File No: STD/1535

May 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1-Propene, 2,3,3,3-tetrafluoro-

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1535	The Chemours Company (Australia) Pty Ltd	1-Propene, 2,3,3,3-tetrafluoro-	Yes	≤ 130 tonnes per annum	Refrigerant

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Flammable gases (Category 1)	H220 - Extremely flammable gas
Gases under pressure (Liquefied gas)	H280 – Contains gas under pressure, may explode if heated

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R12: Extremely flammable

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of its low hazard to the environment and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified under GHS as follows:
  - Flammable gases (Category 1): H220 - Extremely flammable gas
  - Gases under pressure (Liquefied gas): H280 – Contains gas under pressure, may explode if heated

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

- The notified chemical should be classified as follows under the ADG Code:
  - Class 2, Division 2.1 (liquefied Gas, flammable, not otherwise specified (n.o.s.))

- Refrigeration and air conditioning systems and units containing the notified chemical should be marked and labelled in accordance with The Australian Automotive Code of Practice 2008 for *Control of*

Refrigerant Gases during Manufacture, Installation, Servicing or De-commissioning of Motor Vehicle Air Conditioners, Australia and New Zealand Refrigerant handling Code of Practice 2007 and Flammable Refrigerants Safety Guide (AIRAH, 2013) and Flammable Refrigerants - Safety Guide (AIRAH, 2013).

CONTROL MEASURES

Safe Design and Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise the risk from exposure and use of the notified chemical:
  - For use in motor vehicle air conditioning (MVAC) systems, the notified chemical should only be used for passenger cars and light trucks that adhere to all the safety requirements of SAE J639 (adopted December 2011) (SAE, 2011a) or ISO 13043:2011 (ISO, 2011), including requirements for a flammable warning label, high-pressure compressor cut-off switch and pressure relief devices, and unique fittings;
  - Design, installation, operation and maintenance of the notified chemical in stationary air conditioning and refrigeration systems should be in accordance with Flammable Refrigerants - Safety Guide (AIRAH, 2013);
  - Fittings consistent with SAE J2844 (adopted October 2011) (SAE, 2011b) should be used for connections with refrigerant containers in professional servicing of MVAC systems.

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise the risk from exposure and use of the notified chemical:
  - Follow all applicable safety precautions stated in The Australian Automotive Code of Practice 2008 for *Control of Refrigerant Gases during Manufacture, Installation, Servicing or De-commissioning of Motor Vehicle Air Conditioners*, Australia and New Zealand Refrigerant handling Code of Practice 2007 and Flammable Refrigerants Safety Guide (AIRAH, 2013).
  - Ensure all workers carrying out work in relation to refrigeration and air conditioning equipment hold a national Refrigerant Handling Licence;

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - Suitable respiratory equipment in case of insufficient ventilation, such as a positive-pressure supplied-air respirator
  - Face shield and eye protection
  - Protective/cold insulating gloves

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Public Health

- The following measures should be taken by manufacturers, distributors or unit owners, where applicable, to minimise public exposure to the notified chemical:
  - Equipment should be maintained and monitored for leaks, with immediate corrective action taken where leaks are detected.
  - The notified chemical should not be sold to or handled by the public.

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

- The storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012), Flammable Refrigerants Safety Guide (AIRAH, 2013) and/or relevant State or Territory Code of Practice.

Emergency procedures

- Spills or accidental release of the notified chemical should be allowed to evaporate; ventilate enclosed areas until safe for re-entry.

Transport and Packaging

- The transport and packing of the notified chemical should be in accordance with State and Territory laws based on the requirements under the *Australian Code for the Transport of Dangerous goods by Road and Rail* (ADG Code) (NTC, 2014).

- Containers of the notified chemical for use in professional servicing of MVACs should be from 2.3 L to 23 L in size.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the container of the notified chemical for use in professional servicing of MVACs is smaller than 2.3 L or greater than 23 L in size;
- further information on the carcinogenicity, mutagenicity, reproductive toxicity and developmental toxicity of the notified chemical becomes available.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of refrigerant for MVACs and stationary air conditioning and refrigeration systems, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

(Material) Safety Data Sheet
The (M)SDSs of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDSs remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
The Chemours Company (Australia) Pty Ltd (ABN: 90 169 142 750)
7 Eden Park Drive
MACQUARIE PARK NSW 2113

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, impurities, use details, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: melting point/freezing point, boiling point, density, vapour pressure, water solubility, hydrolysis as a function of pH, absorption/desorption, dissociation constant, acute oral toxicity, acute dermal toxicity, skin irritation, eye irritation, skin sensitisation and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
No

NOTIFICATION IN OTHER COUNTRIES
USA and Europe

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Opteon® YF

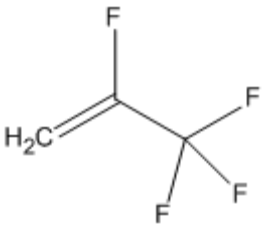
CAS NUMBER
754-12-1

CHEMICAL NAME
1-Propene, 2,3,3,3-tetrafluoro-

OTHER NAME(S)
1,1,1,2-Tetrafluoro-2-propene
1,1,1,2-Tetrafluoropropene
1234yf
2,3,3,3-Tetrafluoro-1-propene
2,3,3,3-Tetrafluoropropene
2,3,3,3-Tetrafluoropropylene
HFC 1234yf
HFO 1234yf
R 1234yf

MOLECULAR FORMULA
$C_3H_2F_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
114.04 Da

ANALYTICAL DATA
Reference FTIR and GC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
≥ 99.5%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: colourless gas

Property	Value	Data Source/Justification
Melting Point/Freezing Point	–152.2 °C	(M)SDS
Boiling Point	–29.4 °C at 101.3 kPa	(M)SDS
Relative Density	4 (Air = 1.0)	(M)SDS
Density	0.0048 g/cm^3 at 20 °C, 101.3 kPa (vapour density)	(M)SDS
Vapour Pressure	583 kPa at 20 °C	(M)SDS
Water Solubility	0.1982 g/L at 20 °C	(M)SDS
Hydrolysis as a Function of pH	Not determined	Not expected as the notified chemical does not contain readily hydrolysable functionality.
Partition Coefficient (n-octanol/water)	log Pow = 2.0 at 25 °C	Measured
Adsorption/Desorption	log K_{oc} = 1.735	Calculated using KOCWIN v2.00 (US EPA , 2011)
Dissociation Constant	Not determined	No dissociable functionality
Flammability Limits	Upper: 6.2% Lower: 12.3%	Measured (M)SDS
Autoignition Temperature	405 °C	Measured
Explosive Properties	Predicted negative	Estimated
Oxidising Properties	Predicted negative	Estimated

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable under normal conditions of use. Due to the low boiling point of the notified chemical (-29.4 °C), it has the potential to cause frostbite burns to human tissue when released from the pressurised form, as it changes from a liquefied gas to a gas.

Thermal decomposition products may include hydrogen fluoride and fluorinated compounds.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of*

Chemicals (GHS), as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Flammable gases (Category 1)	H220 - Extremely flammable gas
Gases under pressure (Liquefied gas)	H280 – Contains gas under pressure, may explode if heated

Dangerous Goods classification
Based on the submitted physico-chemical data in the above table, the notified chemical is classified according to the Australian Dangerous Goods Code (NTC, 2014) as Class 2, Division 2.1 (liquefied gas, flammable, n.o.s.). The data above do not address all Dangerous Goods endpoints. Therefore, consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in the neat form or as a component of refrigerants (at 30% or 60% concentration) in tanks in a shipping container. It may also be imported at ≤ 100% concentration in imported units (motor vehicle air conditioning systems, stationary air conditioning systems and stationary refrigeration systems).

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	10-80	10-80	10-80	10-80	80-130

PORT OF ENTRY
Sydney and Melbourne

IDENTITY OF RECIPIENTS
The Chemours Company (Australia) Pty Ltd

TRANSPORTATION AND PACKAGING
The imported chemical will be repacked from imported bulk tanks to smaller cylinders or bulk storage tanks and will be delivered to customers by road. The notified chemical as a liquefied gas or as a component of liquefied gases will be packaged and transported in accordance with the requirements of Australian Dangerous Goods Code (NTC, 2014).

USE
The notified chemical is proposed for the following uses:
- Refrigerant for motor vehicle air conditioning (MVAC) systems of passenger cars and light trucks; and
- Refrigerant for residential and commercial stationary air conditioning and refrigeration systems.

OPERATION DESCRIPTION

Repackaging
The notified chemical as a liquefied gas or as a component of liquefied gases will be transferred from the bulk imported containers into smaller cylinders, 15 tonne ISO containers or bulk storage tanks in accordance with the requirements of Australian Dangerous Goods Code (NTC, 2014) and Australia and New Zealand Refrigerant Handling Code of Practice 2007 (AIRAH and IRHACE, 2007).

Charging OEM unit –MVAC systems
This operation will not occur in Australia. The notified chemical will be pre-charged in the imported MVAC systems.

Servicing units – MVAC systems

At the sites where MVAC units are serviced, qualified technicians will fill these units with refrigerants containing the notified chemical by transfer from cylinders of 2.3 – 23 L in size with connectors consistent with SAE J2844 (SAE, 2011b). Qualified technicians may also empty the air conditioning units during maintenance and end-of-service life of the units. In these instances, the notified chemical will be captured and returned to a licensed company for destruction or recycling. All these operations are expected to be carried out in accordance with relevant safety requirements such as the requirements in The Australian Automotive Code of Practice 2008 (DEWHA, 2008), Australia and New Zealand Refrigerant Handling Code of Practice 2007 (AIRAH and IRHACE, 2007) and Flammable Refrigerants – Safety Guide (AIRAH, 2013).

Charging OEM units – stationary air conditioning and refrigeration systems
At the sites where stationary air conditioning and refrigeration units are manufactured, the units will be charged with refrigerants containing the notified chemical via production line filling. The operation is expected to be carried out in accordance with relevant safety requirements such as the requirements in Australia and New Zealand Refrigerant Handling Code of Practice 2007 (AIRAH and IRHACE, 2007) and Flammable Refrigerants – Safety Guide (AIRAH, 2013).

The notified chemical may also be pre-charged in the imported stationary air conditioning and refrigeration systems.

Servicing units – stationary air conditioning and refrigeration systems
At the sites where commercial air conditioning and refrigeration units are situated, licenced technicians will top-up or fill these units with refrigerants containing the notified chemical by transfer from cylinders. Qualified technicians may also empty the air conditioning and refrigeration units during maintenance and end-of-service life of the units. In these instances, the notified chemical will be captured and returned to a licensed company for destruction or recycling. All these operations are expected to be carried out in accordance with relevant safety requirements such as the requirements Australia and New Zealand Refrigerant Handling Code of Practice 2007 (AIRAH and IRHACE, 2007) and Flammable Refrigerants – Safety Guide (AIRAH, 2013).

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehousing	2-4	12-24
Repackaging/decanting	0.5-2	50
Refrigeration/air conditioning technicians	0.5-4	200
Motor mechanics	0.5-1	200

EXPOSURE DETAILS
Transport and storage workers are not expected to be exposed to the notified chemical except in the unlikely event of an accidental leakage.

Potential routes of occupational exposure are dermal, ocular and inhalation. However as the notified chemical is a gas at room temperature, inhalation is the main expected route of exposure. Dermal and ocular exposure to the notified chemical as a liquefied gas or as a gas may occur during transfer operations or accidental leakages.

Repackaging
Worker exposure may occur during transfer from imported containers to retail and bulk containers particularly when connecting and disconnecting transfer hoses.

Charging OEM units and servicing units
When used as a refrigerant for residential and commercial air conditioning and refrigeration systems, worker exposure may occur during installation, filling, topping-up and emptying the units, particularly when connecting and disconnecting transfer hoses. The notifier has stated that transfer equipment is expected to use shut-off valve couplers that do not permit release of the gas unless attached to the refrigeration unit. Workers may also be

potentially exposed to the notified chemical if a leakage occurs. This exposure would be highest in the case of any sudden loss of containment. Awareness of exposure to leakage of the notified chemical may not occur, because as a gas it is odourless and colourless.

It is required in Australia that a person who carries out work in relation to refrigeration and air conditioning equipment must hold a national Refrigerant Handling Licence. There are also a variety of safety and use guidelines for employing refrigerants (including the notified chemical), such as The Australian Automotive Code of Practice 2008 (DEWHA, 2008), Australia and New Zealand Refrigerant handling Code of Practice 2007 (AIRAH and IRHACE, 2007) and Flammable Refrigerants – Safety Guide (AIRAH, 2013). Therefore, professional workers are expected to have the proper equipment and knowledge to minimise their risks from exposure to the notified chemical.

