

Guidance on alternative appraisal methods for determining the eye irritation potential of cosmetic raw materials (draft)

1. Introduction

Information on eye irritation potential is one of the required components that must be submitted with the application for the approval of cosmetic products containing new raw materials which have not previously been approved for use in cosmetic products. This is usually obtained by the Draize test, a test that uses rabbits. However, due to concerns over animal welfare, it has been suggested that alternative methods may be used if they are proved to be appropriate as substitutes for the method presently employed.

Several *in vitro* methods have been examined, of which some have correlated well with the results of the Draize test. However, non one test has been able to reliably predict the results of Draize test over the full range of test substances. On the other hand, the results of inter-laboratory validation studies have suggested that some of these alternative methods can identify either non-irritants or strong irritants, or both.

This guidance describes a scheme that uses alternative methods in combination with the Draize test in order to reduce the number of animals required for testing and minimize the suffering of the animals without lowering the reliability of evaluation of the eye irritation potential of cosmetic raw materials. Alternative methods constitute only a part of the evaluation scheme. This is because experience of the utilization of those methods in actual situation seemed insufficient. As further test results are accumulated by testing many cosmetic raw materials by both the Draize test method and alternative methods in accordance with this guidance, revisions to the guidance may be necessary.

The appraisal scheme outlined in this guidance does not necessarily require the same procedure for every substance or at all testing facilities. The emphasis is on selecting the appropriate method according to the purpose of the test, properties of the test substance, experience and equipment available in the testing facilities, etc., while taking into consideration the following points. In some situations, the appraisal may only be conducted by the Draize test.

2. Points to be considered in the appraisal of eye irritation potential of cosmetic raw materials

As cosmetics are used in daily by ordinary people, safety of the products is of great importance. Specifically, cosmetics must demonstrate little or no adverse effects. Most of the cosmetic raw materials are non- or weak irritants, but some of them demonstrate significant irritancy. For the latter group, it is necessary to establish the safe concentration range for their use in cosmetics. Cosmetics are not intended for use in the eye. The risk of injury due to accidental contact with the eye can be minimized by appropriate treatment such as rinsing the eye.

3. Choice of alternative methods for evaluating eye irritation potential

The range of substances that can be examined by alternative methods and

the reliability of the appraisal depend on the mechanism upon which the alternative methods are based and on characteristics such as the sensitivity, reproducibility, correlation of the results with those of the Draize test. Therefore, the method to be employed must be one that has been evaluated objectively to characterize the above mentioned properties by appropriate validation. Proper appraisal of a test substance is possible if the method is appropriately chosen according to the physical/chemical properties and the degree of irritation potential of the test substance, as well as other toxicological information obtained in advance.

A scheme to appraise eye irritation potential should consist of three stages. In the first stage, the decision to conduct the appraisal by alternative methods or by the Draize test is made according to the physical/chemical properties of the test substance. When alternative methods are used, the non- and strong irritants are identified in the second stage. Approximate appraisal of the degree of irritation potential of the irritants is also made for the other substances. In the third stage, the irritation potential is appraised using animals for those substances which cannot be judged as non-irritants. Information regarding the physical/chemical properties of the test substance is also necessary for the appraisal of data obtained at the second stage. Depending on the equipment and experience available in the testing facility, the second stage may be omitted.

Animal tests at the third stage must be conducted in a manner that minimizes the suffering of animals. This may be accomplished by diluting the test substance based on the concentration to be formulated in the products and the results of in vitro tests.

4. Available alternative methods and points to be considered

A test method for which the applicable range, sensitivity, reproducibility and correlation with the Draize test for appropriate test substances that have been determined through validation should be used. As an example of such a method, a cytotoxicity test method may be used to examine the influence of test substances on the viability and proliferation of cultured cells, affording results such as 50% inhibitory concentration, IC₅₀, etc. (Note 1)

5. Scheme of Appraisal

First stage:

Determine whether the test substance can be appraised by alternative methods or by animal test on the basis of the physical/chemical properties such as chemical structure, pH, acidity, alkalinity. If applicable, select appropriate alternative methods. (Notes 2-5)

Second stage:

Along with appraisal of approximate irritation potential, establish whether the test substance can be classified as a non-irritant (Maximum Average Score (MAS) in the eye irritation test is $0 \leq$ and ≤ 5) using only alternative methods.

