

**Irritation Test
(Intracutaneous Reactivity)**

with

**PA 2200 Reused powder (50% virgin + 50% recycled powder from
EOSINT P System)**

Report

Version: Final

Date: 15 February 2010

BSL BIOSERVICE Study No.: 094863

Sponsor:

EOS GmbH Electro Optical Systems

Robert-Stirling-Ring 1

82152 Krailling

Germany

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-The test results relate only to the items tested.-

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Geschäftsführer: Dr. Wolfram Riedel

Amtsgericht München, HRB 109 770

Erfüllung und Gerichtsstand München

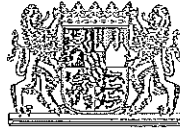
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1. Copy of the GLP Certificate



**BAYERISCHES LANDESAMT
FÜR GESUNDHEIT UND LEBENSMITTELSICHERHEIT,
LANDESINSTITUT FÜR ARBEITSSCHUTZ UND PRODUKTSICHERHEIT**
Pfarrstraße 3 · 80538 München · Telefon (089) 21 84-0

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. Richt-
linie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP
according to Chemikaliengesetz and
Directive 2004/9/EC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

BSL Bioservice Scientific Laboratories GmbH
Behringstrasse 6 - 8
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
9 Sonstige Prüfungen:

a) Mikrobiologische Sicherheitsprüfungen
b) Wirksamkeitsprüfungen an Zellkulturen

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

16./17.09.2008

Die/Der genannte Prüfeinrichtung/Prüfstandort
befindet sich im nationalen GLP-Überwachungs-
verfahren 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 Prüf-
ungen 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, 06.04.2009

Ritter
Leitender Gewerbedirektor



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4. Preface

4.1. Abbreviations

ABS	acrylonitrile-butadiene-styrene
Art.	Artikel (Article)
BGBI.	Bundesgesetzblatt (Federal Law Gazette)
DIN	Deutsches Institut für Normung (German Institute for Standardisation)
Dipl.-Biol.	Diplom Biologe (Biology Diploma)
EC	European Commission
EN	Europäische Norm (European standard)
EWG	Europäische Wirtschaftsgemeinschaft (European Economic Community, EEC)
GLP	Good Laboratory Practice
GmbH	Gesellschaft mit beschränkter Haftung (company with limited liability)
IEC	International Electrotechnical Commission
ISO	International Organisation for Standardisation
NaCl	sodium chloride
NZW	New Zealand White
OECD	Organisation of Economic Cooperation and Development
PII	Primary Irritation Index
PIS	Primary Irritation Score
QA	Quality Assurance
QAU	Quality Assurance Unit
SOP	Standard Operating Procedures
SPF	specific-pathogen free
TVT	Tierärztliche Vereinigung für Tierschutz (Veterinary Association for Animal Welfare)

4.2. General

Sponsor:	EOS GmbH Electro Optical Systems Robert-Stirling-Ring 1 82152 Krailling Germany
Study Monitor:	Ms. Monika Gessler Substitute Mr. Peter Keller
Test Facility:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstraße 6/8 82152 Planegg Germany
BSL BIOSERVICE Study No.:	094863
Test Item:	PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System)
Title:	Irritation Test (Intracutaneous Reactivity) with PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System)

4.3. Project Staff

Study Director:	Dr. Sandra Schmid
Deputy Study Director:	Dr. Patricia Neuenhahn
Management:	Dr. Wolfram Riedel Dr. Angela Lutterbach
Head of Quality Assurance Unit:	Dipl.-Biol. Uwe Hamann

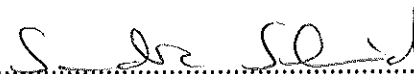
4.4. Schedule

Arrival of the Test Item:	25 November 2009
Date of Final Study Plan:	15 December 2009
Start of Experiment:	08 January 2010
End of Experiment:	14 January 2010
Date of Final Report:	15 February 2010

5. Project Staff Signatures


Study Director

Dr. Sandra Schmid

..... 

Date: 15 Feb 2010

Management

..... 

Print Name: Dr. Angela Lutterbach

Date: 15 Feb 2010

6. Quality Assurance

6.1. GLP Compliance

This study was conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on June 20, 2002 (BGBl. I Nr. 40 S. 2090), revised October 31, 2006 (BGBl. I Nr. 50 S. 2407).