For use in MVAC systems, worker exposure is further mitigated by the safety requirements for design of the units (SAE, 2011a) and requirements for cylinder size and connectors with containers (SAE, 2011b) for use in professional servicing.

6.1.2. Public Exposure

The notified chemical will be used in industrial settings and/or handled by professional technicians. In general, the public is not expected to be exposed to the notified chemical except in the unlikely event of an accidental leakage from the units containing the notified chemical in the vicinity of the public.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute inhalation toxicity	LC50 > 9960 ppm/4 hours; low toxicity
Rat, acute inhalation toxicity	LC50 > 405,800 ppm/4 hours; low toxicity
Mouse, acute inhalation toxicity	LC50 > 99,830 ppm/4 hours; low toxicity
Rabbit, acute inhalation toxicity	LC50 > 102,000 ppm/1 hour; low toxicity
Rat, repeat dose inhalation toxicity – 14 days	NOAEC > 51,690 ppm
Rat, repeat dose inhalation toxicity – 28 days with unscheduled DNA synthesis test and mammalian erythrocyte micronucleus test	NOAEC > 50,031 ppm non-genotoxic
Rat, repeat dose inhalation toxicity – 90 days.	NOAEC > 50,116 ppm
Rabbit, repeat dose inhalation toxicity – 28 days	NOAEC = 500 ppm (males) and 1,000 ppm (females)
Minipig, repeat dose inhalation toxicity – 14 days	cardiotoxicity/skeletal muscle toxicity NOAEC > 10,300 ppm
Minipig, repeat dose inhalation toxicity – 28 days	cardiotoxicity/skeletal muscle toxicity NOAEC > 10,200 ppm
Dog, cardiac sensitisation to adrenaline	NOAEC > 120,189 ppm; no evidence of cardiac sensitisation
Mutagenicity – bacterial reverse mutation	mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test (cultured human lymphocytes)	non-genotoxic
Genotoxicity – *in vivo* mammalian (mouse) erythrocyte micronucleus Test	non-genotoxic
Rat, prenatal developmental toxicity	maternal/developmental NOAEC > 50,315 ppm
Rabbit, prenatal developmental toxicity	maternal NOAEC < 2,500 ppm foetal NOAEC = 4,000 ppm
Rat, two generation reproduction toxicity	NOAEC > 49,958 ppm
Mouse and rat, carcinogenicity	predicted to be non-carcinogenic (female mouse liver and male rat kidney)
Mouse, carcinogenicity	predicted to be similar to other substances found to be carcinogenic in the female mouse lung

Toxicokinetics, metabolism and distribution

In studies on the biotransformation of the notified chemical (Schuster et al., 2008), male Sprague-Dawley rats were exposed by inhalation to levels of 2,000, 10,000, and 50,000 ppm for 6 hours and male B6C3F1 mice were exposed by inhalation to levels of 50,000 ppm for 3.5 hours. Urine was collected for 48 hours after the end of the

exposure time and urinary metabolites were identified by ^{19}F-NMR, LC-MS/MS or GC/MS. The major metabolites identified were two diastereomers of N-acetyl-S-(3,3,3-trifluoro-2-hydroxy-propyl)-l-cysteine. Minor metabolites included trifluoroacetic acid, 3,3,3-trifluorolactic acid, 3,3,3-trifluoro-1-hydroxyacetone, 3,3,3-trifluoroacetone and 3,3,3-trifluoro-1,2-dihydroxypropane.

The quantified amounts of the metabolites excreted with urine suggested a low extent of biotransformation of the notified chemical and most of major metabolites (90%) were excreted within 18 hours after the end of exposure ($t_{1/2}$ = approx. 6 h). The results of the study also suggested that the major metabolic pathway of the notified chemical is likely to be cytochrome P450 2E1-mediated epoxide formation at low rates, followed by glutathione conjugation (Schuster et al., 2008).

A similar study (exposed by inhalation to levels of 2,000, 10,000, and 50,000 ppm for 6 hours) was carried out in rabbits (Schuster et al., 2010). The major urinary metabolite identified was also N-acetyl-S-(3,3,3-trifluoro-2-hydroxy-propyl)-l-cysteine and S-(3,3,3-Trifluoro-2-hydroxypropanyl)mercaptolactic acid, 3,3,3-trifluoro-1,2-dihydroxypropane, 3,3,3-trifluoro-2-propanol and inorganic fluoride were present. The results of the study suggested a low extent of biotransformation of the notified chemical and the same major metabolic pathway as that in rats and mice. Most of major metabolites (95%) were excreted within 12 hours after the end of exposure ($t_{1/2}$ = approx. 9.5 h).

In another study on the biotransformation of the notified chemical (Schmidt et al., 2012; HLS 2011), male, female and pregnant female rabbits were exposed by inhalation to levels of 50,000 and 100,000 ppm for 1 hour. Urine was collected for 48 hours after the end of the exposure time and urinary metabolites were identified by ^{19}F-NMR and LC-MS/MS. The major metabolites identified in rabbit urine were S-(3,3,3-trifluoro-2-hydroxypropanyl)-mercaptolactic acid and N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropanyl)-L-cysteine. No differences in urinary metabolite pattern or quantity of metabolites excreted were noted between the different treatment groups.

An *in vitro* study was carried out to illustrate the relationship between the metabolism of the notified chemical *in vivo* and the metabolism of the notified chemical *in vitro* with metabolic activation (Aroclor-induced rat liver S9) which is normally required in *in vitro* genetic toxicology studies (DuPont 2012a). The study revealed significant differences between the metabolism *in vivo* and that *in vitro* with metabolic activation. The differences included the metabolic activation enhanced *in vitro* bioactivation of the notified chemical, which is distinctly different from *in vivo* metabolism, and the transient epoxide metabolite formed by S9 *in vitro* was readily removed *via* glutathione conjugation, which corroborated the detoxification pathway for the notified chemical *in vivo*.

A physiologically-based pharmacokinetic (PBPK) model was also developed to assess possible toxicological effects of accidental or occupational inhalation exposure of the notified chemical (DuPont 2008a). Breath by breath (BBB) and constant flow (CF) models were developed for adult female humans under acute exposure (80,000 ppm) in a confined space and chronic exposure at a potential Occupational Exposure Limit (OEL = 400 ppm) in the workplace, and compared to equivalent scenarios for pregnant rats and rabbits using experimentally derived NOAECs of 5000 ppm and 4000 ppm, respectively for these species.

As the only route for uptake and elimination in the model is via inhalation and exhalation, the most sensitive parameter in the model is the blood to air partition coefficient, which in humans was determined to be lower than for rats or rabbits, as shown in the table below (DuPont 2008b).

Tissue type	*Blood to air partition coefficient (n = 5) (mean ± standard deviation)*
Female rat blood	0.076 ± 0.010
Male rabbit blood	0.072 ± 0.010
Female human blood	0.038 ± 0.007
Male human blood*	0.035 ± 0.005

* n = 4, one data point eliminated as an outlier

To provide an evaluation of the relative risk of human exposure to the notified chemical, comparisons were made between human and animals based on the peak concentration in arterial blood and the total dose received in blood (area under the curve (AUC) values) (DuPont 2008a). The AUC for this type of exposure is essentially equivalent to the steady-state concentration for the inhalation concentration multiplied by the time of exposure, expressed in units of mg/L·h. The AUC for potential human exposure at the OEL was compared with the AUC values at the experimentally derived NOAECs for the two animal species, to determine a corresponding margin

of safety. The margin of safety ratios for rabbits were calculated to be 14-26, depending on the exposure duration; 14 corresponds to human exposure for 8 h/day and rabbit exposure for 6 h/day. The margin of safety ratios for rats were calculated as 188-349, depending on the exposure duration; 188 corresponds to human exposure for 8 h/day and rat exposure for 6 h/day. The results indicate that higher exposure is required for the human to reach the animal dosimetric, for both the rabbit and rat scenarios. The CF model simulated an improbable, worst case car crash scenario where a pregnant individual was trapped in a vehicle compartment containing the entire refrigerant charge, where no windows were broken, there was no damage to vehicle that would allow outside air exchange and where there was no ventilation for 5 minutes. The AUC in the CF model was calculated as approximately 1.2 mg/L·h. By comparing the AUC of 225.6 mg/L·h for the pregnant rabbit exposed for 28 days to a developmental NOAEC (foetal) it was concluded in the study that the acute exposure scenario was not considered to be a concern.

Another CF PBPK model was developed to assess possible toxicological effects of occupational inhalation exposure to the notified chemical for a longer term (days to weeks) (DuPont 2013). The simulated inhalation exposure scenario for rabbits was daily exposure to 500 ppm (experimentally derived NOAEC) notified chemical in air for 6 h/day for 28 consecutive days. The simulated inhalation exposure scenario for adult female humans was occupational exposure to 500 ppm notified chemical for a typical work week (five consecutive days of exposure followed by two weekend days with no exposure at either 6 h/day or 8 h/day scenarios). The one-day AUC ratio for rabbits versus humans was 1.42 or 1.90, for 6 h/day or 8 h/day assumed for occupational exposure, respectively. The longer-term AUC ratio (exposure periods of 7 days and 28 days) increased from the one-day values of 1.42/1.90 to 1.99/2.65, due to the absence of occupational exposure on weekends.

Acute toxicity
No data on acute oral toxicity or acute dermal toxicity were submitted. Significant oral or dermal absorption is considered to be unlikely.

The notified chemical was found to be of low acute toxicity through inhalation in mice (LC50 > 99,830 ppm/4 h), rats (LC50 > 405,800 ppm/4 h) and rabbits (LC50 > 102,000 ppm/1 h). It was reported that pregnant rabbits were found to be unexpectedly sensitive to an inhalation exposure of 50,000 ppm when compared with pregnant and non-pregnant rats and male rats (HLS 2011). The study (HLS 2011) investigated acute toxicity and biotransformative effects. According to the study authors, the results suggest that the previously seen lethality of the test substance in pregnant rabbits after inhalation exposure was unlikely to be due to changes in biotransformation patterns or capacity of pregnant rabbits to tolerate the notified chemical.

Irritation and sensitisation
No skin or eye irritation data were submitted for the notified chemical. In the gaseous state, the notified chemical is not expected to cause irritation to the skin and eyes or cause skin sensitisation as such effects were not reported in any of the acute or repeated inhalation exposure studies. Furthermore, the notified chemical is expected to rapidly diffuse away from the contact surface. However, in the liquefied state, the notified chemical can cause frostbite upon contact with the skin and eyes.

Repeated dose toxicity
Repeated dose inhalation studies were carried out in rats, with target concentrations of up to 50,000 ppm in a pilot 14-day and in 28-day and 90-day studies. No test substance related mortalities were observed in any of the groups. Macroscopic and microscopic examination at necropsy did not reveal any test substance findings. Some changes in haematology and clinical chemistry parameters were noted but not considered by the study authors to be test substance related.

A repeated dose inhalation study was also carried out in rabbits. Test substance related mortalities occurred to one female rabbit (at 4,500 ppm) and to one male and one female rabbits (at 5,500 ppm). Minimal to slight subacute/chronic myocardial inflammation was observed in male and female rabbits, which recovered after 28 days without exposure to the notified chemical. When compared to the control group increased incidence and/or severity of acute skeletal muscle necrosis was noted in both sexes. The NOAEC was established as 500 ppm in male rabbits and 1,000 ppm in female rabbits.

Further 14 and 28 day studies in minipigs designed to focus on the cardiotoxicity and skeletal muscle toxicity in particular, showed no adverse effects up to the maximum tested doses of 10,300 ppm (14 day study) and 10,200 ppm (28 day study).

Pigs are considered to be a superior model for cardiovascular safety pharmacology in comparison to rabbits (Feldman, 2014). The reasons for this are that in comparison to rabbits pigs are not reported to develop spontaneous myocarditis; pigs have a biochemical profile similar to human hearts in terms of β-adrenergic receptor signalling, calcium cycling and homeostasis, energy production and utilisation, and adenosine signalling; colonies of pigs have not been reported to carry myocarditis-causing bacteria and viruses; pig heart morphology is highly similar to humans; irritants and stress have not been reported to cause myocarditis in pigs; and pigs are hardier animals, less influenced by overcrowding or other stressors. Therefore, in regards to cardiovascular toxicity the pig may be a better model to assess the toxicity of the notified chemical in humans. Based on this, as well as on the absence of adverse treatment related effects in the repeat dose studies with rats, the notified chemical is expected to be of low chronic toxicity to humans following repeated inhalation exposure.

