REPORT

Study Title

PRIMARY SKIN IRRITATION/CORROSION STUDY WITH PERFLUOROHEXANOIC ACID AMMONIUM SALT IN THE RABBIT (SEMI-OCCLUSIVE APPLICATION)

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Laboratory Project Identification

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2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

NOTOX B.V.

Drs. M.S. Teunissen
Study Director

Date: 21 June 2004

W.J.A.M. Frieling, DVM
Director of Toxicology

Date: 21 June 2004

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.
During the on-site process inspections procedures applicable to this type of study were inspected

Type of inspections	Phase / Section	Start Inspection date(s)	End Inspection date(s)	Reporting date
Protocol (Study)		03-FEB-04	03-FEB-04	03-FEB-04
On-site (Process)	SPF Unit	26-JAN-04	06-FEB-04	09-FEB-04
Report (Study)		07-MAY-04	07-MAY-04	07-MAY-04

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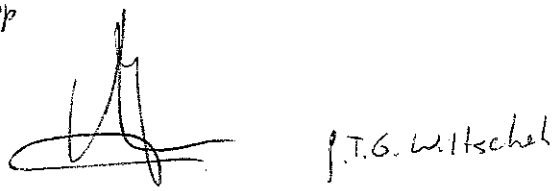

p.T.G. Mitchell

Date: 22 June 2004

4. SUMMARY

Primary skin irritation/corrosion study with Perfluorohexanoic aid Ammonium Salt in the rabbit (semi-occlusive application).

The study was carried out based on the guidelines described in: "Acute Toxicity - Skin irritation", OECD No.404 (2002); EC Commission Directive 92/69/EEC, B.4, "Acute Dermal Irritation/Corrosion" (1992); US EPA, OPPTS 870.2500, Acute Dermal Irritation (1998) and JMAFF, Japanese Test Guidelines (2000).

Initially, one rabbit was exposed to three samples of 0.5 ml of Perfluorohexanoic aid Ammonium Salt applied to separate skin-sites on intact, clipped skin using a semi-occlusive dressing. The exposure periods were 3 minutes, 1 hour and 4 hours, respectively. Observations were made at least once daily for 4 days after treatment and 7 days after exposure. Based on the absence of severe skin reactions, two further animals exposed for 4 hours to Perfluorohexanoic aid Ammonium Salt at a later stage.

Exposure to Perfluorohexanoic aid Ammonium Salt resulted in well defined erythema and very slight oedema in the treated skin-areas of the three rabbits.
The skin irritation had resolved within 7 days after exposure in all animals.

Based on these results and according to the:

- OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998), Perfluorohexanoic aid Ammonium Salt does not have to be classified for skin irritation.

- EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), Perfluorohexanoic aid Ammonium Salt does not have to be classified and has no obligatory labelling requirement for skin irritation.

5. INTRODUCTION

5.1. Preface

Sponsor	Daikin Industries, Ltd. 1-1 Nishi Hitotsuya Settsu-shi OSAKA, 566-8585 Japan
Study Monitor	Mr. H. Iwai, DVM
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. M.S. Teunissen
Study Plan (in-life phase)	Start : 10 February 2004 Completion : 02 March 2004

5.2. Aims of the study

The purpose of this primary skin irritation study was to assess the possible irritation or corrosion potential of a single dose of the test substance when administered to the intact skin of rabbits. This study should provide a rational basis for risk assessment in man.
The absence of skin pigmentation in the albino rabbit facilitates the evaluation of induced skin reactions. The dermal route was selected because the test substance may accidentally come into contact with the skin during manufacture, handling and/or use.

5.3. Guidelines

As required by the Dutch Act on Animal Experimentation, the study protocol was reviewed and agreed by the Article 14-functionary and the Ethical Committee of NOTOX (DEC NOTOX 97-03-09) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in this report were based on the following guidelines:

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.404: "Acute Dermal Irritation/ Corrosion", Paris Cedex, 2002.

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the determination of Toxicity, as last amended by Commission Directive 92/69/EEC, B.4: "Acute Toxicity - Skin Irritation". Official Journal of the European Communities No. L 383, 1992.

United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.2500, Acute Dermal Irritation. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-98-196, August 1998.

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 12 Nousan, Notification No 8147, November 2000, including the most recent partial revisions.

