File No: EX/234 (STD/1360)

May 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Polyfluorinated Chemical in Capstone® FS-60, Capstone® FS-61, Capstone® FS-63 and Capstone® ST 300

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Agriculture, Water and 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:

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Director
NICNAS

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This assessment report is for an extension of the original assessment certificate for Polyfluorinated Chemical in Capstone® FS-60, Capstone® FS-61, Capstone® FS-63 and Capstone® ST 300. Based on the information submitted by the extension notifier, some sections of the original assessment report (STD/1360) have been modified. These modifications have been made under the heading '*Extension Application*' in the respective sections.

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
EX/234 (STD/1360)	Tenaru Timber & Finishes Pty Ltd	Polyfluorinated Chemical in Capstone® FS-60, Capstone® FS-61, Capstone® FS-63 and Capstone® ST 300	Yes	≤ 0.02 tonne per annum	Component of paints and coatings, stone and tile products and commercial floor finishes

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*
Acute Toxicity (Category 1)	H330 – Fatal if inhaled

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:

R26 Very toxic by inhalation

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute (Category 2)	H401 – Toxic to aquatic life
Chronic (Category 1)	H410 – Very toxic to aquatic life with long lasting effects

Human health risk assessment
Provided that the recommended occupational control measures are adhered to, 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.

However, the notified chemical is a potential precursor of perfluorohexanoic acid (PFHxA), which is persistent in the environment. Due to the environmental distribution of PFHxA resulting from the use pattern of the notified chemical, secondary human exposure to PFHxA via the environment may occur. The notified chemical is replacing a longer chain perfluorinated substance, which will result in secondary human exposures to perfluorooctanoic acid (PFOA) and longer chain perfluorocarboxylic acids (PFCAs). PFOA and longer chain

PFCAs are more hazardous to human health and have higher bioaccumulation potential, compared to PFHxA. The overall human health risk posed by the notified chemical is less than that of the substance it replaces.

Environmental risk assessment
On the basis of the PEC/PNEC and assessed use pattern, the notified chemical itself is not considered to directly pose an unreasonable short-term risk to the environment.

However, degradants of the notified chemical, along with associated impurities of the notified chemical, are potential precursors of the very persistent chemical, PFHxA. The assessed use pattern of the notified chemical does not control the release of breakdown products into the environment after disposal and the long-term environmental risk profile of PFHxA is currently unknown. Consequently, the long-term risk profile for the notified chemical and its degradation products is unknown. This situation may change if further data on the environmental behaviour of the notified chemical and its poly- and perfluoroalkyl degradation products (including PFHxA) were to become available.

The notified chemical is a potential precursor for PFHxA in the environment. PFHxA is an environmentally persistent chemical that has potential to be globally distributed. However, the ecotoxicological profile and bioaccumulation potential of PFHxA is considered to be less problematic when compared with long chain (C8 and above) perfluorocarboxylic acids that PFHxA is expected to replace. Nonetheless, the introduction and use of chemicals that degrade to release PFHxA and other very persistent poly- and perfluoroalkyl compounds should be considered a short-term measure until suitable alternatives, with less persistent chemistry, are identified.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute toxicity (Category 1): H330 – Fatal if inhaled

  *Classification of products/mixtures containing the notified chemical should be considered based on the concentration of the notified chemical present.

- Products containing the notified chemical intended for spray use in original equipment manufacturing facilities should carry the following safety directions:
  - May be fatal if inhaled
  - Do not breathe vapours, mists and sprays
  - Use only in enclosed and automated settings

- Products containing the notified chemical that are available to the public should carry the following safety directions:
  - Not suitable for spray application

Safety Data Sheet

- The SDS for products containing the notified chemical should include the following:
  - May be fatal if inhaled
  - Do not breathe vapours, mists and sprays
  - Spray application in original equipment manufacturing facilities should only be conducted in enclosed and automated settings

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical as introduced or in formulated products:
  - Enclosed and automated processes whenever possible
  - Only suitable for spraying in original equipment manufacturing facilities where enclosed and automated processes are utilised

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced or in formulated products:
  - Avoid breathing of vapours, mists and sprays
  - Maintain good hygiene practices

- 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 as introduced or in formulated products:
  - Safety glasses
  - Gloves
  - Coveralls

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

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

- The notified chemical should only be introduced as part of a strategy to phase out the use of long chain perfluoroalkyl chemicals.

- The notifier should seek ways to minimise the level of residual polyfluoroalkyl monomers and impurities in the notified chemical. Such levels should be as low as practicable: where possible, the total weight of these constituents should not exceed the levels attainable utilising international best practice

- The following control measures should be implemented by users of the notified chemical, or products containing the notified chemical, to minimise exposure of the notified chemical to the environment:
  - Best practice on-site treatment of waste streams should be employed to maximise removal of the notified chemical from wastewaters.

Disposal

- If the notified chemical or products containing the notified chemical cannot feasibly be disposed using a technique that will destroy or irreversibly transform the perfluorinated components of the notified chemical, disposal should be to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

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 importation volume exceeds six tonnes per annum notified chemical;
- the use changes from a component of paints and coatings, stone and tile products and commercial floor finishes;
- the notified chemical is intended for use in spray products outside of original equipment manufacturing facilities;
- additional information has become available to the person as to an adverse effect of the polyfluoroalkyl degradation products of the notified chemical (such as perfluorohexanoic acid) to human health and/or the environment;
- additional information has become available to the person as to the environmental fate of the chemical or its polyfluoroalkyl degradation products (such as perfluorohexanoic acid) in relation to degradation or partitioning behaviour, including during water treatment processes;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of paints and coatings, stone and tile products and commercial floor finishes, 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.

AICS Entry

- When the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS) the entry is proposed to include the following statement(s):
  - This chemical has been assessed by NICNAS and there are specific secondary notification obligations that must be met. Potential introducers should contact NICNAS before introduction.

Safety Data Sheet
The SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

Extension Application (EX/234)
The extension applicant has provided SDS for a product containing the notified chemical. The accuracy of the information on the SDS remains the responsibility of the extension applicant.

ASSESSMENT DETAILS

This notification has been conducted under the cooperative arrangement with the United States Environmental Protection Agency (US EPA). Information pertaining to the assessment of the notified chemical by the US EPA was provided to NICNAS and, where appropriate, used in this assessment report. The other elements of the risk assessment, including the recommendations on safe use of the notified chemical, were carried out by NICNAS.

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Holders of Original Assessment Certificate (STD/1360)
The Chemours Company (Australia) Pty Ltd (ABN 90 169 142 750)
7 Eden Park Drive
MACQUARIE PARK NSW 2113

Diversey Australia Pty Limited (ABN 92 080 527 117)
29 Chifley Street
SMITHFIELD NSW 2164

IMCD Australia Limited (ABN 44 000 005 578)
1st Floor, 372 Wellington Road
MULGRAVE VIC 3170

Anderson Dry-Treat Trust & Salmon Dry-Treat Trust (ABN 28 702 168 959)
65 Nicholson Street
ST LEONARDS NSW 2065

Rohm & Haas Australia Pty Ltd (ABN 29 004 513 188)
Level 17, 8 Exhibition Street
MELBOURNE VIC 3000

Applicant for an Extension (EX/234) of the Original Assessment Certificate:
Tenaru Timber & Finishes Pty Ltd (ABN: 25 000 588 358)
Unit 9 & 10, 350 Edgar Street
CONDELL PARK NSW 2200

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: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, 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: flammability, autoignition temperature, explosive properties and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (2009)

2. IDENTITY OF CHEMICAL

The notified chemical is a UVCB substance consisting of several components, each containing a perfluorinated carbon side chain with six perfluorinated carbon atoms.

MARKETING NAMES
TLF-10620 (notified chemical)
Capstone® FS-60, Capstone® FS-61, Capstone® FS-63 and Capstone® ST-300 (up to 40% notified chemical)

MOLECULAR WEIGHT
400 < Mn < 1300 g/mol

ANALYTICAL DATA
Reference NMR, IR, UV spectra were provided

3. COMPOSITION

DEGREE OF PURITY
> 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

DEGRADATION PRODUCTS
Over time, the notified chemical is expected to ultimately degrade into perfluorohexanoic acid (PFHxA) - CAS name: Hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-; CAS No. 307-24-4.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 21.6 °C AND 101.98 kPa: Tan solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Decomposed at 200.2ºC	Measured
Density	1100 kg/m^3 at 22°C	Measured
Vapour Pressure	< 1.067x10^{-2} kPa	Measured
Water Solubility	2.4 g/L at 25 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at pH 4-9, 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = -1.37 to 0.93 at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 1.14 to 3.19 at 25 °C	Measured
Dissociation Constant	pKa_1 = 4.1, pKa_2 = 7.0, pKa_3 = 9.5	Measured
Surface Tension	21.49 mN/m	Measured
Flash Point*	Unable to be determined	The substance decomposes at 200.2 °C. Evolving vapours extinguished the test flame.
Flammability*	Non-flammable	Not expected to be flammable based on the partial fluorination and flash point test.
Autoignition Temperature	Not determined	Not expected to autoignite based on the partial fluorination and flash point test.
Explosive Properties	Not expected to be explosive	Contains no explosophores.
Oxidising Properties	Not expected to be oxidising	Estimated based on structure.

* Test conducted on the notified chemical at 18% concentration in an aqueous dispersion.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties that are not assessed by US EPA, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not 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.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia as an aqueous dispersion at concentrations up to 40%.

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

Original Introduction Volume

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 4	≤ 4	≤ 5	≤ 5	≤ 6

Extension Application Introduction Volume (EX/234)

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02

PORT OF ENTRY
Sydney, Melbourne and Brisbane.

TRANSPORTATION AND PACKAGING
The products containing the notified chemical (up to 40% concentration) will be imported by sea in polyethylene or steel drums in various sizes ranging from 3.6 to 1000 kg, and transported within Australia by road.

USE
The notified chemical is intended to be introduced in order to phase out the use of a partially fluorinated polymer containing fluorinated carbon chain lengths > 6 in various proportions (*i.e.*, existing chemical). The use categories of the notified chemical are identical to those of the existing polymer it replaces, as outlined below.

The notified chemical will be used as a dirt and soil repellent in paints and coatings (up to 0.1% concentration), stone and tile products (up to 4% concentration) and commercial floor finishes (up to 0.02% concentration). Paints and coatings and stone and tile products are expected to be used by professional and domestic users. Commercial floor finishes are intended for use only by professionals.

Extension Application
Paint and coating products containing the notified chemical at concentrations < 0.1%.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. The notified chemical will be imported at up to 40% concentration as a component of Capstone® FS-60, Capstone® FS-61, Capstone® FS-63 and Capstone® ST-300.

Reformulation
Drums of imported formulation containing the notified chemical will be received at reformulation sites and weighed manually or automatically pumped from drums into the mixing vessel (which may be heated) towards the end of the blending process. Once blending is complete, the finished products containing the notified chemical at up to 4% concentration will be automatically dispensed into product containers. The blending and dispensing equipment will be cleaned periodically. Quality control staff may test samples of the finished products.

Paints and coatings

Professional and domestic painters (members of the public) may mix/tint paints containing the notified chemical (up to 0.1% concentration) and apply to various surfaces by brush or roller. Original equipment manufacturers (OEM) may apply the paints and coatings by spray, brush or roller at industrial sites. Sales personnel at paint and hardware stores may open tins of paints containing the notified chemical at up to 0.1% concentration and manually measure and pour tinter into the paint, close the tin and attach to a shaker.

Stone and tile products
Professional and domestic users (members of the public) will apply stone and tile products containing the notified chemical (up to 4% concentration) by brush or roller to stone and tile surfaces in domestic or commercial premises. Application may occur by spray, brush or roller in an OEM setting.

Commercial floor finishes
Professional cleaners will manually dispense/load commercial floor finish products containing the notified chemical (up to 0.02% concentration) into floor polish machines for application to floors, usually in malls and shopping centres. Application may also occur in an OEM setting.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

The notified chemical may undergo slow degradation in the environment. As such, most potential exposure to workers and the public is expected to be to the notified chemical itself, rather than to its degradation products. Exposure to the residual polyfluoroalkylated starting constituents and/or impurities of the notified chemical (discrete polyfluoroalkylated chemicals containing perfluorinated carbon chain lengths ranging from six to ten) is also possible. Such exposure may be limited by the relatively low concentration of polyfluoroalkylated impurities from the notified chemical in imported products (up to 2.5 wt%) or in end-use products (up to 0.1%).

Over time, the notified chemical will break down and release PFHxA into the environment, which is likely to lead to secondary human exposure to PFHxA. This exposure is unquantifiable.

6.1.1. Occupational Exposure

EXPOSURE DETAILS
Transport and storage
Transport and storage workers will only come into contact with the notified chemical (up to 40% concentration) in the unlikely event of an accident.

Reformulation processes
Dermal and ocular exposure to the notified chemical (up to 40% concentration) may occur to workers involved in manually weighing, decanting, blending, quality control testing and cleaning of equipment. Personal protective equipment (PPE) such as protective clothing, goggles and gloves are expected to be worn during these procedures. Inhalation exposures are not likely based on the expected low vapour pressure of the notified chemical and because aerosols are not expected during reformulation processes. The remainder of the formulation process, including packaging, is expected to be mostly automated and exposure is expected to be low.

Original Equipment Manufacture
Dermal and ocular exposure of manufacturing workers to the notified chemical in stone and tile sealants (up to 4% concentration), paints and coatings (up to 0.1% concentration) and floor finishes (up to 0.02% concentration) are expected to be the main routes of exposure during OEM processes, with some potential for inhalation exposure when the product is applied by spray. Isolation controls and enclosed processes, such as spray booths, are expected to be utilised and factory workers are expected to wear PPE such as gloves, goggles and coveralls. Workers may be exposed on a repeated basis.

Paints and coatings application
Dermal exposure of professionals to the notified chemical (up to 0.1% concentration) is expected to be the main route of exposure during professional paint application with brush or roller, with some potential for ocular exposure. Exposure is expected to be minimised by the use of coveralls, gloves and safety glasses. Professional painters may be exposed on a repeated basis.

Stone and tile sealant application

Dermal exposure of professionals to the notified chemical (up to 4% concentration) is expected to be the main route of exposure during professional stone and tile sealant application with brush or roller, with some potential for ocular exposure. Exposure is expected to be minimised by the use of coveralls, gloves and safety glasses. Workers may be exposed on a repeated basis.

Commercial floor finishes
Dermal exposure of professional cleaners to the notified chemical (up to 0.02% concentration) is expected to be the main route of exposure when loading or pouring products into cleaning equipment. Exposure is expected to be minimised by the use of coveralls and gloves. Cleaners may be exposed on a repeated basis.

6.1.2. Public Exposure

Products containing the notified chemical (up to 4% concentration) will be used by the public. Dermal and ocular exposures may occur when applying paints or stone and tile products by brush or roller. Generally, PPE are not expected to be worn by public users, with the exception of normal clothing and possibly gloves. However, exposure to DIY users is expected to occur less frequently than to professional painters.

