Virus Bom

[Lot No:20131024]

Skin Sensitization Study (Maximization Test)

FINAL REPORT

Client: MONEY MARKETING COMMUNICATION LTD.
Testing Institution: SGS Taiwan Ltd
Report No. : UB/2013/A1312A-01
Report Date: 2014.01.24

Note:

1. The content of this report is invalid if it is not presented as the entire report.
2. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted to the fullest extent of the law.
3. The results shown in this test report refer only to the test article(s) tested.
4. This report in the Chinese version of translation UB/2013/A1312A-04

STUDY SCHEDULE

Skin Sensitization Study (Maximization Test):

Virus Bom

Report No.:	UB/2013/A1312A-01
Study Initiation date:	2013.12.03
Experimental starting date:	2013.12.23
Experimental completion date	2014.01.07
Study Completion date	See Study Director's signature date in the report
Study personnel	Yu Jung Pan

Testing Institution

Name: SGS TAIWAN LTD.

Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Subcontract Lab

Name: LEON Biotechnology Company Limited Biocompatibility Testing Laboratory

Address: 4F.-2, No. 288-8, Xinya Rd., Qianzhen Dist., Kaohsiung City 806, Taiwan (R.O.C.)

Client / Sponsor

Name: MONEY MARKETING COMMUNICATION LTD.

Address: 10F-1, NO. 15, SEC 4, JHONGSIAO E, RD.

SGS

SGS **TEST ARTICLE INFORMATION**

INFORMATION FOR ☒ TEST ARTICLE/ ☐ CONTROL ARTICLE

Sponsor Company Name	MONEY MARKETING COMMUNICATION LTD.
Sponsor Address	10F-1, NO. 15, SEC 4, JHONGSIAO E, RD.
Contract study item	☒ Base on the contract ☐ Others _____
Name of Test article/ Control article	Virus Bom
Batch/Lot number	☒ Base on the specific number on the package : 20131024 ☐ Base on the date on the package : _____ ☐ Base on the arrived date ☐ Others : _____
Specification & Amount	50mL/bottle*6 bottles (e.g.10ml / bottle * 6 bottles)
Retention amount (Note 2)	The amount of the same lot is sufficient for ☐ One test ☒ Two test (for retention)
External features	External features: ☒ liquid ☐ powder ☐ tablet ☐ capsule ☐ Other_____ Color : CLEAR
Major components & Purity	Major components: C17H32O4S Purity: 3000 ppm
Solvent and solubility	N/A
Storage condition	☐ Room temperature ☒ 4℃ ☐ Dry ☐ Light sensitive ☐ Others_____
Expiration date(Note 3)	☐ Date: ____/____/____ (YYYY/MM/DD) or ☒ Period : 5 year0 month 0 day
Attachment(Note 4)	☐ Certificate of Analysis ☐ Material Safety Data Sheet ☐ Stability Test Result ☐ Other : _____ ☒ No attachment (Note4)
Sterilization	Has been sterilized ☐ YES ☒ NO (If Yes, please select the following item) Methods ☐ EOsterilization ☐ Gamma sterilization ☐ Steam sterilization ☐ Other_____
Categorization of devices (The column is only for device used)	1. ☐ Contact with intact skin or mucosa (cumulative contact duration) ☐ Short-term (no greater than 4 hr) ☐ Long-term (exceeding 4 hr) Maximum duration is_____hrs 2. ☐ Implanted device
Specific requirement (Note 5)	N/A 本產品非醫療器材

Sponsor Signature/ Date : 張尚敏 / 2013.10.28

Note 1. Above all information is disclosure by the sponsor.
Note 2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.
Note 3. If the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the effective period is longer than 5 years, the test article/control article will be retained for 5 years only.
Note 4. Determination and documentation of identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article are the responsibility of the Sponsor.
Note 5. The test article/control article which has been destroyed or cutting will be discarded after the end of experiment. For retention or return of the kind of test article/control article, please indicate in the "special requirement". The human intake suggests or dose requested by the sponsor also can fill in the "special requirement". Note treatment method after test if the test article need to be retreated
Note 6. The code number of test article is the same as the report number.
Note 7. Note 'N/A' if not applicable. Do not leave blank.

