United States
Consumer Product Safety Commission

Defined Approaches in the GHS: Skin Sensitization

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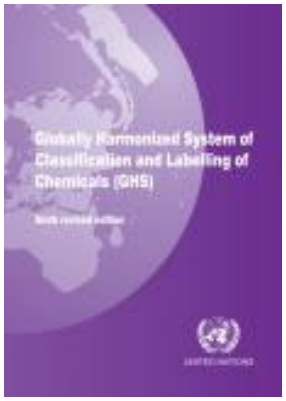
Outline

- Informal working group on the use of non-animal alternatives (NAMs)
- Skin corrosion/irritation (chapter 3.2)
- Serious eye damage/eye irritation (chapter 3.3)
- Defined Approaches for skin sensitization (chapter 3.4)
- Proposed changes to chapter 3.4

Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Use of non-animal testing methods:

- Netherlands and UK proposed several activities for inclusion in the work programme; activities regarding the use of non-animal approaches (*in silico, in vitro, in chemico*) for classifying substances and mixtures.
- Started with skin corrosion and irritation in 2016 (chapter 3.2)



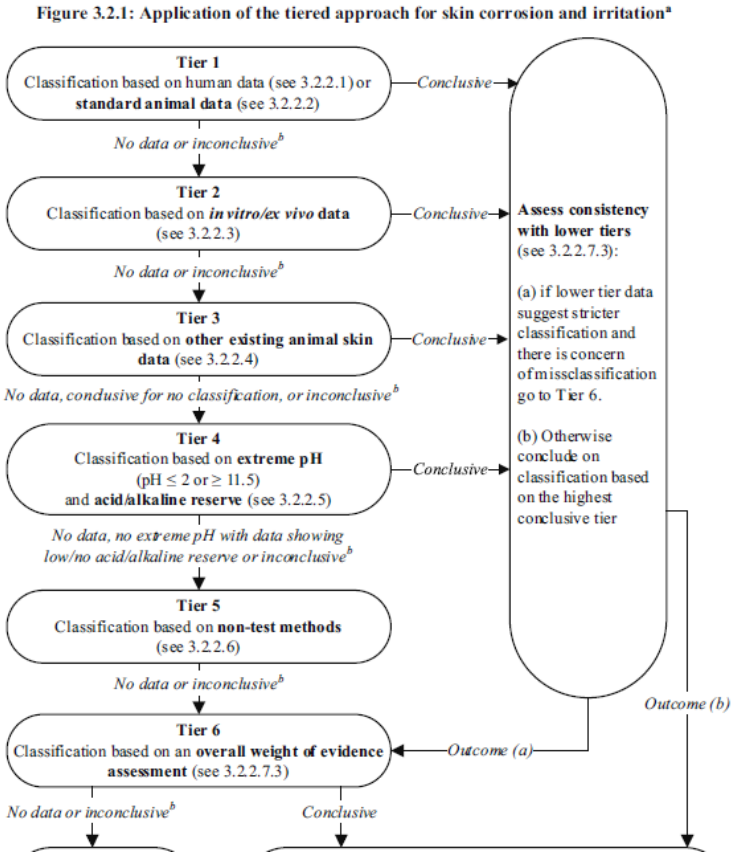
Non-animal Alternative Approaches

- Informal working group on the use of non-animal alternatives
  - Identify and evaluate alternative methods/approaches (*e.g., in vitro*, *in chemico*, read across, grouping, quantitative structure-activity relationships [QSARs]) and guidance useful for classification.
  - Determine whether an integrated or tiered approach should be developed for substances and mixtures; and, whether there is a need for new or modified criteria.
  - Prepare draft amendments and additions that include criteria, notes, decision logics, guidance.

