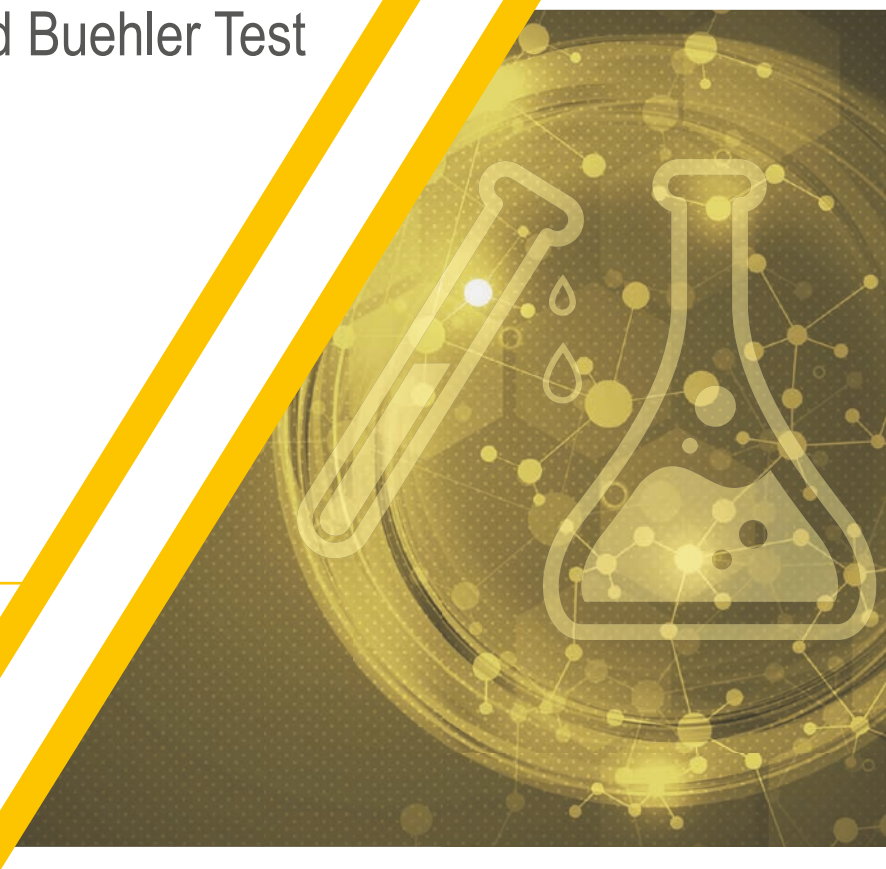
Section 4
Health effects

Test Guideline No. 406
Skin Sensitisation Guinea Pig Maximisation Test and Buehler Test



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OECD Guidelines for the Testing of Chemicals

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OECD GUIDELINE FOR TESTING OF CHEMICALS

Skin Sensitisation Guinea Pig Maximisation Test and Buehler Test

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to reflect the best available science. In the review of this Guideline, special attention was given to possible improvements in relation to animal welfare concerns. This updated version of Guideline 406 (originally adopted in 1981, revised in 1991, and in 2021) takes into account and draws attention to new skin sensitisation Test Guidelines. Although this Test Guideline has been used for sensitisation tests for several decades, users should be aware that other *in chemico, in vitro* and *in vivo* skin sensitisation Test Guidelines are now available and the new TGs, and other information available, should be considered before conducting the guinea pig assay.

2. Both the Buehler and the Guinea pig maximisation tests use animals. For animal welfare reasons, these tests should only be conducted as a last resort, if justified e.g. when other skin sensitisation test methods are not applicable.

3. In the original Guideline 406, four adjuvant tests and three non-adjuvant tests were considered to be acceptable. The present TG describes two types of tests: the Guinea pig maximisation Test (GPMT) of Magnusson and Kligman which uses adjuvant (Freund's Complete Adjuvant (FCA)) to potentiate skin sensitisation (1)(2)(3)(4), and the non-adjuvant Buehler Test (5)(6). Both procedures are presented in detail.

