Final Report

Study Title	Assessment of *in vitro* skin irritation using EpiSkin™
Test articles	PIP silicone gel breast implants: IMGHC-TX-H, batch 16306 IMGHC-TX-H, batch 02808 IMGHC-TX-H, batch 27609 IMGHC-TX-H, batch 41609 IMGHC-TX-S, batch 37908 [redacted]control silicone gel breast implant: [redacted]
Author	[redacted]
Sponsor	Medicines and Healthcare products Regulatory Agency 151 Buckingham Palace Road London SW1W 9SZ UK
Study Monitor	[redacted]
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire HG3 1PY ENGLAND
Covance Study Number	8265941
Report Issued	December 2012
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STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

Assessment of *in vitro* skin irritation using EpiSkin™

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

With the exception of those elements detailed below (*), this study was conducted in compliance with:

- The United Kingdom (GLP Monitoring Authority, Medicines and Healthcare products Regulatory Agency (MHRA)) Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No. 3106, as amended by the Good Laboratory Practice (Codification Amendments etc.) Regulations 2004.

- OECD Principles on Good Laboratory Practice (revised 1997, issued Jan 1998) ENV/MC/CHEM(98)17.

(*) Test article purity was not known at the time of finalisation of this study report.

[redacted]

12 December 2012
Date

[redacted]
Study Director
Covance Laboratories Ltd

QUALITY ASSURANCE STATEMENT

Assessment of *in vitro* skin irritation using EpiSkin™

This study has been reviewed by the GLP Quality Assurance Unit of Covance and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates			Date Reported to SD and SD
From	To	Phase	Management
01 Jun 2012	01 Jun 2012	Protocol Review	01 Jun 2012
13 Jun 2012	13 Jun 2012	Treatment	13 Jun 2012
20 Nov 2012	22 Nov 2012	Draft Report and Data Review	23 Nov 2012
12 Dec 2012	12 Dec 2012	Final Report Review	12 Dec 2012

Process

Inspection Dates			Date Reported to SD and SD
From	To	Phase	Management
06 Jun 2012	06 Jun 2012	Dose Preparation	06 Jun 2012
04 Jul 2012	04 Jul 2012	Data Collation and Transfer	04 Jul 2012
24 Jul 2012	24 Jul 2012	Stock Solution Preparation	24 Jul 2012
26 Jul 2012	26 Jul 2012	Stock Solution Preparation	26 Jul 2012
26 Jul 2012	26 Jul 2012	Dose Preparation	26 Jul 2012
22 Aug 2012	22 Aug 2012	Dose Preparation	22 Aug 2012
06 Sep 2012	06 Sep 2012	Dose Preparation	06 Sep 2012

[redacted]

Quality Assurance Unit

12TH December 2012

Date

RESPONSIBLE PERSONNEL

Assessment of *in vitro* skin irritation using EpiSkin™

The following staff were responsible for key elements of the study:

Formulations	[redacted]
Laboratory Operations	
Quality Assurance	

ARCHIVE STATEMENT

Assessment of *in vitro* skin irritation using EpiSkin™

All primary data, or authenticated copies thereof and specimens will be retained in the Covance Laboratories Limited archives for one year after issue of the final report. At this time the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf and notified of the financial implications of each of these options. One copy of the protocol and final report will be held indefinitely in the Covance Laboratories Limited archives.

CONTENTS

1 SUMMARY

This study was conducted to determine whether the test articles cause irritation in the *in vitro* skin model EpiSkin™. The study used polar and non-polar solvents to extract any leachable inherent or extraneous substances present in or on the test articles.

Extracts of each PIP implant and the [redacted] implant were prepared by immersing the required amount of implant in extract media and incubating at 37ºC for 72 hours, with shaking.

At the end of the extraction period, EpiSkin™ inserts were treated with test article extract, vehicle control, negative control (phosphate buffered saline (PBS)) and positive control (5% w/v sodium dodecyl sulphate (SDS)) for 15 minutes. At the end of the treatment period, the tissues were washed with PBS and placed on an appropriate medium and incubated for 42 hours as a post recovery period. The skin irritation potential was classified according to the remaining cell viability obtained after test article treatment.