GHS Skin Corrosion/Irritation Updates

Key revisions and additions include:

- Sections on *in vitro/ex vivo test methods*: no one single test for corrosion and irritation, some methods cannot distinguish between subcategories, Cat 3 (mild irritants) is not covered by NAMs
- Section on non-test methods (SARs, QSARs, read across, expert systems), use on a case-by-case basis
- Background guidance section



Skin Corrosion/Irritation Guidance Section: A Selection

Table 3.2.6: Skin corrosion criteria for *in vitro/ex vivo* methods

Category	OECD Test Guideline 430 ~~(Transcutaneous~~ Electrical Resistance test method~~)~~	OECD Test Guideline 431 Reconstructed human Epidermis test methods: Methods 1, 2, 3, 4 and 5 as numbered in Annex 2 of OECD Test Guideline 431				OECD Test Guideline 435 Membrane barrier test method	
	Using rat skin discs corrosive chemicals are identified by their ability to produce a loss of normal *stratum corneum* integrity. Barrier function of the skin is assessed by recording the passage of ions through the skin. The electrical impedance of the skin is measured using transcutaneous electrical resistance (TER). A confirmatory test of positive results using a dye-binding step that assesses if an increase in ionic permeability is due to the physical destruction of the *stratum corneum* is performed in case of a reduced TER (less than or around 5 kΩ) in the absence of obvious damage. The criteria are based on the mean TER value in kΩ and sometimes on dye content.	Four similar methods where the test chemical is applied topically to a three-dimensional reconstructed human epidermis (RhE) which closely mimics the properties of the upper parts of human skin. The test method is based on the premise that corrosive chemicals are able to penetrate the *stratum corneum* by diffusion or erosion and are cytotoxic to the cells in the underlying layers. Tissue viability is assessed by enzymatic conversion of the dye MTT into a blue formazan salt that is quantitatively measured after extraction from the tissues. Corrosive chemicals are identified by their ability to decrease tissue viability below defined threshold values. The criteria are based on the percent tissue viability following a defined exposure period.				An *in vitro* membrane barrier test method comprising a synthetic macromolecular bio-barrier and a chemical detection system (CDS). Barrier damage is measured after the application of the test chemical to the surface of the synthetic membrane barrier. The criteria are based on the mean penetration/breakthrough time of the chemical through the membrane barrier.	
						Type 1 chemicals (high acid/alkaline reserve)	Type 2 chemicals (low acid/alkaline reserve)
1	(a) mean TER value ≤ 5 kΩ and the skin discs are obviously damaged (e.g. perforated), or (b) mean TER value ≤ 5 kΩ and (i) the skin discs show no obvious damage (e.g. perforation), but (ii) the subsequent confirmatory testing of positive results using a dye binding step is positive.	Method 1 < 35 % after 3, 60 or 240 min exposure	Methods 2, 3, 4, 5 < 50 % after 3 min exposure; or ≥ 50 % after 3 min exposure and < 15 % after 60 min exposure			≤ 240 min	≤ 60 min
1A	Not applicable	Method 1 < 35 % after 3 min exposure	Method 2 < 25 % after 3 min exposure	Method 3 < 18 % after 3 min exposure	Methods 4, 5 < 15 % after 3	0-3 min.	0-3 min
1B		≥ 35 % after 3 min exposure and < 35 % after 60 min exposure or ≥ 35 % after 60 min exposure and < 35 % after 240 min exposure	≥ 25 % after 3 min exposure and fulfilling criteria for Category 1	≥ 18 % after 3 exposure and fulfilling crite Category 1			
1C							
Not classified as skin corrosive	(a) the mean TER value > 5 kΩ, or (b) the mean TER value ≤ 5 kΩ, and (i) the skin discs show no obvious damage (e.g. perforation), and (ii) the subsequent confirmatory testing of positive results using a dye binding step is negative	≥ 35 % after 240 min exposure	≥ 50 % after 3 min exposure and ≥ 1 exposure				