The public may make dermal contact with surfaces that have had the notified chemical applied in paints, stone and tile sealants or floor finishes. This exposure may be on a long term repeated basis. Once applied, the notified chemical will adhere to the substrate and is not expected to be available for exposure in significant quantities.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted with a test substance containing up to 20% notified chemical, up to 40% analogue chemical 1 or up to 20% analogue chemical 2 are summarised in the following Table. For full details of the studies, refer to Appendix B.

Some of the toxicology studies (see table below) were conducted with formulations containing the notified or analogue chemicals in water. These results have been adjusted for the approximate concentration of notified or analogue chemicals in the tested substance when necessary. It should be noted that the results adjusted for concentration may not accurately reflect the toxicity of the notified or analogue chemicals at 100% concentration, as dosing volume and concentration may affect absorption.

Endpoint	*Result and Assessment Conclusion*
Notified chemical (up to 20% concentration)	
Rat, acute oral toxicity	LD50 > 5000 mg/kg bw; no deaths; low toxicity (equivalent to LD50 > ~1000 mg notified chemical/kg bw)
Rat, acute dermal toxicity	LD50 > 5000 mg/kg bw; no deaths; low toxicity (equivalent to LD50 > ~1000 mg notified chemical/kg bw)
Rat, acute inhalation toxicity	LC50 > 20 and < 47 mg/m^3/4 hours NOAEC(death) = 20 mg/m^3/4 hours NOAEC(histopathology) = 1 mg/m^3/4 hours very toxic/fatal
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose inhalation toxicity – 2 weeks	NOAEC = 1.2 mg/m^3
Rat, repeat dose oral toxicity – 28 days	NOAEL = 125 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non-mutagenic
Genotoxicity – *in vitro* chromosome aberration	non-clastogenic
Analogue chemical 1 (up to 40% concentration)	
Rat, acute inhalation toxicity	LC50 = 57 mg/m^3/4 hours NOAEC(death) = 23 mg analogue chemical/m^3/4 hours very toxic/fatal
Rat, repeat dose inhalation – 14 days	NOAEC = 0.2 mg/m^3/day

Rat, repeat dose dermal toxicity – 28 days	NOAEL = 1000 mg/kg bw/day (equivalent to NOAEL = 400 mg analogue chemical/kg bw/day)
Rat, repeat dose oral toxicity – 90 days	LOAEL = 10 mg/kg bw/day (equivalent to LOAEL = 4 mg analogue chemical/kg bw/day)
	Analogue chemical 2 (up to 20% concentration)
Rat, developmental toxicity	NOAEL(maternal) = 625 mg/kg bw/day (equivalent to NOAEL = 125 mg analogue chemical/kg bw/day) NOAEL(foetal) = 2500 mg/kg bw/day (equivalent to NOAEL = 500 mg analogue chemical/kg bw/day)
Rat, reproductive toxicity	NOAEL(systemic) = 75 mg/kg bw/day (equivalent to NOAEL = 15 mg analogue chemical/kg bw/day) NOAEL(maternal) ≥ 3500 mg/kg bw/day (equivalent to 700 mg analogue chemical/kg bw/day) NOAEL(reproductive) ≥ 3500 mg/kg bw/day (equivalent to 700 mg analogue chemical/kg bw/day) NOAEL(F1 generation) = 500 mg/kg bw/day (equivalent to 100 mg analogue chemical/kg bw/day)

Suitability of the analogue chemicals
The notified chemical is primarily composed of moieties with six perfluorinated carbons, whereas the analogue chemicals are composed of moieties with six or more perfluorinated carbons. The notified and analogue chemicals are comprised of similar non-perfluorinated components, thus the toxicity of the analogue chemicals is expected to be similar to the notified chemical. However, there may be differences in physico-chemical properties and molecular weight distributions between the notified chemical and analogue chemicals, which could lead to differences in toxicokinetic properties, such as absorption across biological membranes. It should also be noted that whilst analogue chemicals 1 and 2 contain similar components, the concentration of each component and their relative proportions in each analogue vary.

Toxicokinetics, metabolism and distribution
The notified chemical is expected to cross biological membranes (skin or gastrointestinal tract) based on its relatively low molecular weight (the majority of components are < 1,000 Da). The tendency of the notified chemical to cross the gastrointestinal tract is supported by the systemic toxicity in a 90-day repeated dose oral study with analogue chemical 1. Dermal absorption was observed in the 28-day dermal toxicity study in rats with analogue chemical 1, supported by fluoride detected in blood and the observations of adaptive effects in the study. Systemic absorption from inhalation of the notified chemical may occur.

Analysis of plasma concentrations of various metabolites and components of the notified chemical following repeated oral administration of the notified chemical (up to 20% concentration) at 5 mg/kg bw/day for 28 days revealed the presence of one metabolite in males and females after cessation of dosing and one month recovery. This could indicate slow clearance of the metabolite after repeated dosing. The metabolite was detected in the liver of both sexes after the recovery period, but not in fat.

Acute toxicity
The notified chemical (up to 20% concentration) was of low acute oral and dermal toxicity in rats.

Inhalation toxicity
Perfluorinated chemicals have been known to cause acute lung injury. Acute lung injury is characterised by respiratory problems ranging from mild to severe effects, including mortality, associated with acute or repeated exposures. Acute lung injury is generally considered to be of most concern when the compound has surface activity (Fischer *et al.*, 2012).

The notified chemical was very toxic/fatal to rats in an acute inhalation study, with 3/5 mortalities in the group exposed to 47 mg/m^3/4 hours, and no mortalities observed at 20 mg/m^3/4 hours, thus the LC50 is between these concentrations. Microscopic examination of the respiratory tract of rats exposed to the notified chemical at 8 and 19 mg/m^3/4 hours included laryngeal changes characterised by erosion and ulceration of the ventral mucosa, with inflammation of the submucosa, degeneration and necrosis of the u-cartilage, the presence of focal aggregates of macrophages and microgranulomas, and minimal regenerative hyperplasia in some animals. Inflammation was also observed in the lungs. Some recovery was observed over 14 days but it was incomplete. The NOAEC for

acute exposures to the notified chemical was established as 1 mg/m^3/4 hours, based on histopathological effects in the lungs at higher doses. This NOAEC is expected to be protective of mortality.

In another acute inhalation study, analogue chemical 1 was also very toxic/fatal by inhalation, with 3/6 and 6/6 mortalities in the groups exposed to 57 and 120 mg/m^3/4 hours, respectively. No deaths were observed in the group exposed to 23 mg/m^3/4 hours.

In a repeated dose inhalation study, the notified chemical was tested in rats at nominal levels of 0, 0.1, 1 and 5 mg/m^3 (actual levels 0, 0.15, 1.2 and 5.2 mg/m^3, respectively), for 6 hours per day over a 2-week period for a total of 12 exposures (nose only). Clinical chemistry, haematology and urinalysis were not conducted in the study. Pathological examinations showed that minimal focal changes were present in the larynx in both males and females in the group treated at 5.2 mg/m^3 notified chemical. These changes included minimal hyperplasia/squamous metaplasia of the ventral laryngeal mucosa, minimal to mild inflammation of ventral submucosa and minimal to mild mineralisation of the U-shaped cartilage. These changes were considered adverse by the study authors. To a lesser extent, similar pathological findings were also noted in the group treated at 1.2 mg/m^3, with 1 of 10 males developing minimal mineralisation of the U-shaped cartilage. The mineralisation of the cartilage was not recoverable within the 4-week recovery period for both the 5.2 and 1.2 mg/m^3 groups. The epithelial changes were resolved after the recovery. Under the conditions of the study, the NOAEC was established at 1.2 mg/m^3 for the notified chemical.

In another repeated dose inhalation study, rats were exposed to analogue chemical 1 at concentrations of 0, 0.2, 2 or 20 mg/m^3 for 6 hours per day for a total of 9 exposures over 14 days. Fluorine levels in the blood were slightly increased in the animals exposed to 20 mg/m^3 indicating systemic absorption following inhalation exposure. At day 10, histopathological effects were observed in animals exposed to 2 or 20 mg/m^3, including mixed inflammatory cells with scattered alveolar lumina in the lung, minimal to mild squamous metaplasia of the mucosal lining of the ventral floor of the larynx. There were no histopathological effects detected after 24 days, demonstrating recovery. The NOAEC was established at 0.2 mg/m^3.

The results from the above inhalation studies seem to be consistent with adverse effects in the lung and larynx, and possibly acute lung injury, particularly the acute inhalation studies where mortalities and histopathological effects were observed in the lungs and larynx following exposure to the notified chemical. These effects demonstrate that the notified chemical is adversely affecting the respiratory system.

Irritation and sensitisation
The notified chemical (up to 20% concentration) was not a skin irritant in rabbits but was a slight eye irritant in rabbits. The notified chemical (up to 20% concentration) was not a skin sensitiser in an LLNA in mice.

Repeated dose toxicity
In a 28-day repeated dose oral study, rats were administered the notified chemical (up to 20% concentration) by gavage at 0, 5, 25 or 125 mg/kg bw/day. The NOAEL was established at 125 mg/kg bw/day, based on the lack of toxicologically adverse effects. This study did not definitively characterise the repeated dose toxicity of the notified chemical, as a LOAEL was not determined.

In a 28-day repeated dose dermal study, rats were administered dermal doses of analogue chemical 1 (up to 40% concentration) at 0, 10, 100, 1000 mg/kg bw/day. A NOAEL was established at 100 mg/kg bw/day (equivalent to 40 mg/kg bw/day of analogue chemical 1), based on the increases in liver enzymes at the higher dose.

In a 90-day repeated dose oral study, rats were administered analogue chemical 1 (up to 40% concentration) by gavage at 0, 10, 60 or 300 mg/kg bw/day, with groups sacrificed at the end of dosing and after recovery for one or three months. Liver toxicity expressed as increased blood levels of liver enzymes (aspartate aminotransferase, alanine aminotransferase, and sorbitol dehydrogenase) was observed in all male dose groups and it was not fully reversible after three months recovery in the groups treated at 60 and 300 mg/kg bw/day. A NOAEL for males could therefore not be established. The NOAEL for female rats was 60 mg/kg bw/day based on elevated liver enzymes and thyroid gland hypertrophy observed in female rats administered 300 mg/kg/day. A LOAEL was established at 10 mg/kg bw/day (equivalent to 4 mg/kg bw/day of analogue chemical 1), based on focal hepatocellular necrosis and elevated liver enzymes in all groups of treated males.

The repeated dose oral toxicity was not adequately characterised for the notified chemical, based on the 28 day oral study in rats, as the tested concentrations did not elicit toxicologically adverse effects to allow for accurate determination of a NOAEL and LOAEL. As such, the true LOAEL for the notified chemical could be based on

toxicity occurring at doses that are considered to be potentially concerning, particularly in a study of longer duration (i.e., of subchronic or chronic duration).

Harmful effects were observed in the liver of males as low as 4 mg/kg bw/day, following repeated oral exposure to analogue chemical 1 for 90 days. It is noted that the repeated dose toxicity of the analogue chemical is likely to be a worst case for the notified chemical. Therefore, although the results of the 90-day oral study with the analogue chemical could be representative of the repeated dose toxicity of the notified chemical, the systemic toxicity for the notified chemical may be less severe and occur at higher doses.

Mutagenicity/genotoxicity
The notified chemical was negative in two bacterial reverse mutation assays and in an *in vitro* chromosome aberration assay.

Reproductive and developmental toxicity
In a developmental toxicity study, rats were administered gavage doses of analogue chemical 2 (up to 20% concentration) at 0, 625, 1250 or 2500 mg/kg bw/day over gestation days 6 to 20. There were no significant developmental effects noted in this study. The maternal NOAEL was established at 625 mg/kg bw/day (equivalent to 125 mg/kg bw/day of analogue chemical 2), based on decreased body weight gains over gestation days 6 to 21; however this had no detrimental effects on the pups. The potential for developmental effects such as litter size or pre implantation loss from reduced weight gain in dams over a longer exposure period cannot be determined from this study; however, no similar weight gain concerns were noted in a longer exposure reproductive toxicity study conducted (see below). Thus, based on the absence of any adverse developmental findings, the foetal NOAEL was established at 2500 mg/kg bw/day (equivalent to 500 mg/kg bw/day of analogue chemical 2).

In a one-generation reproductive toxicity study, rats were administered gavage doses of analogue chemical 2 (up to 20% concentration) at 0, 75, 500 or 3500 mg/kg bw/day. Males were treated for 70 days prior to mating and during mating. Females were treated for 70 days prior to mating, during mating, gestation and lactation. The offspring were not directly treated with the test substance and were maintained until adulthood (sacrificed on postpartum day 60). There was little if any effect on maternal animals and the maternal NOAEL was established as 3500 mg/kg bw/day (equivalent to 700 mg/kg bw/day of analogue chemical 2). Pup body weight gains in the 3500 mg/kg bw/day group were reduced during lactation, with some persistence in males over the remaining observation period. This indicates that the test substance may be administered to the offspring via lactation, but the future developmental or reproductive effects from this exposure are unknown. The offspring NOAEL was established at 500 mg/kg bw/day (equivalent to 100 mg /kg bw/day of analogue chemical 2).

Health 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 following table.

Hazard classification	***Hazard statement***
Acute Toxicity (Category 1)	H330 – Fatal if inhaled

The following cut-off concentrations for products/mixtures containing the notified chemical were derived according to the *GHS* from the Acute Toxicity Estimates for acute inhalation toxicity (dust/mist):

Conc. ≥ 10%: H330
≥ 1% Conc. < 10%: H330
≥ 0.5% Conc. < 1%: H331
≥ 0.1% Conc. < 0.5%: H332

Toxicology of break down products
The notified chemical contains perfluoroalkyl side-chains that are likely to eventually break down to perfluorohexanoic acid (PFHxA; CAS No. 307-24-4), a perfluoroalkyl acid containing a perfluoroalkyl chain of 5 carbons (a short chain perfluoroalkyl substance). The chemical that is proposed for replacement by the notified chemical is expected to break down to perfluorooctanoic acid (PFOA; CAS No. 335-67-1) (consisting of 7 perfluorinated carbons) and other perfluorinated chemicals with longer perfluorinated carbon chain lengths. The

toxicokinetic and toxicological properties of the long chain break down products are generally less favourable compared to the short chain break down products, with properties becoming less favourable with increasing perfluorinated carbon chain length. In addition, it has been established that the bioaccumulation potential of perfluorinated acids increases with fluorinated carbon chain length (Conder *et al.*, 2008; Giesy *et al.*, 2010).

A review of the literature indicates that PFHxA has a less hazardous human health profile, compared to PFOA (refer to Appendix D for details). It is therefore inferred that the human health hazards associated with the expected break down product of the notified chemical (PFHxA) are likely to be similar or less than the human health hazards associated with the expected break down products (PFOA and longer chain perfluorocarboxylic acids) of many perfluorinated chemicals currently on the market and that are intended for replacement by the notified chemical.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical as imported (up to 40% concentration) is very toxic/fatal by inhalation and mortality could occur from a single exposure to low concentrations of the notified chemical, based on effects in rat acute inhalation studies. Inhalation of the notified chemical should therefore be prevented.

Inhalation toxicity is not of concern during reformulation due to the low vapour pressure of the notified chemical and because aerosols will not be generated during reformulation processes. The risk of inhalation toxicity during reformulation is therefore not considered to be unreasonable.

