Magnesium Alloy Specimen
Skin Sensitization Study (Maximization Test)

FINAL REPORT

Sponsor: Metal Industries Research & Development Centre

Testing Institution: SGS Taiwan Ltd.

Report No.: UP/2014/60049A-02

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STUDY SCHEDULE
Skin Sensitization Study (Maximization Test)
Magnesium Alloy Specimen

Report No.:	UP/2014/60050A-04
Experimental starting date:	2014.11.21
Experimental completion date:	2014.12.26
Study completion date:	See Study Director's signature date in the report

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INFORMATION FOR TEST ARTICLE

☒試驗物質/☐對照物質資料表

委託單位名稱	金屬工業研究發展中心	
委託單位地址	82151高雄市路竹區路科五路88號3樓	
委託試驗項目	☒ 以合約為主 ☐ 其它 ：	
試驗物質/對照物質名稱	鎂合金試片	
批號	☐依據特定編號：_____ ☐其它：_____ ☐依據包裝上日期：_____ ☒無批號可提供	
規格數量	45.6cm2/片*11片 (如10mL/瓶*6瓶)	
提供之同批號量 (註2)	☒1次之測試使用 ☐2次以上之測試使用(留樣用)	
外觀型態	型態: ☐液狀☐粉狀☐錠狀☐膠囊狀 ☒其他：片狀	顏色 ：乳白色
主要成分及純度	成分 ：鎂	純度 ：90%以上
適合之溶劑及其溶解度	N/A	
保存條件	保存條件 ：☐室溫 ☐4℃☐避光 ☒其他 真空包裝	
保存期限(註3)	☐有效期限 ：西元_____年_____月_____日 或 ☐保存期間：共 _____年_____月_____日或 ☒依SGS UB之留樣保存期限為主	
檢附之文件(註4)	☐分析證明☐安全資料表 ☐安定性測試結果 ☒無附件(註4) ☐其他：_____	
滅菌	產品是否已滅菌☒是☐否 (如勾選YES請再勾選下方滅菌方法) 滅菌方法是 ☐EO滅菌☒Gamma滅菌 ☐蒸汽滅菌☐其他_____	
醫療器材使用之範疇 (非醫療器材者免填)	1.☐與皮膚或黏膜短期接觸(接觸人體累積時間) ☐短期接觸(不超過4 hr) ☐長期接觸(超過4 hr以上) ，最長累積時數為_____小時 2.☒ 植入式的醫療器材	
特殊需求(註5)	N/A	

客戶簽名/日期：_____ 2014.6.9

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版次：3.3 試驗-對照物質資料表 Information for test article-control article
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SIGATURE OF STUDY PERSONNEL
Skin Sensitization Study (Maximization Test)
Magnesium Alloy Specimen

Study Director:

臺灣檢驗科技股份有限公司

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2014.09.11

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OBJECTIVE

When direct contact with human tissues is anticipated, medical device should be carefully tested for biocompatibility according to the nature and duration of the contact to avoid potential physiological damage caused by hypersensitive substances produced or contaminated during manufacture. In this study, guinea pig skin sensitization study (Maximization test) was conducted to evaluate the possibility of skin sensitization after topical applications of the test article extracts on the skin of guinea pigs. The experiment was performed by following ISO 10993-10 and Leon Biotech Co., Ltd internal document of standard operating procedure SOP-T02.

EXPERIMENTAL DESIGN

1. Test System

A. Species/ Strain: Guinea Pig/ Hartley
B. Resource: National Laboratory Animal Center, Taiwan
C. Reason: According to ISO 10993-10
D. Body weights/Age: 300~500 g or 1~6 month
E. Sex: Female & male. The female guinea pigs were nulliparous and not pregnant
F. Number: 30
G. Quarantine/ acclimation: Once animals were introduced in-house, they were subjected to quarantine and acclimatize before treatment. Animals were selected based on health status by qualified staff..
H. Identification
(1) Individual identification: Animals were identified by ear-marking.
(2) Group identification: Cages were properly labeled for identification including species/ strain, sex, in-housing date, IACUC number, animal I.D. number.
I. Housing condition
(1)Environment temperature: 25±3℃
(2)Humidity: 30-70%
(3)Cage and animal number: 5 animals/cage
(4)Fodder/ Supply: Lab Diet; ad libitum
(5)Drinking water/ Supply: Tape water; ad libitum

2. Reagent

A. 0.9% normal saline (Tai Yu Pharmaceuticla Co., Ltd. Lot No. ML0606)
B. Freund's complete adjuvant (Sigma, F5881 , Lot No. SLBH7316V)
C. Sodium dodecyl sulfate (Sigma, L5750 Lot No. BCBC7174V)

3. Extraction

According to ISO 10993-12 guidelines, the ratio of the test article to the extractant was 0.2g /mL. First extraction for induction I, 1.07g test article was immersed in 5.35mL of 0.9% saline and 2.33g test article was immersed in 11.65ml cottonseed oil. Second extraction for induction II, 1.18g test article was immersed in 5.9ml of 0.9% saline and 2.34g test article was immersed in 11.7mL cottonseed oil Third extraction for challenge, 1.2g test article was immersed in 6ml of 0.9% saline and 2.25g test article was immersed in 11.25mL cottonseed oil. Extract condition of 72 hours at 50℃ with constant agitation were executed in this study. The pH adjustment, filtration and centrifugation were not conducted. After extraction, the appearance of non-polar extract were not different from the control solution, but the appearance of polar extract were

slight yellow.

