REPORT

Study Title

ACUTE EYE IRRITATION/CORROSION STUDY WITH PERFLUOROHEXANOIC ACID AMMONIUM SALT IN THE RABBIT

Author

Drs.

Study completion date

21 June 2004

Test Facility

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The Netherlands

Laboratory Project Identification

NOTOX Project 400927
NOTOX Substance 138276/A

1. CONTENTS

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.

Study Director

Date: 21 June 2004

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

Head of Quality Assurance

Date: June 22, 2004

4. SUMMARY

Acute eye irritation/corrosion study with Perfluorohexanoic acid Ammonium Salt in the rabbit.

The study was carried out based on the guidelines described in: "Acute Toxicity - Eye irritation", OECD No.405 (2002); EC Commission Directive 92/69/EEC, B.5, "Acute Eye Irritation/Corrosion" (1992); US EPA, OPPTS 870.2400, Acute Eye Irritation, (1998) and JMAFF guidelines (2000).

Single samples of 0.1 ml of Perfluorohexanoic acid Ammonium Salt were instilled into one eye of each of three rabbits. Observations were made 1, 24, 48 and 72 hours and 7, 14, 21 and/or 28 days after instillation.

Instillation of the test substance resulted in effects on the cornea, iris and conjunctivae.
The corneal injury consisted of opacity (maximum grade 2) and epithelial damage (maximum 35%, 65% and 50% of the corneal area). As a result of the corneal injury, pannus (neovascularisation of the cornea) was apparent in one animal 7 and 21 days after instillation. The corneal injury persisted until termination.
Iridial irritation grade 1 was observed in all animals and had resolved within 48 hours in one animal and within 7 days in the remaining animals.
The irritation of the conjunctivae consisted of redness, chemosis and discharge and had completely resolved within 21 and 28 days in two animals, respectively, and persisted until termination in the remaining animal.

Reduced elasticity of the eyelids was noted 24 and/or 48 hours and/or 7 days after instillation.

Based on the degree and persistence of the corneal injury, it was concluded that ocular corrosion had occurred by instillation of Perfluorohexanoic acid Ammonium Salt into the rabbit eye in one animal.

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 should be classified as : having irreversible effects on the eyes (Class 1).

- EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), Perfluorohexanoic acid Ammonium Salt should be labelled as: risk of serious damage to eyes (R 41).

5. INTRODUCTION

5.1. Preface

Sponsor	Daikin Industries, Ltd. 1-1 Nishi Hitotsuya Settsu-shi OSAKA, 566-8585 Japan
Study Monitor	
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	
Study Plan (in-life phase)	Start : 16 February 2004 Completion : 05 April 2004

5.2. Aims of the study

The purpose of this acute eye irritation/corrosion study was to assess the possible irritation or corrosion potential when a single dose of the test substance was placed in the conjunctival sac of the rabbit eye.
This study should provide a rational basis for risk assessment in man.
The absence of eye pigmentation in the albino rabbit facilitates the evaluation of induced eye reactions. The ocular route was selected because the test substance may accidentally come into contact with the eyes 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 97-03-10) 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.405, "Acute Eye 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.5: "Acute Toxicity - Eye Irritation". Official Journal of the European Communities No. L 383, 1992

United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-98-195, 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

6.2. Test substance preparation

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

6.3. 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 Males.
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.

6.4. 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: 16.5 – 24.7°C), a relative humidity of 30-70% (actual range: 29 - 78%) and 12 hours artificial fluorescent light and 12 hours darkness per day.
Cleaning procedures in the room might have caused the temporary fluctuations above the optimal maximum level of 70% for relative humidity. Based on laboratory historical data, these

fluctuations were considered not to have affected the study integrity.

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.

6.5. Treatment

All available data relevant to the potential eye irritation/corrosivity of the substance indicated that no severe effects were to be expected. No severe reactions were noted in the skin irritation study (Notox Project 400938). 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 eye irritation study was performed and was started by treatment of a single rabbit (sentinel). The two other animals were treated in a similar manner 4 weeks later, after considering the degree of eye irritation observed in the first animal.

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 eyes, which were free from any abnormality.

Each animal was treated by instillation of 0.1 ml of the test substance in the conjunctival sac of one of the eyes after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The other eye remained untreated and served as the reference control.

Immediately after the 24-hour observation, a solution of 2% fluorescein in water (adjusted to pH 7.0) was instilled into both eyes of each animal to quantitatively determine corneal epithelial damage. When considered necessary, this procedure was repeated to assess recovery. Any bright green stained area, indicating epithelial damage, was estimated as a percentage of the total corneal area.

6.6. Observations

Mortality/Viability	Twice daily.
Toxicity	At least once daily.
Body Weight	Day of treatment (prior to instillation) and at termination.
Irritation	The eyes of each animal were examined approximately 1, 24, 48 and 72 hours and 7, 14, 21 and/or 28 days after instillation of the test substance. The irritation scores and a description of all other (local) effects were recorded.

