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EC Classification of Eye Irritancy

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1. BACKGROUND

Commission Directive 93/21/EEC (1993) was the 18th adaptation to technical progress of Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Annex IV to 93/21/EEC replaced Annex VI, Parts I and II of 67/548/EEC and should have been enforced in EU Member States (MS) by 1 July 1994. The section on ocular lesions (3.2.6.2) of Annex IV (General Classification and Labelling Requirements for Dangerous Substances and Preparations) lays down criteria which lead to the assignment of one of two risk phrases, namely, R36 (Irritating to eyes) or R41 (Risk of serious damage to eyes). Although legally the Directive does not apply to all products, such as pesticide and cosmetic formulations, in practice it is used in the absence of specific EU legislation in these areas.

The replacement of Annex VI to Directive 67/548/EEC by Annex IV to Directive 93/21/EEC introduced minor amendments to existing criteria as well as three entirely new criteria for the classification of substances and preparations based on their potential to cause ocular lesions. The new criteria refer to human experience, colouration and persistence of effects.

Concern was expressed at the European Commission Working Group on the Classification and Labelling of Dangerous Substances (1995) that two of these criteria, colouration and persistence of effects, which are specific to R41, were too severe if interpreted literally. In the absence of additional guidance, the Working Group, industry groups and MS' Competent Authorities have developed their own interpretation of the criteria in 93/21/EEC or considered results on a case-by-case basis. These attempts to clarify the situation or apply pragmatism when interpreting the existing criteria may in practice have increased the potential for inconsistency in the assignment of R41, as interested parties are applying the official criteria with varying degrees of flexibility.

This has led to a significant number of cases where the assignment of the risk phrase R41 has been inconsistent, or has been made with an inadequate degree of confidence. This is an unsatisfactory situation with wide-ranging impact. Those affected by the confusion include experimenters responsible for the conduct and evaluation of eye irritation studies, industry employees responsible for substance or preparation development and marketing, and those accountable for regulation of these substances and preparations.

To address this issue, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) appointed a Task Force in 1997 with the following Terms of Reference:

1. To review criteria for classification of 'ocular lesions' as described in Directive 93/21/EEC so as to identify and clarify areas of confusion or ambiguity, and incompatibility with respect to the EC and OECD guidelines for determination of acute eye irritation;
2. to provide a short, practical guidance document, compatible with the existing test guidelines and the humane treatment of animals, to enable consistent and scientifically-based assignment of the risk phrases R36 (Irritating to eyes) and R41 (Risk of serious damage to eyes).

The document is intended as guidance for all parties involved in testing and classification of substances and preparations for eye irritancy.

2. TEST METHOD AND GUIDELINE: IDENTIFICATION OF AREAS REQUIRING CLARIFICATION

Test Method B.5 Acute Toxicity (Eye Irritation) is included in the Annex to the seventeenth adaptation to technical progress (1992) of the EC "Classification and Labelling" Directive. This method is identical to that in the corresponding OECD test guideline 405 (OECD, 1987).

The Task Force considered that certain points with regard to Test Method B.5, which are relevant to the classification of substances and preparations for eye irritancy, needed clarification. These are discussed below.

2.1 PRINCIPLE OF THE TEST METHOD [Section 1.4]

Under *Initial Considerations* it is stated *inter alia* that "Animals showing severe and enduring signs of distress and pain may need to be humanely killed."

These effects are not currently defined. Where, based on expert judgment, a severe and enduring pain reaction is deemed to occur in any animal, the Task Force agreed that a study should be terminated and that this will result in assignment of R41. If the pain response is less severe, consideration should be given to repeating this element of the test using accepted pain-management techniques (e.g., local anaesthesia) before reaching a decision to terminate a study.

However the potential influence of certain pain-management techniques on the development of ocular lesions must be recognised (Durham *et al*, 1992). For example, tear and blink responses, and hence clearance of a test substance from the eye may be reduced, leading to an increased irritant response and potentially a more serious classification.

Nevertheless the Task Force considered that this approach would enable humane assessment of eye irritation and ensure correct classification and labelling of the test substance.

