HFCPE: Skin Irritation to the Rabbit

<table>
<thead>
<tr>
<th>HLS study number:</th>
<th>VHJ0003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Version ID:</td>
<td>Final</td>
</tr>
<tr>
<td>Issue date:</td>
<td>28 September 2010</td>
</tr>
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# Details of Sponsor and Test Facility

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>ZEON Corporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shin Marunouchi Center Building</td>
</tr>
<tr>
<td></td>
<td>1-6-2 Marunouchi</td>
</tr>
<tr>
<td></td>
<td>Chiyoda-ku</td>
</tr>
<tr>
<td></td>
<td>Tokyo, 100-8246</td>
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<td>JAPAN</td>
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</table>

<table>
<thead>
<tr>
<th>Test facility</th>
<th>Huntingdon Life Sciences</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Eye Research Centre</td>
</tr>
<tr>
<td></td>
<td>Eye</td>
</tr>
<tr>
<td></td>
<td>Suffolk</td>
</tr>
<tr>
<td></td>
<td>IP23 7PX</td>
</tr>
<tr>
<td></td>
<td>UK</td>
</tr>
</tbody>
</table>
Compliance with Good Laboratory Practice

HFCPE: Skin irritation to the rabbit

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and, I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.


These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

P B Rees BSc (Hons) CBiol MSB
Study Director
Huntingdon Life Sciences

Date
28 September 2010
# Quality Assurance Statement

**HFCPE: Skin irritation to the rabbit**

The following inspections and audits have been carried out in relation to this study:

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>Date(s) of Inspection</th>
<th>Date of Reporting to Study Director and Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study details Audit</td>
<td>21 Jun 2010</td>
<td>21 Jun 2010</td>
</tr>
<tr>
<td></td>
<td>28 Sep 2010</td>
<td>28 Sep 2010</td>
</tr>
</tbody>
</table>

**Process based inspections**

At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

<table>
<thead>
<tr>
<th>Process Based Inspections</th>
<th>Date(s) of Inspection</th>
<th>Date of Reporting to Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose administration and</td>
<td>26 May 2010</td>
<td>28 May 2010</td>
</tr>
<tr>
<td>observations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation procedures</td>
<td>28 Jun 2010</td>
<td>29 Jun 2010</td>
</tr>
<tr>
<td>Bodyweights</td>
<td>10 Jun 2010</td>
<td>10 Jun 2010</td>
</tr>
</tbody>
</table>

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.

_H Comm MRQA_  
Unit Head  
Department of Quality Assurance  
Huntingdon Life Sciences

![Signature](signature.png)  
Date: 28 September 2010
Contributing Scientist

HFCPE: Skin irritation to the rabbit

Study management

P B Rees BSc (Hons) CBiol MSB
Study Director
Summary

A study was performed to assess the skin irritation potential of HFCPE to the rabbit. The method followed was that described in:


Three rabbits received a single four hour, semi-occlusive, dermal administration of approximately 0.5 mL of the test substance as supplied and were observed for four days.

Very slight erythema was apparent one hour after bandage removal in a single animal; no other dermal response was apparent at any time during the study.

The means of scores for these reactions at approximately 24, 48 and 72 hours after administration, calculated separately for each animal, are summarised below:

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Erythema</th>
<th>Oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>96</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>97</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

EC trigger values*

|              | ≥2 | ≥2  |

* Classification is triggered if means of scores for either effect are ≥ 2 for two or three animals (or if effects persist to Day 14 in at least two animals).

The Primary Irritation Index was calculated to be 0.0; HFCPE was classified as ‘non-irritant’ according to the criteria of the ECETOC and did not require labelling with the risk phrase R38, “Irritating to skin”, in accordance with Commission Directive 2001/59/EC.
1. Introduction

The study was designed to assess skin irritation potential of HFCPE following a single dermal application to rabbits. The test substance may come into contact with skin during handling or use.

The study was conducted in compliance with the following guidelines:


The albino rabbit was chosen as it has been shown to be a suitable model for skin irritation studies and is the animal recommended in the test guidelines.

The amount of test substance administered was chosen in compliance with the guidelines.

The protocol was approved by the Study Director and Huntingdon Life Sciences Management on 11 June 2010 and by the Sponsor on 15 June 2010.