Mutagenicity/Genotoxicity
When administered as a gas, the notified chemical was found to be mutagenic in a bacterial reverse mutation test. The significance of the positive result in the bacterial reverse mutation study is not clear. Additional information e.g., an in vivo mutation assay in transgenic mice, would be needed to clarify the mutagenic potential of the notified chemical.

There was no evidence of clastogenicity to human lymphocytes *in vitro*, or mouse micronucleus erythrocytes in bone marrow *in vivo*. The genotoxicity (unscheduled DNA synthesis and examination of micronuclei in bone marrow) of the notified chemical was also investigated as part of a 28-day repeated dose inhalation study, where it was found to be non-genotoxic. Based on the weight of evidence from the available studies, the notified chemical is not suspected to be genotoxic.

Cardiac sensitisation
The notified chemical did not induce cardiac sensitisation in beagle dogs at levels up to 120,189 ppm, the highest dose tested.

Developmental toxicity
No significant treatment-related changes indicative of maternal and developmental toxicity were observed in rats in an inhalation study carried out to OECD TG 414. The NOAEC for maternal and developmental toxicity was established as 50,315 ppm, the highest dose tested. A dose-related and statistically significant increase in the foetal and litter incidence of the finding "two or more ribs wavy" in all treatment groups was observed. However, wavy ribs were considered by the study authors to be a reversible pathologic finding. A delayed ossification in foetuses of all treatment groups was not considered to be dose-related by the study authors. The cases of wavy ribs seen in some developing foetuses were considered to be of some concern by US EPA (US EPA 2011). The US EPA also pointed out that the reversibility of the effect was unclear and that the interim results from a two generation reproductive study (TNO 2011) did not find an association between exposure to the notified chemical and skeletal effects (US EPA 2011).

In a developmental toxicity study in rabbits, there were mortalities considered by the study authors to be attributed to the test substance at exposure levels of 5,500 ppm and above, even though the cause of death could not be determined. The maternal NOAEC was established as < 2,500 ppm due to lethality, clinical signs of toxicity and adverse microscopic findings at all doses tested (2,500 ppm, 4,000 ppm, 5,500 ppm and 7,500 ppm). The embryo/foetal developmental NOAEC was established as 4,000 ppm based on malformations in the cardiovascular system of foetuses at the higher doses (5,500 ppm and 7,500 ppm).

Reproductive toxicity
Based on a two-generation reproduction toxicity study in rats carried out to OECD TG 416 on the notified chemical by inhalation, the NOAEC for reproductive toxicity was considered to be 49,958 ppm, the highest dose tested.

There were significant differences in toxicity between the prenatal developmental studies conducted in rats and rabbits. In the absence of a longer term multi-generation study in rabbits to provide further information on the developmental and reproductive toxicity of the notified chemical to rabbits, the prenatal developmental study results in rabbits raise some concerns.

Carcinogenicity
No animal studies for carcinogenicity that were conducted to OECD test guidelines were submitted.

In a submitted toxicogenomic study on the notified chemical, gene expression changes following a 90-day inhalation study were used to assess the carcinogenic potential of the notified chemical in the female mouse liver and male rat kidney. No treatment-related histopathological lesions were observed following exposure at 10,054 ppm and 49,728 ppm. Statistical classification analysis predicted the notified chemical to be non-carcinogenic in both male rat kidney and female mouse liver with 97.8% and 98.5% accuracy, respectively. However, gene expression changes in the male rat kidney suggested potential endocrine-related effects and linkage to changes in body mass index, triglyceride levels, cholesterol and blood pressure status.

In another submitted toxicogenomic study, gene expression changes were examined in the lungs of female mice exposed by inhalation in a 90-day study to 10,054 ppm and 49,728 ppm of the notified chemical, to predict the carcinogenic potential in that organ. Histopathological effects in the lung were limited to minimal inflammation in one of ten mice in each of the dose groups. Statistical classification analysis predicted the notified chemical to be similar to other substances known to induce tumours in the female mouse lung (i.e., the training set) with 77.5% accuracy. Pair-wise gene expression analysis between the air control group and each group exposed to the notified chemical identified multiple differentially expressed genes.

An expert opinion (Dekant, 2009) discussed the relevance of the above study. The adequacy of toxicogenomic assays for mouse lung tumours is in question, as it has been acknowledged in the scientific literature that false positives may be as high as 25%. It is considered that in general toxicogenomic assays for mouse lung carcinogenicity are more suitable for use in a weight of evidence approach, rather than as a stand-alone predictive tool. Several weak points in the study itself were also identified in the expert opinion (e.g., some training chemicals not applied by inhalation route; noncarcinogenic pneumotoxic chemicals not included in the training set; inconsistent data of some training chemicals; genomic data on the notified chemical barely positive). Other available toxicology and metabolism information was considered as part of a weight of evidence approach for carcinogenicity. For the notified chemical, several factors significantly weaken the support for the toxicogenomics-predicted lung carcinogenicity potential: the low rates of metabolism, the absence of positive results in the genotoxicity studies performed, and the absence of rodent lung pathology in the 90-day subchronic inhalation study. The absence of lung toxicity in the subchronic toxicity study in particular is inconsistent with the mode of action delineated for a number of other chemicals inducing mouse lung tumours after inhalation exposures and bioactivation by pulmonary cytochrome P450s. It was suggested in the expert opinion that even if the prediction of mouse lung carcinogenicity potential (by toxicogenomics assay) is correct, the risk most likely cannot be extrapolated to humans because human lung is relatively deficient in the activating enzymes (CYP2E1 and 2F1) and susceptible lung cells (Clara cells) that are crucial for the induction of mouse lung cancer. Furthermore, this assessment is dependent on the assumption that the mode of action of the training set chemicals is applicable to the notified chemical. Overall, the totality of the evidence/data does not support a significant risk for lung tumour induction in humans after inhalation exposures to the notified chemical under realistic exposure conditions. However it is not possible to rule out carcinogenicity potential for the notified chemical.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Physico-chemical hazards
The notified chemical is a gas at room temperature; however, storage and handling will occur with it present as a liquefied gas. It is classified as a Dangerous Good in Class 2, Division 2.1 (liquefied gas, flammable, n.o.s.). Heating of pressurised containers containing the notified chemical may lead to rupture of the container. Vapours of the notified chemical are heavier than air and can reduce the amount of oxygen available for respiration. In addition, contact with rapidly evaporating liquid can cause frostbite to the skin or damage to eyes.

The hazardous decomposition product, hydrofluoric acid (HF) (CAS No. 7664-39-3), may be formed after heating or combustion of the notified chemical.

Although the notified chemical is flammable, extensive testing has indicated that the ignition potential of the chemical is far less than that of other flammable refrigerants and a risk assessment indicated the use of the chemical in motor vehicles would involve an acceptable level of risk (SAE, 2013).

The risk of an individual being exposed to HF above the relevant health-based limit was considered to be extremely low, on the order of 5×10^{-12} events per hour of vehicle operation and a comparative analysis showed that these risks were well below those commonly considered acceptable by the public and regulatory agencies (SAE, 2013). In addition, potential exposure is only expected to occur when a person is in the vicinity when the notified chemical is leaked and subsequently ignited to generate HF.

The risks of flammability and HF generation of the notified chemical are expected to be lower in other proposed uses than the use in MVAC systems and therefore are not considered to be unacceptable.

Health hazards
Toxicological testing carried out on the notified chemical showed a low potential for acute or subchronic toxicity and did not raise a concern for respiratory tract irritation, cardiac sensitisation, genotoxicity or reproductive toxicity.

Eye and skin irritation or skin sensitisation were not tested for. Eye and skin contact with the liquefied notified chemical is known to cause frostbite. However, this risk is expected to be mitigated by the use of protective clothing and eye protection. Contact with the notified chemical in gaseous form is not expected to have significant adverse effects.

Scenarios with high potential exposure include those with poor ventilation and those where there is large accidental or deliberate discharge of the notified chemical. Inhalation exposure to airborne concentrations of the notified chemical can be reduced by the use of the notified chemical in well-ventilated areas. However, if significant inhalation exposure is expected, respiratory protection may be required to reduce exposure.

The results of developmental studies in rats and rabbits raised some concern. In addition, the carcinogenicity potential for the notified chemical cannot be ruled out. However, adverse health impacts to workers are not expected as all operations involving the notified chemical, which may lead to exposure, are regulated by a number of international and Australian standards, codes of practice and industry guides.

Overall, provided that control measures are in place and good practices are followed to limit exposure to the notified chemical and its decomposition product hydrofluoric acid, the risk to the health of workers under the proposed use of the notified chemical is not considered unreasonable.

6.3.2. Public Health
Public exposure to the notified chemical through its industrial use and/or professional handling is expected to be low unless there is accidental release in the vicinity of the public. Considering the units containing the notified chemical are generally situated in well-ventilated areas, which mitigates exposure due to accidental leakage, the risk to public health from these uses is expected to be low.

The worst scenario is considered to be an accidental leakage in a motor vehicle with minimal air exchange and/or HF being generated and released into the vehicle cabin. There are safety and use guidelines such as ISO 13043 (ISO, 2011) and SAE J639 (SAE 2011a) providing guidance to vehicle manufacturers for safe design of MVAC systems. Risk assessments conducted under the SAE International Cooperative Research Program (CPR) concluded that the HF exposure risks associated with use of the notified chemical were extremely low, and on the order of 5×10^{-12} events per hour of vehicle operation (SAE, 2013). The sponsors of the CPR (major automotive manufacturers) have concluded that the notified chemical can be safely accommodated through established industry standards and practices for vehicle design, engineering, manufacturing, and service (SAE, 2009).

Overall, the risk to public health from the proposed uses is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical is not manufactured or reformulated in Australia. Therefore, there will be no releases due to these activities. The notified chemical may be repackaged in Australia but no significant release of the notified chemical is expected during transfer of the notified chemical.

RELEASE OF CHEMICAL FROM USE
The notified chemical may be released to the atmospheric component as a result of accidental leakages when used as a refrigerant for air conditioning and refrigeration systems.

RELEASE OF CHEMICAL FROM DISPOSAL
As a refrigerant, the notified chemical is expected to be recovered during maintenance or at end-of-service life for disposal via an approved product stewardship scheme for either recycling or destruction.

7.1.2. Environmental Fate
The notified chemical is not readily biodegradable (refer to Appendix C) and is expected to be stable to hydrolysis under environmental conditions based on its structure. However, the notified chemical is not expected to bioaccumulate based on its low partition coefficient (log Pow = 2.0). Further, the notified chemical is considered unlikely to be released into or partition to the aquatic compartment in significant quantities based on its reported use pattern and atmospheric fate as elaborated below.

In the atmosphere, the notified chemical is predicted to have a half-life ($t_{½}$) of 1.03 days based on the rate constant for reaction with hydroxyl radicals (k_{OH}) of 8.2×10^{-12} cm^3/molecule·s (AOP v1.92; US EPA 2011). Reaction with ozone is not expected to be a dominant pathway for degradation in the atmosphere ($t_{½}$ = 16.37 days, $kO_3 = 7.0 \times 10^{-19}$ cm^3/molecule·s; AOP v1.91; US EPA 2011).

The notified chemical may be recovered when used as refrigerant, and is expected to be mineralised to water, oxides of carbon and hydrofluoric acid (HF) during destruction.

The notified chemical is expected to degrade in the atmospheric compartment to eventually form trifluoroacetic acid (TFA). Henne *et al*. 2012, modelled notified chemical future emissions after complete conversion to this refrigerant gas in mobile air conditioning units by the year 2020. TFA deposition rates from rainwater were estimated to be a maximum 2.0 kg km^{-2} yr^{-1}. About 30-40% of TFA was deposited over Europe and the remainder over the ocean and other land masses. TFA is ubiquitous in the aquatic environment and has been found at up to 0.2 µg/L in precipitation and 40 µg/L in enclosed lakes, although surface water concentrations are more typically less than 0.6 µg/L (Boutonnet, 1999). TFA has been reported to be present in ocean water at 0.2 µg/L at Noosa Heads, Queensland (Frank *et al*., 1996 and Frank & Klein, 1997 cited in Boutennet, 1999). Environmental concentrations are likely to include natural sources of TFA, such as volcanic emissions, as well as direct and indirect anthropogenic sources of TFA. The IPCC/TEAP (2005) report concludes that cumulative anthropogenic sources of TFA, such as from the degradation of hydrofluorocarbons (HFCs), are smaller than natural sources. Therefore, the introduction and use of the notified chemical is not expected to significantly increase background concentrations of TFA in the aquatic compartment from natural and anthropogenic sources.

