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**Committee of Experts on the Transport of Dangerous Goods  
and on the Globally Harmonized System of Classification  
and Labelling of Chemicals****Sub-Committee of Experts on the Globally Harmonized  
System of Classification and Labelling of Chemicals****Thirtieth session**

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Item 3 (e) of the provisional agenda

**Classification criteria and related hazard communication:  
Miscellaneous****Use of non-animal testing methods for the classification of  
health hazards****Transmitted by the experts from the Netherlands and the United  
Kingdom<sup>1</sup>****Background**

1. The GHS criteria have been applied in the European Union for several years for substances. From 1 June this year, all mixtures must also be classified using the GHS criteria. The experts from The Netherlands and the United Kingdom would like to share with the Sub-Committee some of the practical challenges we have faced in applying non-animal testing methods (read-across and *in-vitro* test methods) for the classification of health hazards. Both read-across approaches and the *in vitro* test methods are mentioned in the GHS as possible ways to classify substances and mixtures based on the given criteria, but in practice this is not possible in many cases. In this document, we address several issues that may need further thought and thorough discussion. If the Sub-Committee decides to put these items onto its programme of work, we are willing to take to lead on these issues (formally or informally). Some are practical classification issues and could be taken forward in the informal correspondence group on practical classification issues (PCI). Here the experts from the Netherlands and the United Kingdom could draft papers for discussion, within the overall lead of the expert from the United States of America.

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<sup>1</sup> In accordance with the programme of work of the Sub-Committee for 2015–2016 approved by the Committee at its seventh session (see ST/SG/AC.10/C.3/92, paragraph 95 and ST/SG/AC.10/42, para. 15).

2. Tests that determine hazardous properties, which are conducted according to internationally recognized scientific principles, can be used for purposes of a hazard determination for health and environmental hazards. The GHS criteria for determining health and environmental hazards are test method neutral, allowing different approaches as long as they are scientifically sound and validated. (GHS par. 1.1.2.5 (b)(i)). Nevertheless, reference is sometimes made to specific OECD test guidelines. For example in tables 3.4.3 and 3.4.4 the criteria for skin sensitization sub-categories 1A and 1B refer to OECD TG 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and OECD TG 429 (Local Lymph Node Assay (LLNA)).
3. Although not explicitly mentioned, it is clear that most classification criteria were originally based on animal test methods because the GHS criteria were at that time based on available (animal) data (GHS par. 1.1.2.5 (b)(ii)).
4. GHS par. 1.3.2.4.6 reads: “The welfare of experimental animals is a concern. This ethical concern includes not only the alleviation of stress and suffering but also, in some countries, the use and consumption of test animals. Where possible and appropriate, tests and experiments that do not require the use of live animals are preferred to those using sentient live experimental animals. To that end, for certain hazards non-animal observations/measurements are included as part of the classification system.”
5. We have encountered several difficulties in applying alternatives to animal testing to classify substances and mixtures, especially read-across approaches and *in vitro* test methods. In this document these difficulties are briefly described. We would like to further reflect on these issues together with experts from the Sub-Committee and to look for possible improvements.

## Discussion

### Limitations and ambiguities in the use of read-across approaches

6. One option to avoid animal testing is to use data from closely analogous substances using structure-activity relationships (SAR) (read-across approach). Several suggestions on how to apply a read-across approach are available. For example, the OECD published a guidance document (Series on testing and assessment, number 102) on the (Q)SAR application toolbox to develop chemical categories according the OECD guidance on grouping of chemicals. The European Chemical Agency (ECHA) recently published its “Read-Across Assessment Framework (RAAF)” that provides a structure for the scientific evaluation of grouping and read-across approaches for mono-constituent substances under REACH dossier evaluation. And in the United States of America, the Environmental Protection Agency has provided the “Analog Identification Methodology (AIM)” to facilitate analog analysis and data identification in support of chemical assessment and read across approaches.
7. In the GHS, the use of read-across within the criteria for health hazards is inconsistent. For some health hazards the criteria state that read-across is allowed without limitations and for other hazard classes there is no mention of read-across. For a third group of health hazards specific criteria on the use of read-across are included (see Annex I for an overview).
8. As shown in Annex I and in paragraphs 9 to 15 below, the use of read-across differs between the health hazards. However, the reasons for this difference are not clear. Given the current state of knowledge, in our opinion in principle read-across can and should be

consistently applied in all health hazard chapters as a stand-alone criterion. Does the Sub-Committee agree that the use of read-across should be consistent for all categories?

