Section A6.1.5 Skin Sensitisation

Annex Point IIA6.1 6.1.5 Skin sensitisation test in Guinea Pigs

(Maximisation test method of Magnusson and Kligman)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1980, PREVENTOL A 4- Study for sensitising effect (Magnusson and Kligman's maximisation test), [redacted], Report No. [redacted], 1980-02-13 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer Chemicals AG
1.2.2	Companies with letter of access	—
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No No guidelines were available at the time the study was performed, but the methods used in this study are comparable to OECD-Guideline 406 (Guinea pig maximisation test method).
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes In addition to the maximum-non-irritant-concentration used for the challenge exposure, a second concentration with a lower content of the active substance was applied. The individual weights of animals at the start and at the end of the study were not documented and the reliability check was not reported. Description, purity, and stability of the test substance were not documented.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	—
3.1.2.2	Purity	—
3.1.2.3	Stability	—
3.1.2.4	Preparation of test substance for application	a) Induction: 10% dichlofluanid, solvent: water b) Challenge: 12.5% dichlofluanid, solvent: water 25% dichlofluanid, solvent: water
3.1.2.5	Pre-test performed on irritant effects	Yes (pilot study, see 4.1 this section)

Section A6.1.5 Skin Sensitisation

Annex Point IIA6.1 6.1.5 Skin sensitisation test in Guinea Pigs
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3.2	**Test Animals**	
3.2.1	Species	Guinea pigs
3.2.2	Strain	Pirbright White
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age: adult Weight: 575 g
3.2.6	Number of animals per group	15
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Adjuvant
3.3.1	Induction schedule	Day 0 – day 7 (see table in appendix)
3.3.2	Way of Induction	Intradermal and topical, occlusive
3.3.3	Concentrations used for induction	1. Intradermal injection 0.1 g test substance/ml 2. Topical application 0.05 g test substance/ml
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	50% in water
3.3.5	Challenge schedule	Day 21
3.3.6	Concentrations used for challenge	0.25 g test substance / ml (maximum non-irritant concentration) or 0.125 g test substance / ml
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24 h, 48 h after challenge
3.3.9	Removal of the test substance	—
3.3.10	Positive control substance	—
3.4	**Examinations**	
3.4.1	Pilot study	Yes
3.5	**Further remarks**	—

Section A6.1.5 Skin Sensitisation

Annex Point IIA6.1 6.1.5 Skin sensitisation test in Guinea Pigs

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4 RESULTS AND DISCUSSION

4.1 Results of pilot studies

The test substance was applied in concentrations of 100%, 50%, 25%, and 12.5% in media described in section 3.3.4 (see above) to various sites of the flanks of four untreated guinea pigs. 24 h after topical application under occlusive dressing, the 12.5% and the 25% concentrations were not skin irritants, the 50% and 100% concentrations averaged slight to moderate skin irritation. The maximum non-irritant-concentration was the 25% dichlofluanid-solution.

4.2 Results of test

4.2.1 24h after challenge

<u>25% Dichlofluanid-solution (maximum non-irritant-concentration):</u>

Test animals:	Control animals:
13/15	0/15

(number of animals with signs of allergic reactions / number of animals)

<u>12.5% Dichlofluanid-solution:</u>

Test animals:	Control animals:
11/15	1/15

(number of animals with signs of allergic reactions / number of animals)

4.2.2 48h after challenge

<u>25% Dichlofluanid-solution (maximum non-irritant-concentration):</u>

Test animals:	Control animals:
13/15	1/15

(number of animals with signs of allergic reactions / number of animals)

<u>12.5% Dichlofluanid-solution:</u>

Test animals:	Control animals:
13/15	0/15

(number of animals with signs of allergic reactions / number of animals)

4.2.3 Other findings

The histopathological examination of the treated skin areas showed slight to moderate intracellular oedema in cells of the stratum granulosum and stratum corneum. In addition. the epidermis of these animals was on average slightly thickened and the corium areas close to the papillae exhibited more severe round cell infiltration than in the case of the controls.

4.3 Overall result

Dichlofluanid proved to be skin sensitising in the guinea pig.

Section A6.1.5 Skin Sensitisation

Annex Point IIA6.1 6.1.5 Skin sensitisation test in Guinea Pigs

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The methods used to perform the study complied with the OECD-Guideline 406 with slight deviations as described in 2.3 (see above). A study for skin sensitisation in guinea pigs was conducted with the test substance Dichlofluanid. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the test substance.
5.2	**Results and discussion**	The observations 24 h and 48 h after challenge exposition revealed to positive reactions in the majority of animals (slight to moderate redness) at both times and with both concentrations.
5.3	**Conclusion**	Dichlofluanid therefore proved to be sensitising under the test conditions described in the Guinea Pig Maximisation Test of Magnusson and Kligman.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/09/04
Materials and Methods	As described above. [IUCLID 5.3]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicants summary and conclusions.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6_1_5-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Inductions	Concentration of test substance in solution	Day of treatment	Application	Post-challenge observations*	
				24 h	48 h
Induction 1	10%	0	intradermal	—	—
Induction 2	5%	7	topical	—	—
Challenge	12.5%	21	topical	0/4/15 1/9/15 2/2/15	0/2/15 1/7/15 2/6/15
	25%	21	topical	0/2/15 1/6/15 2/7/15	0/2/15 1/4/15 2/9/15

*first number = grade of reaction

(0 = no reaction, 1 = in places slight redness, 2 = moderate diffuse redness, 3 = intensive redness and swelling)

second number = number of animals with signs of allergic reactions

third number = number of animals in group

Table A6_1_5-2. Results of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group	
	Control	**Test group**
		12.5% Dichlofluanid-solution
scored after 24h	1 / 15	11 / 15
scored after 48h	0 / 15	13 / 15
		25% Dichlofluanid-solution
scored after 24h	0 / 15	13 / 15
scored after 48h	1 / 15	13 / 15