

BIOSERVICE

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In vitro Skin Corrosion:

Human Skin Model Test

with

Diniobium Pentaoxide

Report

Version: Final

Date: 11 December 2009

BSL BIOSERVICE Study No.: 092568B

Sponsor:

CBMM Europe BV

WTC H-tower

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The Netherlands

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Amtsgericht München, HRB 109 770

Erfüllung und Gerichtsstand München

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

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 toxische 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

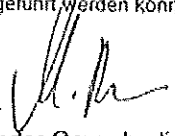
Die/Der genannte Prüfeinrichtung/Prüfstandort
befindet sich im nationalen GLP-Überwachungs-
verfahren und wird regelmäßig auf Einhaltung der
GLP-Grundsätze überwacht.

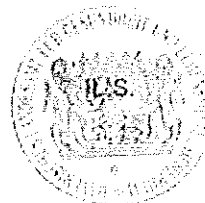
The above mentioned test facility/test site is
included in the national GLP Compliance
Programme and is inspected on a regular basis

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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3. Preface

3.1. Abbreviations

BGBI.	Bundesgesetzblatt
C	Corrosive
DMEM	Dulbecco's Modified Eagle Medium
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
GLP	Good Laboratory Practice
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NC	Non-Corrosive
NHEK	human derived epidermal keratinocytes
OD	Optical Density
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate buffered saline
QAU	Quality Assurance Unit
SOP	Standard Operating Procedure

3.2. General

Sponsor: CBMM Europe BV
WTC H-tower
Zuidplain 96
1077 XV Amsterdam
The Netherlands

Study Monitor: Mr. Jorge Davo
CBMM
Companhia Brasileira de Metalurgia
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Test Facility: BSL BIOSERVICE
Scientific Laboratories GmbH
Behringstrasse 6/8
82152 Planegg
Germany

BSL BIOSERVICE
Study No.: 092568B

Test Item: Diniobium Pentaoxide

Title: *In vitro* Skin Corrosion: Human Skin
Model Test with Diniobium Pentaoxide

3.3. Project Staff

Study Director: Dr. Dominik Stuhlmann
Deputy Study Director: Dipl.-Biol. Dagmar Lehmeier

Management: Dr. Wolfram Riedel
Dr. Angela Lutterbach

Head of Quality Assurance
Unit: Dipl.-Biol. Uwe Hamann

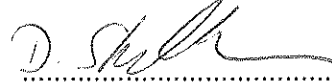
3.4. Schedule

Arrival of the Test Item: 20 July 2009
Date of Final Study Plan: 20 August 2009
Start of Experiment: 20 August 2009
End of Experiment: 27 November 2009
Date of Draft Report: 04 December 2009
Date of Final Report: 11 December 2009

4. Project Staff Signatures

Study Director

Dr. Dominik Stuhlmann



Date: 11 Dec 2009

Management



Print name:

Dr. Angela Lutterbach

Date: 11 Dec 2009

5. Quality Assurance

5.1. 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 20 June 2002 (BGB1. I Nr. 40 S. 2090), revised 31 October 2006 (BGB1. 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, Organization 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 is periodically inspected by the Quality Assurance Unit according to the corresponding SOPs. These inspections and audits are 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.

5.2. Guidelines

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

EEC Directive 2000/33/EC, Annex I, B40, 25 April 2000: "Skin Corrosion" (1).

OECD (2002). OECD Guideline for the Testing of Chemicals. No. 431: *In Vitro* Skin Corrosion: Human Skin Model Test. 13 April 2004 (2).

5.3. Archiving

The following records will be stored in the scientific archives of BSL BIOSERVICE Scientific Laboratories GmbH according to 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, remaining test item will be discarded three months after release of the report.