### **Read-across for carcinogenicity and reproductive toxicity**

9. GHS allows the possibility of read-across from structural analogues to category 1 for carcinogenicity (GHS par. 3.6.2.5.4) and more generally for reproductive toxicity (GHS par. 3.7.2.5.4). However, it is unclear whether the use of read-across from substances classified in category 1A will result in classification in category 1A, 1B or simply category 1. From the description of the hazard categories (GHS figures 3.6.1 and 3.7.1), it could be inferred that for category 1A there should be human evidence on the substance itself. More specifically this is stated in paragraph 3.6.2.4 and 3.6.5.3 (guidance) for carcinogenicity. Consequently, the use of read-across could result in category 1B, but not 1A.

10. We would like to hear the view of the Sub-Committee on the interpretation that read-across from a carcinogenic or reprotoxic substance X (classified as category 1A) to substance Y will result in classification as category 1B.

### **Read across for germ cell mutagenicity**

11. As indicated above, for carcinogenicity and reproductive toxicity the GHS allows the possibility of read-across from structural analogues to category 1 (next to category 2); however for mutagenicity this possibility seems to be excluded, given the note in GHS figure 3.5.1 (Hazard categories for germ cell mutagens): "*Substances which are positive in in vitro mammalian mutagenicity assays, and which also show structure activity relationship to known germ cell mutagens, should be considered for classification as Category 2 mutagens*". A literal interpretation of this note would mean that read-across will always result in category 2 classification, irrespective of whether the structural analog was classified as category 1 or 2. Further, the note limits the use of read-across to substances showing a positive result in the *in vitro* assay. This seems to be inconsistent with the other hazard classes.

12. We would like to know more about the origin and purpose of the note in figure 3.5.1. It is our preference to remove this limitation on the use of read-across in the note in figure 3.5.1.

### **Read across for respiratory and skin sensitization**

13. For respiratory sensitizers, read across is permitted where there is clinical history and data from lung function tests (human evidence) supported by a chemical structure related to substances known to cause respiratory hypersensitivity (GHS par. 3.4.2.1.2.3 (a)(iv)). Therefore read across can only be used as supportive evidence where there is existing human evidence rather than as a stand-alone criterion.

14. For skin sensitization, GHS explicitly allows read-across only as part of a weight of evidence judgement where two or more out of five indicators are fulfilled, with positive results from close structural analogues as one of the five indicators (GHS par. 3.4.2.2.4.3). This means that read-across from known skin sensitizers alone is not sufficient for classification.

15. We would like to hear the view of the Sub-Committee on the application of read-across for respiratory and skin sensitization. Do you agree with our interpretation that

read-across can only be applied as supporting evidence for these hazard classes? Or should classification also be possible based only on read-across evidence, as is the case for skin corrosion/irritation and serious eye damage/eye irritation (see tiered evaluation in Figures 3.2.1 and 3.3.1 respectively).

### **Limitations in the use of *in vitro* methods**

16. Another option to avoid animal testing is the use of *in vitro* test methods as developed by OECD. The availability of currently accepted OECD *in vivo* and *in vitro* test methods for the health hazards is indicated in Annex II.

17. In our experience, the use of *in vitro* test methods for classification of substances and mixtures in several hazard classes is limited because the GHS does not explicitly mention criteria for the results from *in vitro* tests. Several tiered evaluation strategies refer to the use of *in vitro* data, but the GHS criteria are based on *in vivo* effects.