The main potential for inhalation exposure to the notified chemical (up to 4% concentration) will be during OEM manufacture when the substrates are sprayed. Spray booths and other isolation controls are expected to be utilised in industrial manufacturing premises to minimise inhalation exposure. The risk of inhalation toxicity resulting from repeated exposure to the notified chemical is not considered to be unreasonable provided that the recommended isolation, engineering and safe work practices measures are adhered to.

The repeated dose toxicity of the notified chemical has not been adequately characterised, given that toxicity was not elicited in the 28-day oral study. Therefore, as a precaution, it should be assumed that the notified chemical may be toxic following repeated oral exposure, based on the liver toxicity observed as low as 4 mg/kg bw/day in males in the 90 day oral study in rats with analogue chemical 1. Repeated dermal exposure to the notified chemical may result in systemic toxicity, based on increases in liver enzymes observed in the 28-day dermal toxicity study in rats with analogue chemical 1. Workers are expected to wear PPE such as coveralls and gloves to minimise dermal exposure. Overall, the risk to workers from systemic toxicity resulting from exposure to the notified chemical is not considered to be unreasonable.

Workers may also be exposed to polyfluorinated starting constituents and/or impurities of the notified chemical at relatively low concentrations during reformulation and end use operations. It is expected that the engineering controls and personal protective equipment utilised during these operations (as outlined above) will act to mitigate any risk associated with such exposure.

6.3.2. Public Health

The public may use products containing the notified chemical in stone and tile sealants (up to 4% concentration) and paints and coatings (up to 0.1% concentration) by brush or roller. Public exposure will be less frequent than that experienced by professionals. Inhalation exposure and thus toxicity is not expected due to the low vapour pressure of the notified chemical and because aerosols will not be generated when products are applied by brush or roller. The risk to public health from inhalation toxicity is not considered to be unreasonable.

Public exposure from use of products containing the notified chemical (up to 4% concentration) will be infrequent and is unlikely to occur on a repeated basis. Systemic toxicity resulting from repeated exposures are therefore not expected. The risk to public health from repeated exposures to the notified chemical is not considered to be unreasonable. Additionally, the risk to public health from exposures to perfluorinated impurities is not considered to be unreasonable based on their relatively low concentration (< 0.1%) in end-use products.

The public may also be exposed to the notified chemical and low levels of perfluorinated impurities from direct dermal contact with treated articles (after drying onto the article), such as stones and tiles, paints and coatings and floors. This exposure may be on a long term repeated basis. The notified chemical is expected to be of low repeated dermal toxicity and the dermal exposure resulting from contact with treated articles is not expected to

result in adverse effects. The low proportion of the perfluorinated impurities on the treated articles is not expected to result in significant systemic exposure. The risk to public health from repeated dermal exposure to the notified chemical from treated articles is not considered to be unreasonable. The risk to public health from long term repeated dermal exposure to perfluorinated impurities of the notified chemical from treated articles may be mitigated by the relatively low concentrations at which they are present.

The public may be exposed indirectly to the ultimate break down product of the notified chemical, PFHxA, via the environment. Such exposure may increase over time due to the persistence of PFHxA in the environment. A quantitative risk assessment for this exposure was not conducted. However, the available data indicates that PFHxA has a more favourable toxicological profile and bioaccumulation potential than the long chain perfluorinated chemicals that are the ultimate break down products of the majority of perfluorinated chemical currently in Australian commerce (such as PFOA). In particular, it is noted that the chemical being replaced contains perfluorinated carbon chain lengths > 6. It is concluded that the risks to human health from indirect exposure to breakdown products of perfluorinated chemicals will decrease following introduction of the notified chemical, on the basis that the notified chemical is intended to replace a currently available long chain perfluorinated chemical.

It should also be noted that the notified chemical has been approved for the same uses in the US for manufacture/import volumes greater than what is under consideration in Australia.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia; therefore, release to the environment is not expected due to manufacturing activities. Releases to the environment may occur following accidental spills during import, transport or storage. Notified chemical that is spilled is expected to be adsorbed onto a suitable material and collected for disposal in accordance with local regulations.

The notified chemical may either be imported in ready-to-use products or in products for further reformulation in Australia. The notified chemical may enter the wastewater stream during reformulation as a result of rinsing empty import containers, mixing equipment, transfer lines and filling machines. The proportion of the annual import volume of the notified chemical to be locally reformulated has not been specified. Therefore, for the worst-case it is assumed that the entire volume of the notified chemical is will be reformulated in Australia. The notifier's estimate for release of the notified chemical to wastewater due to reformulation activities are as follows:

Use	*Paints*	*Floor wax and polish*	*Stone and tile treatment*
Estimate of the notified chemical annual import volume for each use	74%	18%	8%
Estimate of release to wastewater from reformulation activities	0.32%	0.35%	0.35%
Annual release to wastewater based on a 6 tonne annual import volume	14.2 kg	3.8 kg	1.7 kg
Number of release points for reformulation wastewater	2	2	5

Therefore, a total of 19.7 kg per year of the notified chemical is estimated to be released to wastewater streams across nine potential sites of reformulation.

Reformulation wastes are to be disposed of via waste treatment facilities at the site of reformulation and/or be disposed of by waste disposal contractors. It is presumed that on-site waste treatment facilities are on-site wastewater treatment plants for wastewater streams. It is further assumed that treated water is will be subsequently discharged to sewers. The notifier indicates that reformulation wastes containing the notified chemical may be disposed of by high temperature incineration, in accordance with local regulations.

Notified chemical residues remaining in empty import containers are expected to be minimal as containers will be rinsed prior to disposal, with rinsings expected to be added to the formulated product. Residues in import containers may be thermally decomposed during metals reclamation of metal containers or enter the wastewater streams following plastic container recycling. Alternately, empty containers with residues of the notified chemical may be disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

When used in architectural paints and stone and tile treatments, the notified chemical may enter wastewater as residues in application equipment washings or rinsings from empty product containers. Wastewater containing the notified chemical that is generated by professional and consumer users may be disposed of to sewers. The notified chemical may also enter sewers from the disposal of water in spray booths when products containing the notified chemical are applied by spray by industrial users, such as original equipment manufacturers. The default estimate for release to wastewater of a chemical (with solubility in excess of 100 mg/L) is 5% for both industrial and private use in the paints, lacquers and varnishes industry (European Commission, 2003, pp. 241-242). Approximately 82% of the annual import volume of notified chemical is expected to be used in architectural paints and stone and tile treatments. Therefore, assuming release to wastewater of 5%, up to 0.246 tonnes of the notified chemical is estimated to be released in wastewater to sewers around Australia following its use in architectural paints and stone and tile treatments.

When used in floor wax and polish, the notified chemical may enter wastewater as residues in spent cleaning solution drained from waxing machine tanks, which are expected to be disposed of to sewers by professional users. The default estimate for release to wastewater of a chemical in the public domain with industrial use is 45% (European Commission, 2003, p. 229). Approximately 18% of the annual import volume of notified chemical is expected to be used in floor wax and polish. Therefore, assuming release to wastewater of 45%, up to 0.486 tonnes of the notified chemical is estimated to be released in wastewater to sewers around Australia following its use in floor wax and polish.

It is expected that all three uses will also generate solid wastes containing the notified chemical. These include residues on rags used to wipe drips, on old applicators (brush, roller, mop heads) and in empty product containers. Solid wastes generated during use are expected to be disposed of in accordance with local regulations, most likely to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

Notified chemical applied to painted architectural structures is expected to be physically bound within the inert polymer matrix adhering to the surface of the articles: it is expected to remain associated with the painted articles. Notified chemical applied to treated stone and tile surfaces or polished and waxed floors is expected to adhere to the surface to which it has been applied. However, abrasion of the floor surface by foot traffic is expected to result in some relocation of the notified chemical. Estimates for losses due to abrasion from these uses are not available.

The notified chemical that remains associated with articles to which it has been applied is expected to share the fate of articles. The majority of articles are expected to ultimately be disposed of to landfill. The notified chemical applied to surfaces may also degrade as a result of weathering upon being exposed to environmental conditions after use and after disposal. Degradation may result in the widespread release of degradation products such as PFHxA to surface waters, landfill and landfill leachates, soils, and other regions where release is not foreseen.

7.1.2. Environmental Fate

For details of the environmental fate studies please refer to Appendix C.

The majority of the notified chemical is expected to adhere to the surface to which it is applied. Treated articles and other dried residues containing the notified chemical are expected to ultimately be disposed of to landfill. When associated with the article to which the product containing the notified chemical has been applied, the notified chemical is expected to be released slowly to the environment.

Some of the notified chemical may be released to sewer during reformulation, use and disposal. In general, surfactants have the potential to be removed from influent in sewage treatment plants (STP) via partitioning to phase boundaries. Predictions of the environmental partitioning behaviour of poly- and perfluoroalkylated surfactants such as the notified chemical remain uncertain based on current knowledge because of limited data and their unique properties. In particular, the usual predictive models for partitioning during sewage treatment

are inapplicable for chemicals containing perfluoroalkylated functionality as they assume lipophilicity for hydrophobic functionality, whereas the perfluoroalkylated functionality is both hydrophobic and lipophobic. The assumption that surface activity and/or high molecular weight results in efficient removal by sorption to sludge during conventional wastewater treatment has not been verified by supporting data for this class of chemical. Thus, noting its potential to disperse in water, a significant proportion of the notified chemical, and any associated poly- and perfluoroalkylated impurities, may well remain in the aqueous phase following wastewater treatment. As such, the notified chemical and the poly- and perfluoroalkylated impurities in wastewater have the potential to be released in STP effluent directly to surface waters or reused in the irrigation of agricultural soils throughout Australia.

Over time, the notified chemical is expected to be released slowly from the articles to which it has been applied. The notified chemical is expected to be stable to hydrolysis under environmental conditions with a half-life of greater than one year based on the results of a hydrolysis study. A supporting preliminary hydrolysis study for an analogue confirmed that there was no increase in concentrations of the likely poly- and perfluoroalkylated hydrolysis products under the conditions of the test. Although some degradation of the notified chemical is noted, the notified chemical is not readily biodegradable or inherently biodegradable. However, primary degradation of the notified chemical is expected to occur. The degradation products were not identified in either of the above studies. However, expected degradation products were identified in an inherent biodegradability study for an analogue. The results indicate that the notified chemical can be expected to degrade to form the very persistent degradation product, PFHxA. The formation of PFHxA is consistent with the percentage of degradation observed in the biodegradation studies. Published literature on analogous chemicals also indicates that the notified chemical is likely to undergo primary biodegradation during wastewater treatment processes. The expected metabolite from degradation is volatile but is expected to eventually undergo further degradation in water or soil to form the very persistent degradation product, PFHxA.

The half-life of the notified chemical in the water or soil compartments cannot be extrapolated from the biodegradability results. However, primary degradation of the notified chemical is likely. It is expected that the primary degradation products of the notified chemical will degrade in the environment and release the very persistent degradation product, PFHxA.

In surface waters, agricultural soils and landfill, the notified chemical is expected to eventually degrade to form water, oxides of carbon and nitrogen, inorganic salts, and degradation products containing polyfluoroalkylated functionality. The expected initial poly- and perfluoroalkylated degradation products are assumed to undergo further degradation to form, among other compounds, the very persistent perfluoroalkylated degradation product, PFHxA. It is noted that some volatile degradation intermediates have the potential to undergo long range atmospheric transport and thus may result in translocation of PFHxA in the environment. The notified chemical also contains trace levels of impurities that may degrade to form PFOA and other long-chain perfluorocarboxylic acids.

PFHxA is expected to be recalcitrant in the environment, and potentially undergo long range transport while mainly staying in the water column. In water, it is expected to be very persistent and will not hydrolyse, photolyse or biodegrade.

High-temperature incineration is the preferred method of disposal of perfluoroalkylated chemicals (and polymers) due to the environmental persistence characteristics, when it results in mineralisation of the perfluoralkylated functionality to oxides of carbon and hydrofluoric acid. Disposal of the notified chemical and its degradation products by incineration should only take place at facilities that demonstrate complete combustion of the perfluoroalkylated functionality and have adequate measures in place to control release of hydrofluoric acid.

The n-octanol/water partition coefficient (Pow) of the notified chemical could not be measured directly due to its surface-active properties, but was estimated from its solubility in water and in n-octanol. The log Pow of the three major components of the notified chemical ranged from -1.37 to 0.93. Generally, a log Pow of < 4.2 indicates a low potential for bioaccumulation as high values indicate a tendency to partition to lipids while a low value indicates a tendency to partition to water. However, this assumes lipophilicity of the hydrophobic functionality which does not apply to perfluorinated functionality. Further, it is noted that certain perfluoroalkyl substances are known to accumulate in the blood and liver rather than lipids in biological systems (Danish EPA, 2008). As perfluoroalkyl substances do not follow the usual mechanism for bioaccumulation and are not expected to bioaccumulate in lipids, and because of the notified chemical's surface-active properties, the n-

octanol-water partition coefficient is not considered to be a reliable indicator of bioaccumulation potential for the notified chemical.

Published literature demonstrates that the notified chemical is of a type that may be expected to undergo metabolism in mammals or transformation in the environment, liberating 6:2 fluorotelomer alcohol (6:2 FTOH) and, subsequently, the expected cascade of FTOH metabolites (D'Eon *et al.*, 2007 and Lee *et al.*, 2010). The biotransformation process is expected to occur via enzyme activated hydrolysis to liberate the 6:2 FTOH. A study submitted by the notifier for an analogue chemical indicates that the analogue is not bioaccumulative in fish. While the notified and analogue chemicals are structurally similar, the difference in their compositions may change their properties and potential bioaccumulation. However, the notified chemical is expected to undergo biotransformation more readily than the analogue based on structural considerations, further reducing its potential for bioaccumulation. The probable primary degradation noted in the biodegradation tests also reduces the likelihood that the notified chemical itself is bioaccumulative. Therefore, based on a weight of evidence approach, the notified chemical itself is not expected to bioaccumulate.

The notifier has also submitted multiple study reports regarding 6:2 FTOH toxicology and metabolism in both mammals and fish showing that it is rapidly eliminated from living systems. In addition, biodegradation studies confirm rapid 6:2 FTOH biotransformation in the environment (Liu *et al.*, 2010ab). This transformation process is supported by modelling estimates derived using Catalogic (LMC, 2011). Moreover, the terminal degradation product, PFHxA, has rapid bioelimination (Gannon *et al.*, 2011) and two published studies have shown that PFHxA is not bioaccumulative in aquatic organisms (Martin *et al.*, 2003ab).

In summary, the notified chemical is expected to degrade to form substances (*e.g.*, 6:2 FTOH and PFHxA) that are expected to be rapidly eliminated from living systems and are not bioaccumulative in aquatic organisms. Therefore, the notified chemical and its metabolites are not expected to bioaccumulate in aquatic organisms.

