REPORT

Perlazid® Vlies Duplex 60.03: Systemic Injection Test in Mice

Study Director:	Dr. M. Mallaun
Test Facility:	**Harlan Laboratories Ltd.** Wölferstrasse 4 4414 Füllinsdorf / Switzerland
Sponsor:	**Perlen Converting AG** Perlenring 3 6035 Perlen / Switzerland
Study Identification:	Harlan Laboratories Study **D03717**
Version:	Draft 1
Study Completion Date:	DD-MM-YYYY

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GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Harlan Laboratories Study:	D03717
Test Item:	Perlazid® Vlies Duplex 60.03
Study Director:	Dr. M. Mallaun
Study Title:	Perlazid® Vlies Duplex 60.03: Systemic Injection Test in Mice

The stability of the test item extract under the test conditions is unknown and is therefore excluded from this statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

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

Study Director: Dr. M. Mallaun

.....
Date:

QUALITY ASSURANCE STATEMENT

Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland

Harlan Laboratories Study:	D03717
Test Item:	Perlazid® Vlies Duplex 60.03
Study Director:	Dr. M. Mallaun
Study Title:	Perlazid® Vlies Duplex 60.03: Systemic Injection Test in Mice

The general facilities and activities are inspected at least once a year and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections	Dates of Reports to the Study Director and Test Facility Management

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

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Date:

PREFACE

General Information

Test Item:	Perlazid® Vlies Duplex 60.03
Study Title:	Perlazid® Vlies Duplex 60.03: Systemic Injection Test in Mice
Sponsor:	Perlen Converting AG Perlenring 3 6035 Perlen / Switzerland
Study Monitor:	Dr. S. Bokorny Phone: +41 41 455 8801 Email: stefan.bokorny@perlen.ch
Test Facility:	Harlan Laboratories Ltd. Wölferstrasse 4 4414 Füllinsdorf / Switzerland
QA:	Harlan Laboratories Ltd. Quality Assurance GLP Zelgliweg 1 4452 Itingen / Switzerland

Responsibilities

Study Director:	Dr. M. Mallaun
Deputy Study Director:	Dr. M. Sieber
Laboratory / Technical Coordinator:	C. Weng

Quality Assurance:

Head of QA:	T. Fink

Schedule

Experimental Starting Date:	17-Nov-2010
Termination (Necropsy):	25-Nov-2010
Delivery of Animals:	17-Nov-2010
Acclimatization:	17-Nov-2010 to 21-Nov-2010
Administration / Treatment:	22-Nov-2010
Experimental Completion Date:	25-Nov-2010

Data Requirements / Test Guidelines

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

- ISO 10993-1: 2009 – Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process.
- ISO 10993-11: 2006 – Biological evaluation of medical devices – Part 11: Tests for systemic toxicity.
- ISO 10993-12: 2007 – Biological evaluation of medical devices – Part 12: Sample preparation and reference materials.
- USP 32 – NF27, The United States Pharmacopeia, Inc. (88) Biological Reactivity Tests, in vivo. Systemic Injection Test. Official May 01, 2009.

Animal Welfare

This study was performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 82.

Archiving

Harlan Laboratories Ltd. (4452 Itingen / Switzerland) will retain the study plan, raw data, sample of test item and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

1 SUMMARY

Four groups, each of five female Crl:NMRI (SPF) mice, were treated by intravenous or intraperitoneal injection with the extract of Perlazid® Vlies Duplex 60.03 or with the corresponding blanks as follows:

Test Material	Dose	Application Route	Injection Rate (mL/min)
Polar Extract of Test Item in Sterile Physiological Saline	5 mL/kg	i.v.	0.6
Polar Blank (Sterile Physiological Saline)	5 mL/kg	i.v.	0.6
Non-polar Extract of Test Item in Sesame Oil	50 mL/kg	i.p.	-
Non-polar Blank (Sesame Oil)	50 mL/kg	i.p.	-

The animals were examined for clinical signs immediately and four hours after injection on test day 1 and not earlier than 24, 48 and 72 hours after injection. Mortality/viability was recorded daily during acclimatization, together with the clinical signs on test day 1 and twice daily from test days 2 to 4. Body weights were recorded on test day 1 prior to administration and at termination of the test.

