

## **OECD GUIDELINE FOR TESTING OF CHEMICALS**

**Adopted by the Council on 17<sup>th</sup> July 1992**

### **Skin Sensitisation**

#### **INTRODUCTION**

1. OECD Guidelines for Testing of Chemicals are periodically reviewed in light of scientific progress. In such reviews, special attention is given to possible improvements in relation to animal welfare. This updated version of the original guideline 406, adopted in 1981, is the outcome of a meeting of OECD experts held in Paris in May 1991.
2. Currently, quantitative structure-activity relationships and *in vitro* models are not yet sufficiently developed to play a significant role in the assessment of the skin-sensitisation potential of substances which therefore must continue to be based on *in vivo* models.
3. The guinea pig has been the animal of choice for predictive sensitisation tests for several decades. Two types of tests have been developed: adjuvant tests in which sensitisation is potentiated by the injection of Freund's Complete Adjuvant (FCA), and non-adjuvant tests. In the original guideline 406, four adjuvant tests and three non-adjuvant tests were considered to be acceptable. In this updated version, the Guinea Pig Maximisation Test (GPMT) of Magnusson and Kligman which uses adjuvant (1)(2)(3)(4) and the non-adjuvant Buehler Test (5)(6) are given preference over other methods and the procedures are presented in detail. It is recognised, however, that there may be circumstances where other methods may be used to provide the necessary information on sensitisation potential.
4. The immune system of the mouse has been investigated more extensively than that of the guinea pig. Recently, mouse models for assessing sensitisation potential have been developed that offer the advantages of an endpoint which is measured objectively, short duration and minimal animal treatment. The mouse ear swelling test (MEST) and the local lymph node assay (LLNA) appear to be promising. Both assays have undergone validation in several laboratories (7)(8)(9)(10)(11) and it has been shown that they are able to detect reliably moderate to strong sensitisers. The LLNA or the MEST can be used as a first stage in the assessment of skin sensitisation potential. If a positive result is seen in either assay, a test substance may be designated as a potential sensitiser, and it may not be necessary to conduct a further guinea pig test. However, if a negative result is seen in the LLNA or MEST, a guinea pig test (preferably a GPMT or Buehler Test) must be conducted using the procedure described in this guideline.
5. Definitions used are set out in the Annex.

#### **GENERAL PRINCIPLE OF SENSITISATION TESTS IN GUINEA PIGS**

6. The test animals are initially exposed to the test substance by intradermal injection and/or epidermal application (induction exposure). Following a rest period of 10 to 14 days (induction

period), during which an immune response may develop, the animals are exposed to a challenge dose. The extent and degree of skin reaction to the challenge exposure in the test animals is compared with that demonstrated by control animals which undergo sham treatment during induction and receive the challenge exposure.

#### **ELEMENTS COMMON TO SENSITISATION TESTS IN GUINEA PIGS**

##### **Sex of animals**

7. Male and/or female healthy young adult animals can be used. If females are used they should be nulliparous and non-pregnant.

##### **Housing and feeding conditions**

8. The temperature of the experimental animal room should be 20°C ( $\pm$  3°C) and the relative humidity 30-70 per cent. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. It is essential that guinea pigs receive an adequate amount of ascorbic acid.

##### **Preparation of the animals**

9. Animals are acclimatised to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomised and assigned to the treatment groups. Removal of hair is by clipping, shaving or possibly by chemical depilation, depending on the test method used. Care should be taken to avoid abrading the skin. The animals are weighed before the test commences and at the end of the test.

##### **Reliability check**

10. The sensitivity and reliability of the experimental technique used should be assessed every six months by use of substances which are known to have mild-to-moderate skin sensitisation properties.

11. In a properly conducted test, a response of at least 30% in an adjuvant test and at least 15% in a non-adjuvant test should be expected for mild/moderate sensitisers. Preferred substances are hexyl cinnamic aldehyde (CAS No. 101-86-0), mercaptobenzothiazole (CAS No. 149-30-4) and benzocaine (CAS No. 94-09-7). There may be circumstances where, given adequate justification, other control substances meeting the above criteria may be used.

##### **Removal of the test substance**

12. If removal of the test substance is considered necessary, this should be achieved using water or an appropriate solvent without altering the existing response or the integrity of the epidermis.

