REPORT

Study Title

ASSESSMENT OF ACUTE DERMAL TOXICITY WITH PERFLUOROHEXANOIC ACID AMMONIUM SALT IN THE RAT

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Study completion date

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Test Facility

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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

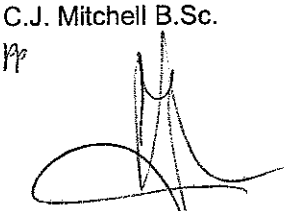
NOTOX B.V., 's-Hertogenbosch, The Netherlands

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)		26-JAN-04	06-FEB-04	09-FEB-04
On-site (Process)		10-FEB-04	19-FEB-04	20-FEB-04
Report (Study)		06-MAY-04	06-MAY-04	06-MAY-04

Head of Quality Assurance
C.J. Mitchell B.Sc.



J.T.G. Wiltschek

Date: June 22, 2004

4. SUMMARY

Assessment of acute dermal toxicity with Perfluorohexanoic acid Ammonium Salt in the rat.

The study was carried out based on the guidelines described in: "Acute Toxicity-Dermal", OECD No.402 (1987); "Acute Dermal Toxicity", EC Commission Directive 92/69/EEC, Part B.3 (1992); Environmental Protection Agency (EPA): Health Effects Test Guidelines OPPTS 870.1200 (1996), "Acute Dermal Toxicity" and JMAFF: Japanese Test Guidelines (2000).

Perfluorohexanoic acid Ammonium Salt was administered to five Wistar rats of each sex by dermal application at 2000 mg/kg body weight for 24 hours. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (day 15).

No mortality occurred.

Flat or hunched posture was noted in the majority of animals and chromodacryorrhoea was noted in three males. The animals had recovered from the symptoms between days 2 and 4.

Scales and/or scabs were seen in the treated skin-area of most animals during the observation period.

The mean body weight gain during the observation period was within the range expected for rats used in this type of study

No abnormalities were found at macroscopic post mortem examination of the animals .

The dermal LD_{50} value of Perfluorohexanoic acid Ammonium Salt in Wistar rats was established to exceed 2000 mg/kg body weight.

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 acute toxicity by the dermal route.

- 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 dermal toxicity.

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 : 30 March 2004 Completion : 13 April 2004

5.2. Aims of the study

The objective of this study was to assess the toxicity of the test substance when administered to rats as a single dermal application.
This study should provide a rational basis for risk assessment in man.
The dermal route was selected as it is a possible route of human exposure during manufacture, handling or use of the test substance.

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 97-03-13) 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. 402, "Acute Dermal Toxicity", Paris Cedex, 1987.

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.3: "Acute Toxicity-Dermal". Official Journal of the European Communities No. L 383, 1992.

United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-96-192, June 1996.

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 dosed undiluted as delivered by the sponsor.

6.2. Test System

Species	Rat, Wistar strain Crl:(WI) BR (outbred, SPF-Quality). Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source: Charles River Deutschland, Sulzfeld, Germany.
Number of animals	5 males and 5 females (females were nulliparous and non-pregnant).
Age and body weight	Young adult animals (approx. 11 weeks old) were selected. Body weight variation did not exceed +/- 20% of the sex mean.
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: 17.5 – 24.2°C), a relative humidity of 30-70% (actual range: 29 - 67%) and 12 hours artificial fluorescent light and 12 hours darkness per day.
Accommodation
Individually housed in labelled Macrolon cages (type III, height 15 cm.) containing purified sawdust as bedding material (Woody-Clean type 3/4; Tecnilab-BMI BV, Someren , The Netherlands). Certificates of analysis were examined and then retained in the NOTOX archives. Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet
Free access to standard pelleted laboratory animal diet (from Altromin (code VRF 1), Lage, Germany). Certificates of analysis were examined and then retained in the NOTOX archives.

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

6.4. Treatment

A health inspection was performed prior to 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 any abnormality.

Method	Dermal application.
Clipping	One day before exposure (day -1) an area of approximately 5x7 cm on the back of the animal was clipped.
Application	The test substance was applied in an area of approx. 10% of the total body surface, i.e. approx. 25 cm^2 for males and 18 cm^2 for females. The test substance was held in contact with the skin with a dressing, consisting of a surgical gauze patch (Surgy 1D)*, successively covered with aluminium foil and Coban elastic bandage*. A piece of Micropore tape* was additionally used for fixation of the bandages in females only. *. Manufacturers: Laboratoires Stella s.a., Liege, Belgium (surgical gauze) and 3M, St. Paul, Minnesota, U.S.A. (Coban & Micropore).
Frequency	Single dosage, on day 1.
Dose level (volume)	2000 mg/kg (1.86 ml/kg) body weight. Dose volume calculated as follows: dose level : density.
Application period	24 hours, after which dressings were removed and the skin cleaned of residual test substance using water.