The experimental start date was 18 June 2010 and the experimental completion date was 29 June 2010.
### 2. Test substance

<table>
<thead>
<tr>
<th>Identification:</th>
<th>HFCPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended use:</td>
<td>Electronic applications</td>
</tr>
<tr>
<td>Description:</td>
<td>Colourless liquid</td>
</tr>
<tr>
<td>Storage conditions:</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Batch number:</td>
<td>Lot No 20090601</td>
</tr>
<tr>
<td>Date of receipt:</td>
<td>22 June 2009</td>
</tr>
<tr>
<td>Expiry date:</td>
<td>End June 2010</td>
</tr>
<tr>
<td>Purity:</td>
<td>99.3%</td>
</tr>
</tbody>
</table>
3. Experimental Procedure

3.1 Animal management

Animals for this study were selected from a stock supply of healthy adult rabbits of the New Zealand White strain. They were in the weight range of 3.65 to 3.93 kg and approximately 41 weeks of age, prior to treatment (Day 1). All rabbits were acclimatised to the experimental environment for a period of 30 weeks prior to the start of the study.

The rabbits were housed individually in plastic cages with perforated floors at the Eye Research Centre, Eye, Suffolk, IP23 7PX.

Each rabbit was offered 125 g of a standard laboratory rabbit diet per day; drinking water was provided \textit{ad libitum}. The batch of diet used for the study was analysed for nutrients, possible contaminants and micro-organisms likely to be present in the diet and which, if in excess of specified amounts, might have an undesirable effect on the test system. The animals were given a dietary supplement of hay.

During the acclimatisation and study period the animals were given small soft white untreated wood blocks for environmental enrichment.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier are made available to Huntingdon Life Sciences Limited.

Animal room environmental controls were set to maintain temperature within the range 16 to 20°C, and relative humidity within 40 to 70%. These environmental parameters were recorded and the permanent record archived with other departmental raw data. Lighting was controlled by means of a time switch to give 12 hours of artificial light (06:00 to 18:00 GMT) in each 24 hour period.

Each animal was identified by a numbered tag placed through the edge of one ear. This identification was unique within the Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study number and animal number.

3.2 Test substance preparation

HFCPE was administered as supplied by the Sponsor.

The absorption of HFCPE was not determined.

The identity, strength and purity of the test substance received, its stability under the storage conditions and the conditions of administration were the responsibility of the Sponsor.

3.3 Treatment procedure

On the day before application of the test substance, hair was removed with clippers from the dorso-lumbar region of each rabbit exposing an appropriate sized area of skin.

Approximately 0.5 mL of the test substance was applied under a 2-ply 25 mm x 25 mm porous gauze pad secured with ‘blenderm’ surgical tape to intact skin sites on three animals.
An additional site was similarly treated with the exception of test substance and acted as a control.

A single animal (number 93) received three exposures of three minutes, one or four hours duration in a step-wise manner and acted as a preliminary screen. In the absence of a severe effect on removal of the dressings the next exposure was initiated. In the absence of a severe response in this animal, two further animals (numbers 96 and 97) were committed to the study each receiving a single four hour exposure.

For exposures of one hour or more each treatment site was covered with cotton wool and "Tubigrip" elasticated bandage dressing for the duration of the exposure period. The animals were returned to their cages immediately after treatment.

At the end of the exposure period the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with lukewarm water (30-40°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

3.4 Serial observations

3.4.1 Clinical signs

All animals were observed daily for signs of ill health or toxicity.

3.4.2 Dermal responses

Examination of the treated skin was made on removal of the dressings (for 3 minute or one hour exposures) and approximately 1, 24, 48 and 72 hours later. Only the data for the four hour exposure are reported, the data from the three minute and one hour exposures are held in the archives.

Local dermal irritation was assessed using the prescribed numerical system:

Erythema and eschar formation:

- No erythema 0
- Very slight erythema (barely perceptible) 1
- Well-defined erythema 2
- Moderate to severe erythema 3
- Severe erythema (beet redness) or eschar formation (injuries in depth) preventing grading of erythema 4

Oedema formation:

- No oedema 0
- Very slight oedema (barely perceptible) 1
- Slight oedema (edges of area well-defined by definite raising) 2
- Moderate oedema (raised approximately 1 millimetre) 3
- Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure) 4
3.4.3 Interpretation of responses

Primary Irritation Index

A primary irritation index (PII) was calculated from the erythema and oedema scores according to the following formula as described in Technical Report No. 66 “Skin irritation and Corrosion: Reference chemicals data bank” (March 1995) ECETOC, Brussels.

\[
\text{PII} = \sum \text{Erythema at 24/48/72 hours} + \sum \text{Oedema at 24/48/72 hours} \\
\text{3 x number of animals}
\]

The maximum possible score was 8.0. The PII was then used to classify the test substance as follows:

<table>
<thead>
<tr>
<th>Primary Irritation Index</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>non-irritant</td>
</tr>
<tr>
<td>&gt;0 - 2.0</td>
<td>mildly irritating</td>
</tr>
<tr>
<td>2.1 - 5.0</td>
<td>moderate irritant</td>
</tr>
<tr>
<td>5.1 - 6.0</td>
<td>moderate to severe irritant</td>
</tr>
<tr>
<td>&gt; 6.0</td>
<td>severe irritant</td>
</tr>
</tbody>
</table>

This classification system is a modification based on “Appraisal of the Safety of Chemicals in Foods Drugs and Cosmetics” published by the Association of Food and Drug Officials of the United States, 1959.

