# Magnesium Alloy Specimen
# White Rabbit Intracutaneous Irritation Test

# FINAL REPORT

**Sponsor:** **Metal Industries Research & Development Centre**

**Testing Institution:** **SGS Taiwan Ltd.**

**Report No.:** **UP/2014/90065**

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3. The results shown in this test report refer only to the test article(s) tested.
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5. The report is the Chinese version of translations UB/2014/90065

# STUDY SCHEDULE
# White Rabbit Intracutaneous Irritation Test
# Magnesium Alloy Specimen

| | |
|---|---|
| Report No.: | UP/2014/90065 |
| Experimental starting date: | 2014.12.05 |
| Experimental completion date: | 2014.12.11 |
| Study completion date: | See Study Director's signature date in the report |

## ADDRESS INFORMATION

**Testing Facility/ Test Site**
**Name:** SGS TAIWAN LTD.
**Address:** No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist.,

**Study Director**
**Name:** Biocompatibility Lab. Of LEON Biotech. Co., Ltd.
**Address:** 4f.-2, No.288-8, Xinya Rd., Qianzhen Dist., Kaohsiung City 806, Taiwan

**Sponsor**
**Name:** Metal Industries Research & Development Centre
**Address:** 3F, No.88, Luke 5th Rd., Luzhu Dist., Kaohsiung City, Taiwan 82151, R.O.C. (Kaohsiung Science Park)

# INFORMATION FOR TEST ARTICLE

## 試驗物質資料表

☒試驗物質/☐對照物質資料表

| | |
|---|---|
| 試驗委託者名稱 | 金屬工業研究發展中心 |
| 試驗委託者地址 | 82151高雄市路竹區路科五路88號3樓 |
| 試驗物質/對照物質名稱 | 鎳合金試片 |
| 批號 | ☐特定編號：_____<br>☒無批號可提供 |
| 數量(註2) | A、 數量/單位：45.6cm2/片*10片 (如10mL/瓶*6瓶)<br>B、☒1次之測試使用(不留樣) ☐2次以上之測試使用(留樣用)<br>C、包裝情況：☐散裝☒完整包裝 |
| 樣品描述 | A、型態:☐液狀☐粉狀☐錠狀☐膠囊狀☒其他：長方狀<br>B、成分:鎳<br>C、純度:99%<br>D、顏色:白色<br>E、適合之溶劑及其溶解度：N.A |
| 儲存環境 | 保存條件：☒室溫☐2~8℃☐-10~-25℃☐其他_____ |
| 有效期限(註3) | ☐有效期限：西元_____年_____月_____日<br>☒無有效期限可提供 |
| 檢附之文件(註4) | ☐分析證明☐安全資料表☐安定性測試結果☐其他：_____<br>☒無附件(註4) |
| 來源 | A、原產地：台灣<br>B、製造/供應商：金屬工業研究發展中心 |
| 滅菌 | 產品是否已滅菌☐否☒是 (如勾選"是"，請再勾選下方滅菌方法)<br>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 | 1.☐與皮膚或黏膜短期接觸(接觸人體累積時間)<br>☐接觸時間：不超過4 hr<br>☐接觸時間：超過4 hr以上，最長累積時數為_____小時<br>2.☒植入式的醫療器材<br>3.☐非醫療器材 |
| 其他 | N.A |

註1. 上述所有資料是由試驗委託者提供並負責。表填列事項即代表試驗委託者無法提供此資訊，亦包含於試驗委託者提供並負責部分。
註2. 需提供同批號之留樣，若試驗委託者無法提供試驗/對照物質每批與代表性之隨備試驗/對照物質，其留存由試驗委託者自行保存與負責。
註3. 試驗物質/對照物質除送來時一次檢測重時，仍應再留一份同批號且可供一次試驗用的關鍵樣品，針對有留樣量之試驗物質/對照物質，試驗物質/對照物質之有效期限少於5年，本公司之留樣保存期限則以試驗委託者提供之有效期限為主；若試驗物質/對照物質之有效期限大於5年，本公司之留樣保存期限則為5年，若有效期限填寫不完整，請以當年度當月之最早期限為主，例如有效期限僅填寫2015年，其有效期限將定義委託者同意為2015.01.01。
註4. 試驗物質/對照物質的本質，包含批次、純度、濃度、成份、合成、製造方法、來源、安定性或其他可適當定義本質的特徵之文件保存為試驗委託者的責任。
註5. 若此空格不適用，請填寫N/A或N.A，勿留空白處。
註6. 請試驗委託者確實填寫資訊，"試驗物質/對照物質資料表"詳呈現在試驗計畫書及報告;請務必仔細填寫，如未填寫，計畫書/報告中將於聲明中書寫排除。