The results were as follows:

Test article	Batch number	% Group mean viability	
		Water extract	DMSO extract
PIP Implant IMGHC-TX-H	16306	86.3	139.7
PIP Implant IMGHC-TX-H	02808	115.3	123.0
PIP Implant IMGHC-TX-H	27609	143.5	148.7
PIP Implant IMGHC-TX-H	41609	175.4	128.1
PIP Implant IMGHC-TX-S	37908	125.7	138.2
[redacted]	[redacted]	129.8	112.9

The PIP implants and the [redacted] implant did not reduce viability of the epidermal tissue to below 50% of the negative control and were therefore considered to be non-irritant to the *in vitro* skin model EpiSkin™.

2 INTRODUCTION

This study was conducted to determine whether the test articles cause irritation in the *in vitro* skin model EpiSkin™. The study used polar and non-polar solvents to extract any leachable inherent or extraneous substances present in or on the test articles.

The extracts were applied topically to a three-dimensional human skin model, comprising a reconstructed epidermis with a functional stratum corneum. Irritant materials are identified by their ability to produce a decrease in cell viability (as determined using the MTT reduction assay) below defined thresholds after a specified exposure period. The principle of the human skin model assay is based on the hypothesis that irritant chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the underlying cell layers.

Study dates

Protocol signed by Study Director:	31 May 2012
Experimental start date:	13 June 2012
Experimental completion date:	29 September 2012

The study completion date is the date the final report is signed by the Study Director.

3 PROTOCOL ADHERENCE

The study was conducted in accordance with the agreed definitive protocol. There were no major deviations from the protocol. Minor deviations, which did not affect the integrity or outcome of the study, are presented in the body of the report.

4 TEST AND CONTROL ARTICLES

The test articles were received at Covance as follows:

Test article	Batch number	Quantity supplied	Expiry Date	Date of receipt at Covance
PIP Implant IMGHC-TX-H	16306	1 x 130 cc implants	April 2011#	23 May 2012
PIP Implant IMGHC-TX-H	02808	2 x 130 cc implants	31 January 2013	23 May 2012
PIP Implant IMGHC-TX-H	27609	2 x 270 cc implants	30 June 2014	23 May 2012
PIP Implant IMGHC-TX-H	41609	2 x 270 cc implants	30 November 2014	23 May 2012
PIP Implant IMGHC-TX-S	37908	2 x 225 cc implants	30 June 2013	23 May 2012
[redacted]	[redacted]	2 x 220 cc implants	31 October 2016*	25 May 2012

- The Sponsor confirmed that this test article was suitable for use in the study despite the package expiry date of April 2011.

* - The expiry date specified in the protocol was January 2016.

Purity values for the test articles were not stated by the Sponsor.

When not in use test articles were stored in sealed containers, at room temperature in the dark.

The negative control substance was phosphate buffered saline supplied by Severn Biotech, Kidderminster, UK.

The positive control substance was 5% w/v sodium dodecyl sulphate supplied by Sigma-Aldrich Co Ltd, Poole, UK.

Positive and negative controls were performed for each study in order to ensure adequate performance of the experimental model.

5 EXPERIMENTAL DESIGN

5.1 Regulatory test guidelines

The study was conducted to meet the known requirements of Method B46 of Commission Regulation (EC) No 761/2009 and OECD Guidelines for Testing of Chemicals Method 439 (adopted 22 July 2010).

Test article preparation was based on BS EN ISO 10993-12: 2007 (Biological Evaluation of Medical Devices – Part 12: Sample preparation and reference materials).

5.2 Assessment of MTT interacting substances

In order to assess the potential non-specific reduction of the test articles, a dose of each test article extract was added to 2 mL of 0.3 mg/mL MTT and colour change assessed after three hours. There was no change in colour therefore the test article extracts did not interact with MTT.

5.3 Application of test and control substances

EpiSkin™ tissues were kept in their packaging at room temperature in a microbiological safety cabinet until the next step. The tissues were set up 24 hours prior to treatment by placing each tissue onto 2 mL pre-warmed maintenance medium (supplied with the EpiSkin™ tissues) in 12-well plates and incubating at 37°C.

The test was performed on a total of three tissues per test article extract, vehicle control, negative control and positive control. Immediately prior to treatment initiation, the media under the tissues was replaced with 2.2 mL of pre-warmed assay medium (supplied with the EpiSkin™ tissues).