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 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 according to the corresponding SOPs. These inspections and audits were carried out by the Quality Assurance unit, personnel independent of staff involved in the study. A signed Quality Assurance Statement, listing all performed audits, is included in the 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.

6.2. Guidelines

This study followed the procedures indicated by internal BSL BIOSERVICE SOPs and the following internationally accepted guidelines and recommendations:

Biological evaluation of medical devices

ISO 10993-1: 2009 "Evaluation and testing within a risk management process"

DIN EN ISO 10993-10: 2007 (ISO 10993-10: 2002 + Amendment 1: 2006) "Tests for irritation and delayed-type hypersensitivity"

ISO 10993-12: 2007 "Sample preparation and reference materials"

6.3. 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 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 study.

If test item is left, a sample will be stored according to the period fixed by the GLP Regulations. Samples that are unstable may be disposed of before that time. No raw data or material relating to the study will be discarded without the Sponsor's prior consent. Unless otherwise agreed upon, the remaining test item will be discarded three months after the release of the report.

7. Statement of Compliance

BSL BIOSERVICE-
Study No.: 094863
Test Item: PA 2200 Reused powder (50% virgin + 50%
recycled powder from EOSINT P System)
Title: Irritation Test (Intracutaneous Reactivity) with
PA 2200 Reused powder (50% virgin + 50%
recycled powder from EOSINT P System)
Study Director: Dr. Sandra Schmid

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 and promulgated on June 20, 2002 (BGBl. Nr. 40 S. 2090), revised October 31, 2006 (BGBl. I Nr. 50 S. 2407).

"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. Sandra Schmid

.....Sandra Schmid.....

Date:12 Feb 2010.....

8. Statement of the Quality Assurance Unit

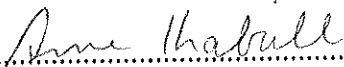
BSL BIOSERVICE-
Study No.: 094863
Test Item: PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System)
Title: Irritation Test (Intracutaneous Reactivity) with PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System)
Study Director: Dr. Sandra Schmid

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

<i>Phases of QAU Inspections</i>	<i>Dates of QAU Inspections</i>	<i>Dates of Reports to the Study Director and Management</i>
Audit Final Study Plan:	18 December 2009	18 December 2009
Audit Experimental Phase (Method Audit):	04 January 2010	04 January 2010
Audit Final Report:	17 February 2010	17 February 2010

This report reflects the raw data.

Member of the
Quality Assurance Unit:

.....
Dipl.oec.troph (FH)
Print Name: Anne Krabiell
Date: 17 Feb 2010

9. Summary

With regard to the data reported it can be stated that the intracutaneous injection of the polar extract of the test item to rabbits caused no signs of irritation compared to the injection sites of the reagent control. Very slight signs of irritation were found for the nonpolar extract as well as the nonpolar reagent control.

Species/strain:	New Zealand White Rabbits CrI: KBL (NZW)
Number of animals:	2
Vehicle and reagent control for the polar extract / Polar reagent control:	physiological saline 0.9% NaCl
Vehicle and reagent control for the nonpolar extract / Nonpolar reagent control:	cottonseed oil
Amount of substance:	0.2 mL / each test item extract 0.2 mL / each reagent control
Number of injection sites per animal:	5 / each test item extract 5 / each reagent control

The intracutaneous injection of the polar test item extract as well as the intracutaneous injection of the polar reagent control caused no signs of irritation.

The Primary Irritation Index (PII) for the polar test item extract and the polar reagent control was **0**.

The intracutaneous injection of the nonpolar test item extract as well as the intracutaneous injection of the nonpolar reagent control caused very slight signs of irritation 24 and 48 h after application and were fully reversible 72 h after application.

The Primary Irritation Index (PII) for the nonpolar test item extract and the nonpolar reagent control was **0 (control-corrected)**.

9.1. Conclusions

Under the conditions of the present study it can be stated that a polar extract of the test item PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System) caused no signs of irritation compared to the corresponding reagent control. Very slight signs of irritation were found for the nonpolar extract of the test item and the nonpolar reagent control.

The test item PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System) met the requirements of the test and will be **classified as not irritant**.

10. Aim of the Study

10.1. Justification for Selection of the Test System

The assessment of the potential of the test item to produce irritation following intracutaneous injection of extracts is performed on albino rabbits, being the recommended species for this type of study.

The degree of irritation can be evaluated after a single exposure (intracutaneous injections of a polar and a nonpolar extract of the test item).