Table 3.2.7: Skin irritation criteria for *in vitro* methods

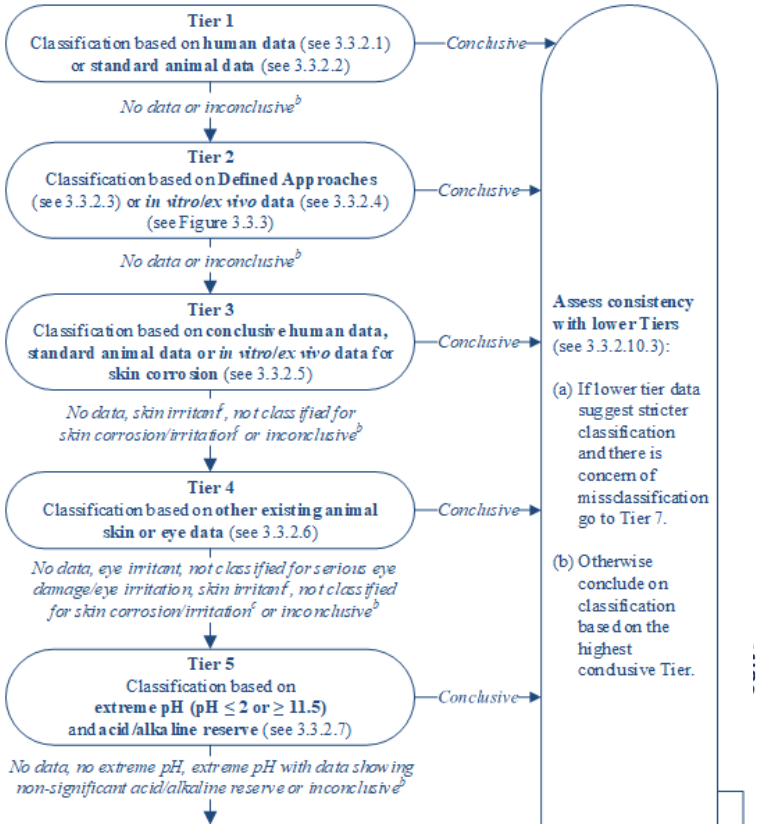
Category	OECD Test Guideline 439 Reconstructed Human Epidermis test methods
	Four similar methods (1-4) where the test chemical is applied topically to a three-dimensional reconstructed human epidermis (RhE) which closely mimics the properties of the upper parts of human skin. Tissue viability is assessed by enzymatic conversion of the dye MTT into a blue formazan salt that is quantitatively measured after extraction from the tissues. Positive chemicals are identified by their ability to decrease tissue viability below defined threshold levels. The criteria are based on mean percent tissue viability after exposure and post-treatment incubation.
1 or 2	Mean percent tissue viability (≤) 50 %. *Note: The RhE test methods covered by this test guideline cannot resolve between GHS categories 1 and 2. Further information on skin corrosion will be required to decide on its final classification (see also the OECD Guidance Document 203).*
2	Mean percent tissue viability ≤ 50 % and the test chemical is found to be noncorrosive (e.g. based on Test Guideline 430, 431 or 435)
Not classified as skin irritant or Category 3	Mean percent-tissue viability > 50 % *Note: The RhE test methods covered by this test guideline cannot resolve between GHS optional Category 3 and not classified as skin irritant. Further information on skin irritation is required for those authorities that want to have more than one skin irritation category.*

GHS Serious Eye Damage and Eye Irritation

Key revisions and additions include:

- Classification based on in vitro/ex vivo test methods
- Classification based on Defined Approaches (DAs)
- Section on non-test methods (SARs, QSARs, read across, expert systems)
- Extensive background guidance section

Figure 3.3.1: Application of the tiered approach for serious eye damage/eye irritation[a]



Proposed GHS Skin Sensitization Updates

Key revisions and additions include:

- Classification based on human data, standard animal data, DAs, *in chemico/in vitro data*, and non-test methods
  - Separate sections for each
  - Non-test methods include computer models predicting qualitative structure activity relationships (structural alerts, SAR) or QSARs, computer expert systems, and read-across using analogue and category approaches
- Classification in a tiered approach
- Extensive background guidance section

