Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 Buehler Test

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1986. Dermal sensitization study in guinea pigs with SY-83. American Biogenics Corporation, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Purac Biochem BV	
1.2.2	Companies with letter of access	No	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: EPA, 1982 (modification of the Buehler closed patch technique)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, during the induction phase (after the second induction application) the concentration of the test substance was reduced from 100% to 30% and a switch was made from the right flank to the left flank. This was done, because of the irritation observed at the 100% application on the right flank.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	SY-83	
3.1.1	Lot/Batch number	Not presented	
3.1.2	Specification	Formulated from Purac HS pharmaceutical grade (USP XX) L(+) lactic acid (88%) by dilution to a concentration of 80% in water.	
3.1.2.1	**Description**	Liquid	
3.1.2.2	**Purity**	SY-83 is formulated from Purac HS pharmaceutical grade by dilution to a concentration of 80% with water: 83.5-76.5% lactic acid in water	
3.1.2.3	**Stability**	As given in section 2	
3.1.2.4	**Preparation of test substance for application**	a) <u>For induction:</u> used as delivered (100% test substance), and also 3, 10 and 30% suspensions in deionized water. b) <u>For challenge:</u> used as delivered.	X
3.1.2.5	**Pretest performed on irritant effects**	Yes (range-finding test on 2 animals)	
3.2	**Test Animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	Hartley	
3.2.3	Source	Charles River Breeding laboratories Inc., Portage, MI facility, USA	
3.2.4	Sex	Female	

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3.2.5	Age/weight at study initiation	Young adult / 272 – 362 gram	
3.2.6	Number of animals per group	10	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	State study type: *Buehler Test*	
3.3.1	Induction schedule	3 times each week (Monday, Wednesday and Friday) until all 9 induction applications had been applied	
3.3.2	Way of Induction	Topical Occlusive	
3.3.3	Concentrations used for induction	100 % test substance, but after 2 induction applications the concentration was reduced to 30% and the test site was changed to the left flank (due to irritation effects seen at 100 % at the right flank)	
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	*state concentration and vehicle (for GPMT only):* 10 % in water or physiological saline	X
3.3.5	Challenge schedule	Two weeks after the ninth induction; see table in appendix	X
3.3.6	Concentrations used for challenge	100 % test substance (usually maximum non-irritant concentration)	
3.3.7	Rechallenge	No	
3.3.8	Scoring schedule	24h, 48h after challenge	
3.3.9	Removal of the test substance	After 6 hours the binders and patches were removed / no information on rinsing	
3.3.10	Positive control substance	Dinitrochlorobenzene	
3.4	**Examinations**		
3.4.1	Pilot study	yes	
3.5	**Further remarks**	-	
		RESULTS AND DISCUSSION	
3.6	**Results of pilot studies**	0.5 mL of test article was applied at 3, 10, 30 and 100% concentration. The 100% test article concentration was selected for induction and challenge since dermal reactions were minimally irritation at this range-finding test site.	
3.7	**Results of test**		
3.7.1	24h after challenge	0/10	
3.7.2	48h after challenge	0/10	

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3.7.3 Other findings	Severe (grade 4 erythema and eschar formation) effects on the skin were observed both 24 and 48 hours after challenge (and also after induction); these reactions were considered irritation reactions, no sensitization reactions, as similar skin effects were observed in the control animals.	
3.8 Overall result	SY-83 was not considered to be a skin sensitizer	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	The test is applied conform EPA, 1982 (modification of the Buehler Closed Patch technique).	
4.2 Results and discussion	Severe (grade 4 erythema and eschar formation) effects on the skin were observed both 24 and 48 hours after challenge (and also after induction); these reactions were considered irritation reactions, no sensitization reactions, as similar skin effects were observed in the control animals.	X
	SY-83 was not considered to be a skin sensitizer	X
4.3 Conclusion		X
4.3.1 Reliability	1	X
4.3.2 Deficiencies	No	X

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/07/16
Materials and Methods	The applicant's version is acceptable with the following changes: 2.1 Similar to OECD 406 2.3 80 % lactic acid (= 100 % SY-83, see remarks) was used for induction and challenge. This concentration proved to be highly irritating (grade 4) in naive as well as induction group animals. Only 10 animals were used in the treatment group instead of 20. 3.1.2.4 3, 10 and 30% suspensions were used only in the range-finding study (30 % in the main study for the last 7 out of 9 inductions). 3.3.4 No adjuvant used (Buehler test) 3.3.5 No corresponding table was included in the appendix.
Results and discussion	The applicant's version is acceptable with the following changes: 4.2 Severe effects (grade 4 erythema (pinpoint pitting, very little redness) and eschar formation) on the skin were observed both 24 and 48 hours after challenge (and also after induction). These reactions were considered irritation reactions, no sensitization reactions, as similar skin effects were observed in the control animals. SY-83 was not considered to be a skin sensitizer
Conclusion	4.3 L-(+)-lactic acid is not sensitising.
Reliability	2
Acceptability	Acceptable with restrictions (see remarks)

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Remarks	The concentrations of all dilutions (10 %, 30 %) in this study relate to 100 % SY-83 which yields 80 % L(+) lactic acid. The L-(+)-lactic acid concentration used for induction and challenge was 80 % and caused severe skin irritation. As stated in OECD guideline 406, "the concentration of test substance used for each induction should be the highest to cause mild irritation. The concentration used for the challenge should be the highest non-irritating dose." Since the quality of the observed skin effects (pitting of the skin, only little redness) differ from those caused by a skin sensitising substance the results of the study can be interpreted as skin irritation. Furthermore, L-(+)-lactic acid is a metabolic intermediate (~130 g/d in humans at rest). A sensitisation potential for endogenous substances which are formed in considerable amounts in the human (or animal) body is highly unlikely. Thus, a sensitisation study is considered not necessary.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	