2.2 NUMBER OF ANIMALS [Section 1.6.2.2]

In relation to numbers of animals, it is stated that "A single-animal test should be considered if marked effects are anticipated. If the results of this test in one rabbit suggest the substance to be

severely irritant (reversible effect) or corrosive (irreversible effect) to the eye using the procedure described, further testing for ocular irritancy in subsequent animals may not need to be carried out. Occasionally, further testing in additional animals may be appropriate to investigate specific aspects.”

It is not clear when a study should be limited to a single animal, as the terms *severely irritant* and *corrosive* are not defined in the EC test method. Moreover, as the method is worded, the implication is that *irreversible* and *corrosive* effects are synonymous. This is incorrect; not all irreversible effects are corrosive. *Corrosive* should only be used where ocular tissue destruction occurs.

In addition, the corresponding EC classification criteria only differentiate between significant (R36) and severe (R41) eye irritation effects and do not mention corrosivity.

The Task Force concluded that the following severe ocular responses, if seen in any animal and clearly treatment-related, warranted termination of a study and assignment of R41:

- corneal perforation or significant corneal ulceration including staphyloma;
- blood in the anterior chamber of the eye;
- corneal opacity (grade 4 as defined in the EC test method) which persists for 48 hours;
- absence of a light reflex (iridial response grade 2 as defined in the EC test method) which persists for 72 hours;
- ulceration of the conjunctival membrane;
- necrosis of the conjunctivae or nictitating membrane;
- sloughing.

2.3 OBSERVATION PERIOD [Section 1.6.2.4]

This section refers to the fact that “The duration of the observation period should not be rigidly fixed. It should be sufficient to evaluate the reversibility or irreversibility of the effects observed, but normally need not exceed 21 days after instillation.”

It is important to recognise that the guideline method does not require a test to be terminated at day 21 but allows for the observation period to be extended beyond 21 days if this is necessary to “evaluate fully reversibility or irreversibility of the effects observed”. This is supported by the classification criteria which do not mention a specific duration for the observation period and this is further discussed under Section 4.4.1 of this Document.

3. CLASSIFICATION CRITERIA IN DIRECTIVE 93/21/EEC

As previously mentioned, there are two categories of classification for irritancy to eyes in Directive 93/21/EEC. For ease of reference these are presented below.

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 lesion 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.*

4. CLASSIFICATION CRITERIA: CLARIFICATION AND GUIDANCE PROPOSED BY THE TASK FORCE

The Task Force identified several aspects of the classification criteria that required clarification. These are detailed below together with recommendations for supplementary guidance which is intended to improve consistency when classifying substances and preparations for ocular lesions.

4.1 CALCULATION OF MEAN SCORES

The criteria for classification as R36 and R41 are restated below in tabular form.

Table 1: Criteria for Classification as R36 and R41

Ocular lesion	Mean of scores for ocular lesions at 24, 48 and 72 hours in studies employing > 3 or 3 animals			
	> 3#		3*	
	R36	R41	R36	R41
Corneal opacity	$\geq 2 < 3$	≥ 3	$\geq 2 < 3$	≥ 3
Iris lesion	$\geq 1 \leq 1.5$	> 1.5	$\geq 1 < 2$	$= 2$
Redness of the conjunctivae	≥ 2.5	na	≥ 2.5	na
Oedema of the conjunctivae	≥ 2	na	≥ 2	na

Classification triggered if overall mean score reaches any of these values

* Classification triggered if any individual mean value is attained by two or three

na Conjunctival effects are not applicable to R41

As an example, the scores for chemosis illustrated in the following table demonstrate how to use these criteria.

Table 2: Examples of Classification Based on Chemosis Scores

Animal	Score for chemosis			Means (3 animal study)	Classification based on scores at 24, 48 and 72 hours
	24 h	48 h	72 h		
1	2	2	1	1.67	
2	2	2	2	2.00	
3	3	2	2	2.33	R36
4	3	2	2	2.33	
5	2	2	1	1.67	
6	2	2	2	2.00	R36
Mean (6-animal study)				2.00	R36

It should be noted that all scores at each of the 24, 48 and 72-hour reading times should be used to calculate the respective mean value, irrespective of the number of animals tested. In a test using more than three animals, a single mean value for each ocular lesion is calculated from the individual scores for all animals whereas in the three-animal test a mean value for each ocular lesion is calculated for each *individual animal*.