18. Although OECD test methods are continuously developing and new *in vitro* methods will become available, it is always very difficult to connect the outcome of the *in vitro* test with the GHS criteria. Currently the possibility of using a specific *in vitro* method for GHS classification is only discussed within the remit of OECD in the discussion of the *in vitro* test guidelines. The use of *in vitro* tests for classification is included in the guideline. Also the use of the results of *in vitro* tests is discussed within guidance documents such as the European Union regulation on the classification, labelling and packaging of substances and mixtures (CLP Regulation) and OECD “Integrated approach on testing and assessment (IATA) for skin corrosion and irritation” guidance. However, such information is missing in the most logical place, the GHS classification criteria.

19. We would like to hear the view of the Sub-Committee on whether GHS should also specifically mention *in vitro* classification criteria (comparable to tables 3.4.3 and 3.4.4 in the GHS for animal test results of specific skin sensitization test methods) or if the Sub-Committee see other options to improve the use of *in vitro* methods.

### ***In vitro* methods for skin corrosion/irritation and eye irritation**

20. For skin corrosion/irritation and eye irritation, GHS refers to the use of (validated and accepted) *in vitro* tests (for example in GHS paragraphs 3.2.2.2.4 and 3.3.2.2.3 and in the tiered testing and evaluation strategy). However, no classification criteria are provided in the GHS for *in vitro* methods. For effects on the eye, it is not possible to classify for all categories in these hazard classes based on *in vitro* data only. The three available *in vitro* OECD test guidelines for serious eye damage/eye irritation (OECD TG 438, OECD TG 437, OECD TG 460) indicate that the test is not applicable for classification in category 2 and that it is expected that only strategic combinations of several tests may be able to fully replace OECD TG 405. We expect that it will be very difficult to develop *in vitro* test guidelines and combinations applicable for all categories, because validation of the *in vitro* method versus the *in vivo* method is expected to be difficult due to the variability between animals in the *in vivo* assay and the currently applied criteria for classification based on reversibility<sup>2</sup>. Guidance has been developed on this issue for example by ECHA. For skin corrosion, some *in vitro* methods do not allow a full sub-categorization into 1A, 1B or 1C. For example, some methods discriminate between category 1A versus 1B or 1C but not

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<sup>2</sup> See also: Adriaens, E., et al., Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the *in vivo* endpoints under UN GHS/EU CLP for the development and evaluation of *in vitro* test methods. Arch Toxicol., 2014.

between 1B and 1C due to the limit set of well-known *in vivo* corrosive sub-category 1C chemicals. Although regional guidance may be available this may lead to global disharmonisation. Another issue may be differences in validation between substances and mixtures.

21. We would like to hear the view of the Sub-Committee if GHS should include criteria based on *in vitro* methods for these hazard classes to increase the global harmonisation.

### ***In vitro* method for skin sensitization**

22. For skin sensitization, there are currently two *in vitro* test guidelines for the direct peptide reactivity assay (DPRA, OECD TG 442C) and the ARE-Nrf2 Luciferase test method (OECD TG 442D). Yet, the GHS does not mention in the criteria or in the specific considerations the possibility of using *in vitro* data for skin sensitization, and the GHS classification criteria are not fitted to the results of *in vitro* tests. Nevertheless the OECD *in vitro* test guidelines indicate that they can be used to support discrimination between skin sensitizers (i.e. GHS Category 1) and non-sensitizers within the context of an integrated approach to testing and assessment. In addition, combinations of several *in vitro* tests are validated for the predictivity for *in vivo* and human skin sensitisation. It is indicated that the *in vitro* guidelines should not be currently used to sub-categorise skin sensitizers into subcategories 1A and 1B.

23. We would like to hear from the Sub-Committee experts whether they accept the use of the above *in vitro* OECD test methods for classification of substances and mixtures in skin sensitization category 1, although GHS table 3.4.2 explicitly mentions evidence in humans or positive results from an animal test. Should the Sub-Committee develop criteria for classification as skin sensitisation based on *in vitro* methods?