Draft Defined Approaches in GHS Chapter 3.4

- Consist of a rule-based combination of data obtained from a predefined set of different information sources (*e.g.*, *in chemico* methods, *in vitro* methods, physico-chemical properties, non-test methods)
- DAs can be useful strategies of combining data for classifying substances (and mixtures) because most single non-animal methods are not able to replace *in vivo* methods fully for most regulatory endpoints
- Results are conclusive for classification for skin sensitization if the criteria of the defined approach are fulfilled (Table 3.4.6)
- Data from a defined approach can only be used for classification when the tested substance is within the applicability domain of the DA used.

Proposed Skin Sensitization GHS Updates - General

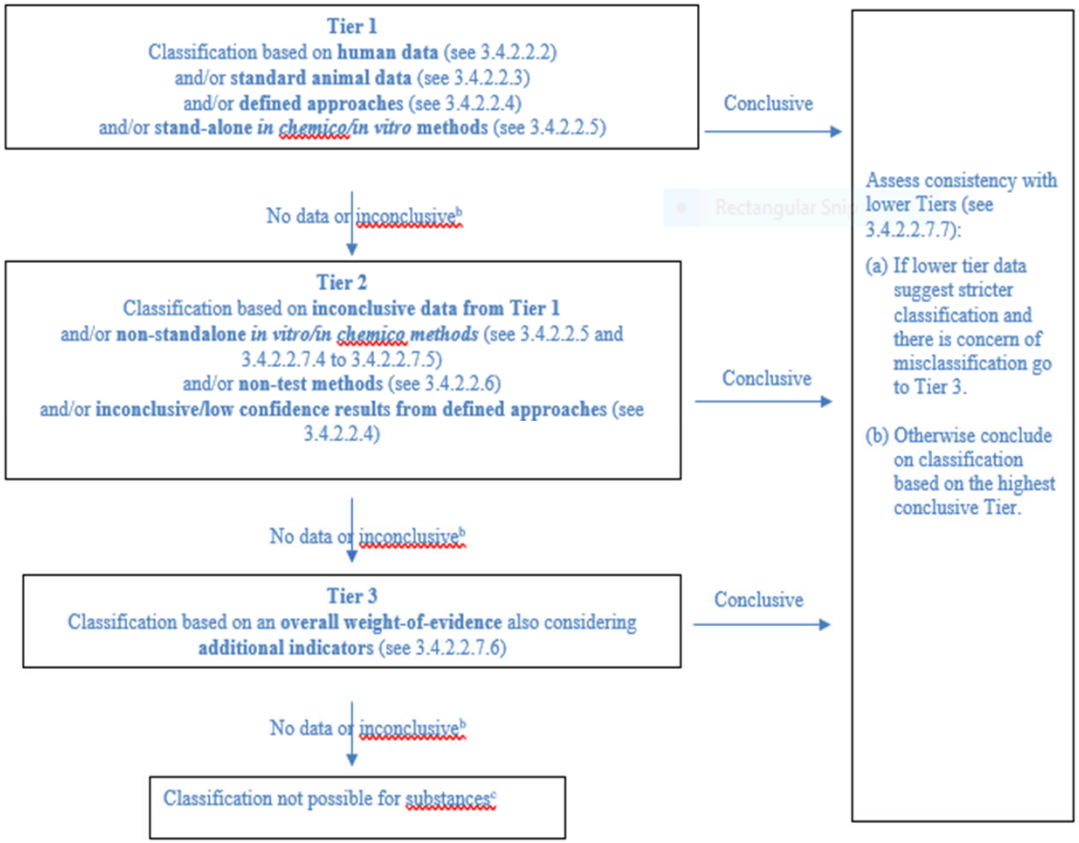
- For classification of skin sensitizers, all available and relevant information is collected and its quality in terms of adequacy and reliability is assessed.
- Classification should be based on mutually acceptable data/results generated using methods and/or DAs that are validated according to international procedures. These include both OECD Guidelines and equivalent methods/DAs.
- *In chemico/in vitro* data can only be used for classification when the tested substance is within the applicability domain of the test method used.