7.1.3. Predicted Environmental Concentration (PEC)
A predicted environmental concentration (PEC) cannot be calculated for the aquatic compartment because the notified chemical is highly volatile and no aquatic exposure is anticipated. A PEC for the atmospheric compartment has not been calculated as there are no available environmental effects endpoints for a PEC to be compared with in a quantitative risk characterisation.

7.2. Environmental Effects Assessment
The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 (96 h) > 197 mg/L	Not harmful to fish up to the limit of its water solubility
Daphnia Toxicity	EC50 (48 h) > 100 mg/L	Not harmful to aquatic invertebrates up to the limit of its water solubility
Algal Toxicity	NOE_rC (72 h) > 100 mg/L	Not harmful to algae up to the limit of its water solubility

The toxicity data to fish, daphnia and alga in the table above suggest that the notified chemical is not harmful to aquatic organisms up to the limit of water solubility. The notified chemical is not expected to be biodegradable in the environment. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is not expected to be harmful to fish, invertebrates and algae on an acute or long term basis and is not formally classified under the GHS.

Atmospheric Compartment
There are no standard ecotoxicological endpoints for evaluating effects in the atmospheric compartment. Generally the effects assessment for this compartment involves the evaluation of the long-range transport potential, global warming potential (GWP) and ozone depleting potential (ODP).

The notified chemical is considered to have long-range transport potential as its half-life in the atmosphere, based on the measured reaction rate with hydroxyl radicals, is 1.03 days.

The notified chemical is phototransformed in the air with ~ 100% degradation observed by 11 days (Hurley *et al*. 2007). In the atmosphere, the notified chemical is predicted to have a half-life (t½) of 1.304 days based on the rate constant for reaction with hydroxyl radicals (k_{OH}) of 8.2034 × 10^{-12} cm^3/molecule·s (AOP v1.92; US EPA 2011). Reaction with ozone is not expected to be a dominant pathway for degradation in the atmosphere (t½ = 16.4 days, kO_3 = 7.00 × 10-19 cm3/molecule·s; AOP v1.92; US EPA 2011). The notified chemical is not expected to deplete the ozone layer (ozone depleting potential = 0).

Vassileios *et al.* (2007) measured the OH reactive coefficient and global warming potential (GWP) of the notified chemical. The atmospheric lifetime was measured to be 12 days solely based on OH reactive loss. The global warming potential was calculated to be < 4.4 for the 100 year time horizon.

7.2.1. Predicted No-Effect Concentration
A predicted no-effect concentration (PNEC) was not calculated for the aquatic compartment as aquatic exposure is not expected.

7.3. Environmental Risk Assessment
The risk quotient (Q = PEC/PNEC) could not be calculated for the notified chemical as no aquatic exposure is expected based on the reported use pattern. The notified chemical is a gas at environmentally relevant temperature and pressure and is expected to be released into the atmospheric compartment following its use or disposal. The notified chemical is of low hazard to aquatic organisms and is not expected to be released to the aquatic compartment. In the atmosphere, the notified chemical may undergo long range transport but is not expected to be a significant contributor to global warming or ozone depletion. Therefore, on the basis of the global warming potential and the assessed use pattern the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Partition Coefficient (n-octanol/water) log Pow = 2.0.at 25 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	CERI (2008)

Flammability Highly flammable

Method	EC Council Regulation No 440/2008 A.11 Flammability (Gases).
Remarks	Ignition was obtained at electrode gap of 3-5 mm and 60 mm above the bottom of the test vessel. Ignition was first noted at a gas concentration of 10% (w/w) although a blue glow was noted at 6% (w/w).
Test Facility	Chilworth (2006)

Flammability Lower: 6.2%

Method	ASTM E681-2004.
Remarks	Test was performed using a spark ignition source. The electrodes were L shaped 1 mm tungsten wire with a gap of ¼ inch. Conducted at 23 ± 3 °C and 50% relative humidity.
Test Facility	Honeywell and DuPont (2008)

Autoignition Temperature 405 °C

Method	EC Council Regulation No 92/69A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	No cool flames were observed. Ignition produced an orange/blue flame.
Test Facility	Chilworth (2006)

Explosive Properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Following examination of the structural formula the study authors concluded that the chemical will not possess explosive properties.
Test Facility	Chilworth (2006)

Oxidizing Properties Non-oxidising

Method	British Standard ISO 10156:2010, Gases and Gas Mixtures – Determination of Fire Potential and Oxidising Ability for the Selection of Cylinder Valve Outlets.
Remarks	The test was to determine if the material would support combustion of a reference combustible gas (ethane) more than a nitrogen mixture containing 23.5 vol.% oxygen.
Test Facility	DuPont (2012b)

Oxidizing Properties Non-oxidising

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties.
Remarks	Following examination of the structural formula the study authors concluded that the chemical does not show any evidence of possessing oxidising properties.
Test Facility	Chilworth (2006)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat/Crl:CD(SD)IGS BR
Vehicle	Air
Method of Exposure	Whole body exposure
Exposure Period	4 hour
Physical Form	Gas
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <ppm> Actual (mean)*	*Mortality*
1	5M	9960	0/5

LC50	> 9,960 ppm/4 hours
Signs of Toxicity	No clinical signs were observed in all study animals.
Body Weights	Two animals displayed a slight weight loss the day after exposure.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	DuPont (2005)

B.2. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat/Spague Dawley
Vehicle	Air
Method of Exposure	Nose-only exposure
Exposure Period	4 hours
Physical Form	Gas
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <ppm>*		*Mortality*	
		Target	*Nominal*	*Actual*	
1	5 per sex	200,000	199,000	201,600 ± 800 (N=48)	0/10
2	5 per sex	400,000	402,000	405,800 ± 1200 (N=49)	0/10

LC50	> 405,800 ppm/4 hours
Signs of Toxicity	Slightly decreased breathing rates and slightly laboured breathing was noted in 2 animals per sex in group 1 and all animals in group 2. No effects were seen after exposure during the 14-day observation period.
Effects in Organs	Gray discoloured lungs were seen at necropsy in two animals of group 1 and 4 animals in group 2. In the absence of a control group the study authors were uncertain whether this is treatment-related.
Remarks - Results	Body weight gain was within the range expected for animals of this strain and age.
CONCLUSION	The notified chemical is of low toxicity via inhalation.

TEST FACILITY TNO (2006a)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD Not specified.
A 26.5 L chamber was used for the exposure with concentrations measured with IR spectroscopy.

Species/Strain	Mouse
Vehicle	Air
Method of Exposure	Whole body exposure
Exposure Period	4 hour
Physical Form	Gas
Remarks - Method	Only a brief report was submitted. There was no indication that GLP standards had been met.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	
1	2 per sex	20,535	23,480	0/4
2	2 per sex	105,787	99,830	0/4

LC50	> 99,830 ppm/4 hours
Signs of Toxicity	The mice were within normal limits during the exposure and during the 7 days after exposure. No lethality or test substance-related clinical signs were noted in any of the test animals.
Effects in Organs	No abnormal signs were noted at gross necropsy in Group 1 and no gross necropsy was performed on Group 2.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY HLS (2004)

B.4. Acute toxicity – inhalation with biotransformation

TEST SUBSTANCE Notified chemical

METHOD In-house

Species/Strain	Rabbit/New Zealand White
Vehicle	Air
Method of Exposure	Whole body exposure
Exposure Period	1 hour
Physical Form	Gas
Remarks - Method	A screening 2-phase study on pregnant and non-pregnant female rabbits with the purpose being to determine whether there is a pregnancy-based or gender-based difference in sensitivity in rabbits with this test substance. Phase 1 had a 2-day post-exposure period and Phase 2 had a 14-day post-exposure period. Urine was collected in 12 hour intervals for 48 hours after the end of the exposure time and urinary metabolites were identified by ^{19}F-NMR and LC-MS/MS.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <ppm>*			*Mortality*
		Target	*Nominal*	*Actual*	

Phase 1	1F (non-pregnant) and 1F (presumed pregnant)	100,000	100,000	110,000	0
Phase 2 – Group1	5M/6F (presumed pregnant)/5F (non-pregnant)	0	N/A	0.0/0.0	0
Phase 2 – Group 2	5M/6F (presumed pregnant)	50,000	150,000/140,000	45,000/47,000	0
Phase 2 – Group 3	5M/6F (presumed pregnant)/5F (non-pregnant)	100,000	150,000/140,000*	100,000/102,000	0

* The nominal concentrations in phase 2 were the combined results of the 2 chambers for each of the separate male and female exposures.

LC50	> 102,000 ppm/1 hour
Signs of Toxicity	No lethality or test substance-related clinical signs were noted in any of the test animals. No substantial differences in urinary metabolite pattern or quantity of metabolites excreted were noted between the different groups. The predominant metabolites were *S*-(3,3,3-trifluoro-2-hydroxypropanyl)-mercaptolactic acid and *N*-acetyl-*S*-(3,3,3-trifluoro-2-hydroxypropanyl)-*L*-cysteine whose signal intensities in ^{19}F-NMR spectra represented more than 78% of total ^{19}F related signals in all urine analysed samples.
Effects in Organs	No test substance-related clinical signs were noted in macroscopic/microscopic examinations in any of the test animals.
Remarks - Results	The results indicated an acceptable agreement between the targeted and actual and nominal concentrations. No differences in body weights were noted. No differences in urinary metabolite pattern or quantity of metabolites excreted were noted between the different groups.

CONCLUSION

The study authors concluded that the results suggested that the previously seen lethality of the test substance in pregnant rabbits after inhalation exposure was unlikely to be due to changes in biotransformation patterns or capacity of pregnant rabbits.

TEST FACILITY

HLS (2011)

B.5. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

METHOD

Species/Strain	Rat/Sprague Dawley
Route of Administration	Inhalation – nose-only exposure
Exposure Information	Total exposure days: 14 days (10 exposure days in total) Dose regimen: 5 days per week Duration of exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Air
Physical Form	Gas
Remarks - Method	For this study, a slight different design of the nose-only exposure units was used, namely, with a cylindrical PVC column (volume of approx. 70 L) surrounded by a transparent hood, test atmosphere inlet at the bottom of the central column, and outlet at the top.

Results

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	
control	5 per sex	0	0	0/10

low dose	5 per sex	6992	4,990 ± 36	0/10
mid dose	5 per sex	20,163	19,599 ± 99	0/10
high dose	5 per sex	50,079	51,690 ± 620	0/10

Clinical Observations
Daily observations did not reveal any treatment-related clinical abnormalities.

No treatment related differences in body weight gain, food consumption and food conversion efficiency were seen.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
The statistically significant higher calcium levels in plasma of mid- and high-concentration males were not considered by the study authors to represent an test substance-related adverse effect as the differences were only slight, did not increase with increasing concentrations, were well within the range of historical control values, were seen in one males only, and were not accompanied by changes in any of the other endpoints examined in this study.

Effects in Organs
Macroscopic and microscopic examinations did not reveal any treatment-related changes.

CONCLUSION
The No Observed Adverse Effect Concentration (NOAEC) was established as > 51,690 ppm in males and females in this study, based on no treatment-related adverse effects in any of the exposure groups.

TEST FACILITY TNO (2005a)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 412 Subacute Inhalation Toxicity: 28-Day Study.
Species/Strain Rat/Sprague Dawley
Route of Administration Inhalation – nose-only exposure
Exposure Information Total exposure days: 28 days
Dose regimen: 5 days per week
Duration of exposure: 6 hours/day
Post-exposure observation period: 14 days
Vehicle Air
Physical Form Gas
Remarks - Method The concentration levels used were established on the basis of a previous 14-day pilot study.

No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	
control	7M/5F	0	0	0/12
low dose	5M/5F	4,905	4,997 ± 7	0/10
mid dose	7M/5F	14,809	15,167 ± 459	0/12
high dose	7M/5F	48,812	50,031 ± 83	0/12
control recovery	5M/5F	0	0	0/10
high dose recovery	5M/5F	0	0	0/10

Clinical Observations
Daily observations did not reveal treatment related clinical abnormalities.

Treatment related differences in body weight gain, food consumption or food conversion efficiency were not seen.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Increases in creatinine concentration in female animals of the high dose group and increases in urea concentration in female of the low dose and high dose groups at the end of the exposure period were considered by the study authors to represent fluctuations rather than real increases.

As no significant differences between groups in red blood cells and coagulation variables or in white blood cell variables were seen, it was not considered necessary by the study authors to perform haematology in the animals of the recovery groups.