4. The Buehler test has shown to be less sensitive than the Guinea pig maximisation test (7)(8). Both the Buehler and the Guinea pig maximisation tests provide data on skin sensitisation potential and limited information on quantitative potency depending on the dose level selection. If information on quantitative potency is required, other test methods (such as the Local Lymph Node Assay (LLNA) or appropriate non-animal methods) should be used to provide the necessary information on sensitisation potency.

5. Definitions used are set out in the Annex to the Guideline.

GENERAL PRINCIPLE OF SENSITISATION TESTS IN GUINEA PIGS

6. The test animals are initially exposed to the test chemical 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 (+ 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 to maintain a healthy condition.

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, no more than six months previously, by use of substances which are known to have mild-to-moderate skin sensitisation properties. Strategies to minimise the animal use for reliability check should be considered by the testing facility. These may include, for example, use of a reduced group size for the reliability check (n=10), integration of the reliability check study into a standard study in order to avoid using a separate negative control group (if vehicle is the same).

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). The concentrations of the sensitisers selected for the sensitivity and reliability check should be close to the threshold for sensitisation. There may be circumstances where, given adequate justification, other control substances meeting the above criteria may be used (9).

Removal of the test chemical

12. If removal of the test chemical 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 chemical 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 chemical 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 non-irritant 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 chemical in an appropriate vehicle at the selected concentration
- Injection 3: the test chemical 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 chemical 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 test chemical 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 chemical 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 chemical is applied to one flank of the animals and, if more application surface is needed, 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 chemical 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 chemical 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^2. 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 (i.e. group receiving the challenge treatment only, see paragraph 6) 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 chemical 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 chemical:

- Mono-constituent substance
  - Chemical identification, such as IUPAC or CAS name(s), CAS number(s), SMILES or InChI code, structural formula, and/or other identifiers, like batch/lot number and expiry date;
  - Physical appearance, water solubility, DMSO solubility, molecular weight, and additional relevant physicochemical properties, to the extent available;
  - Statement on (in)solubility or stable dispersion in exposure media;
  - Purity, chemical identity of impurities as appropriate and practically feasible, etc;
  - Treatment prior to testing, if applicable (e.g. warming, grinding);
  - Concentration(s) tested;

- Storage conditions and stability to the extent available.

- Multi-constituent substance, UVCB and mixture:

  - Characterisation as far as possible by e.g. chemical identity (see above), purity, quantitative occurrence and relevant physicochemical properties (see above) of the constituents, to the extent available;

  - Physical appearance, water solubility, DMSO solubility and additional relevant physicochemical properties, to the extent available;

  - Molecular weight or apparent molecular weight in case of mixtures/polymers of known compositions or other information relevant for the conduct of the study;

  - Statement on (in)solubility or stable dispersion in exposure media;

  - Treatment prior to testing, if applicable (e.g. warming, grinding);

  - Concentration(s) tested;

  - Storage conditions and stability to the extent available.

Vehicle:

- identification data (purity; concentration, where appropriate; volume used)
- 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;
- Justification for the dose level selection (induction and challenge), including the results of the dose range finding study;
- details of test chemical preparation, application and removal;
- vehicle and test chemical concentrations used for induction and challenge exposures and the total amount of test chemical applied for induction and challenge;
- details of food and water quality (including diet type/source, water source);
- details of treatment and sampling schedules;
- methods for measurement of toxicity;
- criteria for considering studies as positive or negative;
- details of any protocol deviations and an explanation on how the deviation affects the study design and results;

Reliability check:

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

Results:

- on each animal including grading system; time course of onset and signs of toxicity, including dermal irritation at site of administration, if any, for each animal;
- a table of individual guinea pig (individual animal approach) or mean/median (pooled treatment group approach);
- narrative description of the nature and degree of effects observed for each animal;
- any histopathological findings.
- statistical analyses, where appropriate;

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 chemical and chemicals used for the reliability and sensitivity check .

LITERATURE

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ANNEX: DEFINITIONS

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 chemical 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 chemical following an induction period, to determine if the subject reacts in a hypersensitive manner.

FCA: Freund's Complete Adjuvant

GPMT: Guinea Pig Maximisation Test