Third stage:

If the test substance has not been established as a non-irritant, appraise the irritation potential of the test substance using the animal test. At this stage, take measures to minimize the suffering of animals, taking into account the concentration of the substance to be formulated in the cosmetics. If the test substance is expected to be at least moderately-irritant from information obtained in advance and/or the test results of the alternative methods, consider diluting the test substance taking into account factors such as the purpose of the test.

6. References

1) Guidebook on application for the manufacturing of cosmetics and quasi-drugs, Third Ed., Supervised by the Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, Yakuji Nippo Co., Ltd., 1996

Note 1)

Methods using cell lines derived from rabbit cornea (SIRC cells), human uterus carcinoma (HeLa cells), etc. in culture medium supplemented with serum generally provide high sensitivity and good reproducibility. These results show a relatively good correlation with the results of the Draize test. Using an appropriate combination of these methods, identification and classification as an irritant or non-irritant, and an approximate appraisal of the degree of irritating potential are possible. Among other cytotoxicity tests and artificial skin models, there are methods that can be used for identification and classification of irritants and non-irritants.

Note 2)

A test substance classified either as a strong acid or a strong alkali with a pH of below 2.0 or above 11.5, respectively, is generally considered to be a strong irritant when their acidity or alkalinity are high.

Note 3)

A test substance showing strong irritancy or corrosive action on the skin is also generally considered to be a strong irritant.

Note 4)

If a test substance cannot be uniformly mixed with the culture medium in the cytotoxicity tests, the results obtained may not properly reflect its cytotoxicity.

Note 5)

The applicability of cytotoxicity tests has not been confirmed for test substances showing strongly acidic or alkaline characteristics, or for volatile substances such as alcohol.

Note 6)

A threshold that is employed for identification of non-irritants based only on the results of a cytotoxicity test should be set at a value that minimize the risk of false-negative results. This value should be higher than the concentration at which the test substance can be regarded as non-irritant under any experimental conditions (for example, when the IC50, in the cytotoxicity test with the culture medium containing serum is higher than 5000 (g/ml) or higher than the value obtained by multiplying by an ample safety factor, the IC50 value of a standard substance that has been clearly established as a non-irritant.

Note 7)

Among the alternative methods, there are some such as cytotoxicity test, in which the IC 50 value is greatly influenced by the kind of cell line used and the culture conditions. In such cases, it is desirable to appraise the validity of the test results by comparing the results with those of several standard substances, including both negative and positive reference substances.

Note 8)

A 10% polyoxyethylene sorbitan monolaurate (20 E.O.) (Tween 20) solution is used as a reference substance for non-irritancy in the appraisal of cytotoxicity of the test substance, and 10% polyoxyethylene octylphenylether (10 E.O.) (Triton X-100) and 10% sodium lauryl sulfate (SLS) solutions are used as positive reference substances. Appropriate substances are selected according to the characteristics of the test method and tested at the same time as the test substance.

The irritation potential of substances that cannot be judged as non irritant based only on the alternative methods are evaluated as follows by comparison with standard and positive reference substances.

Practically non-irritant : Substances with a higher IC50 than that of Tween 20 (MAS around 0)

Slight irritant : Substances with IC50 lower than that of Tween 20 and higher than that of SLS (MAS around 30)

Moderate irritant : Substances with IC50 lower than that of SLS and higher than that of Triton X-100 (MAS around 50)

Strong irritant : Substances with a lower IC50 than that of Triton X-100

Note 9)

If a test substance is found to be a non-irritant on the basis of alternative methods alone and will not be formulated in the products at a concentration in excess of 10%, it may be appraised as a non-irritant without animal tests. Animal tests are necessary for substances which do not meet the above conditions.