10.2. Justification for Selection of the Test Method

No validated *in vitro* method is available for assessing acute irritation.

11. Materials and Methods

11.1. Characterisation of the Test Item

The test item and the information concerning the test item were provided by the Sponsor. All data related to the test item are the responsibility of the Sponsor and have not been verified by the test facility.

Name:	PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System)
Batch no.:	919389
Specifications:	Recycled powder was taken from part cake of an EOSINT P system after build
Sterility:	unsterile
Storage:	at room temperature
Expiry date:	not applicable
Nature of material:	synthetic polymer: Polyamide 12
Safety Precautions:	Routine hygienic procedures were sufficient to assure personnel health and safety.

11.2. Extraction of the Test Item

The extraction of the test item was performed according to ISO 10993-12.

In total a ratio of 60 cm² of sample to 20 mL of extraction medium was used.

Extraction conditions: 37 ± 1 °C for 72 ± 2 h, under agitation

Extraction media:

- physiological saline 0.9% NaCl, B. Braun Melsungen, lot no. 9435A121, expiry date: September 2012
- cottonseed oil, Sigma, lot no. 039K0020, expiry date: 07 February 2010

Up to the administration within the same day the extracts were stored at room temperature.

11.3. Controls

The corresponding extraction media served as intraspecific control and were prepared under agitation at 37 ± 1 °C for 72 ± 2 h.

- physiological saline 0.9% NaCl, B. Braun Melsungen, lot no. 9435A121, expiry date: September 2012
- cottonseed oil, Sigma, lot no. 039K0020, expiry date: 07 February 2010

Up to the administration within the same day the controls were stored at room temperature.

11.4. Test System

Species/strain: Healthy New Zealand White Rabbits, Crl: KBL (NZW)

Source: Charles River Deutschland, 97633 Sulzfeld, Germany

Sex: female

Body weight at the beginning of the study: > 2 kg

Number of animals: 2

The animals were derived from a controlled full-barrier maintained breeding system (SPF). According to Art. 9.2, No.7 of the German Act on Animal Welfare the animals were bred for experimental purposes.

11.4.1. Housing and Feeding Conditions

- Semi barrier in an air-conditioned room
- Temperature: $18 \pm 3^{\circ}\text{C}$
- Relative humidity: $55 \pm 10\%$
- Artificial light, sequence being 12 hours light, 12 hours dark
- Air change: at least 10 x / hour
- Free access to autoclaved hay and to Altromin 2123 maintenance diet for rabbits (lot no. 1046), rich in crude fibre
- Free access to tap water (drinking water, municipal residue control, microbiological control at regular intervals)
- Certificates of food, water and bedding are filed at BSL BIOSERVICE
- Housed in ABS - plastic rabbit cages, floor 4200 cm^2
- Adequate acclimatisation period (at least 5 days)

11.5. Preparation of the Animals

Approximately 6 hours before the test, the fur on the backs of the animals was closely clipped on both sides of the spinal column. Care was taken to avoid mechanical irritation and trauma. Only animals with healthy intact skin were used.

11.6. Experimental Procedure

The rabbits were injected with 0.2 mL of the polar test item extract at five sites on one side of each animal and similarly were injected with 0.2 mL of the polar reagent control at five posterior sites on the same side of each animal.

The procedure described above was repeated for the nonpolar test item extract and the nonpolar reagent control on the other side of each animal.

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

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

Table 1: Grading System for Intracutaneous Reactions

<i>Erythema and Eschar Formation</i>	
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
<i>Oedema Formation</i>	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approx. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
<i>Total possible score for irritation</i>	8

11.7. Evaluation of Results

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

The average erythema and oedema scores for the test were determined at every scoring interval for each rabbit. After the 72 hour scoring, all erythema scores plus oedema scores were totalled separately for each test item injection and reagent control injection.

Each of the totals was divided by 12 (2 animals x 3 scoring periods x 2 scoring categories) to determine the overall mean score for each test item injection versus each corresponding reagent control injection.

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

The requirements of the test are met if the difference between the test sample mean score and the vehicle blank mean score is 1.0 or less.

12. Deviations from the Study Plan

There was no deviation from the study plan.

13. Results and Discussion

The intracutaneous injection of the polar extract of the test item at doses of 0.2 mL caused no signs of irritation compared to the injection sites of the polar reagent control.

