



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

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

° Principle of the test method

Following initial exposure(s) to a test substance, the animals are subsequently subjected, after a period of not less than one week, to a challenge exposure with the test substance to establish whether a hypersensitive state has been induced. Sensitisation is determined by examining the reaction to the challenge exposure.

B. DESCRIPTION OF THE TEST PROCEDURE

Any of the following seven methods is considered to be acceptable. However, it is realised that the methods differ in their probability and degree of reaction to sensitising substances. Periodic use of a positive control substance with an acceptable level of response in test animals is recommended to assess the reliability of a test system.

- Draize Test
- Freund's Complete Adjuvant Test
- Guinea Pig Maximisation Test
- Split Adjuvant Technique
- Buehler Test
- Open Epicutaneous Test
- Mauer Optimisation Test

An additional method which has not, however, been widely used is described in the Annex.

° Preparations

Healthy young adult 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.

° E x p e r i m e n t a l a n i m a l s

Selection of species

The guinea pig is the generally recommended species. If other species are used this should be justified.

Number and sex

The number and sex of animals used will depend on the method employed. If females are used, they should be nulliparous and non-pregnant. Animals may act as their own controls or groups if induced animals can be compared to groups which have received only a challenge exposure.

Housing and feeding conditions

The temperature of the experimental animal room should be 22°C ( $\pm$  3°) 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.

° T e s t c o n d i t i o n s

Dose levels

Depending on the method, the concentration used should produce skin reaction following the induction exposure and should be non-irritating following the challenge exposure. These concentrations can be determined by a small scale (2 or 3 animals) pilot study. The use of a control group is recommended.

Observation period

Skin reactions should be recorded 24 hours and 48 hours after the pertinent exposures. These exposures will vary depending on the method used.

° P r o c e d u r e

The principle features of the seven methods mentioned above are given in Table 1.

### 3. D A T A   A N D   R E P O R T I N G

#### ° T r e a t m e n t   o f   r e s u l t s

Data may be summarised in tabular form, showing for each individual animal the skin reaction results of the induction exposure(s) at 1 and 24 hours, and the challenge exposure(s) at 24 and 48 hours after exposure. As a minimum, the erythema and oedema should be graded. Any unusual finding should be recorded.

#### ° E v a l u a t i o n   o f   t h e   r e s u l t s

Evaluation of the results will provide information on the proportion of each group that became sensitised and the extent (slight, moderate, severe) of the sensitisation reaction in each individual animal.

#### ° T e s t   r e p o r t

The test report should contain the following information:

- a description of the method used (and commonly accepted name);
- species/strain used;
- the number and sex of the animals;
- individual weights of the animals at the start of the test;
- individual weights of the animals at the conclusion of the test;
- a brief description of the grading system; and
- each reading made on each individual animal.

#### ° I n t e r p r e t a t i o n   o f   t h e r e s u l t s

The test results should provide an estimate of the overall sensitisation potential of the test substance, i.e. essentially a non-sensitiser, a weak sensitiser, a moderate sensitiser, or a potent sensitiser.

TABLE 1: PRINCIPAL FEATURES OF TEST METHODS

TEST	DRAIZE	FREUND'S COMPLETE ADJUVANT (FCA)	MAUER OPTIMISATION	BUEHLER	OPEN EPICUTANEOUS TEST	MAXIMISATION	SPLIT ADJUVANT
Species	guinea pig						
Route	intradermal (id)		epicutaneous(ec)		id and ec		
No. in test group	20	8-10	10-10	10-20	6-8	20-25	10-20
No. of test groups	1	1	1	1	up to 6	1	1
No. in control group	20	8-10	10-10	10-20	6-8	20-25	10-20
Induction exposure route	id	id	id	dermal	dermal	id and dermal	id and dermal
no. of exposures	10	5	9	3	20 or 21	1 id, 1 dermal	4
exposure period	-	-	24 hr	6 hr each	continuous	48 hr	48hr each
patch type	-	-	-	closed	open	closed	closed
test group(s)	TS	TS in FCA	TS in FCA	TS	TS	TS, TS+FCA, FCA	TS
control group	FCA only	-	-	-	vehicle (v) only	FCA, FCA+V, V	-
site	L flank	R flank	back	L flank	R flank	shoulder	shoulder
frequency	every 2nd day	0-8d	every other day	every 7 days	daily	0(id), 7d (dermal)	0, 2, 4, 7d
duration	2-10 times	same	0-21d	0-14d	0-20d	0-7d	same
concentration	that of first	throughout	0.1 ml of 0.1%	same	same per group	-	throughout
Challenge exposure route	id	dermal	id	dermal	dermal	dermal	dermal
no. of exposure	1	2	2	1	2	1	1
day(s)	35	22 & 35	14 & 28	28	21 & 35	21	20
exposure period	-	-	24 hr	6 hr	-	24 hr	24 hr
patch type	-	open	closed	closed	open	closed	closed
test group(s)	TS	TS	TS	TS	TS	TS	TS
control group	TS	TS	TS	TS	TS	TS	TS
site	R flank	L flank	back, new site	R flank	L flank	L flank TS	shoulder
concentration	same as first	4 different	0.1 ml of 0.1%	same as induction	4 different	same as induction	half induction
evaluation (hr after challenge)	24, 48	24, 48, 72	24 hr	24, 48	24, 48 and/or 72	24, 48	24, 48
Reference	(2)	(3)	(7)	(4)	(3)	(5)	(6)

A skin sensitisation study thus provides an assessment of whether or not a test substance could be a likely sensitiser. Extrapolation of these results to man is valid only to a very limited degree. The only generalisation that can be made is that substances which are strong sensitisers in guinea pigs also cause a substantial number of sensitisation reactions in man, whereas weak sensitisers in guinea pigs may or may not cause reactions in man.

