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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

In Vitro Skin Corrosion: Human Skin Model Test

INTRODUCTION

1. Skin corrosion refers to the production of irreversible tissue damage in the skin following the application of a test material [as defined by the Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS)] (1). This Test Guideline does not require the use of live animals or animal tissue for the assessment of skin corrosivity.

2. The assessment of skin corrosivity has typically involved the use of laboratory animals (2). Concern for the pain and suffering involved with this procedure has been addressed in the 2002 revision of Guideline 404 and the supplement to guideline 404 that allows for the determination of skin corrosion by using alternative, *in vitro,* methods, avoiding pain and suffering of animals.

3. The principal obstacle to completely replacing *in vivo* testing for skin corrosion in Guideline 404 has been the lack of formal, independent, validation of *in vitro* tests. A first step towards defining alternative tests that could be used for skin corrosivity testing for regulatory purposes was the conduct of prevalidation studies (3). Following this, a formal validation study of *in vitro* methods for assessing skin corrosion (4)(5) was conducted (6)(7)(8). The outcome of these studies and other published literature (9) led to the recommendation that the following tests could be used for the assessment of *in vivo* skin corrosivity (10)(11)(12)(13): the human skin model test (this Guideline) and the transcutaneous electrical resistance test (see Test Guideline 430).

DEFINITIONS

4. Definitions used are provided in the Annex.

INITIAL CONSIDERATIONS

5. Validation studies have reported that tests employing human skin models (3)(4)(5)(9) are able to reliably discriminate between known skin corrosives and non-corrosives. The test protocol may also provide an indication of the distinction between severe and less severe skin corrosives.

6. The test described in this Guideline allows the identification of corrosive chemical substances and mixtures. It further enables the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using other existing information (e.g., pH, structure-activity relationships, human and/or animal data) (1)(2)(13)(14). It does not normally provide adequate information on skin irritation, nor does it allow the subcategorisation of corrosive substances as permitted in the Globally Harmonised Classification System (GHS) (1).

7. For a full evaluation of local skin effects after single dermal exposure, it is recommended to follow the sequential testing strategy as appended to Test Guideline 404 (2) and provided in the Globally

Harmonised System (1). This testing strategy includes the conduct of *in vitro* tests for skin corrosion (as described in this guideline) and skin irritation before considering testing in live animals.

PRINCIPLE OF THE TEST

8. The test material is applied topically to a three-dimensional human skin model, comprising at least a reconstructed epidermis with a functional stratum corneum. Corrosive materials are identified by their ability to produce a decrease in cell viability [as determined, for example, by using the MTT reduction assay (15)] below defined threshold levels at specified exposure periods. The principle of the human skin model assay is based on the hypothesis that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the underlying cell layers.

PROCEDURE

Human skin models

9. Human skin models can be constructed or obtained commercially (e.g., the EpiDerm™ and EPISKIN™ models) (16)(17)(18)(19) or be developed or constructed in the testing laboratory (20)(21). It is recognised that the use of human skin is subject to national and international ethical considerations and conditions. Any new model should be validated (at least to the extent described in paragraph 11). Human skin models used for this test must comply with the following:

General model conditions:

10. Human keratinocytes should be used to construct the epithelium. Multiple layers of viable epithelial cells should be present under a functional stratum corneum. The skin model may also have a stromal component layer. Stratum corneum should be multi-layered with the necessary lipid profile to produce a functional barrier with robustness to resist rapid penetration of cytotoxic markers. The containment properties of the model should prevent the passage of material around the stratum corneum to the viable tissue. Passage of test chemicals around the stratum corneum will lead to poor modelling of the exposure to skin. The skin model should be free of contamination with bacteria (including mycoplasma) or fungi.

Functional model conditions:

11. The magnitude of viability is usually quantified by using MTT or other metabolically converted vital dyes. In these cases the optical density (OD) of the extracted (solubilized) dye from the negative control tissue should be at least 20 fold greater than the OD of the extraction solvent alone [for an overview, see (22)]. The negative control tissue should be stable in culture (provide similar viability measurements) for the duration of the test exposure period. The stratum corneum should be sufficiently robust to resist the rapid penetration of certain cytotoxic marker chemicals (e.g.,1% Triton X-100). This property can be estimated by the exposure time required to reduce cell viability by 50% (ET_{50}) (e.g. for the EpiDerm™ and EPISKIN™ models this is > 2 hours). The tissue should demonstrate reproductivity over time and preferably between laboratories. Moreover it should be capable of predicting the corrosive potential of the reference chemicals (see Table 2) when used in the testing protocol selected.

Application of the test and control substances

12. Two tissue replicates are used for each treatment (exposure time), including controls. For liquid materials, sufficient test substance must be applied to uniformly cover the skin surface; a minimum of 25μL/cm^2 should be used. For solid materials, sufficient test substance must be applied evenly to cover the skin, and it should be moistened with deionisised or distilled water to ensure good contact with the skin. Where appropriate, solids should be ground to a powder before application. The application method should be appropriate for the test substance (see e.g., reference 5). At the end of the exposure period, the test material must be carefully washed from the skin surface with an appropriate buffer, or 0.9% NaCl .