版次：3.1 试验-对照物质资料表 Information for test article-control article
發行日期：2013.06.14

TABLE OF CONTENTS

SGS

STATEMENT OF GLP COMPLIANCE

All study activities performed by SGS Taiwan and LEON Biotechnology Company Limited Biocompatibility Testing Laboratory are carried out in compliance with the GLP (Good Laboratory Practices) for Nonclinical Laboratory Studies (Department of Health, Taiwan, 2006), current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17) and U.S. Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58. The study is conducted in accordance with the protocol and standard operating procedures and monitored in conformity with the protocol. All laboratory data are accurately recorded and verified. SGS Taiwan and LEON Biotechnology Company Limited Biocompatibility Testing Laboratory make no GLP compliance claim for characterization and verification of the test article identity and properties; this is the responsibility of the sponsor.

Study Director:

Howard Kao / SGS Taiwan Ltd. Date Completed: 2014.01.28

Facility Manager:

Yuanmin Wen / SGS Taiwan Ltd. Date Completed: 2014.01.28

QUALITY ASSURANCE STATEMENT

UB/2013/A1312A-01

Skin Sensitization Study (Maximization Test)

Virus Bom

This study was audited by Quality Assurance personnel of LEON Biotechnology Company Limited Biocompatibility Testing Laboratory. The QA inspection report includes review of result of a study-based audit and results of audit of raw data and study report. The audit report was issued upon the completion of each testing. LEON Biotechnology Company Limited Biocompatibility Testing Laboratory was audited by Quality Assurance personnel of SGS Life Science Service.

QA:

Melissa Lin/ SGS Taiwan Ltd.

2014.01.28

Date Completed

ARCHIVING

All the study-related records, protocol and the final report will be kept in archives room of SGS (TAIWAN) LTD and study-related raw data will be kept in archives cabinet of LEON Biotechnology Company Limited Biocompatibility Testing Laboratory for 5 years. Furthermore, retention of the test articles will be in Sample Storage Room of SGS (TAIWAN) LTD for 5 years. All of the records and test articles are handled according to GLP guideline. Agent authorized by the sponsor can apply for the review according to SGS procedure.

Archives Room Address

SGS TAIWAN LTD.: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

LEON Biotechnology Company Limited Biocompatibility Testing Laboratory:

4F.-2, No. 288-8, Xinya Rd., Qianzhen Dist., Kaohsiung City 806, Taiwan (R.O.C.)

Archiving List	
Final Report	Final Report Copy
Raw Data*	Skin Sensitization Study (Maximization Test) Data Sheet
Records	Application Form Information for test article-control article and other supplementary record
Protocol	Protocol

*kept in archives cabinet of LEON Biotechnology Company Limited Biocompatibility Testing Laboratory

ABSTRACT

The study was to investigate the response of skin sensitization of “Virus Bom” on guinea pigs. The experiment was performed by following OECD#406. The test article was applied directly twice in induction phase and once in challenge phase. Approximately 24 hrs and 48 hrs after challenge phase, neither the control nor the test group showed significant skin response on the treated areas, according to the criteria of “Magnusson and Kligman scale”. The results indicated that the “Virus Bom” did not produce skin sensitization in guinea pigs.

PURPOSE

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 on the skin of guinea pigs. The experiment was performed by following OECD #406 and internal document of standard operating procedure SOP-T02.

EXPERIMENTAL DESIGN

1. Test system:

A. Species/ Strain: Guinea Pig/ Hartley

B. Resource: TBT, Taiwan

C. Reason: According to OECD#406

D. Body weights/Age: 300~500g or 1~6 month

E. Sex: Female. The female animals were nulliparous and not pregnant.

F. Numbers: 15

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 hair dyeing.

(2)Group identification: Cages are 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. Reagents

A. 0.9% normal saline (Tai Yu Pharmaceutical Co., Ltd. MH2502)

B. Freud's complete adjuvant(Sigma, F5881, Lot No. SLBC5082)

C. Sodium dodecyl sulfate (Sigma, L5750, Lot No. BCBC7174V)

3. Extraction

The liquid test article was tested directly.