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 10 years after finalization of the report. After this period, the sponsor will be contacted to determine whether raw data and specimens should be returned to them, retained or destroyed on their behalf.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

6. MATERIALS AND METHODS

6.1. Test Substance

6.1.1. Test Substance

The sponsor is responsible for all test substance data unless determined by NOTOX.

Identification	Perfluorohexanoic acid Ammonium Salt
Structure	$C_5F_{11}COONH_4$
Molecular formula	$C_6H_4F_{11}NO_2$
Molecular weight	331
Description	Colourless liquid
Batch	LOT.C15003Z01
Purity	98%
Composition	20 mass%: Perfluorohexanoic acid Ammonium Salt 80 mass%: Water
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	31 January 2005
Stability in vehicle	
Water	Unknown
1% Aq. Carboxymethyl cellulose	Unknown
Corn oil	Unknown
Propylene glycol	Unknown
Polyethylene glycol	Unknown
Methyl ethyl ketone	Unknown
Dimethyl sulphoxide	Unknown
Ethanol	Unknown
Acetone	Unknown
Olive oil	Unknown
Dimethyl formamide	Unknown

6.1.2. Test substance preparation

The test substance was applied undiluted as delivered by the sponsor.

6.2. Test System

Species	Albino Rabbit, New Zealand White, (SPF-Quality) Recognised by international guidelines as the recommended test system (e.g. EC, OECD) Source: Charles River Deutschland, Kisslegg, Germany
Number of animals	3 Animals of one sex.
Age and body weight	Animals used within the study were at least 6 weeks old and body weights were at least 1.0 kg.
Identification	Earmark.

6.3. Animal husbandry

Conditions
Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of 21.0 ± 3.0°C (actual range: 14.9 – 20.6°C), a relative humidity of 30-70% (actual range: 32 - 63%) and 12 hours artificial fluorescent light and 12 hours darkness per day.

Accommodation
Individually in labelled cages with perforated floors (Scanbur, Denmark, dimensions 56x44x37.5 cm).
Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet
Standard laboratory rabbit diet (Charles River Breeding and Maintenance Diet for Rabbits, Altromin, Lage, Germany) approx. 100 g. per day. Certificates of analysis were examined and retained in the NOTOX archives. In addition, pressed hay (BMI, Helmond, the Netherlands) was provided at least three times a week.

Water
Free access to tap-water. Certificates of quarterly analysis were examined and retained in the NOTOX archives.

6.4. Treatment

All available data relevant to the potential dermal irritation/corrosivity of the substance indicated that no severe effects were to be expected. An in-vitro test was considered, but a negative test result was anticipated that still would have to be confirmed in an in-vivo study. Since no severe harm for the animals was to be expected, this in-vivo skin irritation study was performed and was started by treatment of a single rabbit (sentinel). The two other animals were treated in a similar manner 14 days later, after considering the degree of skin irritation observed in the first animal.

Approximately 24 hours before treatment, the dorsal fur was clipped with electric clippers, exposing an area of approximately 150 square centimeters (10x15 cm^2). Whenever considered necessary the treated skin areas were re-clipped at least 3 hours before the observations, to facilitate scoring.

A health inspection was performed prior to the commencement of treatment, to ensure that the animals were in a good state of health. Special attention was paid to the skin to be treated, which was intact and free from abnormalities.

The study was initiated by treatment of one rabbit.
This animal received of 0.5 ml of the test substance to the intact, clipped skin of one flank using a Metalline patch[#] of 2x3 cm. The patch was mounted on Micropore tape[#], which was wrapped around the abdomen and secured with Coban elastic bandage[#].
The dressing was removed 3 minutes after application.
Since no signs of severe skin reactions (i.e. necrosis or corrosion) were observed and it was considered that exposure could be continued humanely, two samples of 0.5 ml of the test substance were then applied to separate skin-sites on the intact, clipped skin of the same animal, using an identical procedure and one sample per dressing.
One of the dressings was removed after a 1-hour exposure.
After similar considerations (i.e. no severe skin reactions, necrosis or corrosion), the other dressing was removed after a 4-hour exposure.
After each removal of a dressing, the treated skin was cleaned of residual test substance using water.

