REPORT

Study Title

ASSESSMENT OF ACUTE ORAL TOXICITY WITH PERFLUOROHEXANOIC ACID AMMONIUM SALT IN THE RAT (ACUTE TOXIC CLASS METHOD)

Author

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Study completion date

21 June 2004

Test Facility

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The Netherlands

Laboratory Project Identification

NOTOX Project 400905
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.

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
On-site (Process)	Pathology	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.

pp

J.T.G. Mitchell

Date: June 22, 2004

4. SUMMARY

Assessment of acute oral toxicity with Perfluorohexanoic acid Ammonium Salt in the rat (Acute Toxic Class Method).

The study was carried out based on the guidelines described in: "Acute Toxicity-Oral, Acute Toxic Class Method", OECD No.423 (2001); "Acute Oral Toxicity"; EC Commission Directive 96/54/EC, Part B.1 tris (1996); Environmental Protection Agency (EPA): Health Effects Test Guidelines OPPTS 870.1100 (2002), "Acute Oral Toxicity - Acute Toxic Class Method" and JMAFF Japanese test guidelines (2000).

Perfluorohexanoic acid Ammonium Salt was administered by oral gavage to two subsequent groups of three female Wistar rats at 3000[1] or 2000 mg/kg body weight. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (day 15).

No mortality occurred.

Hunched posture was noted in all animals on day 1 and/or 2.

The body weight gain shown by the animals over the study period was considered to be normal

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

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

According to the OECD 423 test guideline the LD50 cut-off value was considered to exceed 5000 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 oral 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 oral toxicity.

[1] Inadvertently dosed at 3000 mg/kg (due to a calculation error). In the absence of mortality or severe clinical signs, it was decided to treat the next group at 2000 mg/kg.

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 objective of this study was to assess the toxicity of the test substance when administered in a single dose to female rats at one or more defined dosages. Furthermore, the results of the study allowed the test substance to be ranked according to most classification systems, currently in use.
This study should provide a rational basis for risk assessment in man.
The oral 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 03-42) 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.423, "Acute Oral Toxicity - Acute Toxic Class Method", 2001

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the Determination of Toxicity, anticipating the next adaptation to technical progress (29th ATP), Annex IV B, B.1 tris: "Acute Toxicity (Oral) - Acute Toxic Class Method". Official Journal of the European Communities No. L 248, 1996.

United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-98-190, 2002.

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	6 Females (nulliparous and non-pregnant). Each dose group consisted of 3 animals.
Age and body weight	Young adult animals (approx. 10 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: 15.4 – 21.7°C), a relative humidity of 30-70% (actual range: 29 - 66%) and 12 hours artificial fluorescent light and 12 hours darkness per day.

Accommodation
Group housing of 3 animals per sex per cage in labelled Macrolon cages (type IV; height 18 cm.) containing purified sawdust as bedding material (Woody Clean bedding (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. Study design

The toxicity of the test substance was assessed by stepwise treatment of groups of 3 females. The first group was treated at an intended dose level of 300 mg/kg. However, this group was inadvertently dosed at 3000 mg/kg (due to a calculation error). In the absence of mortality or severe clinical signs, it was decided to treat the next group at 2000 mg/kg. The absence or presence of mortality of animals dosed at one step determined the next step, based on the test procedure defined in the guidelines. The onset, duration and severity of the signs of toxicity were taken into account for determination of the time interval between the dose groups.

6.5. Treatment

A health inspection was performed prior to commencement of treatment, to ensure that the animals were in a good state of health.

Method	Oral gavage, using a stainless steel stomach tube.
Fasting	Food was withheld overnight (for a maximum of 20 hours) prior to dosing until 3-4 hours after administration of the test substance.
Frequency	Single dosage, on day 1.
Dose level (volume)	3000 mg/kg[2] (2.79 ml/kg) body weight. 2000 mg/kg (1.86 ml/kg) body weight. Dose volume calculated as dose level : density.

[2] Inadvertently dosed at 3000 mg/kg (due to a calculation error). In the absence of mortality or severe clinical signs, it was decided to treat the next group at 2000 mg/kg.

6.6. 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 symptoms were graded according to fixed scales and the time of onset, degree and duration were recorded: 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.

6.7. Interpretation

The oral LD_{50} value of the test substance was ranked within the following ranges: 0-5, 5-50, 50-300 or 300-2000 mg/kg b.w. or as exceeding 2000 mg/kg b.w.
No statistical analysis was performed (The method used is not intended to allow the calculation of a precise LD_{50} value).

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.
2. The first set of animals was inadvertently dosed at 3000 mg/kg (due to a calculation error). In the absence of mortality or severe clinical signs, it was decided to treat the next group at 2000 mg/kg. This deviation had no effect on the interpretation of the data and no effect on the study outcome.

The study integrity was not adversely affected by the deviations.

7. RESULTS

	Dose level	Date of treatment
First set of females	3000 mg/kg[3]	10 February 2004
Second set of females	2000 mg/kg	17 February 2004

7.1. Mortality

No mortality occurred.

7.2. Clinical Signs (Table 1)

Hunched posture was noted in all animals on day 1 and/or 2.

7.3. Body Weights (Table 2)

The body weight gain shown by the animals over the study period was considered to be similar to that expected of normal untreated animals of the same age and strain.

7.4. Macroscopic Findings (Table 3)

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

8. CONCLUSION

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

According to the OECD 423 test guideline the LD50 cut-off value was considered to exceed 5000 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 oral route.
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[3] Inadvertently dosed at 3000 mg/kg (due to a calculation error). In the absence of mortality or severe clinical signs, it was decided to treat the next group at 2000 mg/kg.

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
FEMALES 3000 MG/KG																		
ANIMAL 1																		
Posture																		
Hunched posture	(1)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 2																		
Posture																		
Hunched posture	(1)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 3																		
Posture																		
Hunched posture	(1)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 2000 MG/KG																		
ANIMAL 4																		
Posture																		
Hunched posture	(1)	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 5																		
Posture																		
Hunched posture	(1)	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 6																		
Posture																		
Hunched posture	(1)	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
FEMALES 3000 MG/KG				
	1	232	267	269
	2	225	265	270
	3	206	229	235
	MEAN	221	254	258
	ST.DEV.	13	21	20
	N	3	3	3
FEMALES 2000 MG/KG				
	4	228	262	268
	5	226	255	262
	6	223	255	256
	MEAN	226	257	262
	ST.DEV.	3	4	6
	N	3	3	3

TABLE 3 : MACROSCOPIC FINDINGS

ANIMAL ORGAN	FINDING	DAY OF DEATH
FEMALES 3000 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
FEMALES 2000 MG/KG		
4	No findings noted	Scheduled necropsy Day 15 after treatment
5	No findings noted	Scheduled necropsy Day 15 after treatment
6	No findings noted	Scheduled necropsy Day 15 after treatment