One female of group 1 treated with the polar test item extract showed immediately after administration hunched posture, mild signs of ruffled fur, tachypnea and a moderate sedation. The ruffled fur and hunched posture persisted up to test day 2 and had completely reversed by test day 3.

All animals of group 3 and 4 (non-polar extract and non-polar blank) were noted with oily fur on test day one, which had reversed by test day 2.

A loss of body weight (≤ 2 g) was observed in 8 animals. The body weight loss was distributed in 1 animal from the polar extract, in 2 animals from the polar blank, in 4 animals from the non-polar extract and in 1 animal from the non-polar blank. The last animal of the non-polar extract and 1 animal from the polar extract showed a body weight loss of 1 g. Two animals of the polar extract, 1 animal of the polar blank and 4 animals of the non-polar blank (a total of 7 animals) did not loss or gain weight between the treatment start and the termination of the study. Three animals (1 from the polar extract and 2 from the polar blank) exhibited a body weight gain ≥ 2 g. With these results, the body weight losses observed are considered to be tolerated, except in the non-polar extract group.

Based on the results obtained and according to the criteria of the referred guidelines, it can be concluded that Perlazid® Vlies Duplex 60.03 meets the requirements of this test by polar exposure. However, the non-polar sample extract did not meet the requirements of the test.

2 PURPOSE

The purpose of this study was to assess the toxic potential when single doses of different extracts from Perlazid® Vlies Duplex 60.03 are injected intravenously and intraperitoneally.

This study should provide a rational basis for risk assessment in man.

The test item was administered according to the following scheme and levels:

Test Item Extract	Dose	Application Route
Extract of test item in sterile physiological saline	5 mL/kg	i.v.
Extract of test item in sesame oil	50 mL/kg	i.p.

Rationale: Intravenous and intraperitoneal injection have been proven to be efficacious in achieving high rates of test item absorption.

3 MATERIALS AND METHODS

3.1 Test System

Animals:	Mouse, Crl:NMRI (SPF)
Rationale:	Recognized by international guidelines as a recommended test system.
Breeder:	Charles River, Research Models and Services Germany GmbH Sandhofer Weg 7 97633 Sulzfeld / Germany
Number of Animals per Group:	5 females (nulliparous and non-pregnant)
Total Number of Animals:	20 females (nulliparous and non-pregnant)
Age at Treatment:	4-5 weeks
Body Weight at Treatment:	22 – 27 g
Identification:	By unique cage number and corresponding color-coded spots on the tail.
Randomization:	Randomly selected by hand at time of delivery into groups of five.
Acclimatization:	Under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

3.2 Allocation

The animals were distributed as follows:

	Number of Animals per Group	Animal Numbers
1 Polar Extract of Test Item in Sterile Physiological Saline	5	21 - 25
2 Polar Blank (Sterile Physiological Saline)	5	26 - 30
3 Non-polar Extract of Test Item in Sesame Oil	5	31 - 35
4 Non-polar Blank (Sesame Oil)	5	36 - 40

3.3 Husbandry

Room Number:	0106 / Harlan Laboratories Ltd., Füllinsdorf
Conditions:	Air-conditioned with a room temperature of 22 ± 3 °C, a relative humidity between 30-70% and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the daytime light period.
Accommodation:	Groups of five in Makrolon type-3 cages with standard softwood bedding ('Lignocel' J. Rettenmaier&Söhne GmbH&CoKG, 73494 Rosenberg / Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland).
Diet:	Pelleted Teklad Rat-Mouse Diet 2914C (batch no. 30/10, provided by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) available *ad libitum*. Results of analyses for contaminants were archived at Harlan Laboratories Ltd.
Water:	Community tap water from Füllinsdorf, available *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at Harlan Laboratories Ltd.

