EURL ECVAM TEMPLATE FOR CLASSIFICATION OF CHEMICALS FOR SERIOUS EYE DAMAGE / EYE IRRITATION FROM *IN VIVO* DRAIZE EYE TEST DATA

Explanatory document

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Contents

1. General considerations for using the EURL ECVAM template

Before entering the data, it is important to be aware of the following considerations.

1.1. General frame

Yellow cells require your input. Please introduce general information about the chemical (CAS number, data source etc.) and the number of animals used in the study (cell D3) into the header of the spreadsheet. Enter the raw data (scores) in the yellow cells of the appropriate experimental hour or day.

Green cells in contrast contain formulae and/or functions which are executed automatically in order to determine the classification of a chemical. **Green cells must therefore not be modified and for this reason they have been protected**.

1.2. Number of animals

- In order for the template to work correctly it is important to input the correct number of animals used in the study in cell D3.
- For the classification of non-severe effects, EU DSD, EU CLP and US EPA guidelines require at least 3 animals. Most studies in the literature use up to 6 rabbits. Therefore the datasheet was designed for studies using 3 to 6 rabbits.
- OECD, EU and US test guidelines require only 1 animal to be tested if serious eye damage and/or irreversible effects are observed. Therefore, when an animal study with data from less then three animals is entered, the template will automatically assume that the chemical induced serious eye damage and this will consequently result in a EU DSD R41, UN GHS / EU CLP Category 1 and US EPA Category I classification.
- **If less than 3 animals were used and no serious eye damage and/or irreversible effects were observed, the template should not be used.**

1.3. Reversibility of effects

There are two yellow cells for each animal called "Irreversible effects at d21 (N=0, Y=1, unknown=?)" allocated for the following classification systems:

- *EU DSD, EU CLP and UN GHS classification systems*

  The following information should be entered with regard to reversibility of effects:
  - reversible (enter a '0'), when all 4 endpoints of relevance (i.e., corneal opacity, iris lesions, conjunctiva redness and conjunctiva chemosis) have a score of 0 at an observation time up to (and including) day 21 of the study;
  - irreversible (enter a '1'), when at day 21 of the study one or more of the 4 endpoints of relevance (i.e., corneal opacity, iris lesions, conjunctiva redness and conjunctiva chemosis) have a score greater than 0;
  - if this is not possible to determine whether effects are reversible or irreversible based on the data available, enter a '?'.
- *US EPA classification systems*

  The following information should be entered with regard to reversibility of effects:

- reversible (enter a '0'), when scores below 1 for corneal opacity and for iris lesions, and below 2 for conjunctiva redness and for conjunctiva chemosis (oedema) are observed at a certain day up to (and including) day 21 of the study;
- irreversible (enter a '1'), when at day 21 of the study scores for corneal opacity or iris lesions are equal or higher than 1, or that scores for conjunctiva redness or for conjunctiva chemosis (oedema) are equal or higher than 2;
- if this is not possible to determine whether effects are reversible based on the data available, enter a '?'.

The Excel data sheet will automatically assign the day after which effects have fully reversed according to the EU DSD, EU CLP and UN GHS classification systems, with the following possibilities: >21, 21, 14, 7, 3, 1. These possible results are automatically returned in the green box named "effects fully reversed after … days". As mentioned already for green cells in general, these entries must not be modified as they will be relevant for determining the classification of a chemical.

1.4. Incomplete studies

If the data available are not sufficient to allow classification in one or more classification systems, the abbreviation "SCNM", i.e. Study Criteria Not Met, will appear in the green cells of the final classification. This might be the case for example if the observation period of a study with no serious eye damage ends before ocular lesions reverse.

1.5. Compliance with guidelines

- A volume of 0.1 mL for liquids or the weight of chemical corresponding to a volume of 0.1 mL for solids needs to be tested in each rabbit. A study in which a lower quantity was applied to the eye can be accepted for chemical classification, provided that a EU DSD R41, UN GHS / EU CLP Category 1 and US EPA Category I classification is observed.
- Observations of the eye must have been made, at minimum, at 24-, 48-, and 72-hours following chemical application if no serious eye damage was observed.