A volume of 40 µL of each test article extract was added topically to the tissue. A volume of 40 µL of the vehicle control, positive and negative control solutions was used.

Exposure was for 15 minutes after which, the tissues were washed using PBS and dried using cotton wool buds to remove residual material before being transferred to a new well containing 2 mL pre-warmed maintenance medium. The tissues were then incubated at 37°C for the 42 hour recovery time period.

5.4 Cell viability measurements

Upon completion of the 42 hour recovery period, the base of each tissue was rinsed with PBS before being placed on top of 2 mL of 0.3 mg/mL (final concentration) MTT in medium and incubated for three hours (37°C, 5% CO_2). After incubation, the tissues were removed from the MTT solution, their bases rinsed with PBS and then placed on absorbent dry paper. The white ring was discarded and the epidermis was separated from the support layer. Both parts were placed into a sterile, sealable eppendorf containing 0.5 mL isopropanol. The tubes were sealed and vortexed to ensure thorough mixing and left at room temperature overnight protected from light.

At the end of the extraction, the tubes were vortexed to ensure a homogenous colour. Two x 200 µL samples were placed into a 96-well plate per tube for spectrophotometric determination of optical density at 570 nm using extraction solvent as a blank. Tissue viability was calculated for each tissue as a percentage of the mean of the negative control tissue.

6 TEST ARTICLE FORMULATION

Test article extracts were prepared in a polar vehicle (sterile purified water) and a non-polar vehicle (DMSO).

The silicone gel breast implant was pierced to allow sampling of the silicone gel present within the outer shell. An appropriate amount of silicone gel was taken and cut into pieces of approximately 1 cm^3 and the required volume of extraction vehicle was added so that extractions were performed at 200 mg/mL.

The container was then sealed and incubated at 37°C for a period of 72 hours, with shaking.

Containers of extraction vehicle alone were subjected to the same extraction conditions.

6.1 Formulations analysis

Samples of polar and non-polar extracts from each test article and the reference article were taken at the end of the extraction period, consisting of 2 x 1 mL aliquots (random) from all extracts and 2 x 1 mL aliquots from vehicle control.

The samples were stored in uniquely labelled brown glass crimped top vials, at 15 to 25°C. One set of duplicate samples along with any remaining portion of [redacted] control silicone gel breast implant, Model Name [redacted] was sent to LGC, Runcorn, Cheshire, UK.

Any subsequent analysis of these samples was not conducted or reported under this study number.

The reserve samples were discarded on finalisation of the study report.

7 TEST SYSTEM

7.1 Specification

Three-dimensional human skin model, comprising a reconstructed epidermis with a functional stratum corneum, supplied by SkinEthic Laboratories, Lyon France.

7.2 Identification

The test system was appropriately labelled with the study number, date of treatment, duration of treatment, negative/positive/test article.

7.3 General model conditions

Human keratinocytes are used to construct the epithelium. Multiple layers of viable epithelial cells are present under a functional stratum corneum. The stratum corneum is multi-layered with the necessary lipid profile to produce a functional barrier. The containment properties of the model prevent the passage of material around the stratum corneum to the viable model tissue. The skin model is manufactured to defined quality assurance procedures (certified ISO 9001) and supplied free of contamination with bacteria, mycoplasma and fungi.

7.4 Functional model conditions

The magnitude of viability is quantified using MTT. The optical density (OD) of the extracted (solubilized) dye from the negative control tissue is at least 20-fold greater than the OD of the extraction solvent alone. The negative control tissue has been shown to be stable in culture for the duration of the test exposure period. The stratum corneum is sufficiently robust to resist the rapid penetration of certain cytotoxic marker chemicals (eg 5% SDS). The tissue has been shown to demonstrate reproducibility over time.

8 DATA EVALUATION

8.1 Assay acceptance criteria

The assay was considered valid if the following criteria were met:

1. The absolute OD_{570} of the negative control tissues in the MTT test is an indication of the tissue viability in the testing laboratory after the shipping and storage procedure and under specific conditions of the assay. The OD values for the negative controls should be between 0.6 and 1.5, for this tissue model.