However, it should be emphasised that although the EC test method B.5 does not specify the maximum number of animals to be used, classification in the EU can be achieved using no more than three animals. Thus the Task Force recommends that no more than three animals are used, except to clarify equivocal responses and/or to assess the influence of rinsing on irritation, or where specific (non-EU) legislation requires data from additional animals.

4.2 COLOURATION

The Task Force agreed that ocular lesions are severe if substances or preparations cause irreversible colouration of the cornea or iris, and in this situation R41 would apply.

Where colouration is confined to the conjunctivae and persists to the end of the observation period, the Task Force concluded that, whilst this is an irreversible effect, it is inappropriate for this alone to trigger R41 as currently required by Directive 93/21/EEC. The R41 risk phrase is used to describe severe ocular damage likely to impair vision; colouration of the conjunctivae is a cosmetic effect.

Although R36 refers only to reversible effects, the Task Force recommends that R36 is used as an interim measure for those situations where the only effect is persistent colouration of the conjunctivae. In the longer term, the TF recommends that a more appropriate mechanism is found to warn of this effect.

4.3 PRACTICAL EXPERIENCE IN HUMANS

It is stated in the Annex to Directive 93/21/EEC (section 3.2.6.2) that the risk phrases R36 or R41 respectively shall be assigned to substances or preparations which cause significant or severe ocular lesions, based on "practical experience in humans".

The Task Force concluded that the criteria should be amended or supplemented by the following additional criteria:

Where data are available that clearly indicate that, for a particular substance or preparation, a more severe ocular response occurs in humans compared to experimental animals, these data should take precedence for classification purposes.

However, there is evidence that the rabbit eye is more sensitive in its response to many irritants than the human eye (Freeberg *et al*, 1986 Cormier *et al*, 1995; Beckley 1965). Thus if good quality data exist, which show that the substance is less irritant in humans than the animal data indicate, this should be taken into account in classification. Further discussion on the use of human data for the classification of irritation and sensitisation can be found in ECETOC Report XXX (in preparation).

It should be noted that there is no definition in Directive 93/21/EEC of significant or severe ocular effects in humans to allow discrimination between a classification as R36 or R41 based on experience in humans. The Task Force recommended that a separate international group of experts, including experimental toxicologists and clinical ophthalmologists, be commissioned to review this area in greater detail.

4.4 PERSISTENCE OF EFFECTS

4.4.1 Observation Period

As discussed in Section 2.3, neither the EC test method nor the classification criteria stipulate a maximum duration for the observation period.

The Task Force considered that, in some situations, extension of the observation period beyond day 21 is necessary to clarify the reversibility or irreversibility of a persistent lesion, in one or more animals, and hence the appropriateness of assignment of R41. Examples are shown below:

- Persistence of a low grade corneal lesion (i.e., score 1) to 21 days after instillation, in the absence of other lesions such as neovascularisation;
- more marked corneal lesions (i.e., score >1) within 72 hours of exposure which do not trigger R41 but show clear evidence of continuing recovery and do not disappear entirely by day 21.

The Task Force believes that a two-week's extension of the typical 21-day observation period is sufficient to determine the reversibility of these effects.

If the option is taken to extend the observation period and full recovery is demonstrated in *all* animals by the end of that observation period, the effect should not be considered irreversible. In such cases R41 should not be assigned and classification should be based on the mean scores over the first 72 hours.

Where this option has not been taken the classification would normally be R41.

4.4.2 Equivocal Effects

Where significant, severe and/or persistent effects are seen in only one of at least three animals, and are considered to be an artefact (e.g. mechanical damage unrelated to the physical properties of the test material) no further testing should be conducted and the test substance should not be classified. However, in the situation where significant, severe and/or persistent effects are seen in at least one of three animals and they are not considered to be an artefact, then R36 or R41, as appropriate, should be assigned. When the reason for the effects is unclear, another three animals should be tested to clarify the irritant potential of the test material.