Effects in Organs

In male animals, increased absolute and relative liver weights were found in the high dose recovery group. Because of the absence of liver weight data at the end of the exposure period (due to the unscheduled DNA synthesis test), it was not known whether liver weights were also increased at the end of the exposure period. However, the increases in absolute and relative liver weights were considered by the study authors to be fortuitous because of the absence of histopathological and other hepatic changes at the end of the exposure period, the absence of the liver weight changes in female animals at both the end of the exposure and recovery period, and the absence of liver weight changes in concomitantly exposed male and female animals after 90 days of exposure (TNO, 2007a).

CONCLUSION

The No Observed Adverse Effect Concentration (NOAEC) was established as > 50,031 ppm in male and female rats in this study, based on an absence of treatment-related adverse effects in any of the exposure groups.

TEST FACILITY TNO (2006b)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 413 Subacute Inhalation Toxicity: 90-Day Study.

Species/Strain	Rat/Sprague Dawley
Route of Administration	Inhalation – nose-only exposure
Exposure Information	Total exposure days: 97-98 days Dose regimen: 5 days per week Duration of exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Air
Physical Form	Gas
Remarks - Method	The concentration levels used were established on the basis of a previous 14-day pilot study. No deviations from the protocol were reported. The study lasted longer than 90 days to make up 5 public holidays during the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	
control	10 per sex	0	0	0/20
low dose	10 per sex	4,949	4,997 ± 11	1F/20
mid dose	10 per sex	14,853	1,5084 ± 298	0/20
high dose	10 per sex	49,354	50,116 ± 478	0/20

Mortality and Time to Death

One female animal in the 5,000 ppm group was found dead on 71st day of the study. The cause of the death was considered by the study authors to be due to the animal having been dropped inadvertently in the morning of the day.

Clinical Observations
Daily observation of the animals did not reveal treatment-related clinical abnormalities.

Treatment-related differences in body weight gain, food consumption and food conversion efficiency were not seen. Ophthalmoscopic examination near the end of the exposure period did not reveal treatment related abnormalities.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
The differences seen in haematology parameters, i.e. decrease of mean corpuscular volume and mean haemoglobin in red cells of male animals of the low- and mid-dose groups and the decrease in reticulocytes and increase in prothrombin time in female animals of all treatment groups, were not considered by the study authors to be treatment-related due to the absence of a dose-response relationship.

The differences seen in clinical chemistry parameters, i.e. the increase of inorganic phosphate in plasma of the male animals of the low- and high-dose groups, the decrease of aspartate aminotransferase in female animals of the low- and high-dose groups and the decrease of glucose in female animals of the mid- and high-dose groups were also not considered by the study authors to be treatment-related.

Effects in Organs
Treatment-related changes in absolute or relative weights were not noted. Macroscopic examination at necropsy did not reveal any treatment-related findings. Microscopic examination of selected organs including the respiratory tract did not reveal exposure-related findings.

CONCLUSION
The No Observed Adverse Effect Concentration (NOAEC) was established as > 50,116 ppm in males and females in this study, based on an absence of treatment-related adverse effects in any of the exposure groups.

TEST FACILITY TNO (2007a)

B.8. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 412 Subacute Inhalation Toxicity: 28-Day Study.

Species/Strain	Rabbit/New Zealand White
Route of Administration	Inhalation – whole body exposure
Exposure Information	Total exposure days: 7, 14 and 28 days Dose regimen: 7 days per week Duration of exposure: 6 hours/day Post-exposure observation period: 28 days
Vehicle	Air
Physical Form	Gas
Remarks - Method	The concentration levels used in Phase 2 were established on the basis of a 7- and 14-day pilot study in Phase 1. No deviations from the protocol expect that rabbits were selected as test animals instead of rodents. Justification on test animal selection provided in the study report was 'in prior testing via inhalation exposure with this test substance, pregnant rabbits were found to be more sensitive to the test substance when compared with pregnant and non-pregnant rats. No repeated-exposure testing has been conducted in non-pregnant females or males, respectively. This study was designed to evaluate HFO-1234yf repeated-dose toxicity in male and non-pregnant female rabbits'.

RESULTS

Phase1

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	

control	5 per sex	0	0	0/10
low dose	5 per sex	N/A	497	0/10
mid dose	5 per sex	N/A	1508	0/10
high dose (1-6 days)	5 per sex	N/A	5533	1M/10
high dose (7-15 days)	5 per sex	6914	4338	1F/10

Phase 2

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Nominal	*Actual*	
control	10 per sex	0	0	0/20
low dose	10 per sex	N/A	478	0/20
mid dose	10 per sex	N/A	1,010	0/20
high dose	10 per sex	5,938	4,378	1F/20
control recovery	5 per sex	0	0	0/10
high dose recovery	5 per sex	0	0	0/10

Mortality and Time to Death

In Phase 1 study, 1 female animal at the dose level of 5,500 ppm was euthanised on Day 5 due to decreased activity, ano-genital staining, decreased faecal volume and unformed stool. Exposure was stopped on Day 7 for this group and 1 male animal was found dead on Day 7. Exposure resumed on Day 8 at dose level of 4,500 ppm. In Phase 2 study, 1 female animal at the dose level of 4,500 ppm was euthanised on Day 19 due to decreased activity, trembling and pale and decreased faecal volume.

Clinical Observations

There were no treatment-related clinical signs in Phase 1 and 2 studies other than those noted in Mortality for the animals that were euthanized.

There were no treatment related changes in bodyweight or food consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment-related changes in the haematology, coagulation, blood gas or urinalysis parameters were considered to be noteworthy by the study authors. The clinical pathology data were generally unremarkable and comparable between the groups at the end of the recovery period.

Effects in Organs

Subacute/chronic myocardial inflammation was observed in 1 male at 1,000 ppm dose level, 1 male/2 females at 1,500 ppm, 6 males/4 females at 4,500 ppm, 2 males at 5,500/4,500 ppm and 5 males/2 females at 5,500 ppm on Days 8, 15 and/or 29. The lesions were minimal to slight, did not progress over time and recovered after 28 days without exposure to the test substance.

When compared to control animals, increased incidence and/or severity of acute skeletal muscle necrosis was noted in both sexes at ≥ 1,500 ppm on Day 15 and in males at ≥ 1,000 ppm and in females at 4,500 ppm on Day 29. The acute nature of the change was inconsistent with the duration of exposure, suggesting that the change was not a direct effect of the test substance. Minimal to moderate skeletal muscle necrosis was generally associated with elevated myoglobin, total creatine kinase (total CK), isoenzyme CK-MM, heart fatty acid-binding protein (H-FABP), isoenzyme CK-MB, aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) in males at ≥ 1000 ppm and females at ≥ 500 ppm.

Treatment-related increases in liver weight in the 1,500 ppm and 5,500 ppm males, on Day 8 only, had no microscopic correlates and were considered a non-adverse adaptive response by the study authors.

The anatomic and clinical pathology changes fully resolved following 28 days of recovery.

CONCLUSION

The No Observed (Adverse) Effect Concentration (NOAEC) was established as 500 ppm in males and 1,000 ppm in females in this study, based on anatomic pathology findings.

TEST FACILITY HLS (2013a)

B.9. Cardiotoxicity/skeletal muscle toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 412 Subacute Inhalation Toxicity: 14-Day Study.
Species/Strain	Minipig/Göttingen
Route of Administration	Inhalation – whole-body exposure
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Duration of exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Air
Physical Form	Gas
Remarks - Method	The purpose of the study was to assess the toxicity (including cardiotoxicity and/or skeletal muscle toxicity) of the notified chemical when administered via whole body inhalation to minipigs for 14 days. The concentration levels used were established on the basis of previous acute inhalation, subacute inhalation and cardiac sensitisation studies reported in this appendix. No deviations from the protocol were noted, except that the study was conducted in minipigs and only for selected parameters. Justification on test animal selection provided in the study report was 'since cardiotoxicity (as well as skeletal muscle toxicity) was the principal endpoint of interest for this study, the pig was generally accepted to be a sensitive and representative model for humans. In prior testing via inhalation exposures with this test substance, pregnant and non-pregnant rabbits were found to be more sensitive to the test substance when compared to pregnant and non-pregnant rats'.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Target	*Actual*	
control	3 per sex	0	0	0/6
low dose	3M/2F	5,500	5,490	0/5
high dose	3 per sex	10,000	10,300	0/6

Clinical Observations

Daily observation of the animals did not reveal treatment-related clinical abnormalities.

Treatment-related differences in body weight gain and food consumption were not seen.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

The differences seen in hematology parameters were not considered by the study authors to be treatment-related since these were small in magnitude, not dose-related and/or individual values were comparable to pre-test or consistent with normal biological variability.

There were no treatment-related effects on myoglobin, cardiac troponin, creatine kinase (total CK, CK-MM and CK-MB), aspartate aminotransferase or alanine aminotransferase. Any differences seen in clinical chemistry parameters were not considered by the study authors to be treatment-related since these were small in magnitude, not dose-related and/or individual values were comparable to pre-test or consistent with normal biological variability.

Effects in Organs

No treatment-related changes in weights were noted. Macroscopic examination at necropsy did not reveal any treatment-related findings. Microscopic examination of selected organs including the heart (left ventricle, right ventricle and septum) and skeletal muscle (rectus femoris, psoas, soleus and diaphragmatic muscles) did not

reveal treatment-related findings.

CONCLUSION
The No Observed Adverse Effect Concentration (NOAEC) for cardiotoxicity or skeletal muscle toxicity was established as > 10,300 ppm in males and females in this study, based on no treatment-related adverse effects associated with cardiotoxicity or skeletal muscle toxicity in any of the exposure groups.

TEST FACILITY HLS (2013b)

B.10. Cardiotoxicity/skeletal muscle toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 412 Subacute Inhalation Toxicity: 28-Day Study.

Species/Strain	Minipig/Göttingen
Route of Administration	Inhalation – whole-body exposure
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Duration of exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Air
Physical Form	Gas
Remarks - Method	The purpose of the study was to assess the toxicity (including cardiotoxicity and/or skeletal muscle toxicity) of the notified chemical when administered via whole body inhalation to minipigs for 28 days. The concentration levels used were established on the basis of previous acute inhalation, subacute inhalation, cardiac sensitisation and 14-day cardiotoxicity/skeletal muscle toxicity studies reported in this appendix. No deviations from the protocol were noted, except that the study was conducted in minipigs and only for selected parameters. Justification on test animal selection provided in the study report was 'Cardiotoxicity and skeletal muscle toxicity were the principal endpoints of interest for this study and the minipig is considered to be a sensitive and representative model for human responses. In prior testing via inhalation exposures with this test substance, pregnant and non-pregnant female rabbits and male rabbits were found to be more sensitive to the test substance when compared to pregnant and non-pregnant female rats and male rats. This study was designed to determine whether there is a repeated exposure (4 weeks instead of the prior tested 2 weeks) sensitivity in minipigs with this test substance since the minipig is a more appropriate large animal model for safety evaluation for this test substance'.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Target	*Actual*	
control	8 per sex	0	0	0/16
low dose	8 per sex	5,000	5,145	1M/16
high dose	8 per sex	10,000	10,200	0/16

Mortality and Time to Death
One male animal in the 5,000 ppm group was euthanized on Day 16 due to a severely prolapsed rectum. The death was not considered by the study authors to be treatment-related.

Clinical Observations
Daily observation of the animals did not reveal treatment-related clinical abnormalities.

Treatment-related differences in body weight gain and food consumption were not seen.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

The differences seen in hematology parameters were not considered by the study authors to be treatment-related as they were small in magnitude, not dose-related and/or within normal biological variability.

Minimal increases in total creatine kinase and CK-MM isoenzyme noted in 3/8 female animals on Day 21 in the 10,000 ppm group were not considered by the study authors to be treatment-related as they were transient and values were within normal biological variability.

Effects in Organs

Treatment-related, marginal to slightly higher mean absolute and relative liver weights (compared to body and brain weights) were noted at exposure levels ≥ 5,000 ppm in females. Potential morphologic correlates for differences in liver weight were not identified since histopathologic evaluation of the liver was not mandated by the protocol. Statistically significant difference in heart weight to body weight ratio in the 10,000 ppm group males was considered spurious because of the lack of histopathologic findings and the discordance between absolute weights and weights relative to body and brain weights. All other differences in organ weights were considered by the study authors to be incidental and due to normal biological variability.

A few sporadic macroscopic findings were not considered by the study authors to be treatment-related as there were no incidence patterns/trends for these findings to suggest a relationship to test substance exposure.