3.4 Test Item and Extraction Media

3.4.1 Test Item

The following information was provided by the Sponsor:

Identification:	Perlazid® Vlies Duplex 60.03
Description:	Medical device, transparent film
Batch Number:	06.08.10
Purity:	> 98% according to the inorganic substances
Stability of Test Item:	Stable under storage conditions.
Expiry Date:	06-Aug-2012

Stability of Test Item Extracts:	Unknown; excluded from the statement of compliance
Storage Conditions:	At room temperature (range of 20 ± 5 °C, provided by Harlan Laboratories Ltd.), light protected.
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask)

3.4.2 Extraction Media

The following information was provided by Harlan Laboratories Ltd.:

Physiological Saline

Identification:	Physiological saline
Description:	Colorless liquid
Batch Number:	0183A231
Source:	B. Braun Medical AG 6020 Emmenbrücke / Switzerland
Expiry Date:	30-Apr-2013
Storage Conditions:	In the refrigerator (range of 5 ± 3 °C), light protected.
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask)

Sesame Oil

Identification:	Sesame oil
Description:	Slightly yellow clear liquid
Lot Number:	BCBC6505
Source:	Fluka; Sigma-Aldrich Chemie GmbH 9471 Buchs / Switzerland
Stability of the Vehicle:	Stable under storage conditions
Expiry Date:	30-Nov-2015
Storage Conditions:	At room temperature (range of 20 ± 5 °C), light protected.
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask).

3.5 Preparation of Extract

Sample extracts were prepared at Harlan Laboratories Ltd., Füllinsdorf with the following extracting media according to the referred guidelines and as requested by the Sponsor:

Sterile physiological saline (NaCl 0.9%)
Sesame oil (= "vegetable oil").

The test item is an antimicrobial, non-woven material with an acrylate based coating system. Since the test item has an absorption potential, it was extracted with a ratio of 0.1 g/mL, in accordance with the ISO guideline (ISO 10993-12).

The absorption capacity of the test item (mL extraction medium / 0.1g test item) was determined in the two extraction media before the study initiation. This procedure is therefore excluded from the statement of compliance. The following absorption capacities were recorded: 0.70 mL of physiological saline / 0.1g test item and 1.23 mL of sesame oil / 0.1g test item. This additional volume was added to each 0.1g in the extraction mixture.

To prepare the test item extract, an extraction ratio of 0.1 g/mL was used. The absorption capacity of the test item, as determined, was added to the extraction volume.

The samples were extracted by heating at 37 ± 1°C for 72 ± 2 hours. After completion of the extraction period, the extracts were vigorously shaken manually for 1-2 minutes andthe containers were allowed to set at room temperature for approximately 0.5 hours. Each extract was decanted into a separate dry sterile vessel. The extracts were kept at room temperature on the day of application.

The blanks (physiological saline and sesame oil) were prepared in a similar manner as the extracts.

The temperature and pH of the extracts and blanks were measured before application and were found to be as follows:

Test Substance	Temperature in °C	pH
Polar Extract in Sterile Physiological Saline	24.9	5-6
Polar Blank (Sterile Physiological Saline)	23.7	5-6
Non-polar Extract in Sesame Oil	24.8	-
Non-polar Blank (Sesame Oil)	24.7	-

Extracts or blanks with a pH ≤ 2 or ≥ 11.5 must not be administered for animal welfare reasons.

Prior to withdrawal of each injection dose, the homogeneity of the extracts and blanks was maintained during administration using a magnetic stirrer. The extracts were used for testing within 24 hours.

3.6 Test Item Administration

Each test item extract or the corresponding blank was injected into groups of 5 animals by the route described in the table below.