4.4.3 Neovascularisation and Pannus

The Task Force noted that the terms neovascularisation and pannus are often used synonymously leading to the automatic assignment of R41 when either effect is recorded. However some cases of neovascularisation are reversible using standard observation techniques and should not lead to the assignment of R41. Therefore this response should be carefully monitored for a sufficient period to determine whether the intensity increases or decreases with time. In general, poor reversibility may be expected if neovascularisation clearly increases with time. If this occurs and the study is terminated for reasons of animal welfare, R41 should be assigned to the test substance.

In severe cases, neovascularisation of the cornea can lead to the proliferation of vascular connective tissue. This veil of fibrovascular tissue is referred to as pannus (Peiffer, 1983). Pannus will always trigger R41.

4.4.4 Conjunctival Effects

In accordance with the current classification guideline, severity of conjunctival redness and chemosis within 72 hours of exposure is not relevant to the assignment of R41. Similarly, persistence of conjunctival irritation or colouration through to the end of the observation time does not represent a risk of serious damage to eyes.

4.5 SINGLE ANIMAL STUDIES

If an investigation is not progressed beyond a single-animal study due to severity of effects, as described in Section 2.2, the Task Force recommends that R41 is assigned to the test substance.

4.6 EARLY TERMINATION OF STUDIES

If instillation of a substance or preparation results in treatment-related effects, including severe and enduring signs of pain or distress or severe irritancy, as described in Section 2.2, which require an animal to be euthanised before assessment of irritancy has been completed, R41 should be assigned.

4.7 STUDIES WITH RINSING

The test method states that:

The eyes of the test animals should not be washed out for 24 hours following instillation of the test substance. At 24 hours a washout may be used if considered appropriate.

For some substances shown to be irritating by this test, additional tests using rabbits with eyes washed soon after instillation of the substance may be indicated. In these cases it is recommended that three rabbits be used. Half a minute after instillation the eyes of the rabbits are washed for half a minute using a volume and velocity of flow which will not cause injury.

The Task Force agrees that studies in which the test material is removed by rinsing before 24 hours should result in the assignment of R41.

4.8 LETHALITY FOLLOWING OCULAR ADMINISTRATION OF TEST MATERIALS

It is known that certain materials can be significantly absorbed via mucous membranes. In rare cases this can result in lethality in rabbit eye irritation tests, often within a short time (< 1 day) so that an evaluation of the irritancy effects in the eye is not possible. As these are systemic effects, labelling with R36/41 is not appropriate. The effect should be communicated via the safety data sheet and an appropriate S-phrased. This is strongly recommended when the compound is not toxic via other routes (e.g. oral, dermal or inhalation).

4.9 EXISTING STUDIES TERMINATED PREMATURELY

Prior to the publication of the new classification criteria in Directive 93/21/EEC, many studies were terminated before effects had fully resolved. In these cases the study results should be considered and the classification re-evaluated, if appropriate, taking into account the following points:

- Conjunctival effects present at the end of the observation period should not alter the existing classification of the substance or preparation;
- R41 should be applied when irideal effects or significant corneal effects are present at the end of the study;

- where the only data available are from studies in which rinsing has been conducted before 24 hours, an appropriate study without premature rinsing could be considered, otherwise R41 will apply;

- further data, for example from studies on similar test substances, should be taken into account when deciding whether or not there is justification for classification as R41.

If no clear decision can be made with regard to classification, a repeat study with an extended observation period should be conducted. However, a desire to balance appropriate classification and labelling with an aim of avoiding unnecessary use of animals in eye irritation studies must be exercised when considering results of these types of studies.

5. CONCLUSION

In conclusion, the Task Force considers that the aforementioned points of clarification and recommendations for supplementary guidance, when used in conjunction with the existing criteria in Directive 93/21/EEC, will ensure a more consistent, scientifically-based classification for eye irritancy of substances and preparations in the EU. Furthermore, if these points of clarification are accepted by Member States, they could be used in any future revision or amendment to the "Classification and Labelling" Directive.

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