Proposed GHS Table 3.4.6: Criteria for DAs

Category	2o3 approach	ITSv1 and ITSv2
	Based on *in chemico* (KE1-DPRA) and *in vitro* (KE2-KeratinoSens™/KE3-hCLAT). Assays are run for two KEs, and if these assays provide consistent results, then the chemical is predicted accordingly as sensitizer or non-sensitizer. If the first two assays provide discordant results, the assay for the remaining KE is run. The overall result is based on the two concordant findings taking into account the confidence on the obtained predictions as described in the GL.	ITSv1 based on *in chemico* (KE1-DPRA) and *in vitro* (KE3-hCLAT) data, and *in silico* (Derek Nexus) predictions. ITSv2 based on *in chemico* (KE1-DPRA) and *in vitro* (KE3-hCLAT) data, and *in silico* (OECD QSAR Toolbox) predictions. Quantitative results of hCLAT and DPRA are converted into a score from 0 to 3. For the *in silico* prediction, a positive outcome is assigned a score of 1; a negative outcome a score of 0. When these scores have been assessed, a total battery score, ranging from 0 to 7, calculated by summing the individual scores, is used to predict the sensitizing potential (hazard ID; Cat 1 vs. NC) and potency (Cat 1A, Cat 1B and NC).
1	2 out of 3, or 3 out of 3 positive predictions	Total battery score ≥ 2
1A	Not applicable	Total battery score ≥ 6-7
1B	Not applicable	Total battery score ≥ 2-5
Not Classified	2 out of 3, or 3 out of 3 negative predictions	Total battery score < 2

Proposed GHS Tiered Approach

- A tiered approach organizes the available information on skin sensitization into tiers and provides for decision-making in a structured and sequential manner.
- Classification results when the information consistently satisfies the criteria. When available information gives inconsistent and/or conflicting results within a tier, classification is made using a weight-of-evidence assessment within that tier.
- When different tiers give inconsistent and/or conflicting results or where data individually are insufficient to conclude on the classification, an overall weight-of-evidence assessment is used.



Stand-alone and non-Stand-alone methods in the GHS chapter

- When already considered within a DA, non-stand-alone *in chemico/in vitro* methods should not be considered as an additional line of evidence.

- Other non-stand-alone *in chemico/in vitro* methods that are validated according to international procedures (*e.g.,* OECD Test Guidelines 442C (Annex I and II), 442D, 442E) are accepted as supportive evidence and should within Tier 1 only be used in combination with other types of data in DAs.

- Other validated *in chemico/in vitro* test methods accepted by some competent authorities are described in the guidance section. A competent authority may decide which classification criteria, if any, should be applied for these test methods to conclude on classification.

GHS Tier 1 Methods and Approaches

- For classification of a substance, evidence in Tier 1 may include data from any or all of the following lines of evidence:
  - Experimental studies in humans (*e.g.*, predictive patch testing, HRIPT, HMT)
    - see paragraph 1.3.2.4.7, criteria in 3.4.2.2.2.2 (a) and 3.4.2.2.2.3 (a) and guidance 3.4.5.3.2
  - Epidemiological studies (*e.g.*, case control studies, prospective studies) assessing allergic contact dermatitis
  - Well-documented cases of allergic contact dermatitis
  - Appropriate animal studies
  - Defined approaches validated according to international procedures
  - Stand-alone *in chemico/in vitro* methods validated according to international procedures

Proposed GHS Table 3.4.7: Criteria for individual *in chemico/in vitro* methods – an example