The available laboratory (Higgins *et al.*, 2007; Martin *et al.*, 2003ab; Woodcroft *et al.*, 2010) and field (Falandysz *et al.*, 2006; Falandysz *et al.*, 2007, Furdui *et al.*, 2007) evidence indicates that PFHxA is expected to be less bioaccumulative than PFOA and other long chain perfluorinated compounds, which PFHxA-chemistry is replacing (although PHFxA and PFOA are not considered bioaccumulative). However, both are bioavailable and can be detected in wildlife as demonstrated by monitoring studies (Kumar *et al.*, 2009; Ye *et al.*, 2008ab; Wang *et al.*, 2008). In aquatic biota, there is little evidence of increased bioconcentration of PFOA compared with PFHxA although PFOA may generally be expected to be found in aquatic organisms more often than PFHxA. In general, the available evidence indicates that the bioaccumulation potential of perfluorinated compounds is correlated with increasing fluorinated carbon chain length (Giesy *et al.*, 2010). Therefore, PFHxA has a lower bioaccumulation potential than PFOA and other long chain perfluorinated compounds, which PFHxA-based chemistry is replacing.

7.1.3. Predicted Environmental Concentration (PEC)

Aquatic compartment

The notified chemical may be released to the aquatic compartment through the disposal of wastewater generated during its reformulation or use. The predicted environmental concentration (PEC) due to releases from reformulation is calculated assuming point-source release from the site(s) of reformulation. The PEC due to releases from use is calculated assuming diffuse release Australia-wide.

The PEC for the aquatic compartment due to reformulation activities is now calculated. Although up to nine sites of reformulation were nominated, information was not provided on the location or release concentrations, nor was the disposal pathway for wastewater specified (to sewers or to surface waters). Therefore, the worst-case scenario for point-source releases due to reformulation activities, assuming no removal of the notified chemical during wastewater treatment except for precipitation due to saturation, is for direct release of the notified chemical to surface waters at concentrations up to its solubility in water (2.4 g/L).

A less conservative exposure model assumes that wastewater from reformulation point-sources are released to, and diluted in, sewers. In the absence of information to further refine the model, it is assumed that all reformulation occurs at one site and/or all reformulation wastewater is released to one STP. The model assumes the average dry-weather daily flow for the fast primary treatment facility at Malabar in Sydney. This is considered to be the largest STP in Australia and therefore provides the maximum possible dilution of the notified chemical releases due to reformulation activities. It is assumed that there is no removal of the notified chemical in the STP. The combined maximum daily release of the notified chemical due to reformulation activities is calculated as 0.164 kg/day based on the estimated daily release as detailed in the table below.

Use	*Paints*	*Floor wax and polish*	*Stone and tile treatment*
Estimate of annual release to wastewater streams due to reformulation based on a 6 tonne annual import volume	14.2 kg	3.8 kg	1.7 kg
Estimated number of days per annum on which of reformulation occurs (based on reported batch sizes and import volume, limited to 260 work days per annum)	260	260	18
Estimated daily release in wastewater streams	0.055 kg	0.015 kg	0.094 kg

Therefore, the PEC in STP effluent due to release to sewers of the notified chemical in wastewater generated during reformulation ($PEC_{reformulation}$) is calculated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment from Reformulation		
Daily chemical release:	0.164	kg/day
Individual Sewage Treatment Plant Average Daily Flow:	456	ML/day
Removal within STP	0%	
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.36	µg/L
PEC - Ocean:	0.036	µg/L

The PEC of the notified chemical in sewage effluent from releases of the notified chemical to sewers (0.732 tonnes) during use (PEC_{use}) is calculated assuming that releases occur across Australia on 365 days per annum. It is assumed that there is no removal of the notified chemical during STP processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment from Use		
Annual quantity of chemical released to sewer during use	732	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.01	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.44	µg/L
PEC - Ocean:	0.044	µg/L

Based on the above calculations, the maximum PEC for the notified chemical in surface water due to the combined effluent from reformulation and use are 0.80 µg/L for river water and 0.080 µg/L for ocean waters.

Soil compartment

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.80 µg/L may potentially result in a soil concentration of approximately 5.4 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 27 µg/kg and 54 µg/kg, respectively.

PEC for PFHxA and long chain perfluorinated chemicals

The notified chemical is assumed to degrade and ultimately form the persistent degradant, PFHxA. However, the yield and rate of conversion of the notified chemical to PFHxA has not been established as characterisation of the degradation products was not undertaken in the biodegradation study. Environmental monitoring data shows that PFHxA and PFOA, which PFHxA-chemistry is replacing, is widely found in the environment, particularly in fresh water close to industrial sources, but also in some biota. Water appears to be the main

compartment where PFHxA is found. High measured concentrations of both PFHxA and PFOA in surface waters in Germany have been associated with the legal application of waste materials to agricultural soils (Skutlarek *et al.*, 2006) indicating that these chemicals have the potential to enter the aquatic compartment following initial release into the soil compartment.

Some larger available data sets from the literature (McLachlan *et al.*, 2007; Skutlarek *et al.*, 2006; Nakayama *et al.*, 2007; So *et al.*, 2007; Ahrens *et al.*, 2009) include monitoring from a range of rivers in Europe, the USA and China, along with data from the Atlantic Ocean. Using these data ($n \geq 60$), the 10th, 50th and 90th percentile concentrations for PFHxA are 1.0, 6.15 and 22.5 ng/L respectively, while those for PFOA are 2.94, 11.85 and 231.9 ng/L respectively. As use of chemicals that degrade to form PFHxA increases, levels of PFHxA may build up further in the environment.

PFHxA and other fluorochemicals have also been found in landfill leachate, with concentrations of PFHxA ranging from 270 – 790 ng/L (Huset *et al.*, 2011). As landfills are reservoirs of solid waste, and receive waste water treatment plant sludge, which may contain polyfluorinated chemicals, landfills have the potential to continue to release PFHxA and homologues well into the future.

Historically, release of perfluorinated contaminants into the environment has been linked to direct releases of low molecular weight poly- and perfluoroalkylated chemicals, such as poly- and perfluoroalkylated impurities during manufacture and reformulation processes, rather than breakdown of the chemicals themselves. In order to limit the extent of direct release of potential PFHxA precursors to the environment, it is recommended that control measures be implemented to minimise the residual weight percentage of unreacted poly- and perfluoroalkylated impurities in the notified chemical to the extent practicable. Efforts have also been made globally to control releases of perfluorinated contaminants, such as by reducing the presence of poly- and perfluoroalkylated residual constituents and impurities in substances. Where possible, the total weight of residual constituents with polyfluoroalkylated functionality should not exceed 0.1% on a dry weight basis. As the residual weight of these constituents is currently > 0.1% for the notified chemical, it is recommended that the notifier utilise technological advances to further reduce the residual constituents so the total weight of residual constituents with polyfluoroalkylated functionality does not exceed 0.1% on a dry weight basis. The notified chemical is also indicated to contain polyfluoroalkylated impurities, including the above mentioned residual constituents and other polyfluoroalkylated impurities (of different chain lengths). These impurities are recommended to be limited to a level below 0.3% w/w on a dry weight basis.

By reducing the presence of residual polyfluoroalkylated impurities in chemicals, it is expected that indirect releases from the degradation of these poly- and perfluoroalkylated substances will become an insignificant source of persistent perfluoroalkylated compounds in the environment in the future. PFHxA is already being detected in the environment and as the long chain perfluorinated compounds are phased out in preference for PFHxA-based chemistry, the environmental levels of PFHxA are expected to increase.

The notifier expects transformation of the notified chemical into the 6:2 FTOH and its subsequent degradation products. However, degradation products of the notified chemical are unknown and characterisation was not undertaken in the biodegradation study. Therefore, a PEC for indirect releases of PFHxA arising from proposed use and disposal of the notified chemical in Australia cannot be determined.

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		
Fathead minnow	96 h LC50 > 24 mg/L[ab]	- [c]
Rare gudgeon	96 h LC50 > 150 mg/L	Not harmful to rare gudgeon[d]
Rainbow trout	96 h LC50 > 36.4 mg/L	At worst, harmful to rainbow trout[d]
Rainbow trout	90 d NOEC = 2.5 mg/L	Not harmful to rainbow trout

Endpoint	*Result*	*Assessment Conclusion*
Invertebrates Toxicity		
Daphnia magna	48 h EC50 = 3.24 mg/L[bef]	Toxic to aquatic invertebrates[d]
	21 d NOEC = 0.409 mg/L[e]	Very toxic to aquatic invertebrates with long lasting effects[d]
	21 d NOEC = 0.0467 mg/L	Very toxic to aquatic invertebrates with long-lasting effects[d]
Algal Toxicity		
Green algae	72 h EC50 > 24 mg/L[be]	At worst, harmful to algae[d]
	96 h NOEC = 24 mg/L[be]	At worst, harmful to algae with long lasting effects[d]
Other Toxicity		
Inhibition of bacterial respiration	3 h IC50 > 1000 mg/L 3 h NOEC = 1000 mg/L	Not inhibitory to microbial respiration at up to 1000 mg/L
Earthworm	14 d LC50 > 200 mg/kg dry soil	At worst, slightly toxic to earthworms on an acute basis[g]

[a] The results are considered to be unreliable; [b] The results are based on the nominal concentration and were not verified by measured concentrations; [c] An assessment conclusion is not made as the results are not reliable; [d] Classification according to the GHS (United Nations, 2009); [e] The results are considered to be reliable with restrictions; [f] The 95% confidence interval (1.93 mg/L to 17.0 mg/L) is considered wide and is likely to be due to the use of a separation factor that exceeds that recommended by the OECD test guideline; [g] Classification according to Mensink (1995).

Screening studies were provided for the acute effects of the notified chemical to fish (fathead minnow), daphnia and green algae. These screening studies did not demonstrate that constant conditions were maintained for the duration of the test. Notably, the nominal concentrations of the test substance in the test solutions were not verified by analytical measurements. The results based on the nominal concentration of the notified chemical for the fathead minnow test are considered to be unreliable as there was evidence that the test substance was not fully solubilised in the test medium. There was no evidence in the daphnia and algae test of incomplete solubility of the test substance in the test medium. Therefore, the results based on nominal concentrations for daphnia and green algae are considered reliable with restrictions.

Additional acute effects endpoints were provided for a further two fish species. No mortality was observed in the limit test at 150 mg/L for rare gudgeon. Mortality (of 14%) was only observed at the highest tested concentration for rainbow trout. Thus, a defined result for the 96-hour median lethal concentration (LC50) for rainbow trout could not been determined. However, the results of the test qualify that the 96-hour LC50 exceeds 36.4 mg/L. Therefore, the notified chemical is considered, at worst, harmful to fish. A 90-day fish study indicated the notified chemical is not harmful to fish on a chronic basis.

The 48-hour median effect concentration (EC50; immobilisation) for daphnia was determined to be 3.24 mg/L. Although the results of the acute daphnia study are considered reliable with restrictions, the results should be used with caution due to the wide confidence interval associated with this result. On the basis of the measured result for daphnia, the notified chemical is considered toxic to aquatic invertebrates.

Studies were also provided for the chronic effects of the notified chemical to daphnia. In the semi-static study, surface film was observed in the test solutions and there was no statistically significant effect on the number of living offspring at the highest test concentration of 0.169 mg/L. Therefore, the results for the 21-day no-observed effect concentration (NOEC; reproduction - number of dead offspring) for daphnia determined in this study of 0.409 mg/L is used with caution. The flow-through study showed statistically significant reduction in adult survival at the three highest test concentrations. There was no statistically significant effect on the number of living offspring at the lowest test concentration of 0.0467 mg/L and this was determined to be the 21-day NOEC (reproduction - number of living offspring) for daphnia in this study. On the basis of the flow-through long-term daphnia study results, the notified chemical is considered very toxic to aquatic invertebrates with long lasting effects.

No effect on the biomass or growth rate of green algae was observed at any tested concentration in a 72-hour study. With the 72-hour EC50 and NOEC qualified to be greater than or equal to 24 mg/L, the notified chemical is, at worst, harmful to algae.

The 3-hour median inhibition concentration for microbial respiration (IC50) and NOEC indicated that the notified chemical is not expected to be inhibitory to microbial respiration at up to 1000 mg/L.

An acute endpoint is also available for one soil-dwelling organism. The 14-day LC50 for earthworms of greater than 200 mg/kg soil indicates that the notified chemical is, at worst, slightly toxic to earthworms on an acute basis (Mensink, 1995).

Classification under the GHS
The environmental hazard classification of the notified chemical is conducted in accordance with the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). In the absence of defined acute toxicity endpoints for fish and algae, the notified chemical is formally classified under the GHS as 'Acute category 2; Toxic to aquatic life' based on its acute toxicity to aquatic invertebrates.

Adequate chronic toxicity endpoints were available for three trophic levels. Based on its lack of rapid degradability and the chronic endpoint for aquatic invertebrates, the notified chemical is formally classified under the GHS as 'Chronic category 1; Very toxic to aquatic life with long lasting effects'.

Effects of PFHxA and long chain perfluorinated chemicals
There are only limited available toxicity data for PFHxA to organisms, and these are limited to aquatic organisms. Based on the available literature, the most sensitive trophic level is algae. Latala et al., (2009) reported the 72-hour median effect concentrations (72 h EC50) for three marine species as follows: 1.0 mg/L for blue green algae (*Geitlerinema amphibium*); 1.4 mg/L for diatom (*Skeletonema marinoi*); and, 4.0 mg/L for green algae (*Chlorella vulgaris*). The data indicates that PFHxA is toxic to algae on an acute basis. The study also investigated the toxicity of PFOA to the three marine species: 0.25 mg/L for blue green algae; 0.37 mg/L for diatom; and, 0.98 mg/L for green algae. The data indicates that PFOA is very toxic to algae on an acute basis and demonstrate decreased toxicity of PFHxA compared with PFOA to three species tested.

Other data indicate that PFOA is not harmful to fish and aquatic invertebrates on an acute basis with median lethal or effect concentrations (L(E)C50) of greater than 100 mg/L (US FDA, 2009). The majority of the available data for the ammonium salt of PFOA (US EPA, 2002) show this substance is largely expected to be not harmful to fish and aquatic invertebrates, although one reported endpoint (fathead minnow 96 h LC50 = 70 mg/L) is below 100 mg/L.

Giesy *et al.* (2010) reported the relationship between increasing carbon chain length and increasing toxicity. Therefore, PFHxA is expected to have a less problematic ecotoxicological profile than PFOA and other long chain perfluorinated acids it is expected to replace. Long-term effects data that reflect or model the periods over which perfluorinated chemicals are present in the environment are not available for PFHxA or long chain perfluorinated acids. Therefore, the long-term hazard to aquatic organisms has not been adequately established and is unknown.

7.2.1. Predicted No-Effect Concentration

Aquatic Compartment
The Predicted No-Effect Concentration for the aquatic compartment ($PNEC_{water}$) was derived from the lowest chronic endpoint using an assessment factor of 10 as chronic toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (invertebrates)	47	µg/L
Assessment Factor	10	
$PNEC_{water}$:	4.7	µg/L

STP
The PNEC in an STP ($PNEC_{STP}$) is derived from the NOEC for the microbial respiration inhibition test and an assessment factor of 10.

Predicted No-Effect Concentration (PNEC) for the STP		
NOEC (respiration)	> 1000	mg/L
Assessment Factor	10	
$PNEC_{STP}$:	> 100 000	µg/L

Terrestrial Compartment

The $PNEC_{soil}$ is derived from the only acute effects endpoint that is available for soil organisms (earthworm) and an assessment factor of 1000.