Since no signs of severe skin reactions (i.e. necrosis or corrosion) were observed after 4 hours exposure and the irritation was considered reversible in the first animal, two further animals were treated at a later stage. These animals received single samples of 0.5 ml of the test

[#]. Suppliers: Lohmann GmbH, Neuwied, Germany (Metalline) and 3M, St. Paul, Minnesota, U.S.A. (Micropore and Coban).

substance on the intact, clipped skin of one flank applied in a similar manner as in the first animal. Four hours after the application, the dressing was removed and the skin cleaned of residual test substance using water.

6.5. Observations

Mortality/Viability	Twice daily.
Toxicity	At least once daily.
Body Weight	Day of treatment (prior to application) and at termination.
Irritation	In the initially treated animal, the skin reactions of all visible treated sites were assessed immediately after removal of a dressing and approximately 1, 24, 48, 72 hours after the removal of the last dressing and test substance. After the 4 hours exposure in two further animals, the skin reactions were assessed approximately 1, 24, 48, 72 hours after the removal of the dressing and test substance. For the duration of the skin reactions, further observations were made 7 days after exposure. The irritation scores and a description of all other (local) effects were recorded. Adjacent areas of untreated skin of each animal serve as controls.

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given were recorded:

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) *..... 4
*. Where signs of necrosis or corrosion (injuries in depth) prevent erythema scoring, the maximum grade for erythema (= 4) is given.

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 millimeter)..... 3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure) 4

6.6. Histopathology

No histopathology was performed.

6.7. Interpretation

The results were evaluated according to the OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998) and the EC criteria for classification and labelling of dangerous substances and preparations (Council Directive 67/548/EEC and all adaptations to technical progress and amendments of this Directive published in the Official Journal of the European Communities).

6.8. List of protocol deviations

1. Deviations from the minimum level of temperature occurred.
   Evaluation: Based on laboratory historical data these deviations were considered not to have affected the study integrity

The study integrity was not adversely affected by the deviations.

7. RESULTS

7.1. Irritation (Table 1)

Four hours exposure to 0.5 ml of Perfluorohexanoic acid Ammonium Salt resulted in well defined erythema and very slight oedema in the treated skin-areas of the three rabbits. The skin irritation had resolved within 7 days after exposure in all animals.

7.2. Corrosion

There was no evidence of a corrosive effect on the skin.

7.3. Colouration / Remants

No staining of the treated skin by the test substance was observed. No remnants of the test substance were present on the skin.

7.4. Toxicity / Mortality

No symptoms of systemic toxicity were observed in the animals during the test period and no mortality occurred.

8. CONCLUSION

Based on these results and according to the:

- OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances (OECD, 1998), PERFLUOROHEXANOIC ACID AMMONIUM SALT does not have to be classified for skin irritation.

- EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), PERFLUOROHEXANOIC ACID AMMONIUM SALT does not have to be classified and has no obligatory labelling requirement for skin irritation.

Table 1: INDIVIDUAL SKIN IRRITATION SCORES

Animal 842#	3 minutes treatment site			1-hour treatment site			4-hours treatment site		
Time after removal of the bandage	Erythema	Oedema	comments	Erythema	Oedema	comments	Erythema	Oedema	Comments
Immediately	2	0	-	NS	NS	-	NS	NS	-
1 hour	1	0	-	2	1	-	NS	NS	-
3 hours	0	0	-	2	0	-	2	1	-
4 hours	0	0	-	2	0	-	2	1	-
24 hours	0	0	-	1	0	-	2	1	-
48 hours	0	0	-	0	0	-	2	0	-
72 hours	0	0	-	0	0	-	1	0	-
7 days	0	0	-	0	0	-	0	0	-

Animal #	859			863		
Time after exposure	Erythema	Oedema	comments	Erythema	Oedema	comments
1 hour	1	1	-	2	1	-
24 hours	2	1	-	2	1	-
48 hours	2	0	-	2	0	-
72 hours	1	0	-	1	0	-
7 days	0	0	-	0	0	-

Comments:
NS. Not scored according to protocol.

Table 2: MEAN VALUE IRRITATION SCORES AFTER 4 HOURS OF EXPOSURE

Animal #	Mean 24 – 72 hrs	
	Erythema	Oedema
842	1.7	0.3
859	1.7	0.3
863	1.7	0.3

Animal specifications:

Animal no	Sex	Age at start (weeks)	Body weights (grams) prior to application	Body weights (grams) at termination
842	♂	11-13	2577	2777
859	♂	13-15	2788	2892
863	♂	13-15	2693	2799