1 Division 1.2 Division 1.3 Division 1.4 Division 1.5 Division 1.6	Liquid or solid	Explosives	Division 1.1 Division 1.2 Division 1.3 Division 1.4 Division 1.5 Division 1.6	Matching criteria. However, if explosives are un-packed or repacked, they have to be assigned to division 1.1 unless the hazard is shown to correspond to one of the other divisions.
Class 2* – Gases	1 Compressed gas	Gaseous	Gases under pressure	Compressed gas	A correspondence only applies to the

	2 Liquefied gas. 3 Refrigerated liquefied gas 4 Dissolved gas	Gaseous Gaseous Gaseous		Liquefied gas. Refrigerated liquefied gas Dissolved gas	form in which the gas is transported. If it is used in a different form, then the classification has to be amended. Matching criteria with 2.5. Note: Gases may be packaged in other forms such as "chemical under pressure" or "adsorbed gases" that are not considered in the GHS/CLP.
	5 Aerosol dispensers, Class 2.1 Class 2.2	Not relevant (Articles)	Aerosols	Category 1 Category 2 Category 3	The transport classification does not differentiate between Aerosols Category 1 and 2 (both are classified as class 2.1)
	6 Other articles containing gas under pressure	Gaseous	Flammable gases	Category 1	
	7 Non-pressurised gases subject to special requirements	Gaseous	Oxidising gases	Category 1	
	8 Chemicals under pressure***	Not relevant			
	9 Adsorbed gas	Gaseous			
Class 3	Packing group I	Liquid	Flammable liquid	Category 1	
	Packing group II	Liquid	Flammable liquid	Category 2	
	Packing group III	Liquid	Flammable liquid	Category 3	
Class 4.1	Types B-F	Solid or liquid	Self-reactive substances	Types B-F	

Class 4.1 (solid desensitized explosives)	Packing group I	Solid	Solid desensitized explosives		
Class 4.1 (only readily combustible solids)	Packing group II	Solid	Flammable solids	Category 1	
Class 4.1 (only readily combustible solids)	Packing group III	Solid	Flammable solids	Category 2	
Class 4.2 Pyrophoric substances	Packing group I	Liquid	Pyrophoric liquids	Category 1	
		Solid	Pyrophoric solids	Category 1	
Class 4.2	Packing group II	Solid	Self-heating substances and mixtures	Category 1	
Class 4.2	Packing group III	Solid	Self-heating substances and mixtures	Category 2	
Class 4.3	Packing group I Packing group II Packing group III	Liquid or solid	Substances which in contact with water emit flammable gases	Category 1 Category 2 Category 3	
Class 5.1	Packing group I Packing group II Packing group III	Solid	Oxidising solid	Category 1 Category 2 Category 3	
Class 5.1	Packing group I Packing group II Packing group III	Liquid	Oxidising liquid	Category 1 Category 2 Category 3	
Class 5.2	Types B-F	Solid or liquid	Organic peroxides	Types B-F	
Class 8	Packing group III	Liquid or solid	Corrosive to metals	Category 1	Applies only when the substance or mixture is not classified as corrosive to skin and/or eye.

(*) Substances and articles (except aerosols and chemicals under pressure) of Class 2 are assigned to one of the following transport groups according to their hazardous properties, as follows: A asphyxiant, O oxidising, F flammable, T toxic, TF toxic, flammable, TC toxic corrosive, TO toxic, oxidising, TFC toxic, flammable, corrosive, TOC toxic, oxidising, corrosive

(**) Aerosols are assigned to one of the following transport groups according to their hazardous properties, as follows: A asphyxiant, O oxidising, F flammable, T toxic, C corrosive, CO corrosive, oxidising, FC flammable, corrosive, TF toxic, flammable, TC toxic corrosive, TO toxic, oxidising, TFC toxic, flammable, corrosive, TOC toxic, oxidising, corrosive

(***) Chemicals under pressure are assigned to one of the following transport groups according to their hazardous properties, as follows: A asphyxiant, F flammable, T toxic, C corrosive, FC flammable, corrosive, TF toxic, flammable

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