Predicted No-Effect Concentration (PNEC) for the Terrestrial Compartment		
Earthworm, 14 d LC50	> 200	mg/kg soil
Assessment Factor	1000	
$PNEC_{soil}$:	> 200	µg/kg soil

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.80	4.7	0.17
Q - Ocean	0.080	4.7	0.017
Q - STP	0.80	> 100 000	≪ 0.01
Q - Soil	22	> 200	< 0.11

The risk quotients (Q) for river and marine waters are less than 1, indicating the notified chemical will not be present at ecotoxicologically significant concentrations in surface waters. The risk to soil-dwelling organisms and microorganisms in an STP is not unreasonable, as indicated by the $Q_{terrestrial}$ and Q_{STP}. The available data indicates that the notified chemical is harmful to aquatic life on an acute basis and very toxic to aquatic life with long lasting effects.

A narrow safety margin is noted from the calculated PEC/PNEC value. This value has been calculated based on the worst case scenario without any mitigating consideration for the removal of the notified chemical from sewage treatment plants. No information regarding the partitioning behaviour of the notified chemical is available. It is expected that a more accurate PEC may be less than the calculated value, and therefore, the PEC/PNEC value has the potential to be lower.

Based on a weight of evidence approach and analogue data, the notified chemical is not expected to bioaccumulate in aquatic or terrestrial organisms. The notified chemical is also expected to be transformed or degrade to form substances (*e.g.*, 6:2 FTOH and PFHxA) that are expected to be rapidly eliminated from living systems and are not bioaccumulative in aquatic organisms. Therefore, the notified chemical and its metabolites are not expected to bioaccumulate in aquatic organisms.

The main environmental risks associated with perfluoroalkylated chemicals relate to the release of perfluorinated degradation products such as PFHxA. Perfluorinated chemicals, such as the degradation products of the notified chemical, are expected to be very persistent in the environment (*e.g.*, PFOA: $t_{½}$ (hydrolysis) > 200 years; US EPA, 2002). There is limited evidence in the published literature of PFHxA toxicity to aquatic organisms on an acute basis, although it is reported to be toxic to marine algae. However, it is not possible to quantify the long-term risks of PFHxA to the environment due to knowledge gaps both in predicting environmental concentrations from indirect sources of release and its long-term environmental effects. The latter point is considered a critical data gap as aquatic organisms are expected to have long-term exposure to PFHxA due to its persistence in the water compartment. The notified chemical also contains impurities which are assumed to degrade to form PFOA. Therefore, considering the dispersive use pattern of the notified chemical, it is recommended to restrict the impurities in the notified chemical that breakdown to form PFOA.

PFHxA is already wide-spread in surface waters and biota. Continuing release of PFHxA which has no known breakdown mechanism (at least in soil and water) could result in increasing environmental concentrations over time. Hence, there is potential for ecotoxicologically significant concentrations to eventually be reached following its accumulation in the environment. In this eventuality, precursors of PFHxA such as the notified chemical cannot be recalled after release and are a potential source of PFHxA in the environment even long after their use ceases. Thus, use and disposal of the notified chemical increases the environmental risk profile

of PFHxA. The notified chemical also contains impurities which are assumed to degrade to form PFHxA. Therefore, considering the dispersive use pattern of the notified chemical, it is recommended to reduce the impurities in the notified chemical that breakdown to form PFHxA, to the extent possible.

Conclusions
On the basis of the PEC/PNEC ratio and assessed use pattern, the notified chemical itself is not considered to directly pose an unreasonable short-term risk to the aquatic environment, although the safety margin is narrow for this assessed use pattern.

However, degradants of the notified chemical, along with associated impurities of the notified chemical, are potential precursors of the very persistent chemical, PFHxA. The assessed use pattern of the notified chemical does not control the release of breakdown products into the environment after disposal and there are no adequate long-term environmental effects data for PFHxA. Therefore, the long-term environmental implications are unknown. Consequently, the long-term risk cannot be quantified for the degradation products of the notified chemical. In order to inform a detailed assessment, further data needs to be generated. This includes data on longer-term environmental effects, as well as partitioning behaviour and characterisation of the degradation products, for the notified chemical and poly- and perfluoroalkylated degradation products (including PFHxA).

The assumed major degradation product, PFHxA, is environmentally persistent and has potential to be globally distributed. However, the ecotoxicological profile and bioaccumulation potential of PFHxA is considered to be less problematic when compared with long chain (C8 and above) perfluorinated acids that PFHxA is expected to replace. Nonetheless, it is recommended that the introduction and use of chemicals that degrade to release PFHxA and other very persistent poly- and perfluoroalkylated compounds remain a short-term measure until suitable alternatives, with less persistent chemistry, are identified.

In order to limit the extent of direct release of potential PFHxA precursors to the environment, it is recommended that control measures be implemented to minimise the residual weight percentage impurities in the notified chemical to the extent practicable. Where possible, the total weight of residual constituents with polyfluoroalkylated functionality should not exceed 0.1% on a dry weight basis. As the residual weight of these constituents is currently > 0.1% for the notified chemical, it is recommended that the notifier utilise technological advances to further reduce the residual constituents so the total weight of residual constituents with polyfluoroalkylated functionality does not exceed 0.1% on a dry weight basis. The notified chemical is also indicated to contain polyfluoroalkylated impurities, including the above mentioned residual constituents and other polyfluoroalkylated impurities (of different chain lengths). These impurities are recommended to be limited to a level below 0.3% w/w on a dry weight basis.

8. RISK ASSESSMENT FOR EXTENSION APPLICATION

There are no changes under the proposed extension to the use, or to the occupational, public and the environmental exposure. The introduction volume will be decreased. Therefore, the circumstances in the extension are not expected to impact on the original human health and environment risk assessment and recommendations.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Decomposes at 200.2ºC

Method Similar to OECD TG 102 Melting Point/Melting Range.
Remarks The notified chemical decomposes before melting
Test Facility Case Consulting Laboratories, Inc. (2009)

Density 1100 kg/m^3 at 22ºC

Method OECD TG 109 Density of Liquids and Solids.
Remarks Method based on ASTM Method No. E 727
Test Facility Case Consulting Laboratories, Inc. (2009)

Vapour Pressure < $1.067x10^{-2}$ kPa

Method EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined with a Hastings gauge
Test Facility DuPont (2009a)

Water Solubility 2.4 g/L at 25ºC

Method Similar to OECD TG 105 Water Solubility (Flask Method).
Remarks Saturated solutions were prepared in triplicate at a loading rate of 25 g/L and 50 g/L. Samples were shaken at 30ºC for 24, 48 and 72 hours before being equilibrated at 25ºC. The pH of all solutions was 6. Solubility was determined for three components of the UVCB notified chemical at 25ºC. The total water solubility of the notified chemical is 2.4 g/L at 25ºC. The results are based on the average concentration of the samples with a loading rate of 50 g/L only. It was considered that the equilibrium time had been insufficient at the loading rate of 25 g/L as the percent coefficient of variation (%CV) of the three analytes exceeded 15% for the three replicates. Analytical monitoring was by HPLC with evaporative light scattering (ELS) detection.
Test Facility Case Consulting Laboratories, Inc. (2009)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at pH 4-9, 25ºC

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$(25 °C)*
4	50	> 1 year
7	50	> 1 year
9	50	>1 year

Remarks In the preliminary test, less than 10% hydrolysis was observed for test substance in deoxygenated, sterile aqueous buffers of pH 4.0, 6.9 and 9.2 which were incubated in the dark at 50ºC ± 0.5ºC for 5 days. Therefore, the notified chemical is considered hydrolytically stable with a half life greater than 1 year under environmental conditions ($t_{½}$ > 1 year at pH 4-9, 25ºC). Analytical monitoring was by UPLC with MS/MS detection.
Test Facility Key Lab of Pesticide for Environmental Assessment and Pollution Control, MEP (2010a)

Partition Coefficient (n-octanol/water) log Pow = -1.37 to 0.93 at 20ºC

Method OECD TG 107 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Consistent with the recommended method for surface active materials, the n-octanol-water partition coefficient (Pow) was calculated from the individual solubilities in water and n-octanol using the equation in the test guideline above. The water solubilities for the three

main components of the notified chemical, as reported by Case Consulting Laboratories, Inc. (2009), were used in the calculation.

The solubilities in n-octanol of the three main components of the notified chemical were determined at ambient temperature (approximately 20ºC). Analytical determination was by high performance liquid chromatography with mass spectroscopy detection. The n-octanol solubilities of the main components of the notified chemical were: Component 1: 0.1012 g/L; Component 2; 0.2228 g/L; and, Component 3: 0.4112 g/L.

The log Pow calculated for the three main components of the notified chemical were: Component 1: -1.37; Component 2: 0.93; and, Component 3: -0.39.

Test Facility DuPont (2009b)

Adsorption/Desorption log K_{oc} = 1.14 to 3.19 at 25°C

Method OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (2001).
EC Council Regulation No 440/2008 C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks Analysis was by ultra performance liquid chromatography (UPLC) with pulsed amperometric and evaporative light scattering detection. The pH of the test system, and the ionised form of the notified chemical in the test system were not specified. A buffered mobile phase was not used. Two distinct elution peaks were observed for the notified chemical. The soil adsorption coefficients (Koc) for the two fractions were 13.8 and 1549, indicating very high and low mobility in soils, respectively. The major components of each fraction were not identified.

Test Facility Key Lab of Pesticide for Environmental Assessment and Pollution Control, MEP (2010b)

Dissociation Constant pKa_1 = 4.1
pKa_2 = 7.0
pKa_3 = 9.5

Method OECD TG 112 Dissociation Constants in Water.

Remarks Titration method.

Test Facility Case Consulting Laboratories, Inc. (2009)

Surface Tension 21.49 mN/m at 22ºC

Method OECD TG 115 Surface Tension of Aqueous Solutions.

Remarks Concentration: 1 g/L

Test Facility Case Consulting Laboratories, Inc. (2009)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (up to 20% concentration)
METHOD	OECD TG 425 Acute Oral Toxicity: Up-and-Down Procedure
Species/Strain	Rat/Crl:CD(SD)
Vehicle	None
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 F	175	0/1
2	1 F	550	0/1
3	1 F	1750	0/1
4	3 F	5000	0/3

LD50	> 5000 mg/kg bw (equivalent to >1000 mg/kg bw of the notified chemical)
Signs of Toxicity	High posture was observed in one rat treated at 5000 mg/kg bw on the day of dosing.
Effects in Organs	None
CONCLUSION	The notified chemical (up to 20% concentration) is of low toxicity via the oral route.
TEST FACILITY	DuPont (2009c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical (up to 20% concentration)
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M + 5F	5000	0/10

LD50	> 5000 mg/kg bw (equivalent to > 1000 mg/kg bw of the notified chemical)
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	Anogenital staining observed in two males was not considered to be treatment related. One male lost weight over the first week.
Effects in Organs	None
CONCLUSION	The notified chemical (up to 20% concentration) is of low toxicity via the dermal route.
TEST FACILITY	Eurofins (2009)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical (up to 20% concentration)

METHOD — Similar to OECD TG 403 Acute Inhalation Toxicity

Species/Strain	Rat/Crl:CD(SD)
Vehicle	Water
Method of Exposure	Nose-only
Exposure Period	4 hours
Physical Form	Liquid aerosol
Remarks - Method	In the acute lethal concentration study, rats (5/group) were exposed to 20 or 47 mg/m^3/4 hours of the test substances. These groups were sacrificed following 14 days observation. Gross pathological examination was not conducted for the animals. In the pathology study, rats (15/sex/group) were exposed to 0, 0.12, 1.0, 8.0 or 19 mg/m^3/4 hours of the test substance. Rats (5/group/sacrifice point) were then sacrificed on days 1, 7 or 14 post-exposure, then subject to gross pathological and histopathological on selected tissues (lung, larynx/pharynx, trachea and nose).

RESULTS

Group	*Number and Sex of Animals*	*Mean concentration (mg/m^3)*	*Particle size*		*Mortality*
			MMAD ± GSD (µm)	*% <3 µm*	
ALC study					
1	5 M	20 ± 2.3	2.1 ± 2.0	69	0/5
2	5 M	47 ± 3.9	2.1 ± 2.0	70	3/5
Pathology study					
3	15 M	0	-	-	0/15
4	15 M	0.12	0.68 ± 1.5	100	0/15
5	15 M	1.0 ± 0.47	1.0 ± 2.0	66	0/15
6	15 M	8.0 ± 0.51	2.4 ± 2.0	62	0/15
7	15 M	19 ± 0.98	2.3 ± 2.1	66	0/15

LC50	> 20 and < 47 mg/m^3/4 hours
Signs of Toxicity	Nasal discharge was observed shortly after exposure in one animal exposed to 47 mg/m^3/4 hours. Weight loss and laboured breathing occurred in two rats. The three mortalities in group 2 occurred within 2 days post-exposure. No signs of toxicity were observed in the surviving rats exposed to 47 mg/m^3/4 hours or in any rat exposed to 20 mg/m^3/4 hours. There were no clinical signs of toxicity in the pathology exposure groups. There were body weight losses (up to 8 grams) on the day following exposure in most groups, including controls, but this was not considered to be treatment-related as the control group also lost weight.
Effects in Organs	Gross observations in the pathology groups were not considered to be treatment related based on the lack of a dose response and the low incidence. Treatment related microscopic findings were noted in animals exposed to 8 and 19 mg/m^3/4 hours (see following Table) and were similar in the two groups. On day 1, laryngeal changes were characterised by erosion and ulceration of the ventral mucosal, with inflammation of the submucosa, and degeneration and necrosis of the u-cartilage. Minimal regenerative hyperplasia was present in some animals. These changes were mostly limited to the ventral midline, at the base of the epiglottis and at the level of the ventral laryngeal diverticulum. On day 7, changes were mostly limited to the ventral submucosa and were characterised by mineralisation and in some animals sequestration of the u-cartilage, and the presence of focal aggregates of macrophages and microgranulomas (usually associated with mineralised debris). Some recovery was observed by day 14, but it was incomplete, with mineralisation of the ventral cartilage and

microgranuloma/mineralised debris in the ventral submucosa observed at day 14. Inflammation of the lungs was observed in high dose animals at all observation points and at day 14 in the group exposed to 8 mg/m^3/4 hours.

		Test substance concentration (mg/m^3/4 hours)				
	Day	*0*	*0.12*	*1.0*	*8.0*	*19*
Lungs						
Inflammation, perivascular/periobronchiolar	1	1(1.0)	0	0	0	2(1.5)
	7	0	0	0	0	2(1.5)
	14	1(1.0)	0	0	2(1.0)	1(1.0)
Inflammation, focal/multifocal subacute	1	0	0	0	0	2(1.0)
	7	0	0	0	0	2(1.0)
	14	1(1.0)	0	0	1(1.0)	2(1.0)
Pharynx/Larynx						
Erosion/ulcer, ventral mucosa	1	0	0	0	3(2.0)	5(1.8)
	7	0	0	0	0	0
	14	0	0	0	0	0
Inflammation, subacute/chronic	1	0	0	0	4(1.0)	5(1.2)
	7	0	0	0	0	1(1.0)
	14	0	0	0	0	0
Hyperplasia, ventral mucosa	1	0	0	0	1(1.0)	2(1.5)
	7	0	0	0	1(1.0)	1(1.0)
	14	0	0	0	0	0
Degeneration/necrosis, ventral cartilage	1	0	0	0	0	2(1.0)
	7	0	0	0	0	0
	14	0	0	0	0	0
Mineralisation, ventral cartilage	1	0	0	0	0	0
	7	0	0	0	1(1.0)	2(1.5)
	14	0	0	0	1(3.0)	2(2.0)
Microgranuloma/mineralised debris, ventral submucosa	1	0	0	0	0	0
	7	0	0	0	1(1.0)	2(1.0)
	14	0	0	0	3(1.0)	3(1.0)

( ), Average severity of affected animals: 1=minimal, 2=mild, 3=moderate.

