BIOSERVICE

SCIENTIFIC LABORATORIES GmbH

Acute Dermal Irritation/Corrosion

with

Bacoban

Report

BSL BIOSERVICE Project No.: 051691

Sponsor

Ropimex R.Opel GmbH
Geschäftsbereich Hygiene-Systeme Adexano
Bildstocker Straße 12
66538 Neunkirchen - Germany

BSL BIOSERVICE Scientific Laboratories GmbH
Behringstrasse 6 · 82152 Planegg, Germany
Telefon +49-(0)89-899 65 00 · Fax +49-(0)89-899 65 011
e-mail: info@bioservice.com · www.bioservice.com
Geschäftsführer: Dr. Wolfram Riedel
Amtsgericht München, HRB 109 770
Erfüllung und Gerichtsstand München
Raiffeisenlandesbank Oberösterreich, BLZ 740 201 00, Kto. 4 100 002 016, BIC: RZOODE71, Swift: RZOOAT2L999, IBAN: DE31 74020100 4100002016
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GLP zertifiziert

ZLG

Akkreditiert durch
Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
ZLG-P-986.96.01

Copy of the GLP Certificate

BAYERISCHES LANDESAMT FÜR ARBEITSSCHUTZ, ARBEITSMEDIZIN UND SICHERHEITSTECHNIK

Pfarrstraße 3 · 80538 München · Telefon (089) 21 84-0

LfAS

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

BSL Bioservice Scientific Laboratories GmbH
Behringstrasse 6
82152 Planegg

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen auf toxikologische Eigenschaften
3 Prüfungen auf mutagene Eigenschaften (in vitro/in vivo)
9 Sonstige Prüfungen:
a) Mikrobiologische Sicherheitsprüfungen
b) Wirksamkeitsprüfungen an Zellkulturen

Datum der Inspektion/Date of Inspection
(Tag.Monat.Jahr/day.month.year)
11./12.02.2004

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

München, 21.07.2004

I.V.
Ritter
Leitender Gewerbedirektor

BAYERISCHES LANDESAMT FÜR ARBEITSSCHUTZ, ARBEITSMEDIZIN UND SICHERHEITSTECHNIK L.S. 3

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Preface

General

Sponsor:	Ropimex R.Opel GmbH Geschäftsbereich Hygiene-Systeme Adexano Bildstocker Straße 12 66538 Neunkirchen - Germany
Study Monitor:	Dr. med. R. Hanselmann
Test Facility:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstrasse 6 82152 Planegg Germany
BSL BIOSERVICE- Project No.:	051691
Test Item:	Bacoban
Title:	Acute Dermal Irritation/Corrosion with Bacoban

Project Staff

Study Director:	Dr. Ingrid Haist
Deputy Study Directors:	Dr. Daniela Brummer Dr. Achim Albrecht
Management:	Dr. Wolfram Riedel Dr. Angela Lutterbach
Quality Assurance Unit:	Dipl. Biol. Uwe Hamann Dr. Margarete Hoechst Dr. Helga Köhn

Schedule

Arrival of the Test Item:	June 24, 2005
Date of Project Protocol:	June 28, 2005
Start of Study:	July 04, 2005
End of Study:	July 09, 2005
Date of Report:	July 12, 2005

Project Staff Signatures

Study Director: Dr. Ingrid Haist

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Date: 12.07.2005

Management:

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Date: July 12, 2005

Quality Assurance

This study was conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended on May 08, 2001. Published May 14, 2001 in Bundesgesetzblatt 2001 part I no. 21, pp. 844 – 854.

OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1.
Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.

This study was assessed for compliance with the project protocol, the study plan and the Standard Operating Procedures of BSL BIOSERVICE. The study and/or the test facility were periodically inspected by the Quality Assurance Unit and the dates and phases of the inspections and audits are included in this report. These inspections and audits were carried out by the Quality Assurance Unit, personnel independent of staff involved in the study. The final report of the study was audited. A Quality Assurance Statement, signed by the Quality Assurance, is included in this report.