The changes revealed at microscopic examination of selected organs including the heart (left ventricle, right ventricle and septum) and skeletal muscle (rectus femoris, psoas, soleus and diaphragmatic muscles) included cellular infiltration (mononuclear or mixed cell) involving the epicardium, myocardium, and/or endocardium (for heart) and sporadic mononuclear cell infiltration, neutrophil infiltration, histiocytic infiltration, cytoplasmic basophilia of myofibers, and myofiber degeneration involving the rectus femoris, soleus, psoas, and/or diaphragmatic muscle (for skeletal muscle). These changes were considered by the study authors to be incidental and/or to be those that can be routinely observed at the age of the Göttingen minipigs used in this study. The microscopic observations showed no consistent patterns/trends involving incidence or intensity to suggest a relationship to test substance exposure.

Remarks – Result

Daily inhalation administration of the notified chemical at concentrations up 10,200 ppm was not associated with cardiotoxicity or skeletal toxicity. Minipigs responded very differently to inhalation exposure to the notified chemical in this study compared to rabbits studied previously.

CONCLUSION

The No Observed Adverse Effect Concentration (NOAEC) for cardiotoxicity or skeletal muscle toxicity was established as > 10,200 ppm in males and females in this study, based on an absence of treatment-related adverse effects associated with cardiotoxicity or skeletal muscle toxicity in any of the exposure groups.

TEST FACILITY HLS (2014)

B.11. Cardiac sensitisation to adrenaline

TEST SUBSTANCE Notified Chemical

METHOD In house method.

STUDY DESIGN

Species/Strain: Dog/Beagle

Study Design: A group of 6 male dogs was exposed to multiple concentrations of the test substance via muzzle-only inhalation (vapour), at 48 h intervals. The duration of exposure was 10 minutes in each case, and the concentrations tested were 2, 6 and 12% (20,000, 60,000 and 120,000 ppm, respectively). Animals were administered a pre-exposure dose of epinephrine (adrenaline) as a bolus injection via a cephalic vein approximately 5 minutes prior to exposure to the test substance. Five minutes after exposure to the test substance began, the animals were administered a

challenge dose of epinephrine. Dogs were monitored for the development of arrhythmias by means of a continuous electrocardiogram (ECG) tracing. The response to epinephrine was determined for each animal in a pre-test acclimatisation phase, and used to determine the amount of epinephrine administered in the main study. The epinephrine level used for each animal was the highest level that did not elicit significant ECG findings such as premature ventricular contractions (PVCs).

The criteria used to determine whether cardiac sensitisation has occurred include (not exclusively):

- Eleven or more PVCs in 10 seconds, with episodes of confluency
- Ventricular tachycardia
- Fibrillation

Test substance atmospheres were prepared in Tedlar bags and analysed by GC before exposure. At the initiation of the exposure, the three-way valve was turned to the bag position, and during non-exposure periods, it delivered filtered air. Each dog served as its own control, as the same dogs were used for all exposures. After each exposure, the dogs were given at least 2 days of rest before being given the next exposure.

Challenge Procedure

Time	*Event*
0 min	Start ECG recording.
2 min	Pre-exposure epinephrine dose
7 min	Test gas initiated
12 min	Challenge dose of epinephrine
17 min	Termination of exposure/ECG

Remarks - Method No significant protocol deviations. Target concentrations were 20,000, 60,000 and 120,000 ppm.

RESULTS

Summary of Cardiac Response

Dog Number	*Adrenaline Dose (µg/kg)*	*Test Substance Concentration <ppm>*	*Number of Premature Ventricular Contractions (PVCs): 1st Adrenaline Challenge (baseline)*	*Number of Premature Ventricular Contractions (PVCs): 2nd Adrenaline Challenge (exposure)*
1	2	20,159	0	0
		60,522	0	0
		120,189	0	0
2	8	20,159	0	0
		60,522	0	0
		120,189	0	0
3	4	20,159	3 in 25 seconds, occurring 22 seconds after injection	5 in 14 seconds, occurring 40 seconds after injection
		60,522	6 in 21 seconds, occurring 42 seconds after injection	0
		120,189	0	1 occurring 21 seconds after injection
4	8	20,159	4 in 23 seconds, occurring 48 seconds after injection	7 in 30 seconds , occurring 24 seconds after injection
		60,522	6 in 14 seconds, occurring 63 seconds after injection	0
		120,189	2 in 11 seconds, occurring 32 seconds after injection	1 occurring 38 seconds after injection
5	6	20,159	35 in 48 seconds, occurring 29 seconds after injection	0
		60,522	44 in 56 seconds, occurring 23 seconds after injection	39 in 63 seconds, occurring 23 seconds after injection
		120,189	0	0

6	2	20,159	0	0
		60,522	0	0
		120,189	0	0

Signs of Toxicity	All animals survived to study termination. There were no test substance-related clinical observations. All clinical findings in the test substance-related groups were limited to single animals, were not observed consistently and/or common findings for laboratory dogs of this age and breed.
Myocardial Effects	Challenge dosing with epinephrine while the animals were under test substance exposure did not result in the occurrence of arrhythmias such as ventricular fibrillation, tachycardia, or of an increased rate of premature ventricular contractions, compared to the pre-exposure challenge values. The results obtained did not meet the study criteria for cardiac sensitisation.
NOAEC	> 120,189 ppm
Remarks - Results	Body weights were unaffected by test substance administration.

CONCLUSION	There was no evidence of cardiac sensitisation under the conditions of the test.
TEST FACILITY	WIL (2006)

B.12. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA
Metabolic Activation System	S9 mix
Concentration Range in Main Test	a) With metabolic activation: 0, 10, 20, 40, 60, 76% b) Without metabolic activation: 0, 10, 20, 40, 60, 76%
Vehicle	Air
Physical Form	Gas
Remarks - Method	A gas exposure method was used. Agar plates containing bacteria were exposed in modular incubator chambers to various concentrations. To prepare the target concentrations of the test substance, mass flow controlled flows of oxygen, nitrogen, carbon dioxide and the test substance were mixed and used to flush a chamber during 7 min. To prevent infection, a 0.45 μm filter was used. The negative control was clean air and positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene and N-ethyl-N-nitrosourea in the absence of S9 mix and 2-aminoanthracene and benzo[a]pyrene in the presence of S9 mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (%) Resulting in:* *Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test	≥ 10%	Not reported	positive
Present			
Test	≥ 10%	Not reported	positive

Remarks - Results	The test substance was slightly toxic to TA100 at ≥ 10% concentration in the absence of S9 mix. The test substance caused a dose related increase in the mean number of revertants at ≥ 10% concentration and a > 2.5 fold

increase at ≥ 20% concentration to TA100 and WP2uvrA in the presence of S9 mix. A dose-related increase to TA1535 was also observed at ≥ 40% concentration in the presence of S9 mix, however this increase only reached a maximum of 1.5 times the controls.

CONCLUSION The notified chemical was mutagenic to bacteria under the conditions of the test.

TEST FACILITY TNO (2005b)

B.13. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line Cultured human lymphocytes
Metabolic Activation System S9 fraction from Wistar rats treated with Aroclor 1254
Physical Form Gas
Remarks - Method No preliminary test was conducted.

Based on the physico-chemical properties of the test substance, the culture flasks containing the human lymphocytes were exposed in modular incubator chambers to various concentrations of the test substance. The atmosphere in the chamber consisted of 19% O_2, 5% CO_2 and the test substance supplemented with N_2 (i.e. the negative control consisted of 76% N_2, 19% O_2 and 5% CO_2). The chambers were flushed with the test atmosphere using at least 5 times the volume of the chamber.

Following the exposure period, the cells were removed from the chambers (and in the case of cells treated in the presence of metabolic activation, the cells were washed with buffer and supplied with complete medium) and incubated for the relevant time period at ~37 °C in humidified air containing 5% CO_2.

A continuous exposure assay in the absence of metabolic activation was not conducted.

Mitomycin C in the absence of metabolic activation and cyclophosphamide in the presence of metabolic activation were used as positive controls.

Metabolic Activation	*Test Substance Concentration (%)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 10, 20, 40*, 60*, 76*	4	24
Test 2	0*, 10, 20, 40*, 60*, 76*	4	48
Present			
Test 1	0*, 10, 20, 40*, 60*, 76*	4	24
Test 2	0*, 10, 20, 40*, 60*, 76*	4	48

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (%) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 76	> 76	negative
Test 2	> 76	> 76	negative
Present			
Test 1	> 76	> 76	negative

Test 2	> 76	> 76	negative

Remarks - Results

At the harvesting time of 48 hours, in the presence of metabolic activation, the study authors indicated that the test substance appeared to be cytotoxic to the cells at the highest concentration tested (mitotic index 68% at 76% concentration but not at the two lower concentrations analysed (mitotic indices 98% and 84% at 40% and 60% concentration, respectively).

In both the absence and presence of metabolic activation, the test substance did not induce a statistically significant increase in the number of aberrant cells at any of the concentrations and time points analysed, when compared to the number of aberrant cells observed in the negative control cultures.

The concurrent negative and positive controls gave satisfactory responses confirming the validity of the test system, although it is noted that these substances were not tested in the gaseous state.

CONCLUSION

The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

TNO (2005c)

B.14. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/CD-1 albino

Route of Administration: Inhalation – whole body exposure

Vehicle: Air

Physical Form: Gas

Remarks - Method: The positive controls were dosed by oral gavage. The criteria for a positive result was a significant ($p < 0.05$) increase in the mean number of MPE/2000 PE (MPE: micronucleated polychromatic erythrocytes; PE: polychromatic erythrocytes) and for a negative control was within the historical range.

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Sacrifice Time hours*
		Target	*Actual*	
control	5M		0	24
	5M		0	48
low dose	5M	12,500 ppm	13,453 ± 69 ppm	24
mid dose	5M	50,000 ppm	51,922 ± 285 ppm	24
high dose	5M	200,000 ppm	201,803 ± 762 ppm	24
	5M	200,000 ppm	201,803 ± 762 ppm	48
positive control, M	5M	0.75 mg/kg bw (intraperitoneal)		24

M = mitomycin C.

RESULTS

Doses Producing Toxicity: No mortality was seen. It was reported by the study authors that treatment-related clinical signs could not be demonstrated during the performance of the micronucleus test.

Genotoxic Effects: Mice treated with the test substance did not show a statistically significant change in the mean number of MPE/2000 PE over that of the control at either 24 h or 48 h.

Remarks - Results: The concurrent negative and positive controls gave satisfactory responses confirming the validity of the test system, although it is noted that the positive control substance was not administered by inhalation.

	It is not clear whether the notified chemical reached the bone marrow as no toxicity was reported.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this test.
TEST FACILITY	TNO (2005d)

B.15. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus (MN) Test. OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*.
Species/Strain	Rats/Sprague Dawley
Route of Administration	Inhalation – nose-only exposure
Vehicle	Air
Physical Form	Gas
Remarks - Method	The studies were carried out as a satellite to a 28-day repeated dose inhalation study (TNO, 2006b).

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Sacrifice Time hours*
		Nominal	*Actual*	
A control	5M UDS/5M MN		0	24
B low dose	5M MN	4,905 ppm	4,997 ± 7 ppm	24
C mid dose	5M UDS/5M MN	14,809 ppm	15,167 ± 459 ppm	24
D high dose	6M UDS/5M MN	48,812 ppm	50,031 ± 83 ppm	24
E positive control for UDS, 2-AAF	5M	50 mg/kg bw (via gavage)		12-16
F positive control for MN, M	5M	10 mg/kg bw (intraperitoneally)		24

M = mitomycin C.; 2-AAF = N-(9H-fluoren-2-yl)acetamide

RESULTS	
Doses Producing Toxicity	No mortality was seen.
Genotoxic Effects	Both the test substance and the negative control showed NNG (net nuclear grains) ≤ 0. Since exposure to the test substance did not induce NNG ≥ 5, it was considered that the test substance did not induce unscheduled DNA synthesis in rat hepatocytes. Mice treated with the test substance did not show a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes or polychromatic erythrocytes over the frequency of the control in bone marrow cells of male rates.
Remarks - Results	The concurrent negative and positive controls gave satisfactory responses confirming the validity of the test system, although it is noted that the positive control substance was not administered by inhalation. As the study was conducted as part of a 28-day study and effects were seen during the study, it is expected that the liver cells were exposed to the notified chemical. It is not clear whether the notified chemical reached the bone marrow as no toxicity was reported.
CONCLUSION	The notified chemical was not clastogenic under the conditions of these two tests.
TEST FACILITY	TNO (2006b)

B.16. Developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 414 Prenatal Development Toxicity Study.
Species/Strain	Rat/Wistar
Route of Administration	Inhalation – nose only exposure
Exposure Information	Exposure days: day 6 through to day 19 of gestation Duration of exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Air
Physical Form	Gas
Remarks - Method	The purpose of this study was to provide data on the possible effects of the notified chemical on pregnant female rats and on the development of the embryo and foetus. No significant protocol deviations.