Remarks - Results

The MMAD for the group exposed to the test substance at 0.12 mg/m^3/4 hours was below the value of 1 µm recommended by OECD TG 403. The particle size distribution of this group was primarily below 1 µm (84%), compared to the other exposure groups (10-26%).

CONCLUSION

The LC50 for the notified chemical was established at > 20 and < 47 mg/m^3/4 hours.

The NOAEC for death was established at 20 mg/m^3/4 hours.

The NOAEC for histopathological respiratory effects was established at 1.0 mg/m^3/4 hours.

TEST FACILITY

DuPont (2009d)

B.4. Acute toxicity – inhalation, analogue

TEST SUBSTANCE

Analogue chemical 1 (up to 40% concentration)

METHOD

Similar to OECD TG 403 Acute Inhalation Toxicity

Species/Strain	Rat/Crl:CD(SD)IGS
Vehicle	None
Method of Exposure	Nose-only
Exposure Period	4 hours
Physical Form	Liquid aerosol

Remarks - Method	Rats were administered the test substance at 23, 57 or 120 mg/m^3/4 hours (6 males/concentration). Rats were maintained for a 14 day recovery period. Surviving rats were sacrificed after 14 days recovery. Surviving animals exposed to 57 mg/m^3 were subject to a macroscopic examination and the respiratory tract was examined microscopically.

RESULTS

Group	*Number and Sex of Animals*	*Mean concentration (mg/m^3)*	*Particle size*		*Mortality*
			MMAD ± GSD (μm)	*% <10 μm*	
1	6 M	23 ± 3.7	1.4 ± 1.9	100	0/6
2	6 M	57 ± 13	1.8 ± 2.0	100	3/6
3	6 M	120 ± 11	1.3 ± 1.9	100	6/6

LC50	57 mg/m^3/4 hours
Signs of Toxicity	All deaths occurred within the first day. Reduced auditory response was observed in animals treated at 57 and 120 mg/m^3/4 hours. Animals exposed to 120 mg/m^3 exhibited lethargy, lung noise and laboured breathing immediately following the exposure. One rat exposed to 23 mg/m^3/4 hours exhibited slight body weight loss within 1 day of exposure but began to regain weight from day 2 onwards. Rats exposed to 57 mg/m^3/4 hours exhibited slight to moderate body weight losses within 2 days of exposure (up to 14%) but began to regain weight from day 3 onwards.
Effects in Organs	No abnormal macro or microscopic observations were observed in surviving rats. Post mortem examination was not conducted on mortalities.
Conclusion	The LC50 for analogue chemical 1 was established at 57 mg/m^3/4 hours, thus the analogue chemical is very toxic/fatal by the inhalation route. The NOAEC for death was established at 23 mg/m^3/4 hours.
TEST FACILITY	DuPont (2002a)

B.5. Irritation – skin

TEST SUBSTANCE	Notified chemical (up to 20% concentration)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 male
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	Scores of zero were for erythema and oedema formation were noted at all observation points.
CONCLUSION	The notified chemical (up to 20% concentration) is non-irritating to the skin.
TEST FACILITY	DuPont (2009e)

B.6. Irritation – eye

TEST SUBSTANCE	Notified chemical (up to 20% concentration)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain Rabbit/New Zealand White
Number of Animals 3 male
Observation Period 14 days
Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.3	1.3	2	<14 days	0
Conjunctiva: chemosis	0	0	1.0	1	<7 days	0
Conjunctiva: discharge	0	0.7	1.0	2	<14 days	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	<24 hours	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY DuPont (2009f)

B.7. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical (up to 20% concentration)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/ CBA/JHsd (5 females/dose)
Vehicle Propylene glycol
Remarks – Method No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	484	-
5	586	1.21
25	555	1.15
50	752	1.55
100	667	1.38
Positive Control (HCA)		
25	3593	7.42

HCA, hexylcinnamaldehyde.

Remarks - Results The stimulation index values for the test substance groups were <3, indicating the absence of a skin sensitisation response.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical (up to 20% concentration).

TEST FACILITY DuPont (2009g)

B.8. Repeat dose inhalation toxicity

TEST SUBSTANCE Notified chemical (14.10% solids in water)

METHOD Similar to OECD TG 412 Subacute Inhalation Toxicity: 28-Day Study (Actual exposure was set for 2 weeks for this study). The study authors

	stated that, because the purpose of the current study was to examine the subchronic toxicity of the test substance specifically in the lungs, the guidance promulgated by OECD TG 412 was not applicable except for inhalation test conditions.
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Inhalation – nose only
Exposure Information	Dose regimen: 5 days/week over 2 weeks, total of 12 exposures Duration of exposure (inhalation): 6 hours/day
Vehicle	Water
Physical Form	Liquid aerosol
Particle Size	MMAD ± GSD: 1.8-2.7 ± 2.0-2.2 µm
Remarks - Method	Rats (20 males and 20 females for each concentration group) were exposed to the test substance at 0, 0.1, 1 or 5 mg/m^3 (total solids) for 6 hours/day, 5 days/week, for a total of 12 exposures. Body weight of the test animals were observed twice a week during exposure and once a week during the recovery. Mortality, morbidity and clinical signs were observed daily. Following the last exposure, 10 males and 10 females from each group were fasted and sacrificed for anatomic pathology. Following an approximately 4-week recovery period, the remaining 10 males and 10 females for each group were also fasted and sacrificed for the same anatomic pathology examination.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/m^3)*		*Mortality*
		Nominal	*Actual*	
Control	10M + 10 F	0	0	0/20
Low dose	10M + 10 F	0.1	0.15	0/20
Mid dose	10M + 10 F	1	1.2	0/20
High dose	10M + 10 F	5	5.2	0/20
Control recovery	10M + 10 F	0	0	0/20
Low dose recovery	10M + 10 F	0.1	0.15	0/20
Mid dose recovery	10M + 10 F	1	1.2	0/20
High dose recovery	10M + 10 F	5	5.2	0/20

Clinical Observations

All animals survived to their scheduled sacrifice. No test substance-related adverse changes in body weights and body weight gains were noted. No adverse clinical signs of toxicity were observed during the course of the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical chemistry, haematology and urinalysis were not conducted in the study.

Effects in Organs

There was a statistically significant ($p < 0.05$) reduction in brain weight (4.1%) in female animals in the recovery group that were exposed to 5.2 mg/m^3 of the test substance. In the same test group increases in the liver weight relative to brain (13.6%) and body weight (10.1%) were also statistically significant ($p < 0.05$) in comparison to the control animals. As the changes were not replicated in the female animals exposed to the same concentration but sacrificed at the end of the treatment period they were considered to be spurious by the study authors.

One day following the final exposure, minimal focal changes were present in the larynx in both males and females exposed to 5.2 mg/m^3 test substance. These changes included minimal hyperplasia/squamous metaplasia of the ventral laryngeal mucosa, minimal to mild inflammation of ventral submucosa, as well as minimal to mild mineralisation of the U-shaped cartilage. These changes were considered adverse by the study authors.

In animals exposed to 1.2 mg/m^3 of the test substance, changes were limited to minimal mucosal hyperplasia/squamous metaplasia, minimal inflammation of the laryngeal mucosa, and 1 of 10 males demonstrated minimal mineralisation of the U-shaped cartilage. These changes were considered adaptive by the study authors.

No adverse findings were reported in rats exposed to 0.15 mg/m³ of the test substance.

Following the recovery period, the epithelial changes were resolved in the 1.2 and 5.2 mg/m^3 recovery groups; however, mineralisation in the cartilage was still present in 14 of 20 animals from the 5.2 mg/m^3 recovery group and only 1 male from the 1.2 mg/m^3 recovery group.

There were no test substance-related microscopic findings in the larynx of rats in the 0.15 mg/m^3 recovery group.

After the recovery period, it was also noted that the brain weight of the 5.2 mg/m^3 group was statistically smaller than that of the control group ($p < 0.05$) and the relative liver weight of this group was significantly higher than that of the control group ($p < 0.05$). However, these changes were considered by the study authors as spurious and not related to the test substance.

CONCLUSION
Under the conditions of the study, the no-observed-adverse-effect concentration (NOAEC) for the test substance was considered to be 1.2 mg/m^3 (total solids) by the study authors for the rats based on the minimal inflammation and minimal to mild mineralisation of the U-shaped cartilage in animals exposed to 5.2 mg/m^3 (total solids) of the test substance.

TEST FACILITY DuPont (2011a)

B.9. Repeat dose inhalation toxicity - analogue

TEST SUBSTANCE Analogue chemical 1 (up to 40% concentration)

METHOD
- Species/Strain: Rat/Crl:CD(SD)IGS
- Route of Administration: Inhalation – nose only
- Exposure Information: Dose regimen: 9 exposures over 14 days; Duration of exposure (inhalation): 6 hours/day
- Vehicle: None
- Physical Form: Liquid aerosol
- Particle Size: MMAD ± GSD: 0.9-2.1 ± 1.6-2.2 µm
- Remarks - Method: Exposure concentrations were determined based on the results of a range-finding study where six rats were exposed to 18 mg/m^3 for ~6 hours per day for 6 days within an 8 day period. No clinical signs of toxicity were observed during a 3 day rest period after which the rats were exposed to a concentration of 44 mg/m^3 for 5 hours, followed by an exposure to 69 mg/m^3 for 4 hours and 20 minutes. Clinical signs following the final exposure included red nasal discharge, irregular respiration and lethargy. Five rats died within 2 days of the final exposure.

  In the main study, rats (15/concentration) were exposed to the test substance at 0, 0.2, 2 or 20 mg/m^3 for 6 hours per day for a total of 9 exposures over 14 days. Blood was collected on day 10 (10/concentration) and on day 24 (5/concentration) for haematology and clinical chemistry analyses. Animals were sacrificed following the last exposure for anatomic pathology (5/concentration). A recovery group was maintained after exposure until sacrifice on day 24 (5/concentration). Blood was collected from the remaining animals (5/concentration) on days 0, 3 and 9 of the exposure period, then again on days 14 and 24 of the recovery period, for analysis of fluorine content.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/m^3)*		*Mortality*
		Nominal	*Actual*	
control	10M	0	0	0/10
low dose	10M	0.2	0.22	0/10
mid dose	10M	2	1.9	0/10
high dose	10M	20	20	0/10

control recovery	5M	0	0	0/5
low dose recovery	5M	0.2	0.22	0/5
mid dose recovery	5M	2	1.9	0/5
high dose recovery	5M	20	20	0/5

Clinical Observations

There were no treatment related clinical findings or effect on body weight during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Slightly elevated levels of fluorine were detected in the blood in animals exposed to 20 mg/m^3 indicating the presence of the test substance or metabolites in blood.

There were no changes in haematology parameters on days 10 or 24.

There were statistically significant decreases in blood urea nitrogen on days 10 and 24 (both ↓19%) in animals exposed to 20 mg/m^3. This change was considered slight and within expected limits of biological variability. There were no other changes in clinical chemistry parameters.

Effects in Organs

There were no treatment related changes in organ weights.

There was a single macroscopic observation of a red scattered discolouration of the lungs at day 24 in an animal treated at 20 mg/m^3.

Treatment related microscopic findings were observed in the lungs and larynx at day 10 in animals exposed to 2 and 20 mg/m^3 (see following table). Effects in the lung were characterised by mixed inflammatory cells with scattered alveolar lumina. Effects in the larynx were characterised by minimal to mild squamous metaplasia of the mucosal lining of the ventral floor. There were no treatment related microscopic observations at day 24.

	Concentration (mg/m^3)			
Observation (day 10)	*0*	*0.2*	*2*	*20*
Lung				
inflammation, subacute/chronic	0/5	0/5	1/5 (1.0)	3/5 (1.0)
Pharynx/larynx				
squamous metaplasia	0/5	0/5	4/5 (1.0)	5/5 (1.8)

( ), average severity of affected animals: 1=minimal, 2=mild, 3=moderate.

CONCLUSION

The NOAEC was established as 0.2 mg/m^3/day in this study, based on histopathological effects in the lung and larynx.

TEST FACILITY DuPont (2003a)

B.10. Repeat dose oral toxicity

TEST SUBSTANCE Notified chemical (up to 20% concentration)

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents

Species/Strain Rat/Crl:CD(SD)

Route of Administration Oral – gavage

Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week

Vehicle Water

Remarks - Method Rats (15/sex/dose) were administered gavage doses of the test substance at 0, 5, 25 or 125 mg/kg bw/day for 28 days. Rats (10/sex/dose) were sacrificed immediately following exposure with a recovery groups (5/sex/dose) sacrificed after recovery for one month.

An additional group (5/sex) were administered the test substance at 5 mg/kg bw/day for 28 days, then sacrificed after a one month recovery

period. During the dosing period, blood was sampled 2 hours after dosing on days 1, 14 and 28; 24 hours after the final dose; and 1, 2, 3 and 4 weeks after the final dose. The concentration of 9 analytes (notified chemical and potential metabolites) were determined. The plasma, liver and fat were analysed following sacrifice and a glucuronide conjugate metabolite was also monitored. This analysis was conducted by LC/MS/MS.

All animals were subject to clinical and pathological examination. Additional investigations include urinalysis, and plasma and urine fluoride analyses.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M + 10F	0	0/20
low dose	10M + 10F	5	0/20
mid dose	10M + 10F	25	0/20
high dose	10M + 10F	125	0/20
control recovery	5M + 5F	0	0/10
low dose recovery	5M + 5F	5	0/10
mid dose recovery	5M + 5F	25	0/10
high dose recovery	5M + 5F	125	0/10
toxicokinetic	5M + 5F	5	0/10

Mortality and Time to Death

One female treated at 125 mg/kg bw/day died on day 21 due to a dosing accident. This death was not related to treatment of the test substance.

Clinical Observations

There were no treatment related clinical signs of toxicity, or changes in absolute body weight, body weight gain, food consumption or food efficiency.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

At 4 weeks, there were statistically significant decreases in white blood cell count and absolute lymphocyte count in all treated males (~↓20%) and in absolute monocyte count in males treated at 125 mg/kg bw/day (↓32%). Decreases in these haematology parameters were not observed in males following the recovery period or in females at either 4 or 8 weeks. Other statistically significant changes in haematology parameters were sporadic and not considered to be treatment related.

There were no statistically significant changes in prothrombin time or activated partial thromboplastin time measured at 4 weeks.