The test method is part of the BSL BIOSERVICE accreditation scope according to guideline 90/385/EWG, 93/42/EWG and DIN EN ISO/IEC 17025 for testing of medical devices.

Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

Biological evaluation of medical devices:

ISO 10993-1: 2003 "Evaluation and testing"

ISO 10993-10: 2002 "Tests for irritation and delayed-type hypersensitivity"

ISO 10993-12: 2002 "Sample preparation and reference materials"

Archiving

The following records will be stored in the scientific archives of BSL BIOSERVICE Scientific Laboratories GmbH according to the GLP-Regulations:

A copy of the final report, the project protocol, the study plan and a documentation of all raw data generated during the conduct of the study (documentation forms as well as any other notes of raw data, printouts of instruments and computers) and the correspondence with the sponsor concerning the project.

If test item is left over a sample will be stored according to the period fixed by GLP-Regulations. Samples that are unstable may be disposed of before that time. Unless otherwise agreed upon, remaining test item will be discarded three months after release of the report.

Statement of Compliance

BSL BIOSERVICE-Project No.:	051691
Test Item:	Bacoban
Title:	Acute Dermal Irritation/Corrosion with Bacoban
Study Director:	Dr. Ingrid Haist

This study performed in the test facility BSL BIOSERVICE Scientific Laboratories GmbH was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended on May 08, 2001, published May 14, 2001.

"OECD Principles of Good Laboratory Practice (as revised in 1997) ", Paris 1998.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director: Dr. Ingrid Haist

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Date: 19.07.2005

Statement of the Quality Assurance Unit

BSL BIOSERVICE-
Project No.: 051691

Test Item: Bacoban

Title: Acute Dermal Irritation/Corrosion with Bacoban

Study Director: Dr. Ingrid Haist

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates:

Phases of QAU Inspections	*Dates of QAU Inspections*	*Dates of Reports to the Study Director and Management*
Audit Project Protocol/ Study Plan:	June 28, 2005	June 28, 2005
Experimental Phase Audit (Method Audit):	May 31, 2005	May 31, 2005
Report Audit:	July 18, 2005	July 18, 2005

This report reflects the raw data.

Member of the
Quality Assurance Unit: .....

Date: 19. July 2005

Summary

The patch with 0.5 mL of the test item was applied directly on an area of 2.5 cm x 2.5 cm on both sides of the spinal column of albino rabbits and was held in contact with surgical tape. Two patches, moistened with isotonic saline, serving as intraspecific control were applied similarly, one each side.

Test items and controls were placed in such a way, that at one side the test item was placed cranial and the control caudal. At the other side the test item was placed caudal and the control cranial.

The application sites were covered with a semi - occlusive bandage for a 4 hour contact time.

Observations were recorded and compared to the control sites immediately, 24, 48 and 72 hours after patch removal.

The test item showed no irritant effects on the intact skin after a contact time of 4 hours.

The mean Primary Irritation Index was 0.

Classification according to ISO 10993-10:

Negligible

No other clinical signs of toxicity were found.

Conclusions

Considering the reported data of this irritation study it can be stated that the test item Bacoban : showed no irritant/corrosive effects and is classified into the lowest response category.

Introduction

The assessment of the potential of the test item to produce irritation is performed in albino rabbits, being the recommended species for this type of study.

The degree of irritation can be evaluated in a single exposure.

No valid *in vitro* method is available to determine acute irritation.

Materials and Methods

Characterisation of the Test Item

The test item and the information concerning the test item were provided by the sponsor.

Name:	Bacoban
Batch No.:	20050617_life_sme
Storage:	at room temperature
Expiry date:	2007-06
Safety precautions	Routine hygienic procedures were sufficient to assure personnel health and safety.

Preparation of the Test Item

The liquid test item was tested as provided.

Test Animals

New Zealand White Rabbits Crl:KBL (NZW). Bodyweight at the commencement of the study > 2.0 kg.

3 female animals were used.