4. Grouping

Test group	Control group
10 animals	5 animals
Test article(Virus Bom 3000ppm)	0.9% Saline

5. Test Method

A. Prior to the study, the 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. The clipped area will be about 8 cm^2.

B. Induction phase I

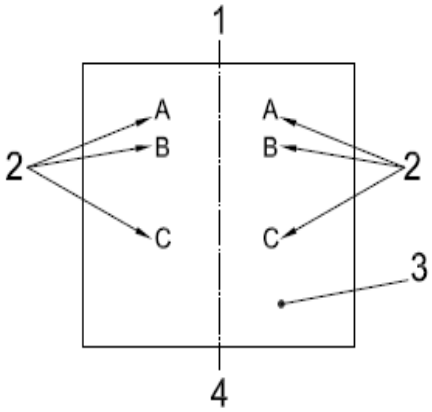
(1) Three kinds of solutions or emulsions were prepared from the control solution or test article as follow:

a. Emulsion of Freund's complete adjuvant (Sigma F5881, Lot No.SLBC5082) in 0.9% saline (Tai Yu Pharmaceutical Co., Ltd. Lot No.MH2502) and volume ratio 1:1 (50% FCA).

b. Solution of either test article or 0.9% saline.

c. Emulsion of either test article or 0.9% saline in 50% FCA in volume ratio 1:1.

(2) 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.



C. Induction phase II

(1) 7±1 days later, the injection sites were applied with 10% of sodium dodecyl sulfate (Sigma, L5750, Lot No.BCBC7174V) for 24±2 h.

(2) Then, an appropriate absorbent gauze patch was saturated (about 8 cm^2) with the test article 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.

D. 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 and then secured with an occlusive dressing.

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

E. Observation

(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.

RESULTS

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

Group	Sex	Number of animals	24 hrs. after challenge phase	48 hrs. after challenge phase
Control "0.9% saline"	female	G-131206-01	0	0
		G-131206-02	0	0
		G-131206-03	0	0
		G-131206-04	0	0
		G-131206-05	0	0
Mean score			**0**	**0**
Test "Virus Bom 3000ppm"	female	G-131122-01	0	0
		G-131122-02	0	0
		G-131122-03	0	0
		G-131122-04	0	0
		G-131122-05	1	1
	female	G-131122-06	0	0
		G-131122-07	0	0
		G-131122-08	0	0
		G-131122-09	0	0
		G-131122-10	0	0
Mean score			**0.1**	**0.1**

3 Individual weights of animals at the start and at the conclusion of the test

Group	Sex	Number of animals	Start (g)	Conclusion (g)
Control “0.9% saline”	female	G-131206-01	363	474
		G-131206-02	363	442
		G-131206-03	336	408
		G-131206-04	349	444
		G-131206-05	345	440
Test “Virus Bom 3000ppm”	female	G-131122-01	342	486
		G-131122-02	299	394
		G-131122-03	385	442
		G-131122-04	356	455
		G-131122-05	394	473
	female	G-131122-06	433	492
		G-131122-07	380	445
		G-131122-08	411	471
		G-131122-09	342	384
		G-131122-10	370	429

CONCLUSION

The results indicated that the “Virus Bom” did not produce skin sensitization in guinea pigs.

RELIABILITY CHECK

The positive control study was finished at 2014/01/03. 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 1~2, had a 100% incidence and 1.2 severity (24 hour score) are indicated that is positive sensitization reaction.

REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58. United States Food and Drug Administration.
2. Current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17).
3. Acute oral toxicity/ Acute toxic class method, OECD guideline for the testing of chemicals. #423 (2001) OECD.
4. Biological evaluation of medical devices- Part 10: Tests for irritation and skin sensitization. ISO 10993-10:2010.
5. Biological evaluation of medical devices- Part 12: Sample preparation and reference materials. ISO 10993-12:2012.
6. Biological evaluation of medical devices- Part 2: Animal welfare requirements. ISO 10993-2:2006.

TABLE

1. Magnusson and Kligman Scale (OECD# 406)

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

UB/2013/A1312

UB/2013/A1312