#### 4. L I T E R A T U R E

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(2) Draize, J.H., Food Drug Cosmets. Law J., 10, 722, 1955.

(3) Klecak, G. et al, J. Soc. Cosm. Chem., 28, 53, 1977.

(4) Buehler, E.V., Toxicol. Appl. Pharmacol. 6, 341, 1964, also Arch. Dermatol. 91, 171, 1965.

(5) Magnusson B. and Kligman A.M., J. Invest. Dermatol., 52, 268, 1969; also Allergic Contact Dermatitis in the Guinea Pig, publ. Thomas, Springfield, Illinois, 1970.

(6) Maguire, A.C., Immunol. Commun., 1973, 1, 239, J. Soc. Cosm. Chem. 24, 151, 1973; also Animal Models in Dermatology, (ed. Maibach), publ. Churchill Livingstone, Edinburgh, 1975.

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5. ANNEX

SKIN SENSITISATION TEST

1. I N T R O D U C T O R Y I N F O R M A T I O N

° P r e r e q u i s i t e s

- Solid or liquid test substance
- Chemical identification of test substance
- Purity (impurities) of test substance
- Solubility characteristics
- pH (where appropriate)
- Melting point/boiling point

° S t a n d a r d d o c u m e n t s

There are no relevant international standards.

2. M E T H O D

Skin sensitisation (allergic contact dermatitis) is a condition occurring in man and some other animals in which skin reactions are exacerbated after two or more exposures to sensitising materials.

FOOTPAD TECHNIQUE FOR EVALUATING SENSITISATION POTENTIAL IN GUINEA PIGS

A 1.0 per cent mixture (w/v) of the test material is prepared in Freund's Complete Adjuvant (FCA) and stirred gently at room temperature for three hours.

After being allowed to settle for a few minutes, 0.05 ml of the mixture is injected into the front footpad of ten white guinea pigs which have not previously been exposed to test materials by any route.

One week later the test material is dissolved at a concentration of 1.0 per cent in a solvent system of guinea pig fat\*:dioxane:acetone, 1:2:7. Each previously injected animal is challenged by dropping 0.3 ml on the clipped (not depilated) skin of the lower back. (If a 1.0 per cent solution produces moderate irritation, an 0.1 per cent solution is used.)

An equal number of control guinea pigs which have not been previously exposed to test material, but were injected with FCA, are challenged in an identical manner.

Twenty-four hours after the "drop on" exposure, the stubble of the exposed area is removed with a suitable depilatory\*\* followed by a wash with warm (approximately 37°C) tap water.

Approximately three hours after depilation, the challenged skin site is scored under uniform fluorescent lighting for irritation (erythema and oedema) compared to untreated skin areas.

The grading of redness is as follows: 0 = normal; 1 = slight, just detectable; 2 = moderate, easily seen, 3 = definite deep red, usually hot, not haemorrhagic; 4 = dark red, may show haemorrhagic areas, usually accompanied by marked swelling and increase in temperature of the skin.

The degree of swelling of the skin is determined by picking up a fold of skin about 1 cm in length, feeling it between thumb and forefinger, and grading as follows: 0 = normal; 1 = slight, just detectable; 2 = moderate, easily felt; 3 = marked, difficult to pick up a fold of skin, often visible without feeling the skin

The total irritation score for each animal equals the sum of redness and swelling and ranges from zero for no irritation to seven for severe irritation.

The exposed skin sites are again graded 24 hours later (48 hours after the "drop on").

The difference between the control guinea pigs (primary irritation) and the injected guinea pigs (sensitisation response) is considered to be a measure of the degree of skin sensitisation.

#### \* Method of Preparing Guinea Pig Fat

Any method that produces clean guinea pig fat should be suitable. The following method has consistently provided an adequate product.

1. Strip fat from large, preferably obese, guinea pigs.
2. Freeze.
3. Grind frozen fat with a kitchen-style meat grinder.



4. Add acetone to ground fat (approximately 10:1, acetone: fat). Stir well and heat by placing flask in a hot water bath.
5. Filter (Whatman No. 4 paper or similar, through a Buchner funnel).
6. Add and stir with Norite - warmed.
7. Filter through Whatman No. 4 and Super Cel, pour into beaker.
8. Freeze, fat will solidify on bottom of beaker.
9. Decant acetone and discard.
10. Gently warm fat and vacuum distill off acetone.
11. Pour warmed fat into vials of a size which allow use of one vial per group of sensitisations.
12. Freeze all vials and use as needed. Frozen fat will remain acceptable for at least one year.

**\*\* Depilatory**

Any depilatory can, if misused, cause serious skin burns and death and therefore should be handled and used correctly. The following depilatory is preferred because of easy handling and excellent results: 6 parts soluble starch, 6 parts talc, 6 parts barium sulphide, 2.7 parts of a granular non-irritant anionic surfactant.

Add the parts in the order listed and mix well. Add cold water to make a viscous paste. Apply to the clipped skin of guinea pigs and allow to remain for about four minutes. Rinse off all traces of depilatory.