3.7 Amounts and Routes of Systemic Injection of Extract and Blank

Test Material	Dose	Application Route	Injection Rate (mL/min)
Polar Extract of Test Item in Sterile Physiological Saline	5 mL/kg	i.v.	0.6
Polar Blank (Sterile Physiological Saline)	5 mL/kg	i.v.	0.6
Non-polar Extract of Test Item in Sesame Oil	50 mL/kg	i.p.	-
Non-polar Blank (Sesame Oil)	50 mL/kg	i.p.	-

According to the USP 32 and ISO 10993-11, 2006 the dose volume requested (USP 32) or proposed (ISO) for i.v. injection was 50 mL/kg. According to the journal of applied toxicology 21, 15-23 (2001) "A good practice guide to the administration of substance and removal of blood, including routes and volumes", the highest recommended dose for mice is 5 mL/kg.

3.8 Observations

Mortality / Viability:	Daily during the acclimatization period, together with the clinical signs on test day 1 and twice daily from test days 2 to 4.
Body Weights:	On test day 1 and at termination of the test.
Clinical Signs:	Daily during the acclimatization period. The animals were observed immediately and 4 hours after injection on test day 1 and than not earlier than 24, 48 and 72 hours after injection.

3.9 Pathology

3.9.1 Necropsy

No intercurrent deaths occurred, hence no necropsy was performed. All animals were sacrificed at the end of the observation period by carbon dioxide asphyxiation and discarded.

3.10 Statistical Analysis

No statistical analysis was used.

3.11 Data Compilation

Body weights were recorded on-line.

Clinical signs were recorded on data sheets.

Mortality/viability was recorded on data sheets.

The RCC Tox Computer System (RCC-Tox-Lims) had been validated with respect to data collection, storage and retrievability.

3.12 Interpretation

If during the observation period none of the animals treated with the extract of the samples shows a significantly greater biological reactivity than the animals treated with blank, the sample meets the requirements of this test. If two or more animals die, or if abnormal behavior such as convulsions or prostration occurs in two or more animals or if a body weight loss greater than 2 g occurs in three or more animals, the sample does not meet the requirements of the test.
If any animal treated with the sample shows slight signs of biological reactivity or dies, the test is repeated using groups of 10 animals each. In the repeated test, the requirements are met if none of the animals treated with an extract shows a significantly greater reaction than the animals treated with the respective blank.

4 RESULTS AND DISCUSSION

4.1 Mortality / Viability and Macroscopic Findings

No deaths occurred during the course of the study, hence no necropsy was performed.

4.2 Body Weights

(See Summary Tables on p. 20 and Individual Tables on p. 22)

A loss of body weight (≤ 2 g) was observed in 8 animals. The body weight loss was distributed in 1 animal (No. 22) from the polar extract, in 2 animals (Nos. 26 and 30) from the polar blank, in 4 animals (Nos. 31, 32, 34, 35) from the non-polar extract and in 1 animal(No. 37) from the non-polar blank. The last animal (No. 33) of the non-polar extract and 1 animal (No. 21) from the polar extract showed a body weight loss of 1 g. Two animals (Nos. 23 and 24) of the polar extract, 1 animal (No. 29) of the polar blank and 4 animals (Nos. 36, 38 – 40) of the non-polar blank (a total of 7 animals) did not loss or gain weight between the treatment start and the termination of the study. Three animals (1 from the polar extract, No. 25 and 2 from the polar blank, Nos. 27 - 28) exhibited a body weight gain ≥ 2 g. With these results, the body weight losses observed are considered to be tolerated, except in the non-polar extract group.

4.3 Clinical Signs

(See Individual Tables on p. 23)

One female (No. 21) of group 1 treated with the polar test item extract showed immediately after administration hunched posture, mild signs of ruffled fur, tachypnea and a moderate sedation. The ruffled fur and hunched posture persisted up to test day 2 and had completely reversed by test day 3.

4.4 Other Findings

All animals of group 3 and 4 (non-polar extract and blank) were noted with oily fur on test day one, which had reversed by test day 2.