RESULTS

Group	*Number of Animals*	*Dose/Concentration* <*ppm*>		*Mortality*
		Nominal	*Actual*	
Control	25F	0	0	0/25
Low dose	25F	5,135	5,000 ± 20	0/25
Mid dose	25F	14,856	15,106 ± 290	0/25
High dose	25F	49,614	50,315 ± 935	0/25

Effects on Dams

Daily clinical observations during the gestation period did not reveal any remarkable findings in the animals' appearance, general condition or behaviour between the dosing and control groups.

No effect was seen on the mean body weight. Statistically significant increases and reductions in food consumption were not considered by the study authors to be adverse or related to the test substances.

In each group, 25 females were mated of which 24, 21, 24 and 25 female animals of the control and low-, mid- and high-dose group, respectively, appeared to be pregnant and had live foetuses at caesarean section. One female animal in the low-dose group had an early delivery. No statistically significant differences were observed in the female fecundity index, gestation index among the groups. The number of corpora lutea was statistically significantly increased in the mid-dose group. No differences were observed in the number of implantation sites, pre- and post-implantation loss, live and dead foetuses, resorptions and sex ratio between the groups.

The carcass weight was increased in the mid-dose group (statistically significant) and high-dose group as compared to the control group and as a result the net weight change from GD 6 was less in the mid- and high-dose groups. In all groups, however, the carcass weight was lower that the body weight at GD 6. No effect was seen on the weight of gravis uterus, empty uterus and ovaries. Macroscopic findings at necropsy did not reveal any remarkable or treatment related findings among the dosing and control groups.

Effects on Foetus

No statistically significant differences in the incidences of foetal external observations and/or placental findings were observed. No dose relationship was established for the statistically significant increase in placental weights in male animals, female animals and both sexes together in all treatment groups compared to the control group. No treatment related effects were observed at visceral examination. A dose-related and statistically significant increase in the foetal and litter incidence of the finding "two or more ribs wavy" in all treatment groups was observed. However, wavy ribs were considered by the study authors to be a reversible pathologic finding. A delayed ossification in foetuses of all treatment groups was not considered to be dose-related by the study authors.

CONCLUSION
The No Observed (Adverse) Effect Concentration (NOAEC) was established as > 50315 ppm for maternal and developmental toxicity in this study, based on an absence of adverse maternal or developmental effects.

TEST FACILITY TNO (2007b)

B.17. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 414 Prenatal Development Toxicity Study.
Species/Strain Rabbit/New Zealand White
Route of Administration Inhalation – whole body exposure
Exposure Information Exposure days: day 6 through to day 28 of gestation
Duration of exposure: 6 hours/day
Post-exposure observation period: none
Vehicle Air
Physical Form Gas
Remarks - Method The purpose of this study was to assess gross maternal and/or embryo-foetal toxicity of notified chemical during the critical period of organogenesis in a non-rodent species, the rabbit.
Groups were divided into 2 phases (P1 and P2) due to spatial limitations of the exposure chambers. Target exposure concentrations were 0, 2,500, 4,000 and 7,500 ppm in P1 and 0, 2,500, 5,500 and 7,500 ppm in P2. The mid dose was increased from 4,000 ppm in P1 to 5,500 ppm in P2 as no toxicity was noted in P1. The test substances from 2 suppliers were tested at the mid dose (mid dose 1 and mid dose 2), to determine if there was any difference in the substances.

RESULTS

Group	*Number of Animals*	*Dose/Concentration* <*ppm*>		*Mortality*
		Nominal	*Actual*	
Control	12F per phase	0	0	0/24
Low dose	12F per phase	2,328P1/2,363P2	2,504P1/2,479P2	0/24
Mid dose 1	12F per phase	3,745P1/5,203P2	3,982P1/5,408P2	0/24 (P1)
Mid dose 2	12F per phase	3,635P1/5,407P2	4,013P1/5,479P2	4/24 (P2)
High dose	12F per phase	7,055P1/7,078P2	7,512P1/7,441P2	7/24

Mortality and Time to Death
In the 5,500 and 7,500 ppm groups 4 and 7 female animals respectively, were found dead or were euthanized in extremis during the study. Exposure-related clinical findings, including laboured and/or decreased respiration and/or hypoactivity, were observed in 2 and 3 female animals in the 5,500 and 7,500 ppm groups, respectively, prior to death or euthanasia. The mortalities and moribundity were considered by the study authors to be attributed to the test substance.

Effects on Dams
In addition to the mortalities and moribundity mentioned above, 1 and 3 female animals in the 5,500 and 7,500 ppm groups, respectively, aborted, and 1 female animal in the 7,500 ppm group delivered on gestation day 29.

Lower (statistically significant) mean body weight gain was noted in the 7,500 ppm group during gestation days 12-20, with corresponding occasional reductions in mean daily food consumption. Because the most sensitive females died or were euthanized prior to the scheduled necropsy, mean net body weight and net body weight change were not significantly different from the control group. A slightly lower mean body weight gain and a mean body weight loss were observed in the 5,500 ppm group during gestation days 12-20 and 20-29, resulting in a lower mean body weight gain when the entire exposure period (gestation days 6-29) was evaluated and a mean net body weight loss; the majority of the differences were statistically significant. Correspondingly lower mean food consumption was observed in this group during gestation days 20-29 (statistically significant).

Slightly (not statistically significant) lower mean body weight gains were observed in the 4,000 ppm group during gestation days 12-20 and 20-29, resulting in a lower mean body weight gain (statistically significant) when the entire exposure period (gestation days 6-29) was evaluated. However, there were no test substance-related effects on mean food consumption, mean net body weight or net body weight change in this group. Therefore, the lower mean body weight gain noted in the 4,000 ppm group was not considered adverse by the study authors.

The study authors concluded that there were no test substance related macroscopic effects in any of the surviving animals that were euthanized on gestation day 29. However, in animals that died during the study the following effects were noted: dark red areas in the lungs, thymus gland, spleen and kidney, green contents in the uterus, brown discoloration in the lungs and red fluid and white material in the thoracic cavity. In the animals that aborted or delivered early during the study the following effects were noted: dark red areas in the lungs, swollen spleen and mottled liver.

Microscopic examination revealed subacute inflammation in the heart in the 2,500, 4,000, 5,500 and 7,500 ppm groups, coagulation necrosis of the heart in the 7,500 ppm group, and renal tubular necrosis in the 5,500 and 7,500 ppm groups. All of these changes were considered related to test substance administration and considered adverse by the study authors.

Effects on Foetus

Heart and great vessel malformations (bulbous aorta, stenotic pulmonary trunk, interventricular septal defects [absent septa], absent tricuspid valve and/or interrupted aortic arch) were noted in 2 and 3 foetuses in the 5,500 and 7,500 ppm groups, respectively. The mean litter proportions for these findings exceeded the maximum mean value in the WIL historical control data for those findings that have been observed in the historical control data. Because of the increase compared to historical control and the similarity of the findings, the malformations in the cardiovascular system of foetuses in the 5,500 and 7,500 ppm group were considered to be test substance-related. The malformations in these groups were noted in the presence of maternal toxicity. No test substance-related effects on intrauterine growth and survival were noted at any exposure level.

CONCLUSION

The No Observed Adverse Effect Concentration (NOAEC) was established as < 2,500 ppm for maternal toxicity in this study, based on treatment-related mortality, moribundity, abortions, premature delivery, lower mean body weight gain, mean body weight loss and/or lower food consumption observed at 5,500 and 7,500 ppm, adverse microscopic findings noted in all exposure groups that consisted of subacute inflammation of the heart at ≥ 2,500 ppm, coagulation necrosis of the heart at 7,500 ppm, and renal tubular necrosis at ≥ 5,500 ppm.

The NOAEC was established as 4,000 ppm for embryo/foetal developmental toxicity in this study, based on treatment-related visceral malformations in the heart and/or great vessels observed in the 5,500 and 7,500 ppm groups in the presence of maternal toxicity.

TEST FACILITY	WIL (2011)

B.18. Toxicity to reproduction – two generation study

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 416 Two-Generation Reproduction Toxicity.
Species/Strain	Rat/Wistar Crl:WI(WU)
Route of Administration	Inhalation – nose only exposure and whole body exposure
Exposure Information	Exposure period – males of F0-generation: 6 hours/day and 5 days/week for at least 10 weeks prior to mating (nose only) and daily during mating for 6 hours/day until sacrifice (nose only). Exposure period – females of F0-generation: 6 hours/day and 5 days/week for at least 10 weeks prior to mating (nose only) and daily during mating and up to gestation day (GD) 19 for 6 hours/day (nose only). From day 5 of lactation onwards, females were exposed daily for 6 hours/day to the test substance by whole body exposure until the end of the lactation period (postnatal, PN day 21) or the sacrifice of females soon after.

	From postnatal (PN) day 22 up to approximately 6 weeks of age, the F1-generation male and female pups were exposed by whole body exposure for 6 hours/day and 5 days/week. Subsequently, F1-generation males were exposed by nose-only exposure until the end of premating period for 6 hours/day and 5 days/week, and daily for 6 hours/day during mating and up to sacrifice. F1-generation females were exposed by nose-only exposure until the end of the premating period for 6 hours/day and 5 days/week, and daily during mating and up to GD 19 for 6 hours/day. From PN day 5 onwards, F1-generation females were exposed daily 6 hours/day to the test substance by whole body exposure until sacrifice on or shortly after day 21 of lactation. Non-mated females of both generations were exposed (nose-only) until the end of the nose-only exposure period. Non-pregnant females were exposed by nose-only exposure until GD 19 of the presumed gestation period; then the exposure was not resumed.
Vehicle	Air
Physical Form	Gas
Remarks - Method	Protocol deviations were considered not to have affected the validity of the study.

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*	
		Target	*Actual*
1	28 per sex	0	0
2	28 per sex	5,000	4,995 (± 44)
3	28 per sex	15,000	15,013 (± 107)
4	28 per sex	50,000	49,958 (± 311)

RESULTS

Mortality and Time to Death

F0-generation

A female animal of the control group and a male of the mid-dose group were found stuck in their tubes and dead on premating (PM) day 1 and PM day 6 respectively and were replaced by surplus animals. A female animal of the high-dose group was found dead just before the start of the exposure on PM day 28.

F1-generation

A female animal of the high-dose group was killed in poor health condition on PM day 2 and was replaced by a spare pup selected from the same dose group. At autopsy the intestines were filled with air. A male animal of the mid-dose group was killed in poor health condition on PM day 7 with the cause of the poor health condition not being able to be determined. The animal was replaced by a spare pup selected from the same dose group. Two female animals of the control group and low-dose group were found dead on PM days 24 and 35 respectively as they got stuck in the tube after trying to turn around. A male animal of the mid-dose group was killed in poor health condition on PM day 67 and showed piloerection and a swollen abdomen. Necropsy revealed very severe hydronephrosis of the right kidney.

The study authors concluded that the mortalities and clinical findings observed in the animals of both generations were common findings in rats of this strain and age or occurred as individual fortuitous findings. Furthermore, they were about equally distributed amongst the different treatment groups or occurred in only one or a few animals. Therefore, they were not considered by to be treatment-related.

Effects on Parental (F0) animals and 1st Filial Generation (F1)

Statistically significant decreases of body weight, and body weight change and food consumption were observed in the treatment groups during the PM period. In F1-females, there were test substance-related reductions in mean body weights at all doses tested during the first three weeks of the PM period. Mean body weights on approximately PN days 28, 35, and 42 (week 0, 1 and 2 of the F1-generation, respectively) were up to 13, 15, and 14% lower than controls at 5,000, 15,000, and 50,000 ppm, respectively. This period of time is toxicologically relevant to the onset of puberty, these reductions were not considered adverse by the study authors because they were transient and by the end of the premating period mean body weights for all groups were within 3% of the control mean. The study authors considered the effects to be non-

adverse due to a lack of a strong dose-related response, the relative low magnitude of the change, and the fact that the body weight data were consistent with the food consumption data.