#### **DESCRIPTION OF THE GUINEA-PIG MAXIMISATION TEST METHOD**

##### **Number of animals**

13. A minimum of 10 animals is used in the treatment group and at least 5 animals in the control group. When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitiser, testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended.

**Dose levels**

14. The concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest nonirritant dose. The appropriate concentrations can be determined from a pilot study using two or three animals. Consideration should be given to the use of FCA-treated animals for this purpose.

**Induction: Intradermal Injections****Day 0 - treated group**

15. Three pairs of intradermal injections of 0.1 ml volume are given in the shoulder region which is cleared of hair so that one of each pair lies on each side of the midline.

Injection 1: a 1:1 mixture (v/v) FCA/water or physiological saline

Injection 2: the test substance in an appropriate vehicle at the selected concentration

Injection 3: the test substance at the selected concentration formulated in a 1:1 mixture (v/v) FCA/water or physiological saline.

16. In injection 3, water soluble substances are dissolved in the aqueous phase prior to mixing with FCA. Liposoluble or insoluble substances are suspended in FCA prior to combining with the aqueous phase. The concentration of test substance shall be equal to that used in injection 2.

17. Injections 1 and 2 are given close to each other and nearest the head, while 3 is given towards the caudal part of the test area.

**Day 0 - control group**

18. Three pairs of intradermal injections of 0.1 ml volume are given in the same sites as in the treated animals.

Injection 1: a 1:1 mixture (v/v) FCA/water or physiological saline

Injection 2: the undiluted vehicle

Injection 3: a 50% w/v formulation of the vehicle in a 1:1 mixture (v/v) FCA/water or physiological saline.

**Induction: Topical Application****Day 5-7 - treated and control groups**

19. Approximately twenty-four hours before the topical induction application, if the substance is not a skin irritant, the test area, after close-clipping and/or shaving is painted with 0.5 ml of 10% sodium lauryl sulphate in vaseline, in order to create a local irritation.

**Day 6-8 - treated group**

20. The test area is again cleared of hair. A filter paper (2 x 4 cm) is fully-loaded with test substance in a suitable vehicle and applied to the test area and held in contact by an occlusive dressing for 48 hours. The choice of the vehicle should be justified. Solids are finely pulverised and

incorporated in a suitable vehicle. Liquids can be applied undiluted, if appropriate.

**Day 6-8 - control group**

21. The test area is again cleared of hair. The vehicle only is applied in a similar manner to the test area and held in contact by an occlusive dressing for 48 hours.

**Challenge: Topical Application**

**Day 20-22 - treated and control groups**

22. The flanks of treated and control animals are cleared of hair. A patch or chamber loaded with the test substance is applied to one flank of the animals and, when relevant, a patch or chamber loaded with the vehicle only may also be applied to the other flank. The patches are held in contact by an occlusive dressing for 24 hours.

**Observations - treated and control groups**

23. - approximately 21 hours after removing the patch the challenge area is cleaned and closely-clipped and/or shaved or depilated if necessary;
- approximately 3 hours later (approximately 48 hours from the start of the challenge application) the skin reaction is observed and recorded according to the grades shown below;
- approximately 24 hours after this observation a second observation (72 hours) is made and once again recorded.

Blind reading of test and control animals is encouraged.

**TABLE: MAGNUSSON AND KLIGMAN GRADING SCALE FOR THE EVALUATION OF CHALLENGE PATCH TEST REACTIONS**

0 = no visible change

1 = discrete or patchy erythema

2 = moderate and confluent erythema

3 = intense erythema and swelling

**Rechallenge**

24. If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e. a rechallenge), where appropriate with a new control group, should be considered approximately one week after the first one. A rechallenge may also be performed on the original control group.

**Clinical observations**

25. All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, e.g.

histopathological examination, the measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

### **DESCRIPTION OF THE BUEHLER TEST METHOD**

#### **Number of animals**

26. A minimum of 20 animals is used in the treatment group and at least 10 animals in the control group.

#### **Dose levels**

27. The concentration of test substance used for each induction exposure should be the highest to cause mild irritation. The concentration used for the challenge exposure should be the highest non-irritating dose. The appropriate concentration can be determined from a pilot study using two or three animals.