At 4 weeks, there were statistically significant increases in blood urea nitrogen in males treated at 25 (↑10%) and 125 mg/kg bw/day (↑19%) and statistically significant decreases in creatinine in males treated at 125 mg/kg bw/day (↓13%). Changes in these parameters were not observed in males following recovery. In females, there were statistically significant decreases in blood urea nitrogen (↓16%) and creatinine (↓12%) in animals treated at 125 mg/kg bw/day in the recovery groups. There was a statistically significant decrease in cholesterol levels in males treated at 125 mg/kg bw/day (↓29%) with only minimal non-statistically significant decreases were observed in males treated at 5 and 25 mg/kg bw/day (↓14% and ↓18%, respectively). There were no cholesterol decreases in males following recovery or in treated females. The decreased cholesterol in males treated at 125 mg/kg bw/day was possibly related to treatment but is likely to be non-adverse. There was a statistically significant increase in glucose levels in males treated at 125 mg/kg bw/day (↑16%) that was not considered to be adverse. Glucose levels were not analysed following recovery. Aspartate aminotransferase (AST) was increased in males treated at 125 mg/kg bw/day (↑33%) following recovery.

Overall, changes in haematology and clinical chemistry were minimal with no indication of a dose response, no consistency between sexes and thus are not considered to be toxicologically significant effects.

There were no treatment related changes in urinalysis parameters.

Plasma fluoride level increases reached statistically significant levels in males and females treated at 25 (males only) and 125 mg/kg bw/day at 4 weeks, but there were no changes following recovery. Urine fluoride levels were statistically increased at all treatment levels in males and females at 4 weeks, with statistically significant increases in urine fluoride levels in males and females treated at 125 mg/kg bw/day following recovery.

Effects in Organs

There was a statistically significant increase in relative liver weights in males (↑10%) and females (↑7%) treated at 125 mg/kg bw/day at 4 weeks. This finding was considered non-adverse as there were no associated microscopic observations in the liver. Liver weight changes were not observed following recovery. There was a statistically significant increase in relative kidney weights in males treated at 125 mg/kg bw/day (↑10%) at 4 weeks but these changes were not observed following recovery and there were no associated histopathological changes. Overall, the liver and kidney weight changes are not considered to be toxicologically significant effects, in the absence of histopathological findings, the small magnitude of the increases and the recovery observed after one month.

There was an increased incidence of minimal to mild follicular cell hypertrophy in the thyroid in males treated at 125 mg/kg bw/day (4/10). There were no similar observations in the treatment recovery groups, but there were observations in the recovery control group (2/5). This effect is a common background finding in rats and is of low toxicological significance.

A statistically significant increase in relative thymus weights in males treated at 125 mg/kg bw/day (↑27%) was observed following recovery. This finding is not considered to be toxicologically significant in the absence of increased thymus weights at 4 weeks and the lack of associated histopathological effects.

Toxicokinetic Evaluation

The concentrations of detected analytes (out of the 9 possible) in plasma are presented in the table below. With the exception of analyte 5, no other analytes (including the parent compound) were detected following cessation of dosing. Analyte 5 was the only analyte detected in the liver of males (45±8 ng/g) and females (94±75 ng/g) at day 55. In fat, all analytes were below the limit of quantitation at day 55. Plasma concentrations of analyte 5 and 8 were similar in males and females but more analytes were detected in males.

The glucuronide conjugate was detected in males up to and including day 28 and in females up to and including day 27. Levels detected in females were approximately half of those detected in males. The glucuronide conjugate was not detected in the liver or fat.

Pharmacokinetic analysis of analyte 8 revealed that a steady state in plasma was achieved by day 13 and that the compound was rapidly eliminated as the analyte was not detected following cessation of dosing. The elimination half-life for analyte 5 was 21 days in male rats but the half-life in females could not be established due to the poor fit of the regression line.

	Concentration (ng/mL) on day:							
	0	*13*	*27*	*28*	*34*	*41*	*48*	*55*
Males								
Analyte 1	<LOQ	28	33±8	33±10	<LOQ	<LOQ	<LOQ	<LOQ
Analyte 3	<LOQ	22	26	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Analyte 4	<LOQ	27±8	36	35	<LOQ	<LOQ	<LOQ	<LOQ
Analyte 5	30±6	109±31	139±12	129±31	71±23	59±30	44±20	37±10
Analyte 8	177±74	526±174	553±240	394±225	<LOQ	<LOQ	<LOQ	<LOQ
Females								
Analyte 5	31±7	94±36	211±133	157±118	77±58	84±24	69±53	91±95
Analyte 8	114±20	446±88	477±72	350±99	<LOQ	<LOQ	<LOQ	<LOQ

Data presented as mean±standard deviation
< LOQ, less than limit of quantitation

CONCLUSION

The NOAEL was established at 125 mg/kg bw/day in this study, based on the lack of toxicologically significant effects at any of the doses tested. This study did not definitively characterise the repeated dose toxicity of the notified chemical as a LOAEL was not determined.

Analysis of the parent compounds and metabolites in plasma revealed that one metabolite persisted in plasma one month after cessation of dosing.

TEST FACILITY DuPont (2011b)

B.11. Repeat dose dermal toxicity – analogue

TEST SUBSTANCE Analogue chemical 1 (up to 40% concentration)

METHOD OECD TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study
Species/Strain Rat/Crl:CD(SD)IGS
Route of Administration Dermal – semi-occluded
Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Duration of exposure: 6 hours/day
Vehicle None
Remarks - Method No significant protocol deviations.

An additional collection of blood was conducted on day 21 for determination of total fluorine content.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M	0	0/10
low dose	10M	10	0/10
mid dose	10M	100	0/10
high dose	10M	1000	0/10

Clinical Observations
There were no treatment related clinical observations or effect on body weight during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Fluorine levels on day 21 were increased in all treatment groups with the highest level of 5.96 ppm detected in one male treated at 10 mg/kg bw/day. All other values above the limit of quantification were < 1.5 ppm, with the remainder below the limit of quantification (< 0.5 ppm). All control group observations were less than the limit of quantification.

There were statistically significant decreases in haemoglobin and haematocrit concentration in males treated at 100 and 1000 mg/kg bw/day, and statistically significant increased eosinophils in males treated at 1000 mg/kg bw/day.

Clinical chemistry changes indicative of liver toxicity including aspartate (AST) and alanine aminotransferase (ALT) were statistically increased in males treated at 100 (AST ↑33%: ALT ↑100%) and 1000 mg/kg bw/day (AST ↑44%: ALT ↑122%). Sorbitol dehydrogenase was also increased in rats in rats in the 100 and 1000 mg/kg bw/day dose groups. Alkaline phosphatase and bilirubin were increased in males treated at 1000 mg/kg bw/day. Cholesterol levels were statistically increased at all treatment levels (up to ↑33%) with a statistically significant increase in triglycerides in males treated at 1000 mg/kg bw/day (↑87%). Although there was no histological correlations seen in the liver the study authors considered the increases in the AST and ALT parameters to be potentially adverse due to the magnitude of the changes.

Urine osmolality was statistically decreased in males treated at 1000 mg/kg bw/day with slight non-statistically significant increases in urine volumes, with an associated decreased urine protein concentration.

Plasma fluoride levels were similar in treated and control groups. However, urine fluoride levels were statistically increased in males treated at 1000 mg/kg bw/day, indicating exposure to and metabolism of a fluoride containing compound.

Effects in Organs
Organ weights were similar in treated and control groups. There were no macro or microscopic pathological changes.

Remarks – Results
The changes in clinical chemistry parameters and fluoride detected in the urine were indicative of systemic absorption following dermal application of the test substance. Despite the absence of associated functional or pathological changes the increases in liver enzymes were considered by the study authors to be potentially adverse due to the magnitude of the changes.

CONCLUSION
The NOAEL was established at 100 mg/kg bw/day in this study (equivalent to 40 mg/kg bw/day for analogue chemical 1), based on the increases in liver enzymes at the higher dose.

TEST FACILITY	DuPont (2003b)

B.12. Repeat dose oral toxicity – analogue

TEST SUBSTANCE	Analogue chemical 1 (up to 40% concentration) in water and isopropanol
METHOD	US EPA OPPTS 870.3100 90-Day Oral Toxicity in Rodents
Species/Strain	Rat/Crl:CD(SD)BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week
Vehicle	None
Remarks - Method	In a range-finding study, rats were administered the test substance by gavage at 10, 100, 1000 or 3000 mg/kg bw/day for 45 days. Males treated at 3000 mg/kg bw/day exhibited body weight gain decreases (↓36%) over the first month and the treatment level was decreased to 2000 mg/kg bw/day for the remainder of the study. Body weight gain decreases were also observed in animals treated at 100 and 1000 mg/kg bw/day. Absolute and relative liver weight increases were observed in animals treated at 1000 and 2000/3000 mg/kg bw/day. In the main study, rats were administered the test substance by gavage at 0, 10, 60, or 300 mg/kg/day. Additional groups of male and female rats were administered 60 mg/kg/day isopropanol to control for the approximate amount of isopropanol administered to the 300 mg/kg bw/day group from the test substance. Animals were sacrificed at 10 and 90 days and following recovery for one and three months. Clinical pathology endpoints were evaluated during the dosing period at weeks 7 and 13, and then after one and three months recovery. Neurobehavioral assessments were performed prior to dosing and during week 12 of the dosing period. After 10 days of dosing, rats (5/sex/dose) were sacrificed and evaluated for hepatic β-oxidation as a measure for peroxisome proliferation. Hepatic β-oxidation was also measured at the sacrifices at 90 days and after the one and three month recovery periods. After 90 days of dosing, rats (10/sex/dose) were sacrificed and subject to gross and microscopic pathological examination. After a one month recovery period, rats (10/sex/dose) were sacrificed from the 0 and 300 mg/kg bw/day groups. Following a three month recovery period, rats (5/sex/dose) were sacrificed from all doses. The isopropanol control group was maintained only until the 90 day sacrifice.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M + 10F	0	1/20
control (isopropanol*)	10M + 10F	0	0/20
low dose	10M + 10F	10	0/20
mid dose	10M + 10F	60	0/20
high dose	10M + 10F	300	2/20
control recovery	15M + 15F	0	1/30
low dose recovery	5M + 5F	10	1/10
mid dose recovery	5M + 5F	60	0/10
high dose recovery	15M + 15F	300	0/30

*60 mg/kg bw/day isopropanol

Mortality and Time to Death

There were no treatment related mortalities at any dose level. One male control rat was accidentally killed (reason not given), the death of one male treated at 300 mg/kg bw/day was attributed to dosing. One female control animal was killed during blood collection, and one female treated at 300 mg/kg bw/day was found dead (cause of death unknown). One female treated at 10 mg/kg bw/day was sacrificed *in extremis* during the three month recovery period possibly due to a mammary tumour.

Clinical Observations

There were no treatment related findings following ophthalmological examination prior to dosing and at day 81. There were slight increases of hair loss in males treated at 60 and 300 mg/kg bw/day and of hyperreactivity in males and females treated at 300 mg/kg bw/day.

Absolute body weights were statistically decreased in males treated at 300 mg/kg bw/day from day 42 onwards, with statistically significant decreases in the absolute body weights at the end of dosing (↓6%) and body weight gain over the dosing period (↓11%) in males at this treatment level. There were associated statistically significant decreases in food consumption and efficiency in males treated at 300 mg/kg bw/day during the dosing period. Absolute body weights were similar in controls and males treated at 300 mg/kg bw/day after recovery for one month. Males treated at 300 mg/kg bw/day then gained weight at a higher rate than controls (↑67%) over the recovery period, demonstrating reversal of the test substance related effect on body weight. The food efficiency was also higher in males treated at 300 mg/kg bw/day during the recovery period. There was no treatment related effect on body weight in treated females rats.

There were no treatment related changes in forelimb or hindlimb grip strength, sensory function observation or motor activity.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were statistically significant decreases in red blood cell count (up to ↓10%), haemoglobin (up to ↓9%) and haematocrit (up to ↓9%) in males, mostly in the groups treated at 300 mg/kg bw/day, with some smaller but statistically significant decreases in haemoglobin (up to ↓5%) and haematocrit (up to ↓6%) in males treated at 60 mg/kg bw/day. There was some indication of recovery but complete reversibility was not reached over three months. These effects were considered to be treatment related and adverse. There were no similar changes in female haematology. Other statistically significant changes in haematology parameters in males and females were either sporadic or minimal and were therefore considered incidental.

Treatment related changes in clinical chemistry parameters in males are summarised in the table below. Statistically significant increases in AST and ALT levels occurred mostly in males treated at 300 mg/kg bw/day (also with changes in the lower dose groups) with persistence of these effects after the recovery period, indicating hepatocellular injury. Increases in AST and ALT were mostly small and non-statistically significant in females. Alkaline phosphatase (ALP) was also increased in males treated at 60 and 300 mg/kg bw/day during the dosing period but no changes were observed following recovery.

There were statistically significant decreases in bilirubin in females treated at 60 and 300 mg/kg bw/day at day 39 with a statistically significant decrease in females treated at 300 mg/kg bw/day at day 93. These decreases did not persist during recovery. There were statistically significant decreases in total protein in all male treatment

level (up to ↓7%), mostly at day 92. The study authors noted that this was due to decreases in globulin. Other clinical chemistry changes were not considered to be treatment related or adverse due to the lack of a dose response, or due to the sporadic or minimal nature of the changes.

	Males (mg/kg bw/day)				
	0	*0^{IPA}*	*10*	*60*	*300*
Aspartate aminotransferase (U/L)					
day 38	86±18	90±21	90±15	138*±50 (↑60%)	142*±35 (↑65%)
day 92	74±9	75±7	128*±82 (↑73%)	327*±164 (↑336%)	148*±50 (↑100%)
day 122	89±13	-	-	-	178*±142 (↑100%)
day 183	95±23	-	110±60	137*±12	416*±414 (↑338%)
Alanine aminotransferase (U/L)					
day 38	31±5	34±4	34±8	94*±44 (↑203%)	109*±34 (↑252%)
day 92	29±5	31±6	88*±93 (↑203%)	291*±166 (↑903%)	133*±60 (↑359%)
day 122	37±9	-	-	-	121*±123 (↑227%)
day 183	38±6	-	57±38	76±25	312*±371 (↑745%)
Alkaline phosphatase (U/L)					
day 38	134±31	144±31	139±25	175*±35 (↑31%)	222*±36 (↑66%)
day 92	85±19	98±28	100±22	178*±48 (↑109%)	228*±44 (↑168%)
day 122	87±35	-	-	-	105±27
day 183	81±14	-	101±24	99±15	105±21

Data presented as mean ± standard deviation (percentage change)
*Statistically significant difference to control group ($p < 0.05$)
IPA, 60 mg/kg bw/day isopropanol control

Plasma fluoride levels were increased in males treated at 300 mg/kg bw/day at day 92 but not at day 122. Plasma fluoride was not increased in females. Urine fluoride was increased in males treated at 60 and 300 mg/kg bw/day at day 92 and males treated at 300 mg/kg bw/day at day 122. In females, urine fluoride was increased in females treated at 60 and 300 mg/kg bw/day at day 93 but not during the recovery period.

