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TESTING
CNAS L2954

Testing Center of Radiological Medical Research Institute, Soochow University Test Report

Report Number: SDFY-2007-2509

Sample Name: Soft silicon housing

Testing Item: Biocompatibility Test

Sample Supplier: Justec Shenzhen Co., Limited

Supplementary Explanation

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2. Testing report is void without analysis center seal.
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Cytotoxicity Test

Summary

The test article, Soft silicon housing was evaluated for cytotoxicity test in accordance with the ISO 10993-5:1999: Tests for in vitro cytotoxicity. The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 2 and 4 days. Observe the morphology and the cell growing on the culture bottle wall. The RGR (Relative Growth Rate) are 102 % and 107 % respectively by calculation on the basis of cell concentrations of different groups. The RGR of testing group is determined as above Grade 0. This means the testing sample has no toxicity to L-929 cells.
Date completed: Oct 13, 2007

Tested by: Liu Fenju Checked by: Zhang Tonghang

Introduction

The test article, Soft silicon housing were evaluated for cytotoxicity test in accordance with the ISO 10993-5:1999: Tests for in vitro cytotoxicity .The purpose of this study was to determine the potential cytotoxicity of the testing article to L-929 cell.

Materials

1. Test Sample:

1.1Sample Name: Soft silicon housing
1.2 Lot No: 2007-9-4
1.3 Material No: S1001
1.4 Materials:

Chemical material	Content
Methyl vinyl silicone rubber	81.25%
SiCO2	15.10%
Hydroxy terminated Dimethyl Siloxane (Low Molecuiar Weight)	1.50%
Stearate	0.15%
Pigment Grey 1002	1%
Vulcanizing Agent	1%

1.5 Receiving Date: Sep 30, 2007
1.6 Equipment: Autoclaves、Co_2 Incubator、Inverted microscope、Super clean working desk

2. Sample and Control Preparation:

2.1 Testing sample group

The testing sample is treated in accordance with experiment requirements followed by sterilization in autoclave.

24 hours prior to experiment, add liquid culture medium in the proportion of 1ml culture medium to 0. 2g testing sample, and then incubated at 37±1℃ for 24 ±2hours. Thus, the sample solution is obtained.

2.2 For the positive controls, add con. 0.5% phenylhydroxide solution.
2.3 For the negative controls, add fresh 1640 liquid culture medium.

3. Culture medium:

RPMI 1640, calf serum, PBS, Pancreatin and double antibiotic.

Test method

1. Cell Strain: Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).
2. Cell Culture

2.1 Take growing-well cell of strain L-929 to prepare cell soliquiod with concentration of 4×10^4 counts/ml in culture bottles. Proceed to next step after the cell grows up to monolayer.

2.2 Discard the liquid in culture bottles. In these culture bottles. Take testing group, add con. 50% sample solution, the positive control solution and the negative control solution. They are all incubated at 37℃. After 2 and 4 days incubation respectively, conduct the morphology

evaluation and cell counting.

Results

The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 2 and 4 days. Observe the morphology and the cell grow well sticking on the culture bottle wall. The RGR (Relative growth Rate) are 102% and 107% respectively by calculation on the basis of cell concentrations of different groups (the negative and positive controls).

Conclusion

By the experiment incubating L-929 cell using culture medium with sample solution, the RGR of testing group is determined as above Grade 0. This means the testing sample has no toxicity to L-929 cell.

Table 1. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	severe	Nearly complete destruction of the cell layers

According to USP , test articles scoring "0", "1",or "2" will be considered NON-TOXIC. Test articles scoring "3" or "4" will be considered TOXIC.

The positive control sample must have a score of "3" or "4" and the negative control sample must have a score of "0" for a valid test.

Delayed Contact Sensitization Study (A Maximization Method) In the Guinea Pig

Summary

A guinea pig maximization test (ISO10993-10: 2002) of sample Soft silicon housing was conducted to evaluate the potential for delayed dermal contact sensitization .The method of Magusson and Kligman (1970) was adapted for alcohol in a 0.9% sodium chloride USP solution (AS) test article extract.