28. For water soluble test materials, it is appropriate to use water or a dilute non-irritating solution of surfactant as the vehicle. For other test materials 80% ethanol/water is preferred for induction and acetone for challenge.

#### **Induction: Topical application**

##### **Day 0 - treated group**

29. One flank is cleared of hair (closely-clipped). The test patch system should be fully loaded with test substance in a suitable vehicle (the choice of the vehicle should be justified; liquid test substances can be applied undiluted, if appropriate). The test patch system is applied to the test area and held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 hours.

30. The test patch system must be occlusive. A cotton pad is appropriate and can be circular or square, but should approximate 4-6 cm<sup>2</sup>. Restraint using an appropriate restrainer is preferred to assure occlusion. If wrapping is used, additional exposures may be required.

##### **Day 0 - control group**

31. One flank is cleared of hair (closely-clipped). The vehicle only is applied in a similar manner to that used for the treated group. The test patch system is held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 hours. If it can be demonstrated that a sham control group is not necessary, a naive control group may be used.

##### **Days 6-8 and 13-15 - treated and control groups**

32. The same application as on day 0 is carried out on the same test area (cleared of hair if necessary) of the same flank on day 6-8, and again on day 13-15.

#### **Challenge**

##### **Day 27-29 - treated and control groups**

33. The untreated flank of treated and control animals is cleared of hair (closely-clipped). An occlusive patch or chamber containing the appropriate amount of test substance is applied, at the maximum non-irritant concentration, to the posterior untreated flank of treated and control animals.

When relevant, an occlusive patch or chamber with vehicle only is also applied to the anterior untreated flank of both treated and control animals. The patches or chambers are held in contact by a suitable dressing for 6 hours.

**Observations - treated and control groups**

34. - approximately 21 hours after removing the patch the challenge area is cleared of hair;
- approximately three hours later (approximately 30 hours after application of the challenge patch) the skin reactions are observed and recorded according to the grades shown in the Guinea-Pig Maximisation Test (see paragraph 23);
- approximately 24 hours after the 30 hour observation (approximately 54 hours after application of the challenge patch) skin reactions are again observed and recorded.

Blind reading of test and control animals is encouraged.

**Rechallenge**

35. If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e. a rechallenge), where appropriate with a new control group, should be considered approximately one week after the first one. The rechallenge may also be performed on the original control group.

**Clinical observations**

36. All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, e.g. histopathological examination, measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

**DATA AND REPORTING (GPMT and Buehler Test)**

**Data**

37. Data should be summarised in tabular form, showing for each animal the skin reactions at each observation.

**Test report**

38. The test report must include the following information:

Test substance:

- physical nature and, where relevant, physicochemical properties;
- identification data.

Vehicle:

- justification of choice of vehicle.

Test animals:

- strain of guinea-pig used;

- number, age and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start and at the conclusion of the test.

Test conditions:

- technique of patch site preparation;
- details of patch materials used and patching technique;
- result of pilot study with conclusion on induction and challenge concentrations to be used in the test;
- details of test substance preparation, application and removal;
- vehicle and test substance concentrations used for induction and challenge exposures and the total amount of substance applied for induction and challenge.

Reliability check:

- a summary of the results of the latest reliability check including information on substance, concentration and vehicle used.

Results:

- on each animal including grading system;
- narrative description of the nature and degree of effects observed;
- any histopathological findings.

Discussion of the results.

If a screening assay is performed before the guinea pig test the description or reference of the test, including details of the procedure, must be given together with results obtained with the test and reference substances.

## **LITERATURE**

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ANNEXDEFINITIONS

Skin sensitisation (allergic contact dermatitis) is an immunologically mediated cutaneous reaction to a substance. In the human, the responses may be characterised by pruritis, erythema, oedema, papules, vesicles, bullae or a combination of these. In other species the reactions may differ and only erythema and oedema may be seen.

Induction exposure: an experimental exposure of a subject to a test substance with the intention of inducing a hypersensitive state.

Induction period: a period of at least one week following an induction exposure during which a hypersensitive state may develop.

Challenge exposure: an experimental exposure of a previously treated subject to a test substance following an induction period, to determine if the subject reacts in a hypersensitive manner.