6. Statement of Compliance

BSL BIOSERVICE

Study No.: 092568B

Test Item: Diniobium Pentaoxide

Title: *In vitro* Skin Corrosion: Human Skin Model
Test with Diniobium Pentaoxide

Study Director: Dr. Dominik Stuhlmann

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 20 June 2002 (BGB1. I Nr. 40 S. 2090), revised 31 October 2006 (BGB1. 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. Dominik Stuhlmann



Date: 4 Jan 2010

7. Statement of the Quality Assurance Unit


BSL BIOSERVICE
Study No.: 092568B
Test Item: Diniobium Pentaoxide
Title: *In vitro* Skin Corrosion: Human Skin Model
Test with Diniobium Pentaoxide
Study Director: Dr. Dominik Stuhlmann

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

Audit	Dates of QAU Inspections	Dates of Reports to the Study Director and Management
<i>Study Plan</i>	20 August 2009	20 August 2009
<i>Experimental Phase (Project Audit)</i>	21 August 2009	21 August 2009
<i>Report</i>	21 December 2009	21 December 2009

This report reflects the raw data.

Member of the
Quality Assurance Unit:


.....
Print name: Dipl.oec.troph (FH)
Anne Krabiell
Date: 21 Dec 2009.....

8. Summary

In the present study the skin corrosivity potential of Diniobium Pentaoxide was analysed. Since corrosive chemicals are cytotoxic after a short time exposure to the stratum corneum of the epidermis the cytotoxic effects of the test item on EpiDerm™, a reconstituted three-dimensional human epidermis model, were determined. Hereby, the test item was applied topically. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT after a 3 min. and 60 min. exposure period and compared to those of the concurrent negative controls.

In this study under the given conditions the test item showed no corrosive effects. The relative mean tissue viability after 3 min. treatment was $\geq 50\%$ and after 60 min. treatment $\geq 15\%$. The test item is therefore classified as “non corrosive“.

9. Introduction

Skin corrosion refers to the production of irreversible tissue damage in the skin following the application of a test material. The two major mechanisms of skin corrosion are the destruction (erosion or solubilisation) of the skin penetration barrier (stratum corneum) including the viable cells underneath, and the rapid penetration of highly cytotoxic chemicals through the skin barrier without involving its destruction (3).

In order to replace *in vivo* testing on skin corrosion (4, 5, 6, 7) (pre-) validation studies on alternative *in vitro* methods were conducted under the auspices of ECVAM (8, 9, 10). Some alternative methods employed human skin models. It was concluded that the EpiDerm™ human skin model can be used for distinguishing between corrosive (C) and non-corrosive (NC) chemicals (11, 12). The EpiDerm™ skin corrosivity test (13) gives an excellent prediction for a wide spectrum of chemicals, shows reproducible results and provides good *in vivo* - *in vitro* correlation (10, 14, 15).

The present test is based on the experience that corrosive chemicals show cytotoxic effects following short-term exposure of the *stratum corneum* of the epidermis. The test material is applied topically to EpiDerm™, a three-dimensional human skin model comprising a reconstructed epidermis with a functional stratum corneum. Corrosive materials are identified by their ability to produce a decrease in cell viability as determined by using the MTT reduction assay (16) below defined threshold levels after a 3 min. and 60 min. exposure period.

5.1. Aim of the Study

This *in vitro* method is designed to predict and classify the skin corrosivity potential of a chemical by assessment of its effect on EpiDerm™, a reconstituted three-dimensional human epidermis model. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT after a 3 min. and 60 min. exposure period.

5.2. Justification for Selection of the Test System

The EpiDerm™ Skin Model is a well established organotypic, three-dimensional model of the human epidermis and is used for *in vitro* experiments since many years. It is known for its similarity to human skin.

5.3. Justification for Selection of the Test Method

This *in vitro* method allows the identification of corrosive chemical substances and mixtures. It further allows the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using non-animal methods.

10. Materials and Methods

10.1. Characterization 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:	Diniobium Pentaoxide
Product:	Niobium Pentoxide (Nb ₂ O ₅)
CAS no.:	1313-96-8
Batch no.:	AD/4199
Chemical name:	Niobium pentoxide
Density:	~4.5 g / cm ³
Active components:	Nb ₂ O ₅ - >98.5%
Colour:	pale yellow
Physical state:	solid
Storage:	at room temperature
Safety Precautions:	Routine hygienic procedures were sufficient to assure personnel health and safety

10.2. Preparation of the Test Item

25 mg of the test item were applied directly atop the EpiDerm™ tissue using an application spoon avoiding compression of the skin model. To ensure good contact with the skin the test item was moistened with 25 µL H₂O.

10.3. Controls

Negative control

Distilled water (A. dest.)