4. Grouping

Test group	Control group
10 animals	5 animals
Polar extract of test article	0.9% Saline

5. Test Method

A. Induction phase I

(1) Three kinds of solutions or emulsions were prepared from the control solution or test article extract as follow:

Polar group

I. Emulsion of Freund's complete adjuvant (Sigma F5881) in 0.9% saline (Tai Yu Pharmaceutical Co., Ltd.) and volume ratio 1:1 (50% FCA).

II. Solution of either test article extracts or 0.9% saline (Tai Yu Pharmaceutical Co., Ltd.).

III. Emulsion of either test article extracts or 0.9% saline (Tai Yu Pharmaceutical Co., Ltd.) in 50% FCA in volume ratio 1:1.

Non-polar Group

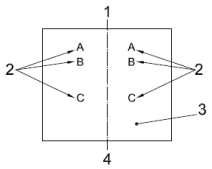
IV. Emulsion of Freund's complete adjuvant (Sigma F5881) in cottonseed oil (Sigma C7767) and volume ratio 1:1 (50% FCA).

V. Solution of either test article extracts or cottonseed oil (Sigma C7767).

VI. Emulsion of either test article extracts or cottonseed oil (Sigma, C7767) in 50% FCA in volume ratio 1:1.

B. Furs of animal's backside were clipped with an electric animal shaver. Animals with scratches or skin diseases on the clipped skin surfaces were rejected from the study.

C. Injections sites were paired, and there were six injection sites in the clipped zone of



each animal (see figure below). Each solution was injected into injection sites matches A, B and C. The injected volume was 0.1 ml at each injection sites.

D. Induction phase II

(1) 7±1 days later, the injection sites were applied with 10% of sodium dodecyl sulfate (Sigma, L5750) for 24±2 h.

(2) Then, an appropriate absorbent gauze patch was saturated (about 8 cm2) with the test article extract or control solution, and applied to the clipped skin under an occlusive dressing secured by a wrap around the torso of the animal for another 48±2 h.

E. Challenge phase (14±1 days after induction phase II)

(1) The furs of flank of the animals were clipped. An appropriate site of this hairless area were selected and applied by the patches that soaked with the control solution or test article extract and then secured with an occlusive dressing.

(2) The dressings and patches were removed after 24±2 h.

F. Observation and evaluation

(1) The appearance of the challenge skin sites of the test and control animals were observed at 24±2 h and 48±2 h after removal of the dressings.

(2) Skin reactions for erythema and oedema were graded according to the Magnusson and Kligman grading given in Table 1.

(3) Grades greater than 1.0 in the test group generally indicate sensitization while grades of control animals are less than 1.0. If grades greater than 1.0 are noted in control animals, the reactions of test animals which exceed the most severe reaction are presumed to be due to sensitization.

RESULT

1. Approximately 24±2 hours and 48±2 hours after challenge phase, neither the control nor the test group showed significant skin response on the treated areas. None of the test or control groups had a mean score increase of 1.0 or more in the observation period.
2. Individual Animal Grades skin reaction

polar group

Group	Sex	Number of animals	24 hrs. after challenge phase	48 hrs. after challenge phase
Control "0.9% saline"	female	G-160725-01	0	0
		G-160725-02	0	0
		G-160725-03	0	0
		G-160725-04	0	0
		G-160725-05	0	0
Mean score			**0**	**0**
Test "Laparoscopic electrocautery electrode Extracts (saline)"	male	G-160801-46	0	0
		G-160801-47	0	0
		G-160801-48	0	0
		G-160801-49	0	0
		G-160801-50	0	0
		G-160801-51	0	0
		G-160801-52	0	0
		G-160801-53	0	0
		G-160801-54	0	0
		G-160801-55	0	0
Mean score			**0**	**0**

RELIABILITY CHECK

The positive control study was finished at 2014/07/04. According to ISO 10993-10 guidelines, positive control shall be performed at least once every six month. α-hexylcinnamicaldehyde (Sigma 291285, Lot. No. MKAA2596) were used for positive control substances. The method for the positive control assay are identical to the method described above in this study. For the induction phase, 0.5% and 85% α-hexylcinnamicaldehyde was used. For the challenge phase, 85% α-hexylcinnamicaldehyde was used. Animals in the positive control group exhibited discrete erythema to confluent erythema at the challenge site. All reactions in the positive control group scored of 1, had a 100% incidence and 1.0 severity (24 hour score) are indicated that is positive sensitization reaction.

CONCLUSION

The results indicated that the polar and non-polar extracts of “Magnesium Alloy Specimen” did not produce skin sensitization in guinea pigs.

REFERENCES

1. Skin Sensitisation, OECD guideline for the testing of chemicals. #406 (1992) OECD.
2. Biological evaluation of medical devices- Part 10: Tests for irritation and skin sensitization. ISO 10993-10:2010.
3. Biological evaluation of medical devices- Part 12: Sample preparation and reference
4. materials. ISO 10993-12:2012.
5. Biological evaluation of medical devices- Part 2: Animal welfare requirements. ISO 10993-2:2006.

TABLES

Magnusson and Kligman Scale (ISO 10993-10)

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

TEST ARTICLE PHOTO