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

CORNEAL IRRITATION
Opacity: degree of density (area most dense taken for reading)
No ulceration or opacity (may include slight dulling of normal lustre) 0
Scattered or diffuse areas of opacity, details of iris clearly visible 1
Easily discernible translucent area, details of iris slightly obscured 2
Nacreous area, no details of iris visible, size of pupil barely discernible 3
Opaque cornea, iris not discernible through the opacity 4

Area of cornea involved:
No ulceration or opacity 0
One quarter or less but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

IRIS
Normal 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive) 1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAL IRRITATION
Redness (refers to palpebrae and sclera, excluding cornea and iris):
Blood vessels normal 0
Some blood vessels definitely hyperaemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible 2
Diffuse beefy red 3

Chemosis (refers to lids and/or nictitating membranes):
No swelling 0
Any swelling above normal (includes nictitating membranes) 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed 3
Swelling with lids more than half closed 4

Discharge:
No discharge (may include small amounts observed in inner canthus of normal animals) 0
Any amount different from normal and/or lacrimation 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs (considerable area around the eye) 3

Where standard lighting was considered inadequate for observing minor effects, eye examinations were performed using an ophthalmic examination lamp.
In cases of equivocal results when comparing the treated and untreated eyes, the illustrated guide from the Consumer Product Safety Commission, Washington, D.C. 20207 was used for additional control purposes.

6.7. Interpretation

The results will be 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 for relative humidity occurred and 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. Irritation and corrosion (Table 1)

Instillation of 0.1 ml of Perfluorohexanoic acid Ammonium Salt into one eye of each of three rabbits resulted in effects on the cornea, iris and conjunctivae.
The corneal injury consisted of opacity (maximum grade 2) and epithelial damage (maximum 35%, 65% and 50% of the corneal area). As a result of the corneal injury, pannus (neovascularisation of the cornea) was apparent in one animal 7 and 21 days after instillation. The corneal injury persisted until termination.
Iridial irritation grade 1 was observed in all animals and had resolved within 48 hours in one animal and within 7 days in the remaining animals.
The irritation of the conjunctivae consisted of redness, chemosis and discharge and had completely resolved within 21 and 28 days in two animals, respectively, and persisted until termination in the remaining animal.

Reduced elasticity of the eyelids was noted 24 and/or 48 hours and/or 7 days after instillation.

7.2. Colouration / Remnants

No staining of (peri) ocular tissues by the test substance was observed and no test substance remnants were seen.

7.3. Toxicity / Mortality

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

8. CONCLUSION

Based on the degree and persistence of the corneal injury, it was concluded that ocular corrosion had occurred by instillation of Perfluorohexanoic acid Ammonium Salt into the rabbit eye in one animal.

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 should be classified as : having irreversible effects on the eyes (Class 1).

- EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), Perfluorohexanoic acid Ammonium Salt should be labelled as: risk of serious damage to eyes (R 41).

Table 1: INDIVIDUAL EYE IRRITATION SCORES

Time after dosing	Cornea: Opacity	Cornea: Area	Cornea: Fluor area (%)	Iris	Conjunctivae: Redness	Conjunctivae: Chemosis	Conjunctivae: Discharge	Comments
♂ No 886# (Sentinel)								
1 hour	1	1		0	2	4	2	-
24 hours	2	1	35	0	2	3	3	-
48 hours	1	2		1	3	2	2	f
72 hours	1	1	15	1	3	1	1	f
7 days	0	0	0	0	2	0	0	-
14 days	0	0		0	1	0	0	-
21 days	0	0		0	1	0	0	-
28 days	0	0		0	0	0	0	-
♂ No 916#								
1 hour	1	3		1	2	3	2	-
24 hours	1	3	65	1	3	3	2	-
48 hours	1	3		1	3	2	2	-
72 hours	1	2	35	0	3	1	1	f
7 days	0	0	0	0	2	0	0	-
14 days	0	0		0	1	0	0	-
21 days	0	0		0	1	0	0	-
♂ No 918#								
1 hour	1	3		1	2	3	2	-
24 hours	1	2	50	1	3	2	2	-
48 hours	1	2		1	3	2	2	-
72 hours	1	2	35	1	3	1	1	f
7 days	1	1	10	0	2	0	0	f, p
14 days	1	1	5	0	1	0	0	-
21 days	1	1	5	0	0	0	0	p

Fluor area (%): green staining (percentage of total corneal area) after fluorescein treatment.

#. See next page.

Comments:
p Pannus, neovascularisation of the cornea.
f Reduced elasticity of the eyelids.

Table 2: MEAN VALUE EYE IRRITATION SCORES

Animal #	Mean 24 – 72 hours: Corneal opacity	Iris	Conjunctivae: Redness	Conjunctivae: Chemosis
886	1.3	0.7	2.7	2.0
916	1.0	0.7	3.0	2.0
918	1.0	1.0	3.0	1.7

#. See next page.

Animal specifications:

Animal no	Sex	Age at start (weeks)	Body weights (grams) prior to application	Body weights (grams) at termination
886	♂	9-11	2330	3018
916	♂	9-11	2049	2476
918	♂	9-11	2251	2710