6.5. Observations

Mortality/Viability	Twice daily.
Body weights	Days 1 (pre-administration), 8 and 15.
Clinical signs	At periodic intervals on the day of dosing (day 1) and once daily thereafter, until day 15. The time of onset, degree and duration were recorded and the symptoms graded according to fixed scales: Maximum grade 4: grading slight (1) to very severe (4) Maximum grade 3: grading slight (1) to severe (3) Maximum grade 1: presence is scored (1).
Necropsy	At the end of the observation period, all animals were sacrificed by asphyxiation using an oxygen/carbon dioxide procedure and subjected to necropsy. Descriptions of all internal macroscopic abnormalities were recorded.

6.6. 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).

6.7. List of protocol deviations

1. Deviations from the minimum and maximum 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. Mortality

No mortality occurred.

7.2. Clinical Signs Table 1)

Flat or hunched posture was noted in the majority of animals and chromodacryorrhoea was noted in three males. The animals had recovered from the symptoms between days 2 and 4.

Scales and/or scabs were seen in the treated skin-area of most animals during the observation period.

7.3. Body Weight (Table 2)

The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore considered not indicative of toxicity.

7.4. Macroscopic Findings (Table 3)

No abnormalities were found at macroscopic post mortem examination of the animals .

Cysts in ovaries, found in one female, are commonly noted among rats of this age and strain and was therefore considered not toxicologically significant.

8. CONCLUSION

The dermal LD_{50} value of Perfluorohexanoic acid Ammonium Salt in Wistar rats was established to exceed 2000 mg/kg body weight.

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 acute toxicity by the dermal route.

- 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 dermal toxicity.

TABLE 1 : CLINICAL SIGNS

TEST DAY HOURS AFTER TREATMENT	MAX GRADE	1 0	1 2	1 4	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MALES 2000 MG/KG																		
ANIMAL 1																		
Posture																		
Flat posture	(1)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 2																		
Posture																		
Hunched posture	(1)	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	1	1	1	1	1	1	-	-	-	-	-	-	-
Scabs (Treated skin)	(3)	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-
ANIMAL 3																		
Posture																		
Hunched posture	(1)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales	(3)	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 4																		
Posture																		
Hunched posture	(1)	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	1	1	1	1	1	1	1	1	1	-	-	-	-
ANIMAL 5																		
Posture																		
Flat posture	(1)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 2000 MG/KG																		
ANIMAL 6																		
Posture																		
Hunched posture	(1)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	1	1	1	1	1	1	1	1	1	-	-	-	-
ANIMAL 7																		
Posture																		
Hunched posture	(1)	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	-	1	1	1	1	1	1	-	-	-	-	-	-
Scabs (Treated skin)	(3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
ANIMAL 8																		
Posture																		
Hunched posture	(1)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	1	1	1	1	1	1	1	1	1	-	-	-	-
ANIMAL 9																		
No clinical signs noted		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 10																		
Posture																		
Flat posture	(1)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur / plumage																		
Scales (Treated skin)	(3)	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

TABLE 2 : BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 8	DAY 15
MALES 2000 MG/KG				
	1	405	408	430
	2	462	470	502
	3	405	413	440
	4	431	433	466
	5	456	461	488
	MEAN	432	437	465
	ST.DEV.	27	28	31
	N	5	5	5
FEMALES 2000 MG/KG				
	6	266	273	290
	7	254	257	267
	8	298	301	318
	9	254	246	259
	10	261	267	281
	MEAN	267	269	283
	ST.DEV.	18	21	23
	N	5	5	5

TABLE 3 : MACROSCOPIC FINDINGS

ANIMAL	ORGAN	FINDING	DAY OF DEATH
MALES 2000 MG/KG			
1		No findings noted	Scheduled necropsy Day 15 after treatment
2		No findings noted	Scheduled necropsy Day 15 after treatment
3		No findings noted	Scheduled necropsy Day 15 after treatment
4		No findings noted	Scheduled necropsy Day 15 after treatment
5		No findings noted	Scheduled necropsy Day 15 after treatment
FEMALES 2000 MG/KG			
6		No findings noted	Scheduled necropsy Day 15 after treatment
7		No findings noted	Scheduled necropsy Day 15 after treatment
8		No findings noted	Scheduled necropsy Day 15 after treatment
9		No findings noted	Scheduled necropsy Day 15 after treatment
10	Ovaries	Left side: cyst(s), watery-clear.	Scheduled necropsy Day 15 after treatment