2. The viability of the tissues treated with the positive control should be ≤40%.

3. The standard deviation (SD) for tissues should be less than 18%.

The following results did not meet the acceptance criteria specified in the protocol:

For the purified water extracts, only two of the individual OD values for the negative control aliquots were within the range specified above and the standard deviations for the vehicle control (extracted water), PIP implant batch 27609 and [redacted] implant batch [redacted], were >18%.

For the DMSO extracts, only one of the individual OD values for the negative control aliquots was within the range specified above and the standard deviations for all PIP implant extracts were >18%.

These deviations from protocol were considered not to have affected the integrity or outcome of the study as no equivocal results were obtained and all the results from all test articles were clearly negative.

8.2 Interpretation of results

Test substances (test articles) that reduce the viability of the epidermal tissue to below 50% of the negative control are predicted as IRRITANT. Test substances (test articles) that do not reduce the viability of the epidermal tissue to below 50% of the negative control are predicted as NON-IRRITANT.

9 MAJOR COMPUTER SYSTEMS

The following computerised systems were used during the conduct of this study:

Scheduling	CMS (Covance Management System)
Formulations	Talisman
Data capture	SoftMax Pro GxP
Report generation	Microsoft Office / Adobe Acrobat

Version numbers of the systems are held on file at Covance.

10 RESULTS

Individual results can be found in Table 1 and Table 2.

The results are as follows:

Test article	Batch number	% Group mean viability compared to negative control	
		Water extract	DMSO extract
PIP Implant IMGHC-TX-H	16306	86.3	139.7
PIP Implant IMGHC-TX-H	02808	115.5	123.0
PIP Implant IMGHC-TX-H	27609	143.5	148.7
PIP Implant MGHC-TX-H	41609	175.4	128.1
PIP Implant IMGHC-TX-S	37908	125.7	138.2
[redacted]	[redacted]	129.8	112.9

11 CONCLUSION

The PIP implants and the [redacted] implant did not reduce viability of the epidermal tissue to below 50% of the negative control and were therefore considered to be non-irritant to the *in vitro* skin model EpiSkin™.

TABLES

Table 1
Cell viability measurements – Water Extract

Substance	Tissue replicate	OD_{570}		Mean	Corrected mean	% Relative survival		SD	CV
		Aliquot 1	Aliquot 2			Tissue	Mean		
Negative control (PBS pH 7.4)	A	0.297	0.539	0.418	0.418	81.6	100.0	16.50	16.50
	B	0.534	0.539	0.537	0.537	104.8			
	C	0.650	0.513	0.581	0.581	113.6			
Vehicle control (extracted water)	A	0.639	0.611	0.625	0.625	122.0	101.6	28.57	28.10
	B	0.329	0.377	0.353	0.353	69.0			
	C	0.380	0.786	0.583	0.583	113.8			
PIP Implant IMGHC-TX-H batch 16306	A	0.582	0.478	0.530	0.530	103.6	86.3	15.15	17.55
	B	0.351	0.471	0.411	0.411	80.3			
	C	0.390	0.379	0.385	0.385	75.2			
PIP Implant IMGHC-TX-H batch 02808	A	0.651	0.544	0.597	0.597	116.7	115.3	6.83	5.90
	B	0.584	0.521	0.552	0.552	107.9			
	C	0.592	0.650	0.621	0.621	121.3			
PIP Implant IMGHC-TX-H batch 27609	A	0.701	0.757	0.729	0.729	142.4	143.5	18.47	12.90
	B	0.635	0.651	0.643	0.643	125.5			
	C	0.806	0.857	0.832	0.832	162.4			
PIP Implant IMGHC-TX-S batch 37908	A	0.561	0.588	0.575	0.575	112.2	125.7	11.91	9.50
	B	0.656	0.675	0.666	0.666	130.0			
	C	0.676	0.705	0.690	0.690	134.8			
[redacted]	A	0.739	0.809	0.774	0.774	151.2	129.8	18.56	14.30
	B	0.621	0.599	0.610	0.610	119.2			
	C	0.650	0.568	0.609	0.609	119.0			
Positive control (5% SDS)	A	0.026	0.032	0.029	0.029	5.7	5.5	3.48	62.91
	B	0.040	0.051	0.046	0.046	8.9			
	C	0.007	0.014	0.010	0.010	2.0			
Blank		0.000	0.000						

The data in these tables was computer generated. In this system individual and derived figures are rounded. Thereby recalculation of derived values from the individual data will, in some instances, yield minor variations.

