

The Regulatory Acceptance Board Report on the fluorescein leakage test method for evaluation of ocular irritation potency

JaCVAM Regulatory Acceptance Board

October 1th, 2012

The subject of this report is the fluorescein leakage test method for evaluation of ocular irritation potency as described in OECD Test Guideline 460 (TG 460).^{1, 2}

This test method measures the quantity of sodium fluorescein (Na-FL) that permeates a monolayer of cells as an alternative means of evaluating ocular irritation and ocular corrosion potency.^{3, 4, 5} Having received the Peer Review Panel's report² on a test method for evaluation of ocular irritation potency, we hereby report our evaluation of the following 10 items.

The Item Discussed

1. For which existing test methods is this test method an alternative and what kinds of toxicity will it be used to evaluate or predict?

This test method is an alternative means of detecting severe ocular irritants and ocular corrosives that comprise a portion of the toxicities conventionally evaluated using the Draize rabbit eye test.

OECD TG 460 describes test substances that give positive results under this test method as being either a severe ocular irritant or ocular corrosive, but also implies that negative results under this test method are not sufficient to identify a test substance as not being a severe ocular irritant or ocular corrosive.

This test method is used as part of a Top-Down approach* to detecting severe ocular irritants or ocular corrosives.⁶

2. What kind of scientific connection is there between this test method and existing test methods?

This test method involves growing a monolayer of cells on the inner surface of an insert (see diagram) and exposing the top of the cell layer to the test substance, after which fluorescein is added from the top of the cell layer. Damage to the cells caused by the test substance and any weakening of intercellular junctions are evaluated by measuring the quantity of fluorescein leakage at the bottom of the cell layer. In contrast, results in the Draize rabbit eye test are evaluated on a 110-point scale, comprising a maximum score of 80 points for damage to the cornea, 20 points for damage to the conjunctiva, and 10 points for damage to the iris.

The Draize test therefore evaluates a more diverse range of damage that extends beyond the damage to intercellular junctions and the cells themselves evaluated by this test method.

3. Have this test method and the supporting validation data been subjected to a transparent and independent peer review process?

Data demonstrating the validity of this test method was summarized in a background review document (BRD) prepared by J. Gartlon and R. Clothier, and the validity of that data was the subject of a transparent and independent review by ECVAM.

4. As an alternative to an existing test, what substances or products will this test method be used to evaluate?

This test method will be used to identify as ocular irritants or ocular corrosives any substances or products that are conventionally tested by a Draize eye irritation test, including drugs, quasi drugs, cosmetics, agrochemicals, or medical devices.

5. Does this test method generate data useful for hazard or risk assessment purposes?

This test method is used to identify severe ocular irritants and ocular corrosives and therefore is useful for hazard assessment but not useful for risk assessment.

6. Is this test method capable of assessing toxicity of the subject substances and products? In which case, have the application parameters for this test method been clarified?

This test method is used as an initial step within a Top-Down approach to identifying severe ocular irritants and ocular corrosives.

Positive results obtained with this test method are sufficient to identify the presence of toxicity but negative results are not sufficient to identify the absence of toxicity. For substances that yield negative results with this test method, further testing with other test methods is required to identify the absence of toxicity.

This test method is applicable only to water-soluble substances. Limitations for this test method exclude strong acids and bases, cell fixatives, and highly volatile substances.

7. Is this test method robust against minor changes in protocol?

For the following reasons, we consider this test method to be robust against minor changes in protocol.

- 1) The Madin-Darby Canine Kidney (MDCK) cell line used in this test method is well established and commonly used.
- 2) Fluorescein leakage is a convenient means of confirming that the monolayer of cells.
- 3) There are neither special parameters required by TG 460 nor special precautions specified in the BRD.

8. Is this test method easily transferable among adequately trained and experienced personnel? Does this test method require special equipment?

Other than the growing of a monolayer of cells, there are no special skills or any special equipment needed.

The growing of a monolayer of cells is explained in detail in TG 460 and is easily learned by adequately trained and experienced personnel.

9. Is this test method time and cost effective relative to conventional test methods?

Compared with the Draize eye irritation test, this test method is time and cost effective.⁵

10. Is this test method likely to be useful in a regulatory context as an alternative means of evaluating the toxicity of subject substances and products from the perspective of both science and animal welfare?

This test method enables the identification of severe ocular irritants and ocular corrosives without using animals, which is useful from the perspective of animal welfare.

As an alternative means of evaluating the toxicity of subject substances and products, it is likely to be useful in a regulatory context.

There are limitations on this usefulness, however, insofar as this test method is only useful as part of a Top-Down approach and only applicable to water soluble substances.

Bibliography

- 1) Yoshimura, I., Yamamoto, N.: FL *shikenhou no gaiyou* (Summary of the fluorescein leakage test method), JaCVAM Regulatory Acceptance Board (4 June, 2012)
- 2) Proposal for a New Test Guideline 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants.
- 3) Gartlon, J., Clothier, R.: Fluorescein Leakage Assay Background Review Document as an Alternative Method for Eye Irritation Testing. Available under Validation Study Documents, Section Eye Irritation at [<http://ecvam.jrc.it>]
- 4) Gartlon, J., Clothier, R.: Fluorescein Leakage Assay Background Review Document as an Alternative Method for Eye Irritation Testing: Appendices and Annexes. Available under Validation Study Documents, Section Eye Irritation at [<http://ecvam.jrc.it>]
- 5) Fluorescein Leakage (FL) Test DB-ALM Protocol No. 71. Available under Validation Study Documents, Section Eye Irritation at [<http://ecvam.jrc.it>]
- 6) ECVAM Retrospective Validation Study on Cytotoxicity/Cell-Function Based in vitro Assays for the prediction of Eye Irritation: ESAC Peer Review. Available under Validation Study Documents, Section Eye Irritation at [<http://ecvam.jrc.it>]

Glossary of Terms

Top-Down approach

An approach to testing that comprises the use of multiple successive tests to evaluate a single test substance. Testing is concluded at the point where toxicity is found, but continues to a subsequent test when no toxicity is found, thereby confirming whether the substance is truly non-toxic.