## Annex I

## Read across for health hazards of substances in GHS (Rev.6)

Chapter	Use of read-across	Relevant paragraph
3.1 Acute toxicity	Not stated	
3.2 Skin corrosion/irritation	Unconditional	3.2.2.2.6 In some cases sufficient information may be available from structurally related substances to make classification decisions.
3.3 Serious eye damage/eye irritation	Unconditional	3.3.2.2.5 In some cases sufficient information may be available from structurally related substances to make classification decisions.
3.4 Respiratory or skin sensitization	Conditional	3.4.2.1.2.3 The evidence referred to above could be: (a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include: (i) <i>in vivo</i> immunological test (e.g. skin prick test); (ii) <i>in vitro</i> immunological test (e.g. serological analysis); (iii) studies that may indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects; <b>(iv) a chemical structure related to substances known to cause respiratory hypersensitivity;</b> (b) data from positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction.  3.4.2.2.4.3 If none of the above mentioned conditions are met, the substance need not be classified as a skin sensitizer. However, a combination of two or more indicators of skin sensitization as listed below may alter the decision. This shall be considered on a case-by-case basis. (a) Isolated episodes of allergic contact dermatitis; (b) Epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence; (c) Data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant; (d) Positive data from non-standard methods; <b>(e) Positive results from close structural analogues.</b>
3.5 Germ cell mutagenicity	Conditional	Figure 3.5.1 Note Substances which are positive in <i>in vitro</i> mammalian mutagenicity assays, and which also show structure activity relationship to known germ cell mutagens, should be considered for classification as Category 2 mutagens.
3.6 Carcinogenicity	Unconditional, based on expert judgement	3.6.2.5.4 A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1 or Category 2 based on tumour data from a structural analogue together with substantial support from

Chapter	Use of read-across	Relevant paragraph
		consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes.
3.7 Reproductive toxicity	Unconditional, based on expert judgement	3.7.2.5.4 Evidence from <i>in vitro</i> assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. 3.7.2.3.1 Evaluation of substances chemically related to the material under study may also be included, particularly when information on the material is scarce.
3.8 Specific target organ toxicity single exposure	Unconditional, based on expert judgement	3.8.2.1.10.3 A substance that has not been tested for specific target organ toxicity may in certain instances, 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.
3.9 Specific target organ toxicity repeated exposure	Unconditional, based on expert judgement	3.9.2.10.3 A substance that has not been tested for specific target organ toxicity may in certain instances, 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.
3.10 Aspiration hazard	Not stated	

## Annex II

### Availability of *in vivo* and *in vitro* OECD test methods (health hazards)

- Acute toxicity: many OECD *in vivo* methods are available (TG 402, 403, 420, 423, 425, 436), no *in vitro* methods.
- Skin corrosion/irritation: *in vivo* method TG 404 is available, several *in vitro* methods (TG 430, 431, 435, 439) are available, sub-categorization in 1A, 1B or 1C is not always possible with the *in vitro* methods.
- Serious eye damage/eye irritation: *in vivo* method TG 405 is available, three *in vitro* methods (TG 437, 438, 460, 491, 492) are available, but they allow only classification in category 1 or no classification.
- Respiratory sensitization: both *in vivo* and *in vitro* methods are not available.
- Skin sensitization: *in vivo* methods TG 406 and 429 are available. Also *in vivo* methods TG 442A and 442B for non-radiolabelled LLNA are available, but they are only suitable for category 1 and no classification. Two *in vitro* methods (TG 442C, 442D) are available, but they also allow only classification in category 1 or no classification.
- Germ cell mutagenicity: both several *in vivo* and *in vitro* methods are available.
- Carcinogenicity: *in vivo* methods TG 451 and 453 are available, no *in vitro* methods.
- Reproductive toxicity: many *in vivo* methods are available (TG 414, 415, 416, 421, 422, 426, 443), no *in vitro* methods.
- Specific target organ toxicity – single exposure: both no specific *in vivo* and *in vitro* methods are available.
- Specific target organ toxicity – repeated exposure: many *in vivo* methods are available (TG 407, 408, 409, 410, 411, 412, 413, 415, 416, 419, 422, 443, 452, 453), no *in vitro* methods.
- Aspiration hazard: viscosity test for liquids (TG 114) is used for this hazard class.