The AS extract of the test article was intradermally injected and occusively patched to ten guinea pigs in an attempt to induce sensitization. Following a recovery period, the original ten test and five previously untreated control animals received a challenge patch of the test article extract and the control vehicle .In addition the test article was applied the same animals. All sites were scored at 24 and 48 hours after patch removal.

Under the conditions of this study, the AS test article extract and the test article showed no signification evidence of causing delayed dermal contact sensitization in the guinea pig.

Date completed: Nov 13, 2007

Tested by: Wang Hongyun Checked by: LiuYanbin

Introduction

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. The test article was received on Sep 30, 2007. The method of Magnusson and Kligman, as reported in Allergic Contact Dermatitis in the Guinea Pig, 1970, was employed with adaptations for a test article extract. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at TCRSU with this method under lab number SDFY-2007-2441 completed on Sep 29, 2007.

Materials

The sample provide by the sponsor was identified and handled as follows:

Test Article: Soft silicon housing
Lot No: 2007-9-4
Material No: S1001
Materials:

Chemical material	Content
Methyl vinyl silicone rubber	81.25%
SiCO2	15.10%
Hydroxy terminated Dimethyl Siloxane (Low Molecuiar Weight)	1.50%
Stearate	0.15%
Pigment Grey 1002	1%
Vulcanizing Agent	1%

Equipment: Incubator
Storage Conditions: Room temperature
Vehicle: alcohol in saline 1:20 solution (AS)

Preparation: For each phase of this test, a ratio of 0.2g: 1ml(test article to volume of vehicle)was used for the test extract . The test article was extracted in AS at 37 ±1℃ for 24 ±2hours. For the challenge phase ,the vehicle (without test article)was similarly prepared to serve as the control .In addition ,the test article (as received)was cut into 2×2cm sections at the challenge phase .

Condition of Extracts:	TEST	CONTROL
	Induction I: clear with test article particulates*	Not applicable
	Induction II: clear with test article particulates	Not applicable
	Challenge: clear with test article particulates	Clear

*Filtered with a 0.8um filter disc to yield a clear particulate free extract

Additional Materials: Freund's Complete Adjuvant (FCA) was used at induction I, and a 10%(w/w)sodium lauryl sulfate (SLS)suspension in petrolatum was used for induction II. These materials were provided by the test facility .

Method

Test System:

Species: Albino Guinea pig
Source: Provided by Animal Center, TCRSU 〈Permit Code: SCXK(SU)2002-0008〉
Acclimation Period: Minimum 5days
Number of Animals: 15

Justification of Test System:

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman ,1970) . The guinea pig is believed to be the most sensitive animal model for this type of study .the susceptibility of the guinea pig to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at TCRSU with this method .

Animal Management:

Husbandry : Refer to ISO 10993-10-2002 Annex C: Animal and husbandry.
Food: All-nutrient animal food Provided by Suzhou (Twin-lion) Experimental Animal Food Science & Technology Service Co .Ltd.

Water: Drinking water met the sanitary standard

Housing: Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number , animal numbers, test code , sex, animal code and first treatment date .

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused animals were selected .

Intradermal induction phase Ⅰ :

Make a pair of 0.1ml intradermal injections of each of the following, into each animal, at the injection sites(A,B and C) as shown in Figure 1 in the clipped intrascapular region.

Site A: A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent. Use physiological saline (equivalent) for water-soluble alone.

Site B: The test sample (undiluted extract); inject the control animals with the solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%); inject the control animals with an emulsion of the blank liquid with adjuvant.

Topical induction phase Ⅱ :

Seven days(±1day) after completion of the intradermal induction phase, administer the test sample by topical application to the intrascapular region of each animal, using a patch of area approximately 8cm^2 (filter paper or absorbent gauze),so as to cover the intradermal injection sites. Use the concentration selected in Intradermal induction phase Ⅰ for Site B. If the maximum concentration that can be achieved in Intradermal induction phase Ⅰ does not produce irritation, pretreat the area with 10% sodium dodecyl sulfate massaged into the skin 24h±2h before the patch is applied. Secure the patches with an occlusive dressing. Remove the dressings and patches after 48h±2h.