Positive control

8 N Potassium Hydroxide (KOH; CAS No.: 1310-58-3; Lot 121K6154, Sigma)

10.4. Dose Groups

- | | |
|---------------------|--------------------------------|
| 1. Negative control | 50 µL distilled water |
| 2. Positive control | 50 µL 8 N KOH |
| 3. Test Item | 25 mg + 25 µL H ₂ O |

The test was performed on a total of 4 tissues per dose group, 2 replicates for each treatment period (3 min. and 60 min. exposure time).

10.5. Test System

The test was carried out with the reconstituted three-dimensional human skin model EpiDerm™ (MatTek). This skin model consists of normal (non-cancerous), human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis. The NHEK are cultured on chemically modified, collagen-coated cell culture inserts (Millicell®). The EpiDerm™ skin model exhibits *in vivo*-like morphological and growth characteristics which are uniform and highly reproducible. It consists of organised basal, spinous, granular and cornified layers analogous to those found *in vivo*.

10.6. Provided Materials

The EpiDerm™ tissues were provided as kits (EPI-200; MatTek), consisting of the following components relevant for this study:

- 1 sealed 24-well plate containing 24 inserts with tissues on agarose (Lot 12290 Kit L)
- 2 24-well plates
- 4 6-well plates
- 1 bottle of serum-free DMEM-based medium (Lot 111909 TTA)

For MTT reduction assay the MTT medium was prepared by diluting a MTT stock solution of 5 mg/mL in PBS buffer (Applichem/8F006298) in a 1:5 ratio with assay medium.

10.7. Pre-Experiment

To check the MTT-reducing capability of the test item 30 mg of the test item were mixed with 1 mL MTT medium and incubated for 1 h at room temperature. If the mixture turns blue/purple, the test item is presumed to have reduced MTT. This check can only be used to explain unexpected results, but it can not be used for quantitative correction of results.

10.8. Experimental Procedure

Upon receipt of the EpiDerm™ - kit, the tissues were placed in the refrigerator (2 – 8 °C) over two nights. Then the EpiDerm™ tissues were transferred into 6-well plates containing 900 µL prewarmed assay medium per well. The 6-well plates were pre-incubated in a humidified incubator at 37 ± 1 °C, 5.0% CO₂ / 95% air for at least 1 h. Then the medium was replaced by 900 µL fresh assay medium. The 6-well plate used for the 3 min. experiment was placed back into the incubator. The other plate was used for the 60 min. treatment. About 1 h before the end of the first treatment period the MTT medium was prepared and pre-warmed

60 min. experiment: the tissues were treated with each dose group in duplicate, starting with the negative control. Start time was recorded with dosing of the first tissue. Then the 6-well plate was incubated at 37 ± 1 °C, 5.0% CO₂ / 95% air.

3 min. experiment: the tissues were treated with each dose group in duplicate, starting with the negative control. Start time was recorded with dosing of the first tissue. A constant time interval of 20 sec. was kept between dosing.

After 3 min. of application, with forceps, the first insert was removed from the 6-well plate. Using a wash bottle the tissue was gently rinsed about 20 times with PBS (phosphate buffered saline) to remove any residual test item. Excess PBS was removed by gently shaking the insert and blotting bottom with blotting paper. The insert was placed in a prepared 24-well "holding plate" containing 300 µL prewarmed assay medium per well. All inserts were treated in the same manner.

Then the inserts were transferred into a prepared 24-well "MTT assay plate" containing 300 µL prewarmed MTT medium. The plate was incubated for 3 h at 37 ± 1 °C, 5.0% CO₂ / 95% air.

60 min. experiment: after 60 min. application, with forceps, the first insert was removed from the 6-well plate. Using a wash bottle the tissue was gently rinsed about 20 times with PBS to remove any residual test item. Excess PBS was removed by gently shaking the insert and blotting bottom with blotting paper. The insert was placed in a prepared 24-well "holding plate" containing 300 µL prewarmed assay medium per well. All inserts were treated in the same manner.

Then the inserts were transferred into a prepared 24-well “MTT assay plate” containing 300 µL prewarmed MTT medium. The plate was incubated for 3 h at 37 ± 1 °C, 5.0% CO₂ / 95% air.