EU Classification

The Official Journal of the European Communities (Directive 2001/59/EC) contains the following criteria for classification of irritants and corrosives to skin.

Substances and preparations shall be classified corrosive if, when applied to healthy intact animal skin, full thickness destruction of skin tissue on at least one animal occurs as a result of up to four hours exposure. The following risk phases shall be assigned in accordance with the following criteria:

R35: Causes severe burns

- if, when applied to healthy intact animal skin, full thickness destruction of the skin occurs as a result of up to three minutes exposure.

R34: Causes burns

- if, when applied to healthy intact animal skin, full thickness destruction of the skin occurs as a result of up to four hours exposure.

Non-corrosive substances and preparations shall be classified irritant if, when applied to healthy intact animal skin for up to four hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean value of the scores for either erythema (including eschar formation) or oedema formation observed in two or more animals is equivalent to the value of two or more. All scores at 24, 48 and 72 hours should be used in calculating the respective mean values.
Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation period. Particular effects e.g. hyperplasia, scaling, discolouration, fissures, scabs and alopecia should be taken into account.

The following risk phrase shall be assigned:

R38: Irritating to skin

3.4.4 Termination

Following completion of the observation period the animals were humanely killed by an intravenous injection of sodium pentobarbital.

3.5 Archives

All raw data arising from the performance of this study at Huntingdon Life Sciences is the property of the Sponsor and will be lodged together with a copy of the final report in the Huntingdon Life Sciences Archive.

Such records will be retained for a minimum of one year from the date on which the Study Director signs the final report. At the end of the retention period the Sponsor will be contacted and advice sought on the return, disposal or further retention of the records.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

3.6 Deviations from protocol

The following deviation from protocol occurred:

Section 3.1 (Animal management) of the Study Protocol indicates that the animals to be employed on this study would be within the approximate age range of 12 to 40 weeks of age on the day of dosing.

The dates of birth and dose administration for the animals used on the study are given below:

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Date of Birth</th>
<th>Date of administration</th>
<th>Age at administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>9 September 2009</td>
<td>21 June 2010</td>
<td>41 Weeks</td>
</tr>
<tr>
<td>96</td>
<td>8 September 2009</td>
<td>23 June 2010</td>
<td>41 Weeks</td>
</tr>
<tr>
<td>97</td>
<td>8 September 2009</td>
<td>23 June 2010</td>
<td>41 Weeks</td>
</tr>
</tbody>
</table>

The animals were healthy young adult animals and the use of slightly older than intended animals had no impact upon the study.
4. **Results**

4.1 **Clinical signs**

There was no sign of toxicity or ill health in any rabbit during the observation period.

4.2 **Dermal responses**

Very slight erythema was apparent one hour after bandage removal in a single animal; no other dermal response was apparent at any time during the study.
5. Conclusion

The Primary Irritation Index was calculated to be 0.0; HFCPE was classified as ‘non-irritant’ according to the criteria of the ECETOC and did not require labelling with the risk phrase R38, “Irritating to skin”, in accordance with Commission Directive 2001/59/EC.
### Table 1  Mean values for erythema and oedema

24, 48 and 72 hours after removal of dressings *

<table>
<thead>
<tr>
<th>Animal number and sex</th>
<th>Erythema</th>
<th>Oedema</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>93 M</td>
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<tr>
<td>96 M</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>97 M</td>
<td>0.0</td>
<td>0.0</td>
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</table>

* 4 hour exposure

M Male
<table>
<thead>
<tr>
<th>Animal number and sex</th>
<th>Type of Response</th>
<th>1 hour</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td>Test site</td>
<td>Control site</td>
<td>Test site</td>
<td>Control site</td>
</tr>
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<td>93 M #</td>
<td>Erythema</td>
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<td>0</td>
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<td>Oedema</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>96 M</td>
<td>Erythema</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Oedema</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>97 M</td>
<td>Erythema</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oedema</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

M Male
#
Sentinel animal
Annex 1  Weight of Evidence

Prior to undertaking in-vivo skin irritation testing the Study Director conducted a weight-of-the-evidence analysis to ensure that the in-vivo testing was sufficiently justified. The following factors have been taken into consideration:

1. **Nature of test substance.**

A colourless liquid for use in electronic applications. The Sponsor indicated that the test substance has the following:

Molecular weight: 194.05  
Chemical name: 1,3,3,4,4,5,5-Heptafluorocyclopentene  
Log Pow: 2.4  
Structure:

![Structure](image)

2. **Evaluation of existing human and animal data (including dermal toxicity)**

Searches for the test material failed to yield any data.