A statistically significant increase of the mean cycle length was observed in the females of the mid-dose group when compared to the control group, and the length of the longest cycle was statistically significantly decreased in the females of the low- and mid-dose groups of the F0-generation. This effect was not considered to be a treatment-related effect by the study authors as no effect was observed in the high-dose group of the F0-generation and no effect on oestrus cycle was observed in the treatment groups of the F1-generation.

The number of pregnant F0-females was 25, 25, 28 and 25 in the control, low-, mid- and high-dose groups, respectively. The number of pregnant F1-females was 27, 25, 26 and 27 in the control, low-, mid- and high-dose groups, respectively. Pre-coital time although slightly increased in the treatment groups of both generations, was not considered to be adversely affected by the study authors. All females were mated within 2 oestrus cycles (8-10 days) and the highest mean pre-coital time was 3.5 days. Duration of the gestation period was slightly increased in the treatment groups and was statistically significant in the low- and high-dose groups of the F10-generation and in the mid- and high-dose groups of the F1-generation. In none of the F0- and F1-females a gestation period of more than 22 days was detected and no difficulties during parturition were observed. All F0- and F1-dams, except one animal of the mid-dose group, delivered live pups; this dam was sacrificed on presumed gestation day 24. In both generations, there was no statistically significant difference between the postimplantation loss in the treatment groups and the control groups.

The number of pups delivered and the number of stillborn pups were not considered to be adversely affected by treatment by the study authors. The statistically significant increase of pup mortality (8.5%) as observed in the high-dose group of the F1-generation on PN 4 was within the historical range (0-20.7%) and therefore not considered to be an adverse effect. The effect on sex ratio on PN 1 of the F0-generation was not considered to be an adverse effect as no other effects such as sex ratio on PN 1 in the F1-generation and anogenital distance in the F1-generation were observed.

Two dams of the high-concentration group gave birth to 3 pups with malformations. As no malformations were observed in the prenatal developmental toxicity study with the test substance (TNO, 2007b), this finding was not considered to be a treatment-related effect.

In both generations, pup body weights and body weight changes were considered not to be affected by exposure to the test substance.

In F1-females, there was an apparent delay in the onset of puberty evident as a delay in days to achievement of vaginal opening. These apparent delays were not considered by the study authors to reflect a direct effect on this endpoint but rather, were considered secondary to previously described test substance-related reductions in body weight and food consumption parameters that were evident during the first three weeks of exposures and concurrent with the onset of vaginal patency.

Sperm analysis did not reveal a treatment-related effect.

Macroscopic observation of the F0-and F1-pups selected for necropsy did not reveal any treatment-related effect. No differences were observed between pup brain and spleen weights.

Microscopic observation of the thymus of the control and high-dose groups of the F1-generation, F2-pups, did not reveal any treatment-related effects. For that reason the decrease detected in absolute and relative thymus weight of the F2-pups, F1-generation, of the high-dose group was not considered by the study authors to be a relevant effect. In addition, the decrease in absolute and relative thymus weight (F1-pups, F0-generation) and in relative thymus weight (F2-pups, F1-generation) of the low-dose group were considered not to be treatment-related by the study authors as no relation with concentration was observed. Examination of the ribs of the F1-pups did not reveal a treatment-related effect.

No relation to the concentration of the test substance was observed on the effects on organ weights. The decrease in absolute organ weights and the decrease and increase in relative organ weights were considered

to be related to the decreased terminal body weights of the treatment groups.

CONCLUSION
The No Observed Adverse Effect Concentration (NOAEC) was considered to be > 49,958 ppm, in this study based on an absence of treatment related adverse effects at the highest dose tested.

TEST FACILITY TNO (2011)

B.19. Chronic toxicity/carcinogenicity

TEST SUBSTANCE Notified chemical

METHOD Toxicogenomic assessment designed in-house
Species/Strain Mouse/B6C3F1/Crl and Rat/F344/CrlBR
Route of Administration Inhalation – whole body exposure
Exposure Information Total exposure: 90 days
Dose regimen: 5 days per week
Duration of exposure: 6 hours/day.
Vehicle Air
Physical Form Gas
Remarks - Method Gene expression changes in the rat kidney and mouse liver were evaluated following a 90-day exposure to the test substance. Gene expression microarray analysis together with standard histopathology was performed on the liver and kidney. Statistical tools were then used to identify a predictive set of gene expression changes and compare expression patterns from the test substance to the previously tested control chemicals. Control chemicals used and vehicle controls were tetrafluoroethylene (TFEL), 1-amino-2,4-dibromoanthraquinone (ADBQ), tris (2,3-dibromopropyl) phosphate (TDPP), trichlorofluoromethane (TCFM), 1,1,2,2-tetrafluoroethane (TFEA), iodoform (IODO), N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), air (ACON), corn oil (CCON) and feed (FCON).

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Target	*Actual average daily)*	
1	10 female mice	10,000	10,054	0/10
2	10 female mice	50,000	49,728	0/10
3	10 male rats	10,000	10,054	0/10
4	10 male rats	50,000	49,728	0/10

Clinical Observations
Rats in both dose groups and mice in the 50,000 ppm group showed significant decreases in terminal body weight compared to the vehicle controls.

Effects in Organs
Rats in the 10,000 ppm group showed significantly increased relative kidney weights. No treatment-related histopathological lesions were noted.

Prediction of Rat Kidney Carcinogenic Potential
The gene expression changes for the test substance were significantly different from the positive controls (except TFEL) and were more similar to the negative controls, supporting the classification of the test substance as non-carcinogenic in the male rat kidney. The overall peak accuracy was 97.8% with 100 genes with a sensitivity and specificity of 90% and 100% respectively when excluding the positive control TFEL. The exclusion of TFEL was due to it produced a statistically significant increase in male rat kidney tumours only when an extended histological evaluation using step sections was performed while other positive controls showed a statistically significant increase in tumours using the standard histological evaluation methods with only a single section.

Prediction of Mouse Liver Carcinogenic Potential

The gene expression changes for the test substance were significantly different from the positive controls and were more similar to the fluorinated negative control TFEA treatment group and the air control ACON treatment group, supporting the classification of the test substance as non-carcinogenic in the female mouse liver. A peak accuracy of 98.5% was obtained with 50 genes with a specificity of 97.2% and 99.2% respectively.

Remarks – Results

The study authors noted that gene expression changes in the male rat kidney suggested potential endocrine-related effects and were consistent with a reduction in circulating androgens. In addition, a significant upregulation of the SA rat hypertension-associated gene (*Sah*) was noted in the male rate kidney. Increased expression of the human homolog of this gene has been linked to changes in body mass index, triglyceride levels, cholesterol and blood pressure status.

CONCLUSION

Statistical classification analysis predicted the test substance to be non-carcinogenic in both female mouse liver and male rat kidney.

TEST FACILITY HIHS (2007)

B.20. Chronic toxicity/carcinogenicity

TEST SUBSTANCE Notified chemical

METHOD Toxicogenomic assessment designed in-house

Species/Strain	Mouse/B6C3F1/Crl
Route of Administration	Inhalation – whole body exposure
Exposure Information	Total exposure: 90 days Dose regimen: 5 days per week Duration of exposure: 6 hours/day.
Vehicle	Air
Physical Form	Gas
Remarks - Method	Gene expression changes in mouse lung were evaluated following a 90-day exposure to the test substance (HIHS study No. 06041). Gene expression microarray analysis together with standard histopathology was performed on the lung. Statistical tools were then used to identify a predictive set of gene expression changes and compare expression patterns from the test substance to the previously tested control chemicals. Control chemicals used and vehicle controls were 1-amino-2,4-dibromoanthraquinone (ADBQ), benzofuran (BFUR), methylene Chloride (MECL), N-Methylolacrylamide (MACR), 1,5-naphthalenediamine (NAPD), tris(2,3-dibromopropyl)phosphate (TDPP), 2,2-bis(bromomethyl)-1,3-propanediol (BBMP), 1,2-dibromoethane (DBET), Ethylene Oxide (ETOX), naphthalene (NPTH), vanadium pentoxide (VANP), Benzene (BENZ), coumarin (COUM), 1,2,3-trichloropropane (TCPN), 1,4-dichlorobenzene (DCBZ), propylene glycol mono-*t*-butyl ether (PGBE), tetrafluoroethylene (TFEL), 2-chloromethylpyridine hydrochloride (CMPH), diazinon (DIAZ), iodoform (IODO), malathion (MALA), N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), 4-nitroanthranilic acid (NAAC), pentachloronitrobenzene (PCNB), tetrafluoroethane (TFEA), trichlorofluoromethane (TCFM), air (ACON), corn oil (CCON), feed (FCON) and water (WCON).

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration <ppm>*		*Mortality*
		Target	*Actual average daily)*	
1	10 female mice	10,000	10,054	0/10

2	10 female mice	50,000	49,728	0/10

Effects in Organs

In the 10,000 ppm group 1/10 mice had minimal lung inflammation and in the 50,000 ppm group 1/10 mice also had minimal lung inflammation in. No control animals had inflammation in the lung.

CONCLUSION

Statistical classification analysis based on the gene expression changes predicted the test substance to be similar to other substances found to be carcinogenic in the female mouse lung. The average predictive accuracy of the top five models under honest five-fold cross-validation was 77.5% with an average sensitivity and specificity of 71.3 and 83.0%, respectively. Pair-wise gene expression analysis between the air control group and each group exposed to the test substance identified multiple differentially expressed genes.

TEST FACILITY HIHS (2009)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Gas chromatography
Remarks - Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	15	7	57
14	14	14	68
21	0	21	Nd
28	0	28	Nd

Nd = not determined

Remarks - Results	All validity criteria for the test were satisfied. The results of the microbial activity control show the expected degradation of sodium acetate (> 60% within 14 days). The maximum degradation found in the toxicity control was 39% (measured after 14 days). As this is higher than 25%, the test substance is not considered to be inhibitory to the inoculums. At an average measured initial concentration of 3.29 mg/L of the test substance, a maximum biodegradability of 15% was reached after 7 days of incubation.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	TNO (2007c)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks - Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*

5	< 5	5	67
10	< 5	10	75
15	< 5	15	78
20	< 5	20	79
28	< 5	28	83

Remarks - Results

All validity criteria for the test were satisfied.

The results obtained from the toxicity control showed that the test substance did not inhibit the biodegradation of sodium benzoate, as the total oxygen consumption in the toxicity control bottles was similar to the total of oxygen consumptions by the test substance.

The test substance indicated negligible biodegradation (< 5%), and therefore it is classified as not readily biodegradable.

CONCLUSION

The notified chemical is not readily biodegradable.

TEST FACILITY

AstraZeneca (2008)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 203 Fish, Acute Toxicity Test - Static

Species	Carp (*Cyprinus carpio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not given
Analytical Monitoring	Gas chromatography

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*			
Mean measured		*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0
197	7	0	0	0	0

LC50	> 197 mg/L
NOEC	197 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied.

The results were calculated and reported based on the mean measured concentrations. Under the test conditions, the notified chemical had no lethal effect on carp at the average measured concentration of 197 mg/L.

CONCLUSION

The notified chemical is not harmful to fish.

TEST FACILITY

NOTOX B.V (2006a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L

Analytical Monitoring	Gas chromatography
Remarks - Method	A stock solution was prepared by bubbling the test substance (gas) through dilution water for 3 hours at the rate of 20 mL/minute.

RESULTS

Concentration mg/L Geometric mean	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
83	20	0	0

EC50	> 83 mg/L at 48 hours
NOEC	83 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. Chemical analysis showed an initial concentration of 102 mg/L. At the end of the test period the concentration was 68 mg/L. Hence the geometrical mean value was calculated to be 83 mg/L. Under the test condition, the EC50 for Daphnia exposed to test substance was > 83 mg/L. Since no effect was recorded at this level, it can be concluded that 48 h EC50 for Daphnia is > 100 mg/L.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates.
TEST FACILITY	NOTOX B.V (2006b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	Nominal: … mg/L Actual: … mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOE_bC mg/L at 72 h*	*E_rC50 mg/L at 72h*	*NOE_rC mg/L at 72 h*
> 75	75	> 75	75

Remarks - Results	Based on growth rate over the test period, the lowest observed effect concentration (LOEC) was determined to be 75 mg/L. The E_rC50 is, therefore, concluded to be > 75 mg/L. Since no effect was recorded at > 75 mg/L and at a maximum average exposure of 114 mg/L in one of the treated solutions, it can be concluded that that the EC50 for algal growth is > 100 mg/L. The results were calculated and reported based on the mean measured concentrations. All validity criteria for the test were satisfied.

CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	NOTOX B.V (2006c)

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