試驗委託者簽名/日期：_____ 2014.9.4

試驗物質編號 UP/2014/90065 此欄由本公司之收樣人員標示

版次：3.4 試驗物質/對照物質資料表 Information For Test Article/Control Article
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# SIGATURE OF STUDY PERSONNEL
## White Rabbit Intracutaneous Irritation Test
## Magnesium Alloy Specimen

Study Director:

劉錦誠 / 台灣檢驗科技股份有限公司　　2014.12.19 日期

Facility Manager:

文元民 / 台灣檢驗科技股份有限公司　　2014.12.19 日期

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# OBJECTIVE

The constituent materials of medical devices are considered potentially produce irritation. 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 irritative substances produced or contaminated during manufacture. Test procedure of in vivo is described in ISO 10993-10; albino rabbit use is suggested to evaluate the possibility of irritant reaction. The test was performed following ISO 10993-10 and Leon Biotech. Co. Ltd. internal document of standard operating procedure SOP-T03, to investigate the response of intracutaneous irritation of “Magnesium Alloy Specimen” extract on New Zealand White Rabbits.

# EXPERIMENTAL DESIGN

## 1. Test System

A. Species/ Strain: New Zealand White Rabbit (NZW)
B. Resource: Taiwan Livestock Research Institute
C. Reason: According to ISO 10993-10
D. Body weights/Sex/Age: >2 kg /female/ 2~12 month
E. Sex: Female
F. Number: 3
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: 20~26℃
(2)Humidity: 30-70%
(3)Cage and animal number: 1 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.PE 1401)
B. Cottonseed oil (SIGMA C7767, Lot No.MKBN8713V)

## 3. Extraction

According to ISO 10993-12 guidelines, the ratio of the test article to the extractant was 3cm2/mL. In this study, 22.8 cm2 test article was immersed in 7.6 mL of 0.9% saline and 22.8 cm2 test article was immersed in 7.6 mL cottonseed oil. Extract condition of 72 hours at 50℃ with constant agitation wereexecuted in this study. The pH adjustment, filtration and centrifugation were not conducted. After polar and non-polar extraction, the appearance of extracts were not different from the control solution.

## 4. Grouping

| Test group | Control group |
|---|---|
| 3 animals | |
| Polar extract of test article | 0.9% Saline |
| Non-polar extract of test article | Cottonseed oil |

## 5. Test Method

A. Administration of test article extract

(1) Furs of NZW rabbit backside from scapula to middle back were clipped with an electric animal shaver. Clipped zone are about 8cm x 15cm to exposure skin surface along the spine to execute intra-dermal injection. Animals with scratches or skin diseases in the clipped zone were rejected from study.

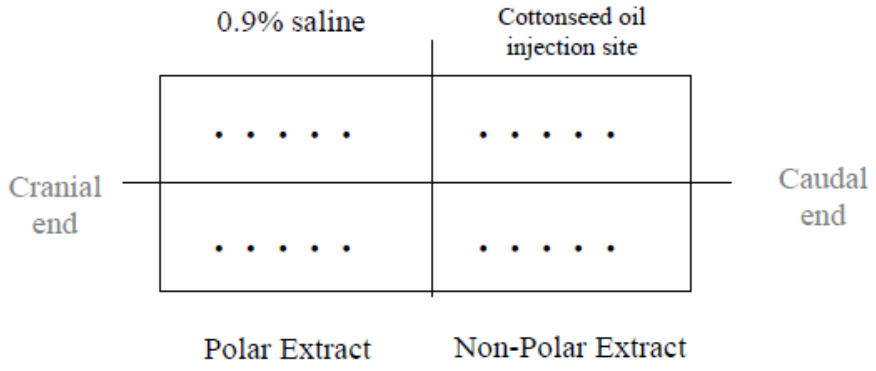
(2) A maker pen was used to divide clipped zone into four regions, and there were five injection site of each test and control group (see figure below).