Table 1 continued
Cell viability measurements – Water Extract

Substance	Tissue replicate	OD_{570}		Mean	Corrected mean	% Relative survival		SD	CV
		Aliquot 1	Aliquot 2			Tissue	Mean		
Negative control (PBS pH 7.4)	A	0.435	0.465	0.450	0.450	85.6	100.0	13.47	13.47
	B	0.518	0.555	0.537	0.537	102.2			
	C	0.577	0.602	0.590	0.590	112.3			
Vehicle control (extracted water)	A	0.902	1.047	0.975	0.975	185.5	176.4	9.20	5.20
	B	0.906	0.949	0.927	0.927	176.5			
	C	0.836	0.920	0.878	0.878	167.1			
PIP Implant IMGHC-TX-H batch 41609	A	0.863	0.996	0.930	0.930	176.9	175.4	6.71	3.82
	B	0.958	0.946	0.952	0.952	181.2			
	C	0.857	0.908	0.883	0.883	168.0			
Positive control (5% SDS)	A	0.060	0.083	0.072	0.072	13.6	22.7	14.29	62.88
	B	0.078	0.084	0.081	0.081	15.4			
	C	0.203	0.209	0.206	0.206	39.2			
Blank		0.000	0.000						

The data in these tables was computer generated. In this system individual and derived figures are rounded. Thereby recalculation of derived values from the individual data will, in some instances, yield minor variations.

Table 2
Cell viability measurements – DMSO Extract

Substance	Tissue replicate	OD_{570}		Mean	Corrected mean	% Relative survival		SD	CV
		Aliquot 1	Aliquot 2			Tissue	Mean		
Negative control (PBS pH 7.4)	A	0.435	0.465	0.450	0.450	85.6	100.0	13.47	13.47
	B	0.518	0.555	0.537	0.537	102.2			
	C	0.557	0.602	0.590	0.590	112.3			
Vehicle control (DMSO)	A	0.848	0.867	0.857	0.857	163.2	166.0	13.18	7.90
	B	0.798	0.825	0.812	0.812	154.5			
	C	0.918	0.977	0.948	0.948	180.4			
PIP Implant IMGHC-TX-H batch 16306	A	0.643	0.675	0.659	0.659	125.4	139.7	21.73	15.56
	B	0.660	0.694	0.677	0.677	128.9			
	C	0.844	0.887	0.865	0.865	164.7			
PIP Implant IMGHC-TX-H batch 02808	A	0.563	0.573	0.568	0.568	108.1	123.0	21.26	17.30
	B	0.587	0.606	0.596	0.596	113.5			
	C	0.763	0.785	0.774	0.774	147.3			
PIP Implant IMGHC-TX-H batch 27609	A	0.806	0.841	0.823	0.823	156.7	148.7	27.95	18.80
	B	0.601	0.635	0.618	0.618	117.7			
	C	0.892	0.913	0.903	0.903	171.8			
PIP Implant IMGHC-TX-H batch 41609	A	0.557	0.587	0.572	0.572	108.9	128.1	41.53	32.40
	B	0.546	0.501	0.523	0.523	99.6			
	C	0.903	0.944	0.923	0.923	175.7			
PIP Implant IMGHC-TX-S batch 37908	A	0.498	0.533	0.515	0.515	98.1	138.2	37.46	27.10
	B	0.749	0.765	0.757	0.757	144.0			
	C	0.876	0.935	0.905	0.905	172.3			
[redacted]	A	0.543	0.602	0.572	0.572	108.9	112.9	16.79	14.90
	B	0.507	0.527	0.517	0.517	98.5			
	C	0.672	0.708	0.690	0.690	131.3			
Positive control (5% SDS)	A	0.060	0.083	0.072	0.071	13.6	22.7	14.29	62.88
	B	0.078	0.084	0.081	0.081	15.4			
	C	0.203	0.209	0.206	0.206	39.2			
Blank		0.000	0.000						

The data in these tables was computer generated. In this system individual and derived figures are rounded. Thereby recalculation of derived values from the individual data will, in some instances, yield minor variations.