13. Concurrent positive and negative controls should be used for each study to ensure adequate performance of the experimental model. The suggested positive control substances are glacial acetic acid or 8N KOH. The suggested negative controls are 0.9% NaCl or water.

Cell viability measurements

14. Only quantitative, validated, methods can be used to measure cell viability. Furthermore, the measure of viability must be compatible with use in a three-dimensional tissue construct. Non-specific dye binding must not interfere with the viability measurement. Protein binding dyes and those, which do not undergo metabolic conversion (e.g. neutral red) are therefore not appropriate. The most frequently used assay is MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue; CAS number 298-93-1)] reduction, which has been shown to give accurate and reproducible results (5) but others may be used. The skin sample is placed in an MTT solution of appropriate concentration (e.g. 0.3 – 1 mg/mL) at appropriate incubation temperature for 3 hours. The precipitated blue formazan product is then extracted using a solvent (isopropanol), and the concentration of the formazan is measured by determining the OD at a wavelength between 540 and 595 nm.

15. Chemical action by the test material on the vital dye may mimic that of cellular metabolism leading to a false estimate of viability. This has been shown to happen when such a test material is not completely removed from the skin by rinsing (9). If the test material directly acts on the vital dye, additional controls should be used to detect and correct for test substance interference with the viability measurement (9)(23).

Interpretation of results

16. The OD values obtained for each test sample can be used to calculate a percentage viability relative to the negative control, which is arbitrarily set at 100%. The cut-off percentage cell viability value distinguishing corrosive from non-corrosive test materials (or discriminating between different corrosive classes), or the statistical procedure(s) used to evaluate the results and identify corrosive materials, must be clearly defined and documented, and be shown to be appropriate. In general, these cut-off values are established during test optimisation, tested during a prevalidation phase, and confirmed in a validation study. As an example, the prediction of corrosivity associated with the EpiDerm™ model is (9):

17. The test substance is considered to be corrosive to skin:
i) if the viability after 3 minutes exposure is less than 50%, or

ii) if the viability after 3 minutes exposure is greater than or equal to 50 % and the viability after 1 hour exposure is less than 15%.

18. The test substance is considered to be non-corrosive to skin:

i) if the viability after 3 minutes exposure is greater than or equal to 50% and the viability after 1 hour exposure is greater than or equal to 15%.

DATA AND REPORTING

Data

19. For each tissue, OD values and calculated percentage cell viability data for the test material, positive and negative controls, should be reported in tabular form, including data from replicate repeat experiments as appropriate, mean and individual values.

Test report

20. The test report must include the following information:

Test and Control Substances:
- Chemical Name(s) such as IUPAC or CAS name and CAS number, if known;
- Purity and composition of the substance or preparation (in percentage(s) by weight);
- physico-chemical properties such as physical state, pH, stability, water solubility relevant to the conduct of the study;
- treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding);
- stability, if known.

Justification of the skin model and protocol used.

Test Conditions:
- cell system used;
- calibration information for measuring device used for measuring cell viability (e.g. Spectrophotometer);
- complete supporting information for the specific skin model used including its validity.
- details of test procedure used;
- test doses used;
- description of any modifications of the test procedure;
- reference to historical data of the model;
- description of evaluation criteria used.

Results:
- tabulation of data from individual test samples;
- description of other effects observed.

Discussion of the results.

Conclusion.

LITERATURE

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Table 2: Reference Chemicals

1,2-Diaminopropane	CAS-No. 78-90-0	Severely Corrosive
Acrylic Acid	CAS-No. 79-10-7	Severely Corrosive
2-tert. Butylphenol	CAS-No. 88-18-6	Corrosive
Potassuim hydroxide (10%)	CAS-No. 1310-58-3	Corrosive
Sulfuric acid (10%)	CAS-No. 7664-93-9	Corrosive
Octanoic acid (caprylic acid)	CAS-No. 124-07-02	Corrosive
4-Amino-1,2,4-triazole	CAS-No. 584-13-4	Not corrosive
Eugenol	CAS-No. 97-53-0	Not corrosive
Phenethyl bromide	CAS-No. 103-63-9	Not corrosive
Tetrachloroethylene	CAS-No. 127-18-4	Not corrosive
Isostearic acid	CAS-No. 30399-84-9	Not corrosive
4-(Methylthio)-benzaldehyde	CAS-No. 3446-89-7	Not corrosive

Most of the chemicals listed are taken from the list of chemicals selected for the ECVAM international validation study (4). Their selection is based on the following criteria:

i) equal number of corrosive and non-corrosive substances;

ii) commercially available substances covering most of the relevant chemical classes;

iii) inclusion of severely corrosive as well as less corrosive substances in order to enable discrimination based on corrosive potency;

iv) choice of chemicals that can be handled in a laboratory without posing other serious hazards than corrosivity.

ANNEX

DEFINITIONS

Skin corrosion *in vivo:* is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

Cell viability: parameter measuring total activity of a cell population (e.g., as ability of cellular mitochondrial dehydrogenases to reduce the vital dye MTT), which, depending on the endpoint measured and the test design used, correlates with the total number and/or vitality of the cells.