The animals were derived from a controlled full barrier maintained breeding system (SPF).

Source: Charles River Deutschland, D-88353 Kisslegg

According to Art. 9.2, No.7 of the German Act on Animal Welfare the animals were bred for experimental purposes.

Animal Husbandry

- Semi-barrier in an air conditioned room
- Temperature: 18 ± 3 °C
- Rel. humidity: 55 ± 10%
- Artificial light, sequence being 12 hours light, 12 hours dark
- Air change: at least 10 x / hour
- Free access to Altromin 2123 maintenance diet for rabbits, rich in crude fibre, totally pathogene free (TPF)

- Free access to tap water (drinking water, municipal residue control, microbiol. controlled periodically)
- Housed in ABS - plastic rabbit cages, floor 4200 cm^2
- Certificates of food, water and bedding are filed at BSL Bioservice
- Adequate acclimatization period

Preparation of the Animals

Approximately 24 hours before the test, fur was removed from the dorsal area of the trunk by clipping. Care was taken to avoid mechanical irritation and trauma.

Experimental Procedure

0.5 mL of the test item were applied on a patch first. Then this moistened patch was applied directly on an area of 2.5 cm x 2.5 cm on both sides of the spinal column. It was held in contact with surgical tape (Blenderm, surgical tape hypoallergen, # 1525-2, 3M Health Care). Two patches were moistened with isotonic saline (B. Braun Melsungen AG, Lot 4291A195), serving as intra-specific control. They were applied similarly, one each side.

Test items and controls were placed in such a way, that at one side the test item was placed cranial and the control caudal. At the other side the test item was placed caudal and the control cranial.

The test sites were covered with a bandage (Acrylastic, Beiersdorf AG).

Contact time was 4 hours.

Tissue reaction for erythema and oedema were graded according to the classification system given in table 1.

Clinical Observation

Animals were examined for signs of erythema and oedema until 72 hours after patch removal. Dermal irritation was scored and recorded according to the grades in the table below.

Table 1 Classification system

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

Evaluation of Results

Individual values for each animal were recorded according to the grading system described above.

The Primary Irritation Score (PIS) was calculated for each animal for test and control sites.

$$PIS = \frac{(S\ 24 + 48 + 72)}{n}$$

S = Sum of irritation grades 24, 48 and 72 hours after removal of test item

n = Sum of scoring sites

The Primary Irritation Index (PII) were determined by adding the control-corrected Primary Irritation Score (PIS_{cc}) for each animal and dividing the total score by the number of animals.

$PIS_{CC} = PIS_T - PIS_C$

CC = control-corrected

T = Test

C = Control

According to ISO 10993-10 only the 24, 48 and 72 h observations were used for calculations.

The test item were classified according to primary irritation response categories (table 2).

Table 2

Mean Score(PII)	*Response Category*
0.0 to 0.4	negligible
0.5 to 1.9	slight
2.0 to 4.9	moderate
5.0 to 8.0	severe

Deviation to the Project Protocol

There was no deviation to the project protocol:

Results

The test item showed no irritant effects on the intact skin after a contact time of 4 hours.

Animals showed normal weight development.

Scoring

For individual data see table 3.

Individual values for each animal were recorded according to the grading system described above.

The mean Primary Irritation Index was 0.

Classification according to ISO 10993-10:

Negligible

Table 3

Animal number	Application site	Irritation (hours after application)																PIS	PIS cc
		1 hour				24 hours				48 hours				72 hours					
		Ery-thema		Oedema		Ery-thema		Oedema		Ery-thema		Oedema		Ery-thema		Oedema			
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L		
1	Test Item	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	Test Item	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	Test Item	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

R= Right; L= Left

PII **0**

Distribution of the Report

Sponsor	1x (original)
Study Director	1x (copy)

References

OECD Guidelines for Testing of Chemicals
Section 4: Health Effects
Acute Dermal Irritation/Corrosion, (2002)
Organisation for Economic Co-Operation and Development, Paris