The intracutaneous injection of the nonpolar extract of the test item at doses of 0.2 mL caused very slight signs of irritation as well as the injection of the nonpolar reagent control 24 and 48 h after application. The signs of irritation were fully reversible 72 h after application.

Scoring

For individual data see Tables 2 and 3 in the appendix.

The Primary Irritation Index (PII) for the polar test item extract compared to the polar reagent control was 0.

The Primary Irritation Index (PII) for the nonpolar test item extract compared to the nonpolar reagent control was 0.

13.1. Body Weight Development

There were no significant body weight changes during observation period (Table 4 in the appendix).

13.2. Conclusions

Under the conditions of the present study it can be stated that a polar extract of the test item PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System) caused no signs of irritation compared to the corresponding reagent control. Very slight signs of irritation were found for the nonpolar extract of the test item and the nonpolar reagent control.

The test item PA 2200 Reused powder (50% virgin + 50% recycled powder from EOSINT P System) met the requirements of the test and will be **classified as not irritant**.

14. Distribution of the Report

1 original (paper):

Sponsor

1 copy (paper):

BSL BIOSERVICE

15. References

BSL BIOSERVICE, Standard Operating Procedures (SOP) No. 11-2-2

Draize, J.H. (1965)
Appraisal of the Safety of Chemicals in:
Foods, Drugs and Cosmetics - Dermal Toxicity pp. 49-52
Assoc. of Food and Drug Officials of the United States, Topeka, Kansas

Draize, J.H. (1955)
Dermal Toxicity; pp. 46-59
Association of Food and Drug Officials of the U.S., Washington D.C.

ISO 10993-1: 2009 "Evaluation and testing within a risk management process"

DIN EN ISO 10993-10: 2007 (ISO 10993-10: 2002 + Amendment 1: 2006) "Tests for irritation and delayed-type hypersensitivity"

ISO 10993-12: 2007 "Sample preparation and reference materials"

16. Appendix – Individual Data

**Table 2: Intracutaneous Reactivity in the Rabbit –
Extract with Physiological Saline 0.9% NaCl**

Test Item: PA 2200 Reused powder
(50% virgin + 50% recycled powder from EOSINT P System)

Test Item

Animal number	Injection site	Post injectionem		24 hours after injection		48 hours after injection		72 hours after injection		PIS Test
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	
1	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	
2	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	

Control

Animal number	Injection site	Post injectionem		24 hours after injection		48 hours after injection		72 hours after injection		PIS Control
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	
1	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	
2	1	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	

PII = PIS Test – PIS Control

PII:

0

**Table 3: Intracutaneous Reactivity in the Rabbit –
Extract with Cottonseed Oil**

Test Item: PA 2200 Reused powder
(50% virgin + 50% recycled powder from EOSINT P System)

Test Item

Animal number	Injection site	Post injectionem		24 hours after injection		48 hours after injection		72 hours after injection		PIS Test
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	
1	1	0	0	1	0	1	0	0	0	1.67
	2	0	0	1	0	1	0	0	0	
	3	0	0	1	0	1	0	0	0	
	4	0	0	1	0	1	0	0	0	
	5	0	0	1	0	1	0	0	0	
2	1	0	0	1	0	1	0	0	0	
	2	0	0	1	0	1	0	0	0	
	3	0	0	1	0	1	0	0	0	
	4	0	0	1	0	1	0	0	0	
	5	0	0	1	0	1	0	0	0	

Control

Animal number	Injection site	Post injectionem		24 hours after injection		48 hours after injection		72 hours after injection		PIS Control
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	
1	1	0	0	1	0	1	0	0	0	1.67
	2	0	0	1	0	1	0	0	0	
	3	0	0	1	0	1	0	0	0	
	4	0	0	1	0	1	0	0	0	
	5	0	0	1	0	1	0	0	0	
2	1	0	0	1	0	1	0	0	0	
	2	0	0	1	0	1	0	0	0	
	3	0	0	1	0	1	0	0	0	
	4	0	0	1	0	1	0	0	0	
	5	0	0	1	0	1	0	0	0	

PII = PIS Test – PIS Control

PII:

0

Table 4: Absolute Body Weights in kg

<i>Animal no.</i>	<i>1</i>	<i>2</i>
<i>Start of study (weight kg)</i>	3.8	3.7
<i>End of study (weight kg)</i>	3.8	3.8