B. Test article and control solution administration

(1) Control solution was conducted in the right side of clipped zone with 0.9% saline in the anterior and cottonseed oil in the posterior region.

(2) Test article extract was conducted in the left side of clipped zone with polar extract in the anterior and non-polar extract in the posterior region.

(3) Each injection site was injected 0.2 ml solution.




C. Irritant reaction evaluation

(1) The dermal reactions at the treated areas were observed and recorded based on "Grading system for intracutaneous reaction" (see Table 1) at the time points of 24th, 48th and 72nd hours after administration.

(2) The observation items included erythema, oedema, irritation, corrosion recovery

and other toxicity reactions.

D. Determination of dermal reaction

(1) In the end of the test, each region in one rabbit, grades of erythema and oedema were summed and then divided by 15 (3 grading time points multiply 5 injection sites). To determine the mean score for each test sample and each corresponding blank, add the scores for the three animals and divide by three. The requirements of the test will be met if the differs between test article and control solution mean scores less than 1.0.

(2) If at any observation period the average reaction to the test article is questionably greater than the average reaction to control side, the test will repeat using three additional rabbits.

# RESULT

During the study, there were no significant clinical signs and gross findings in either the control or test group, and there were no mortalities also. Grades were recorded as the table below.

1. Grades in clinical observation of individual rabbit were as below (polar group).

| Treated article | Sex | Animal No. | Items for Grading | Clinical Observation (time point/h) | | |
|---|---|---|---|---|---|---|
| | | | | 24 | 48 | 72 |
| Test article Polar extract | F | RB-140626-01 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-140626-04 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-141002-05 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| 0.9% normal saline (control solution) | F | RB-140626-01 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-140626-04 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-141002-05 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |

2. Grades in clinical observation of individual rabbit were as below (Non-polar group).

| Treated article | Sex | Animal No. | Items for Grading | Clinical Observation (time point/h) | | |
|---|---|---|---|---|---|---|
| | | | | 24 | 48 | 72 |
| Test article Non-polarextract | F | RB-140626-01 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-140626-04 | Erythema and eschar formation | 3 | 4 | 4 |
| | | | Oedema formation | 2 | 1 | 0 |
| | F | RB-141002-05 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 2 | 0 | 0 |
| Cottonseed oil (control solution) | F | RB-140626-01 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 0 | 0 | 0 |
| | F | RB-140626-04 | Erythema and eschar formation | 3 | 5 | 3 |
| | | | Oedema formation | 2 | 1 | 0 |
| | F | RB-141002-05 | Erythema and eschar formation | 0 | 0 | 0 |
| | | | Oedema formation | 1 | 0 | 0 |

F:Female

Erythema and oedema were recorded in non-polar group, but there were no significant difference between test and control group. It could be the background reaction of cottonseed oil.

$$Final\ Scores = \frac{Total\ Scores\ of\ test\ group}{15 * 3} - \frac{Total\ Scores\ of\ control\ group}{15 * 3}$$

Final calculated score value was less than 1.0 in both test groups (polar group: 0/45-0/45=0; non-polar group: 16/45-15/45=0.02), which indicated negative results.

## CONCLUSION

The study results showed that a single application of “Magnesium Alloy Specimen” extract induced neither significant clinical signs nor dermal gross changes on New Zealand White Rabbits at each time point. Furthermore, the final score value of test article extract was less than 1.0. Therefore, single intracutaneous application with 0.2 mL of “Magnesium Alloy Specimen” extract did not cause irritation on New Zealand White Rabbits.