5 CONCLUSION

Based on the results obtained and according to the criteria of the referred guidelines, it can be concluded that Perlazid® Vlies Duplex 60.03 meets the requirements of this test by polar exposure. However, the non-polar sample extract did not meet the requirements of the test.

6 REFERENCES

1. Diel K.H. et al. A good practice guide to the administration of substance and removal of blood, including routes and volumes. J. Appl. Toxicol. 21, 15-23 (2001).

7 SUMMARY TABLES

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 EXTRACT IN NACL 0.9% I.V	GROUP 2 NACL 0.9% BLANK I.V
DAY 1 WEEK 1	MEAN	24	24
	ST.DEV.	0.8	1.3
	MINIMUM	24	22
	MAXIMUM	26	25
	N	5	5

	GROUP 3 EXTRACT IN SESAME OIL I.P	GROUP 4 SESAME OIL BLANK I.P
MEAN	26	25
ST.DEV.	1.3	1.4
MINIMUM	24	23
MAXIMUM	27	26
N	5	5

		GROUP 1 EXTRACT IN NACL 0.9% I.V	GROUP 2 NACL 0.9% BLANK I.V
DAY 4 WEEK 1	MEAN	25	23
	ST.DEV.	3.4	1.5
	MINIMUM	23	21
	MAXIMUM	31	25
	N	5	5

	GROUP 3 EXTRACT IN SESAME OIL I.P	GROUP 4 SESAME OIL BLANK I.P
MEAN	24	25
ST.DEV.	0.7	1.6
MINIMUM	23	23
MAXIMUM	24	26
N	5	5

8 INDIVIDUAL TABLES

BODY WEIGHTS (GRAM) INDIVIDUAL
FEMALES

	TREATMENT	
DAYS	1	4
WEEKS	1	1
ANIMAL		
GROUP 1 (EXTRACT IN NACL 0.9% I.V)		
21	24	23
22	26	23
23	24	24
24	24	24
25	24	31
GROUP 2 (NACL 0.9% BLANK I.V)		
26	24	22
27	23	25
28	22	24
29	25	25
30	25	21
GROUP 3 (EXTRACT IN SESAME OIL I.P)		
31	26	23
32	26	24
33	24	23
34	27	24
35	26	24
GROUP 4 (SESAME OIL BLANK I.P)		
36	26	26
37	25	23
38	25	25
39	23	23
40	26	26

Clinical Signs

Group	Animal No.	Sex	Signs	Test days				
				1		2	3	4
				0 h*	4 h*	24 h	48 h	72 h
1	21	F	No clinical signs				√	√
			Hunched Posture	√	√			
			Sedation	2				
			Tachypnea	√				
			Ruffled fur		1	1		
	22	F	No clinical signs	√	√	√	√	√
	23	F	No clinical signs	√	√	√	√	√
	24	F	No clinical signs	√	√	√	√	√
	25	F	No clinical signs	√	√	√	√	√
2	26	F	No clinical signs	√	√	√	√	√
	27	F	No clinical signs	√	√	√	√	√
	28	F	No clinical signs	√	√	√	√	√
	29	F	No clinical signs	√	√	√	√	√
	30	F	No clinical signs	√	√	√	√	√
3	31	F	No clinical signs	x	x	√	√	√
	32	F	No clinical signs	x	x	√	√	√
	33	F	No clinical signs	x	x	√	√	√
	34	F	No clinical signs	x	x	√	√	√
	35	F	No clinical signs	x	x	√	√	√
4	36	F	No clinical signs	x	x	√	√	√
	37	F	No clinical signs	x	x	√	√	√
	38	F	No clinical signs	x	x	√	√	√
	39	F	No clinical signs	x	x	√	√	√
	40	F	No clinical signs	x	x	√	√	√

*Observations were made immediately (0 hours) and 4 hours after application.

Key: √ = noted, grade 1= slight, grade 2=moderate, x = oily fur at the injection site

No clinical signs were evident in any animal during the acclimatization period.