2. Criteria applied with regard to the EU DSD classification system

The EU DSD classification and labelling system was applied in accordance to the Commission Directive 2001/59/EC (EC, 2001) and is based on the eye irritation testing method B.5 of Annex V as reported in the Commission Directive 2004/73/EC (EC, 2004), which is equivalent to the OECD TG 405 (OECD, 2002). The EU criteria for eye irritation classification are summarized in Table 1 and reported below as quoted in the Commission Directive 2001/59/EC (EC, 2001):

"The following risk phrases shall be assigned in accordance with the criteria given:

R36 Irritating to eyes

- Substances and preparations which, when applied to the eye of the animal, cause significant ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test cited in Annex V have any of the following values:

- cornea opacity equal to or greater than 2 but less than 3,
- iris lesions equal to or greater than 1 but not greater than 1.5,
- redness of the conjunctivae equal to or greater than 2.5,
- oedema of the conjunctivae (chemosis) equal to or greater than 2,

or, in the case where the Annex V test has been completed using three animals if the lesions on two or more animals, are equivalent to any of the above values except that for iris lesion the value should be equal to or greater than 1 but less than 2 and for redness of the conjunctivae the value should be equal to or greater than 2.5.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

- Substances or preparations which cause significant ocular lesions, based on practical experience in humans.
- Organic peroxides except where evidence to the contrary is available.

R41 Risk of serious damage to eyes

- Substances and preparations which, when applied to the eye of the animal cause severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are severe if the means of the scores of the eye irritation test in Annex V have any of the values:

- cornea opacity equal to or greater than 3,
- iris lesion greater than 1.5.

The same shall be the case where the test has been completed using three animals if these lesions, on two or more animals, have any of the values:

- cornea opacity equal to or greater than 3,
- iris lesion equal to 2.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Ocular lesions are also severe when they are still present at the end of the observation time.

Ocular lesions are also severe if the substance or preparation causes irreversible colouration of the eyes.

- Substances and preparations which cause severe ocular lesions, based on practical experience in humans."

Table 1: EU DSD criteria for classification (EC, 2001). All scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values

	R36 Irritant to the eyes[1]	**R41 Risk of serious damage to eyes[2]**
Corneal opacity	2.0 ≤ mean score < 3.0 or 2.0 ≤ 2 animals (of 3) < 3.0	mean score ≥ 3.0 or 2 animals (of 3) ≥ 3.0
Iris lesion	1.0 ≤ mean score ≤ 1.5 or 1.0 ≤ 2 animals (of 3) < 2.0	mean score > 1.5 or 2 animals (of 3) = 2.0
Redness of conjunctivae	mean score ≥ 2.5 or 2 animals (of 3) ≥ 2.5	
Oedema of conjunctivae (chemosis)	mean score ≥ 2.0 or 2 animals (of 3) ≥ 2.0	

[1] R36 classification also applies to:

- Organic peroxides (except if evidence to the contrary)
- Substances causing significant ocular lesions in humans

[2]R41 classification also applies to:
- Ocular lesions still present at the end of the observation time (a maximum of 21 days or until lesions clear but not earlier than 3 days according to method B.5 of annex V)
- Substances causing irreversible colouration to eyes
- Substances causing severe ocular lesions in humans

It has to be noted that clearance of lesions (or reversibility of effects) was considered when scores of 0 were observed for all 4 endpoints of relevance (i.e., corneal opacity, iris lesions, conjunctiva redness and conjunctiva chemosis) at a certain day up to (and including) day 21 of the study.

3. Criteria applied with regard to the UN GHS / EU CLP classification system

The EU has implemented the United Nations Globally Harmonized System (UN GHS) for the classification and labelling of hazardous chemicals with Directive 1272/2008 (EU CLP) (EC, 2008). The guidelines provided in UN GHS (UN, 2009) and adopted in the EU CLP (EC, 2008) are briefly summarized in Table 2.