3 min. and 60 min. experiment: after the 3 h MTT incubation period the MTT solution was aspirated. The wells were refilled with PBS and the PBS was aspirated. The rinsing was repeated twice and the tissues were dried. Then the inserts were transferred into new 24-well “extraction plates“. 2 mL of isopropanol (Applichem, Lot: 9P004553) were pipetted into each insert, thus the insert was covered from both sides. The extraction plates were sealed in zip-bags to inhibit isopropanol evaporation. Extraction was carried out at least 2 h with shaking at room temperature.

After the extraction period the inserts were pierced with an injection needle to allow the extracts to run through the tissues into the corresponding wells. Then the inserts were discarded and the extraction plates were placed on a shaker for 15 min.

Per each tissue 3 x 200 µL aliquots of the extract were transferred into a 96-well plate and OD was measured at 550 nm without reference wavelength in a plate spectrophotometer.

10.9. Data Analysis

Corrosivity potential of the test item was predicted from the relative mean tissue viabilities obtained after 3 min. treatment compared to the negative control tissues concurrently treated with A. dest (= 100%). A test item is classified “corrosive“ (C) in any case, if the relative tissue viability after 3 min. treatment is decreased below 50%.

In addition, those test items classified as “non-corrosive“ (NC) after 3 min. (viability \geq 50%) are classified as “corrosive“ if the relative mean tissue viability after 60 min. treatment compared to the concurrent negative control tissues is decreased below 15% (Prediction Model 2 (10)).

Mean tissue viability (% negative control)	Prediction C / NC
3 min: < 50%	C
3 min: \geq 50% and 60 min: < 15%	C
3 min: \geq 50% and 60 min: \geq 15%	NC

10.10. Test Acceptance Criteria

The test meets acceptance criteria if:

- mean OD_{550 nm} of the two negative control tissues of the 3 min. and 60 min. treatment period is ≥ 0.8 ,
- mean relative tissue viability of the two positive control tissues of the 3 min. treatment period is $\leq 30\%$,
- maximum inter tissue viability difference between two tissues treated identically is $\leq 30\%$.

11. Deviations from the Study Plan

Concerning:

10.2. Preparation of the Test Item

Before:

25 mg of the test item will be applied directly atop the EpiDerm™ tissue using an application spoon avoiding compression of the test item.

New:

25 mg of the test item were applied directly atop the EpiDerm™ tissue using an application spoon avoiding compression of the skin model.

Reason for alteration:

Typing error.

Concerning:

10.6. Provided Materials: MTT assay kit

Before:

For MTT reduction assay the MTT assay kit (MTT-100; MatTek) is provided, consisting of:

- 1 vial MTT concentrate
- 1 vial MTT diluent (supplemented DMEM)
- 1 bottle extractant solution (Isopropanol)

New:

For MTT reduction assay the MTT medium was prepared by diluting a MTT stock solution of 5 mg/mL in PBS buffer (Applichem/8F006298) in a 1:5 ratio with assay medium.

Reason for alteration:

The MTT assay kit was not provided by MatTek.

Concerning:

10.8. Experimental Procedure

Before:

Upon receipt of the EpiDerm™ - kit, the tissues were placed in the refrigerator (2 – 8 °C) over night. On the following day the EpiDerm™ tissues were transferred into 6-well plates containing 900 µL prewarmed assay medium per well.

New:

Upon receipt of the EpiDerm™ - kit, the tissues were placed in the refrigerator (2 – 8 °C) over two nights. Then the EpiDerm™ tissues were transferred into 6-well plates containing 900 µL prewarmed assay medium per well.

Reason for alteration:

Repetition of the test was necessary.

Concerning:

10.8. Experimental Procedure

Before:

MTT solution

New:

MTT medium

Reason for alteration:

Typing error.

These deviations did not affect the quality and integrity of the study.

12. Results

12.1. Pre-Experiment

The mixture of 30 mg test item per 1 ml MTT medium showed no reduction of MTT compared to the solvent. No influence on the assay was detectable. The OD₅₅₀ was 0.687 (solvent control: 0.778).