Category	OECD TG 442C Key event-based Test Guideline for *in chemico* skin sensitization assays addressing the AOP Key Event on covalent binding to proteins		
	Method described in Appendix I **The Direct Peptide Reactivity Assay (DPRA)[a]**	**Method described in Appendix II** **The Amino acid Derivative Reactivity Assay (ADRA)[a]**	**Method described in Appendix III** **The kinetic Direct Peptide Reactivity Assay (kDPRA)[b]**
	Methods: in chemico methods addressing the process of haptenation by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine (DPRA and kDPRA) or towards model synthetic amino acid derivatives containing either cysteine (NAC) or lysine (NAL) (ADRA). The criteria are based on the mean of cysteine and lysine peptides percent depletion (DPRA), kinetic rates of cysteine depletion (kDPRA) and mean NAC and NAL percent depletion value (ADRA). Predictions models based on the cysteine or NAC percent depletion value alone in case the unreacted lysine peptide or NAL cannot be reliably measured can be applied for the DPRA and ADRA.		

1	The mean cysteine/lysine % depletion > 6.38% Or the mean cysteine % depletion > 13.89 %	The mean NAC and NAL % depletion ≥ 4.9% Or NAC% depletion ≥ 5.6%	Not applicable

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Proposed GHS Table 3.4.7: Criteria for individual *in chemico/in vitro* methods – an example (cont.)

Category	OECD TG 442C Key event-based Test Guideline for *in chemico* skin sensitization assays addressing the AOP Key Event on covalent binding to proteins		
	Method described in Appendix I The Direct Peptide Reactivity Assay (DPRA)[a]	Method described in Appendix II The Amino acid Derivative Reactivity Assay (ADRA)[a]	Method described in Appendix III The kinetic Direct Peptide Reactivity Assay (kDPRA)[b]
	Methods: in chemico methods addressing the process of haptenation by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine (DPRA and kDPRA) or towards model synthetic amino acid derivatives containing either cysteine (NAC) or lysine (NAL) (ADRA). The criteria are based on the mean of cysteine and lysine peptides percent depletion (DPRA), kinetic rates of cysteine depletion (kDPRA) and mean NAC and NAL percent depletion value (ADRA). Predictions models based on the cysteine or NAC percent depletion value alone in case the unreacted lysine peptide or NAL cannot be reliably measured can be applied for the DPRA and ADRA.		
1A	Not applicable		log kmax ≥ -2.0
1B	Not applicable	Not applicable	Not applicable
Not classified	The mean cysteine/lysine % depletion ≤ 6.38% or the mean cysteine % depletion ≤ 13.89 % %	The mean NAC and NAL % depletion < 4.9% Or NAC% depletion < 5.6%	Not applicable

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GHS Informal working group on the use of non-animal alternatives

- US core members:
  - Paul Brigandi
  - Janet Carter
  - Marianne Lewis
  - Joanna Matheson