Searches for related materials yielded:

**OFCPE (1,2,3,3,4,4,5,5-Octofluorocyclopentane):** skin irritation study in rabbits (HLS study ZCE037): no dermal irritation. Eye irritation study in rabbits (HLS study ZCE038): irritating to the eye

**HFCPA (1,1,2,2,3,3,4-Heptafluorocyclopentane):** skin irritation study in rabbits (HLS study ZCE034): no dermal irritation. Eye irritation study in rabbits (HLS study ZCE035): non-irritating to the eye

3. **Analysis of structure activity relationships (SAR).**

None available.

4. **Physicochemical properties.**

pH (as measured at Huntingdon Life Sciences) – 5.0

5. **Results from in-vitro or ex-vivo tests.**

EPISKIN (HLS study VHJ0002): predicted as non-irritant to the skin

BCOP (HLS study VHJ0004): no severe eye irritancy or corrosivity prediction
Annex 2  GLP Compliance Statement

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM
GOOD LABORATORY PRACTICE
STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

<table>
<thead>
<tr>
<th>TEST FACILITY</th>
<th>TEST TYPE</th>
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<tbody>
<tr>
<td>Huntingdon Life Sciences</td>
<td>Analytical/Clinical Chemistry</td>
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<td>Ecosystems</td>
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<td>Occold</td>
<td>Environmental Fate</td>
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<td>Eye</td>
<td>Environmental Toxicity</td>
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<tr>
<td>Suffolk</td>
<td>Mutagenicity</td>
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<tr>
<td>IP23 7PX</td>
<td>Physico-chemical Testing</td>
</tr>
<tr>
<td></td>
<td>Residue Studies</td>
</tr>
<tr>
<td></td>
<td>Toxicology</td>
</tr>
</tbody>
</table>

DATE OF INSPECTION
26 January 2010

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority
HFCPE: Skin irritation to the rabbit

Date Report issued: 28 September 2010             Date of amendment: 1 October 2010

This amendment was audited by Huntingdon Life Sciences Quality Assurance Department on 1 October 2010.

Helen Comb, MRQA
Unit Head, Department of Quality Assurance

I declare that this amendment is accurate and correct.

Peter Rees, BSc, CBiol, MSB
Study Director

<table>
<thead>
<tr>
<th>Details of amendment</th>
<th>Reason for amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Page 18: chemical name of OFCPE</td>
<td>Correct a typographical error in Annex 1 (Weight of Evidence)</td>
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Annex 1  Weight of Evidence

Prior to undertaking in-vivo skin irritation testing the Study Director conducted a weight-of-the-evidence analysis to ensure that the in-vivo testing was sufficiently justified. The following factors have been taken into consideration:

1. Nature of test substance.

A colourless liquid for use in electronic applications. The Sponsor indicated that the test substance has the following:

Molecular weight: 194.05  
Chemical name: 1,3,3,4,4,5,5-Heptafluorocyclopentene  
Log Pow: 2.4  
Structure:

2. Evaluation of existing human and animal data (including dermal toxicity)

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**HFCPA (1,1,2,2,3,3,4-Heptafluorocyclopentane)**: skin irritation study in rabbits (HLS study ZCE034): no dermal irritation. Eye irritation study in rabbits (HLS study ZCE035): non-irritating to the eye

3. Analysis of structure activity relationships (SAR).

None available.

4. Physicochemical properties.

pH (as measured at Huntingdon Life Sciences) – 5.0

5. Results from in-vitro or ex-vivo tests.

EPISKIN (HLS study VHJ0002): predicted as non-irritant to the skin  
BCOP (HLS study VHJ0004): no severe eye irritancy or corrosivity prediction
HFCPE: Skin irritation to the rabbit

Date Report issued: 28 September 2010  Date of amendment: 8 October 2010

This amendment was audited by Huntingdon Life Sciences Quality Assurance Department on 8 October 2010.

Helen Comb, MRQA  
Unit Head, Department of Quality Assurance

I declare that this amendment is accurate and correct.

Peter Rees, BSc, CBiol, MSB  
Study Director

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pH (as measured at Huntingdon Life Sciences) – 5.0

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