Treat the control animals similarly, using the blank liquid alone.

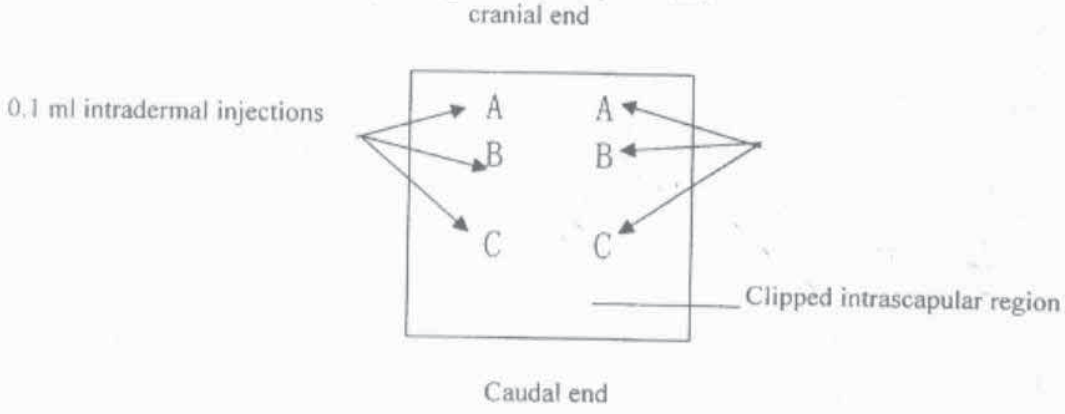

Figure 1-Location of intradermal injection sites

Challenge phase :

At 14days(±1day) after completion of the topical induction phase, challenge all test and control animals with the test sample. Administer the test sample and a vehicle control by topical application to sites that were not treated during the induction stage, such as the upper flank of each animal, using appropriate patches or chambers soaked in the test sample at the concentration selected in Intradermal induction phase Ⅰ for site C. Dilutions of this concentration may also be applied to other untreated sites in a similar manner. Secure with an occlusive dressing. Remove the dressings and patches after 24h±2h.

Observation of animal:

Observe the appearance of the challenge skin sites of the test and control animal 24h and 48h after removal of the dressings. Use of natural or full-spectrum lighting is highly recommended to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in Table 1 for each challenge site and at each time interval .It is highly recommended that reading be done without knowledge of the treatment ,in order to minimize bias in the evaluation of the results.

Evaluation of results:

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization ,provided grades of less than 1are seen in control animal .If grades of 1 or greater are noted in control animal, then the reactions of test animal which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge. The outcome of test is presented as the frequency of positive challenge results in test and control animal.

Table 1– Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Result

Individual results of dermal scoring for the challenge appear in Table 1. No evidence of sensitization was observed.

Clinical Observations: All animals appeared clinically normal throughout the study .

Conclusion

Under the conditions of this study , the as test article extract and the test article showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

Table 2 GUINEA PIG SENSITIZATION DERMAL REACTIONS – CHALLENGE

Animal Number/ Group	Hours following patch removal（h）		weight（g）	
	24	48	Before injection	After experiment
1 Test	0	0	336	386
2 Test	0	0	342	389
3 Test	0	0	346	390
4 Test	0	0	350	394
5 Test	0	0	341	388
6 Test	0	0	352	396
7 Test	0	0	345	391
8 Test	0	0	328	378
9 Test	0	0	337	387
10Test	0	0	343	389
11control	0	0	335	385
12control	0	0	346	392
13control	0	0	353	398
14control	0	0	348	396
15control	0	0	342	393

Skin Irritation Test

Summary

The test article, Soft silicon housing was evaluated for primary skin irritation in accordance with the ISO 10993- 10:2002: Tests for irritation and delayed-type hypersensitivity. Observe and write down the skin responses on injection sites in 24, 48 and 72 hours respectively after injection. The skin responses include erythema and oedema. Grade the tissue reaction for erythema and oedema according to the classification system given in Table 1. According to what observed, the response of skin on testing side does not exceed that on the control side. The primary irritation index for the test article was calculated to be 0. The test result shows that leached solution of sample does not induce irritation to rabbit skin.