Thank you

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Extra slides

Proposed GHS Table 3.4.7: Criteria for individual *in chemico/in vitro* methods

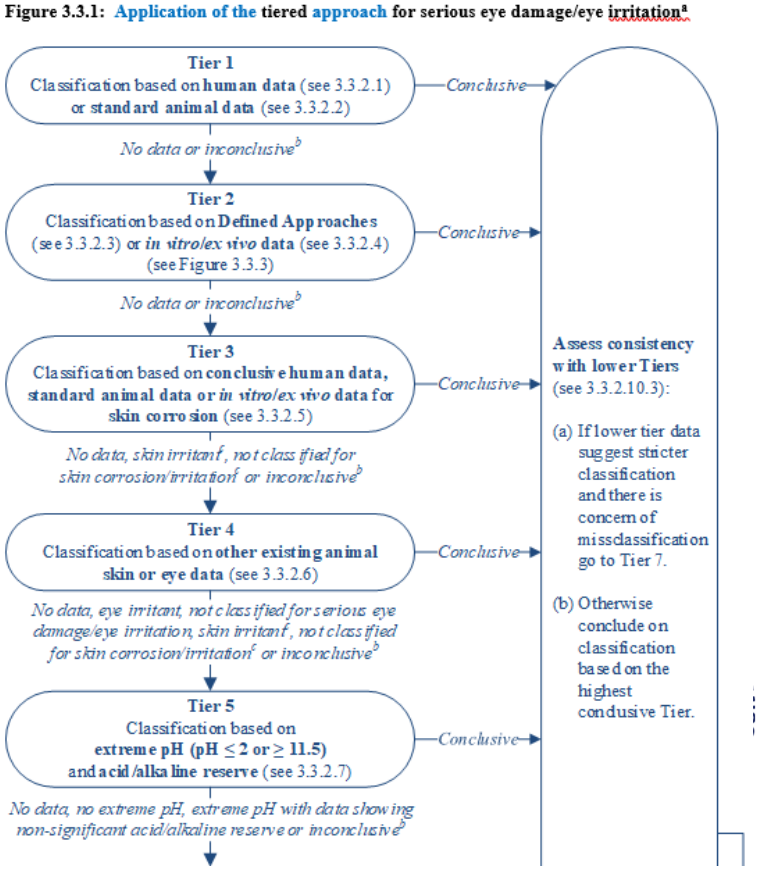
Category	OECD TG 442C Key event-based Test Guideline for *in chemico* skin sensitization assays addressing the AOP Key Event on covalent binding to proteins			OECD TG 442D Key event-based Test Guideline for *in vitro* skin sensitization assays addressing the AOP Key Event on keratinocyte activation ARE-Nrf2 luciferase methods		OECD TG 442E In vitro skin sensitization assays addressing the AOP Key Event on activation of dendritic cells			
	Method described in Appendix I The Direct Peptide Reactivity Assay (DPRA)[a]	Method described in Appendix II The Amino acid Derivative Reactivity Assay (ADRA)[a]	Method described in Appendix III The kinetic Direct Peptide Reactivity Assay (kDPRA)[b]	Method described in Appendix 1A KeratinoSens™[a]	Method described in Appendix 1B Lusens[a]	Method described in Annex I human Cell Line Activation Assay (h-CLAT)[a]	Method described in Annex II U937 Cell Line Activation Test[a]	Method described in Annex III IL-8 Luc assay[a]	Method described in Annex IV GARD skin ™
	Methods: *in chemico* methods addressing the process of haptenation by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine (DPRA and kDPRA) or towards model synthetic amino acid derivatives containing either cysteine (NAC) or lysine (NAL) (ADRA). The criteria are based on the mean of cysteine and lysine peptide percent depletion (DPRA), kinetic rates of cysteine depletion (kDPRA) and mean NAC and NAL percent depletion values (ADRA). Prediction models based on the cysteine or NAC percent depletion value alone in case the unreacted lysine peptide or NAL cannot be reliably measured can be applied for the DPRA and ADRA.			Methods: cell-based methods addressing the process of keratinocyte activation, by assessing with the help of luciferase, the Nrf2-mediated activation of antioxidant response element (ARE)-dependent genes following exposure of the cells to the test chemical. Cell viability is quantitatively measured in parallel by enzymatic conversion of the dye MTT. The criteria are based on the induction of the luciferase gene above a given threshold, quantified at subtoxic concentrations. Criteria should be met in 2 of 2 or in 2 of 3 repetitions.		Methods: ~~three~~ cell-based methods ~~are~~ addressing the process of monocytes/dendritic cell activation by either quantifying the change in the expression of cell surface marker(s) (e.g. CD54, CD86) or the change in IL-8 expression or the transcriptional patterns of an endpoint-specific genomic biomarker signature following exposure of the cells to the test chemical. Criteria should be met in 2 of 2 or in at least 2 of 3 repetitions for test methods described in Annexes I, II and III or in three valid biological replicates for test method described in Annex IV.			
1	The mean cysteine/lysine % depletion > 6.38% Or	The mean NAC and NAL % depletion ≥ 4.9% Or	Not applicable	The following 4 conditions are all met in 2 of 2 or in the same 2 of 3 repetitions: 1. Imax equal or higher than (≥) 1.5 fold and statistically significantly different to the	The following conditions are all met in 2 of 2 or in the same 2 of 3 repetitions: 1. A luciferase induction above or	At least one of the following conditions is met in 2 of 2 or in at least 2 of 3 independent runs: The Relative	The following condition is met in 2 of 2 or in at least 2 of 3 independent runs: The stimulation index of CD86 is equal or	The Ind-IL8LA is equal or higher than (≥) 1.4 and the lower limit of the 95% confidence	The mean Decision Value (DV) is ≥0