Table 2: UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008). Scores are calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material

	Category 2[1] **Irritating to eyes**	**Category 1[2]** **Irreversible effects on the eye**
Corneal opacity	at least 2 of 3 animals ≥ 1.0; and/or	at least 2 of 3 animals ≥ 3.0; and/or
Iritis	at least 2 of 3 animals ≥ 1.0; and/or	at least 2 of 3 tested animals > 1.5
Conjuctival redness	at least 2 of 3 animals ≥ 2.0; and/or	
Conjunctiva oedema (chemosis)	at least 2 of 3 animals ≥ 2.0	

[1] All effects have to fully reverse within an observation period of normally 21 days. UN GHS provides the option to distinguish this single hazard category into two optional sub-categories (not implemented in EU CLP): **'Category 2A' (irritating to eyes) when the eye effects listed above are not fully reversible**

within 7 days of observation; 'Category 2B' (mildly irritating to eyes) when the eye effects listed above are fully reversible within 7 days of observation.

[2] Category 1 also applies to:
- At least in one animal effects that are not expected to reverse, or have not fully reversed within an observation period of normally 21 days.
- Animals with grade 4 cornea lesions and other severe reactions (e.g., destruction of cornea) observed at any time during the test, as well as discoloration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight.

As the UN GHS classification system does not consider the cases where more than 3 animals are used, the decision criteria recommended for the EU CLP were used when available. In cases the CLP did not foresee specific decision criteria (e.g., corneal opacity scores of 4, or subcategories 2A and 2B) data was expanded in a proportional way to studies where more than 3 rabbits were used. In more details, the following rationales were applied:

- for ocular effects where 2 tested animals out of 3 are needed to assign a certain category (i.e., corneal opacity, iritis, conjunctiva redness and chemosis scores), a minimum of 67% of the animals was required for applying the same scoring systems, which means 3 tested animals out of 4, 3 out of 5 and 4 out of 6.
- A single animal showing irreversible or otherwise serious effects consistent with corrosion (i.e., a corneal score of 4 observed at any time) is sufficient to assign classification as serious eye damage Category 1, irrespective of the number of animals used in the study, which means 1 tested animal out of 3, 1 out of 4, 1 out of 5 and 1 out of 6.

Full reversibility was considered when scores of 0 were observed for all endpoints of relevance, i.e., corneal opacity, iris lesions, conjunctiva redness and conjunctiva chemosis at a certain day up to and including day 21 of the study.

4. Criteria applied with regard to the US EPA classification system

The U.S. Environmental Protection Agency (US EPA) classification and labelling system was performed in accordance to the guidelines given in the Label Review Manual (US EPA, 2003), and based on the test method described in the Acute Eye Irritation Health Effect Test Guideline (US EPA, 1998). The US EPA criteria for eye irritation classification are summarized in Table 3.

Table 3: US EPA Criteria for Classification (US EPA, 2003)

US EPA Category	Classification criteria
Category I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days
Category II	Corneal involvement or other eye irritation clearing[1] in 8-21 days
Category III	Corneal involvement or other eye irritation clearing in 7 days or less
Category IV	Minimal effects clearing in less than 24 hours

[1]Clearing is defined as corneal opacity or iritis < 1 and conjunctiva redness or chemosis < 2 (US EPA, 1998).

According to US EPA (1998), the following ocular scores are considered as positive:

Corneal opacity ≥ 1

Iritis ≥ 1

Conjunctiva redness ≥ 2

Conjunctiva chemosis ≥ 2

An animal is considered positive if any of the above-mentioned grades occurs at any of the grading periods, and 1 positive animal out of generally a maximum of 6 is required for the assignment to an irritant category (OECD, 1999).

When below the scores above, ocular effects can be considered as having reversed, in contrast to the EU and GHS classification systems where scores of 0 are required.

5. References

US EPA (1998). Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195. US Environmental Protection Agency, Washington, DC, USA.

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OECD (1999). Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries. *OECD Series on Testing and Assessment, number 14*. ENV/JM/MONO (99) 4. Organisation for Economic Cooperation and Development, Paris, France.

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