12.2. Experiment

3 min.	Negative Control		Test Item		Positive Control	
	tissue 1	tissue 2	tissue 1	tissue 2	tissue 1	tissue 2
absolute OD ₅₅₀ -values	1.696	1.797	1.532	1.777	0.154	0.154
	1.721	1.829	1.527	1.654	0.146	0.151
	1.763	1.774	1.442	1.728	0.154	0.156
OD ₅₅₀ (mean of 3 aliquots)	1.727	1.8	1.5	1.72	0.151	0.154
OD ₅₅₀ (mean of 2 replicate tissues)	1.763*		1.61		0.152	
mean relative tissue viability [%]	100		91		9**	
inter tissue viability difference [%]***	4.2		13.6		1.5	

* mean OD₅₅₀ ≥ 0.8

** mean relative tissue viability of the 3 min. positive control ≤ 30%

*** inter tissue viability difference ≤ 30%

60 min.	Negative Control		Test Item		Positive Control	
	tissue 1	tissue 2	tissue 1	tissue 2	tissue 1	tissue 2
absolute OD ₅₅₀ - values	2.045	2.028	1.389	1.268	0.133	0.130
	2.021	1.984	1.4	1.298	0.133	0.126
	0.068	2.021	1.397	1.262	0.135	0.131
OD ₅₅₀ (mean of 3 aliquots)	2.045	2.011	1.395	1.276	0.134	0.129
OD ₅₅₀ (mean of 2 replicate tissues)	2.028*		1.336		0.131	
mean relative tissue viability [%]	100		66		6	
inter tissue viability difference [%]**	-1.7		8.9		-3.7	

* mean OD₅₅₀ ≥ 0.8

** inter tissue viability difference ≤ 30%

13. Discussion

The potential of the test item to induce skin corrosion was analysed by using the three-dimensional human skin model EpiDerm™, comprising a reconstructed epidermis with a functional stratum corneum.

In the present study Diniobium Pentaoxide was applied topically to the EpiDerm™ tissue for 3 min. and 60 min. followed by immediate determination of cytotoxic effects via MTT reduction assay.

Corrosivity potential of the test item was predicted from the relative mean tissue viabilities obtained after both treatment periods compared to the corresponding negative control tissues concurrently treated with A. dest.

The test item showed no corrosive effects. The mean relative tissue viability (% negative control) was $\geq 50\%$ (91%) after 3 min. treatment and $\geq 15\%$ (66%) after 60 min. treatment.

The controls confirmed the validity of the study. The mean OD₅₅₀ of the two negative control tissues was ≥ 0.8 for each exposure period. The mean relative tissue viability (% negative control) of the positive control was $\leq 30\%$ (9%) after 3 min. treatment. The maximum inter tissue difference of replicate tissues of all dose groups was $\leq 30\%$ (1.5% - 13.6%).

13.1. Conclusions

In this study under the given conditions the test item showed no corrosive effects. The test item is classified as “non corrosive“.

14. Distribution of the Report

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15. References

- (1) EC (2000). Annex I to Commission Directive 2000/33/EC adapting to technical progress for the 27th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities* **L136**, 91 – 97.
- (2) OECD (2002). OECD Guideline for the Testing of Chemicals. No. 431: *In Vitro* Skin Corrosion: Human Skin Model Test. 13 April 2004.
- (3) European Centre for the Validation of Alternative Methods (ECVAM) (2002). EPIDERM™ Skin Corrosivity Test. Last update October 2002. Supplied 27 January 2003. Available: <http://ecvam-sis.jrc.it/>
- (4) Worth, A.P., Fentem, J.H., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J. & Liebsch, M. (1998). An evaluation of the proposed OECD testing strategy for skin corrosion. *ATLA* **26**, 709 – 720.
- (5) Botham, P. (1999). Skin Corrosion and Irritation: Toward Complete Replacement. *ATLA* **27**, 108.
- (6) Worth, A. & Balls, M. (2002). Alternative (Non-Animal) Methods for Chemicals Testing: Current Status and further Prospects. Chapter 5: Local Toxicity: Acute Dermal and Ocular Effects. *ATLA* **30**, Suppl. 1, 35 – 47.
- (7) Robinson, M.K., Cohen, C., de Fraissinette Ade, B., Ponec, M. & Fentem, J.H. (2002). Non-animal testing strategies for assessment of skin corrosion and skin irritation potential of ingredients and finished products. *Food Chem Toxicol.* **40**, 573-592.
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