Date completed: Oct 19, 2007

Tested by: Wang Hongyun Nov 14, 2007

Checked by: Liu Yanbin Nov 14, 2007

Signed by: Zhang Tongchao Nov 14, 2007

Testing Center of Radiological Medical Research Institute, Soochow University

Introduction

The test article, Soft silicon housing, was evaluated for primary skin irritation in accordance with the guidelines of the ISO 10993-10:2002: Tests for irritation and delayed-type hypersensitivity. This study was to determine the potential skin irritation after the injection of sample solution into the animal back. The test article was received on Sep 30, 2007. Injections were applied on Oct 15, 2007 and the observations were concluded on Oct 19, 2007.

Materials

Test Article: Soft silicon housing
Lot No: 2007-9-4
Material No: S1001
Materials:

Chemical material	Content
Methyl vinyl silicone rubber	81.25%
SiCO2	15.10%
Hydroxy terminated Dimethyl Siloxane (Low Molecuiar Weight)	1.50%
Stearate	0.15%
Pigment Grey 1002	1%
Vulcanizing Agent	1%

Equipment: Incubator
Storage Conditions: Room temperature
Sample and Control Preparation

The samples are rinsed with redistilled water and blotted up with filter paper. In a container with cut sample, add the leaching solution (0.9% NaCl injection solution) in the proportion of 1ml leaching solution to 0. 2g sample. Seal up the container at 37±1℃ for 24 ±2hours. The solution so prepared is termed as leached solution of sample. The control solution (0.9% NaCl) is obtained by same way but absent of sample.

Method

Test System:

Species: Three rabbits.

Breed: New Zealand white (single strain)

Source: Provided by Animal Center, TCRSU<Permit Code: SCXK (SU)2002-0008>

Body Weight Range: Not less than 2 kg

Age: Young adult

Acclimation Period: Minimum 5 days.

Number of Animals: Three

Justification of Test System:

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current ISO testing standards. The rabbit is widely used for this purpose and relative ranking of irritant scores can be determined.

Animal Management:

Husbandry: Refer to ISO 10993-10: 2002 Annex C: Animal and husbandry

Food: All-nutrient animal food provided by Suzhou (Twin-lion) Experimental Animal Food Science & Technology service Co., Ltd.

Water: Provided by Sanitary Standard for drinking water.

Housing: Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused, animals free from irritation or other dermatological lesions that could interfere with the test were selected.

Experimental Procedure

Clean the rabbit's naked skin with 75% alcohol. Choose ten points at 2cm intervals on one side of rabbit back and inject 0.2ml leached solution at each point. Similarly, on the other side of rabbit back. Choose five points at 2cm intervals and inject 0.2ml control solution at each point.

Observe and write down the skin responses of injection sites in 24h, 48h and 72h respectively after injection. The skin response include erythema, odema and necrosis as well. From weak to serious, the responses are differentiated by grade 0, 1, 2, 3, 4 on the basis of its extent. See table 1.

Result

According to what observed, the response of skin on testing side does not exceed that on the control side. Thus, it is identified as grade 0. See table 2.

Conclusion

The test result shows that leached solution of sample does not induce irritation to skin.

Record Storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated SUFY archive files.

TCRSU was awarded Metrology Accreditation Certificate (No. S0174) by National Technology Supervision Bureau in Nov 1, 2004.

Table.1 Classification System for Skin Reaction

Reaction	Numerical Grading
Erythema and Eschar Formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more than 1mm and extending beyond exposure area)	4
Total possible score for irritation 8	

Irritation Response Categories in the Rabbit

Response Category	Mean score
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

NOTE: Other adverse changes at the skin sites shall be recorded and reported

Table 2. Dermal Observations

Rabbit No			Interval (hours)		
			24	48	72
1	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0
2	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0
3	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0