Proposed Table 3.4.7: Criteria for individual *in chemico/in vitro* methods (cont.)

	the mean cysteine % depletion > 13.89 %	5.6%		2. The cellular viability is higher than (>) 70% at the lowest concentration with induction of luciferase activity equal or above 1.5 fold 3. The $EC_{1.5}$ value is less than (<) 1000 µM (or < 200 µg/mL for test chemicals with no defined MW) 4. There is an apparent overall dose-dependent increase in luciferase induction	as compared to the solvent control is observed in at least 2 consecutive non-cytotoxic tested concentrations (i.e. cellular viability is equal or higher than (≥) 70%) 2. At least three tested concentrations should be non-cytotoxic (cellular viability equal or higher than (≥) 70%).	Intensity of CD86 is equal to or greater than 150% at any tested concentration (with cell viability ≥ 50%) or the Relative Fluorescence Intensity of CD54 is equal to or greater than 200% at any tested concentration (with cell viability ≥ 50%).	and/or interference is observed	IL8LA is equal or higher than (≥) 1.0 in at least 2 out of a maximum of 4 independent runs	
1A	Not applicable		log kmax ≥ -2.0	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
1B	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Not classified	The mean cysteine/lysine % depletion ≤ 6.38% or the mean cysteine % depletion ≤ 13.89 %	The mean NAC and NAL % depletion < 4.9% Or NAC% depletion < 5.6%	Not applicable	At least one of the conditions for Category 1 is not met	At least one of the conditions for Category 1 is not met	None of the conditions for Category 1 is met	The stimulation index of CD86 is < 150% at all non-cytotoxic concentrations (cell viability ≥ 70%) and if no interference is observed	The Ind-IL8LA is < 1.4 and/or the lower limit of the 95% confidence interval of Ind-IL8LA is < 1.0 in at least 3 out of a maximum of 4 independent runs	The mean Decision Value (DV) is <0

GHS Serious Eye Damage and Eye Irritation DAs

- TG467 adopted by OECD 6/30/22
- Can discriminate between Cat 1 (serious), Cat 2 (irritation) and NC
  - Cannot subclassify into Cat 2A or Cat 2B
- DAL-1: based on physico-chemical properties and *in vitro* data
  - Is for neat liquids, but not surfactants
- DAL-2: based on *in vitro* data
  - Is for neat liquids, not surfactants; and liquids and solids dissolved in water



GHS Serious Eye Damage and Eye Irritation DAs

	DAL-1 (VRM1)	DAL-1 (VRM2)	DAL-2
Physico-chemical properties	1 (water solubility) or a combination of 3 physchem properties (LogP, VP, ST)	1 (water solubility) or a combination of 3 physchem properties (LogP, VP, ST)	NA
In vitro methods	BCOP-LLBO (TG437)	BCOP-LLBO (TG437)	BCOP-LLBO (TG437)
	RhCE - EpiOcular EIT (TG492)	RhCE - SkinEthic HCE EIT (TG492)	STE (TG491)
Performance overall	69.20%	75.20%	74.30%
Performance for Cat 1 and NC, respectively	76.5% and 70.5%	76.5% and 79.7%	81.2% and 85.3%