Urine pH was decreased in males treated at 300 mg/kg bw/day at the one month recovery period and total protein was decreased in females treated at 300 mg/kg bw/day also after one month recovery. The relevance of these findings is unclear given that they only occurred during the recovery period.

Increased hepatic β-oxidation activity (nmol/min/mg protein) were observed in males and females treated at 60 and 300 mg/kg bw/day. The increase remained significant after one and three month recovery in females at both dose levels, and in 300 mg/kg bw/day group males. The study authors did not consider these increases to be adverse effects.

Effects in Organs

There were statistically significant increases in absolute liver weights in males treated at 300 mg/kg bw/day (↑28%) at the end of dosing, with statistically significant increases in relative liver weights in males treated at 60 (↑7%) and 300 mg/kg bw/day (↑35%). Relative liver weights were statistically increased in males treated at 300 mg/kg bw/day (↑17%) after the one month recovery period (noting that organs at the low and middle doses were not weighed). No statistically significant liver weight increases were observed in males after the three month recovery period. Centrilobular hepatocellular hypertrophy was observed in 10/11 males treated at 300 mg/kg bw/day at the end of dosing and after recovery for one month (6/9 males) but not after the three month recovery period. Hepatocellular focal necrosis at the end of dosing was only observed in animals treated at 10 and 60 mg/kg bw/day (4/10 and 3/10, respectively) and not at 300 mg/kg bw/day, with observations in animals treated at 300 mg/kg bw/day at one month (1/9) and at three months in animals treated at 60 and 300 mg/kg bw/day (1/5 and 3/5, respectively). In females, relative liver weights were statistically increased in the 300 mg/kg bw/day group (↑9%) at the end of dosing but there were no statistically significant changes during the recovery period. There were no microscopic findings in the liver of treated females.

There were statistically significant increases in absolute kidney weights in males treated at 60 (↑12%) and 300 mg/kg bw/day (↑21%) with associated increases in relative kidney weights at these treatment levels (↑14% and ↑29%, respectively). After one month, there was a statistically significant increase in relative kidney weights in males treated at 300 mg/kg bw/day (16%) but there were no statistically significant increases after the three month recovery period. Tubular hypertrophy was observed in all males treated at 300 mg/kg bw/day at the end

of dosing but was not observed during the recovery period. In females, there was a non-statistically significant increase in absolute kidney weights in the 300 mg/kg bw/day group at the end of dosing but there was no associated change in relative weights. There were no histopathological findings in the kidneys of treated females and there were no statistically significant changes in kidney weights during the recovery period.

There were statistically significant increases in relative thyroid weight in females treated at 300 mg/kg bw/day after recovery for one month. Non-statistically significant increases were observed at other observations points at this treatment level. There were no thyroid weight changes in males but increased incidences of hypertrophy were observed in males treated at 300 mg/kg bw/day at the end of dosing and after recovery for one month, with minimal occurrences in males treated at 60 mg/kg bw/day at the end of dosing. Also in the thyroid were increased incidences of altered colloid, in males treated at 60 and 300 mg/kg bw/day at the end of dosing and in females treated at 300 mg/kg bw/day after recovery for one month. No thyroid effects were observed after the three month recovery period. The study authors noted that there was no clear association between hypertrophy and altered colloid.

Remarks – Results

The isopropanol control group was similar to the standard control group in all measured endpoints and therefore the effects observed in the treated groups are not likely to be attributable to the isopropanol component of the test substance.

CONCLUSION

A LOAEL was established as 10 mg/kg bw/day (equivalent to 4 mg/kg bw/day for analogue chemical 1), based on the focal hepatocellular necrosis and elevated liver enzymes observed in all treated males that was not reversible after three months. A NOAEL could therefore not be established for male rats. The NOAEL for female rats was 60 mg/kg bw/day based on elevated liver enzymes and thyroid gland hypertrophy observed in female rats administered 300 mg/kg/day.

TEST FACILITY DuPont (2002b)

B.13. Toxicokinetics

TEST SUBSTANCE Notified chemical (up to 20% concentration)

METHOD Non-guideline study

STUDY DESIGN AND OBJECTIVE

The study aimed to determine toxicokinetic parameters, including half-life ($t_{1/2}$), area under the curve (AUC), maximum concentration (C_{max}) and the time of maximum concentration (t_{max}). Rats (Crl:CD(SD)) were administered single oral doses of the test substance in water at 10 or 30 mg/kg bw (3/sex/dose). The dosing method was not specified in the study report but it was assumed to be gavage. Blood was collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 96, 120, 144, and 168 hours. The plasma concentrations of 10 analytes were determined by LC/MS. Fat and liver concentration was determined at sacrifice.

RESULTS

The toxicokinetic parameters for the detected analytes in plasma are presented in the table below. Analyte 8 was detected in the liver in both sexes treated at 10 mg/kg bw (mean 718 and 2627 ng/g, m/f) and 30 mg/kg bw (mean 1417 and 3177 ng/g, m/f) but was not detected in plasma at this observation point (*i.e.*, below the limit of quantitation). Analyte 5 was detected at low levels in the liver in both sexes treated at 10 mg/kg bw (<30 ng/g) and at slightly higher levels in both males (69 ng/g) and females (43 ng/g) treated at 30 mg/kg bw. There were no analytes detected in fat.

		Toxicokinetic parameter (plasma)				
	Sex	*$t_{1/2}$ (hr)*	*t_{max} (hr)*	*C_{max} (ng/mL)*	*AUC_{last}*	*AUC_{inf}*
10 mg/kg bw						
Analyte 3	M	4±3	2±0.6	47±18	93±68	269±29
	F	ND	ND	ND	ND	ND
Analyte 5	M	4±1	3±1.2	171±23	920±139	1272±251
	F	7±5	7±5	154±34	1946±249	2436±761
Analyte 8	M	102±88	17±11.5	156±12	6797±1705	21052±14126
	F	153±140	4±3.5	254±119	16804±4145	37978±6556

		Toxicokinetic parameter (plasma)				
Analyte 10	M	19±10	1±0.9	63537±29748	236732±110604	257889±115750
	F	12±14	0±0.1	63917±16323	151009±36730	163711±54632
30 mg/kg bw						
Analyte 3	M	4±1	2±0.0	81±28	396±264	532±257
	F	ND	ND	ND	ND	ND
Analyte 5	M	93±13	5±2.3	316±13	11142±3205	12343±77
	F	94±25	4±3.5	403±121	9295±5113	17290±4011
Analyte 8	M	50±10	33±34.9	424±114	24617±7401	36250±3491
	F	47±9	23±22	319±110	21625±10696	27699±10912
Analyte 10	M	32±24	1±0.8	136433±81598	846881±327030	1033489±268232
	F	12±9	2±0.9	64183±8472	372289±119156	384472±125175

Data presented as mean±standard deviation.
ND, non-detect

CONCLUSION
The results should be interpreted with caution due to the low group numbers and high variability in the data.

TEST FACILITY DuPont (2010a)

B.14. Genotoxicity – bacteria (1)

TEST SUBSTANCE Notified chemical (up to 20% concentration)

METHOD OECD TG 471 Bacterial Reverse Mutation Test – Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
Vehicle	Water
Remarks - Method	No significant protocol deviations.

RESULTS

Metabolic Activation	*Test substance concentration (µg/plate) resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 5000	> 5000	Negative
Test 2	> 5000	> 5000	Negative
Present			
Test 1	≥ 5000	> 5000	Negative
Test 2	> 5000	> 5000	Negative

CONCLUSION The notified chemical (up to 20% concentration) was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY BioReliance (2009a)

B.15. Genotoxicity – bacteria (2)

TEST SUBSTANCE Notified chemical (up to 20% in water)

METHOD Standards for Mutagenicity Tests Using Microorganism (Ministry of Labour, Japan; Similar to OECD TG 471 Bacterial Reverse Mutation Test)

– Preincubation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA

Metabolic Activation System	S9 (manufactured from phenobarbital and 5,6-benzoflavone induced rat liver by Oriental Yeast, Co., Ltd., Japan)
Concentration Range in Main Test	a) With metabolic activation: 156-5000 µg/plate b) Without metabolic activation: 156-5000 µg/plate
Vehicle	Water
Remarks - Method	Purity of the test substance was adjusted to 100% when preparing the samples for the study. No significant protocol deviations.

RESULTS

Metabolic Activation	*Test substance concentration (µg/plate) resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 5000	> 5000	Negative
Test 2	≥ 5000	> 5000	Negative
Present			
Test 1	≥ 5000	> 5000	Negative
Test 2	≥ 5000	> 2500	Negative

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BML (2010)

B.16. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (up to 20% concentration)
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test
Cell Type/Cell Line	Human peripheral blood lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Water
Remarks - Method	A preliminary study was conducted at concentrations up to 5000 µg/plate, treated for 4 hours in the presence and absence of metabolic activation, and continuously for 20 hours in the absence of activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 1250*, 2500*, 5000*, 0.6 MMC*	4 hours	20 hours
Test 2	0*, 1250*, 2500*, 5000*, 0.3 MMC*	20 hours	20 hours
Present			
Test 1	0*, 250*, 500*, 1250*, 10 CPA*	4 hours	20 hours

*Cultures selected for metaphase analysis.
MMC, mitomycin-C. CPA, cyclophosphamide.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	>5000	>5000	Negative
Test 2	>5000	>5000	>5000	Negative
Present				
Test 1	≥1500	≥1250	>1250	Negative

CONCLUSION	The notified chemical (up to 20% concentration) was not clastogenic to human peripheral blood lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	BioReliance (2009b)

B.17. Developmental toxicity – analogue

TEST SUBSTANCE	Analogue chemical 2 (up to 20% concentration)
METHOD	US EPA OPPTS 870.3700 Prenatal Developmental Toxicity Study
Species/Strain	Rat/Crl:CD(SD)IGS
Route of Administration	Oral – gavage
Exposure Information	Exposure days: gestation days 6 to 20
Vehicle	Water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	22 F	0	0/22
low dose	22 F	625	0/22
mid dose	22 F	1250	0/22
high dose	22 F	2500	0/22

Effects on dams

There was a statistically significant weight loss in maternal animals treated at 2500 mg/kg bw/day over gestation days 6-8, with statistically significant decreases in absolute body weights in this group from gestation days 8 to 14. Absolute body weights at gestation day 21 were similar in control and treated groups but maternal body weights were statistically significantly decreased after the foetuses were removed, which indicates that the maternal body weights were affected by treatment (although it was not specified whether the maternal body weights were adjusted for litter size or weight). There were statistically significant decreases in body weight gains from gestation days 6 to 21 in animals treated at 1250 and 2500 mg/kg bw/day (↓22% and ↓26%, respectively) indicating maternal toxicity at these treatment levels. There were statistically significant decreases in body weight gain over various measured two day intervals in animals treated at 625 mg/kg bw/day, but as there was no statistically significant decrease in overall body weight gain, body weights were not affected in animals treated at 625 mg/kg bw/day.

There were statistically significant decreases in food consumption at various two day intervals up to day 12 in animals treated at 2500 mg/kg bw/day but overall food consumption was only slight decreased (↓8%) in this group. There were statistically significant decreases in food consumption over gestation days 10 to 12 in animals treated at 625 and 1250 mg/kg bw/day.

There were no treatment related clinical observations or post mortem findings in maternal animals.

Effects on the foetus

There were no foetal mortalities or increased resorptions. There was a slight non-statistically significant decrease in foetal body weights (↓4%) in the 2500 mg/kg bw/day group.

There were no treatment related foetal malformations. Caudal angensis was observed in one foetus from the 2500 mg/kg bw/day but this was not considered to be treatment related based on the low incidence.

There was an increased incidence of foetuses and litters with supernumerary ribs in the 2500 mg/kg bw/day group, with an increased number of foetuses with this variation in the 1250 mg/kg bw/day group (see following table). Historical control data for the laboratory for six developmental toxicity studies showed that this malformation was a common finding in control groups (5-11 litters and 10-22 foetuses affected per study). The finding in the current study was within the range of provided historical control data and is therefore unlikely to be related to treatment.

	Dose (mg/kg bw/day)			
	0	*625*	*1250*	*2500*
Supernumerary rib				
litters	3/22 (14%)	6/21 (29%)	5/22 (23%)	11/22* (50%)
foetuses	3/288 (1%)	9/271 (3%)	16/303 (5%)	15/309 (5%)

*Statistically significant difference to control group ($p < 0.05$)

CONCLUSION

The foetal NOAEL was established at 2500 mg/kg bw/day (equivalent to 500 mg/kg bw/day for analogue chemical 2), based on the absence of adverse effects at this dose. The maternal NOAEL was established as 625 mg/kg bw/day in this study (equivalent to 125 mg/kg bw/day for analogue chemical 2), based on decreased body weight gain. However, this effect had no noticeable adverse effects on the foetuses.

TEST FACILITY DuPont (2002c)

B.18. Toxicity to reproduction – one generation study, analogue

TEST SUBSTANCE Analogue chemical 2 (up to 20% concentration)

METHOD Similar to OECD TG 415 One Generation Reproduction Toxicity Study

Species/Strain Rat/Crl:CD(SD)IGS

Route of Administration Oral – gavage

Vehicle Water

Remarks – Method The P generation (20/sex/dose) were treated for 70 days prior to cohabitation and for up to 2 weeks for mating. Rats that showed no evidence of copulation continued to be dosed until the end of the cohabitation period. Females showing evidence of copulation were dosed throughout gestation and lactation, and were sacrificed at weaning. P generation males were sacrificed at the birth of the litters. At postpartum day 21, F1 weanlings (20/sex/dose) were randomly assigned to become F1 adults and sacrificed on postpartum day 60. Weanlings not assigned to become F1 adults were sacrificed. The F1 generation were not treated.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	20M + 20F	0	1/40
low dose	20M + 20F	75	0/40
mid dose	20M + 20F	500	1/40
high dose	20M + 20F	3500	2/40

Mortality and Time to Death

Mortalities in two animals treated with 3500 mg/kg bw/day and one treated with 500 mg/kg bw/day were likely due to trauma during gavage administration. A control group animal was killed *in extremis* and observations prior to sacrifice included body weight loss and a sore on the shoulders/neck.

Effects on Parental (P) animals:

Noisy respiration, salivation, stained nose and stained perineum were observed sporadically and at low incidence, mostly after dosing, in males treated at 3500 mg/kg bw/day. Sporadic salivation was observed in females treated at 3500 mg/kg bw/day during premating but not during gestation or lactation.

There were statistically significant decreases in absolute body weights in males treated at 3500 mg/kg bw/day at most weekly observations and a statistically significant decrease in premating (↓31%) and overall body weight gain (↓36%). There were statistically significant decreases in body weight in some weekly intervals in males treated at 500 mg/kg bw/day but the premating (↓9%) and overall (↓12%) body weight gain decreases were slight and non-statistically significant. In males, there is a clear effect on body weight in the 3500 mg/kg bw/day group, with only slight changes in the 500 mg/kg bw/day group. There were associated statistically significant decreases in mean food consumption (↓7%) and food efficiency (↓27%) in males treated at 3500 mg/kg bw/day.

There were statistically significant decreases in body weight gain at two weekly observations during premating in females treated at 3500 mg/kg bw/day but the overall body weight gains were similar in treated and control groups. There were statistically significant decreases in body weight gains over the first two weeks of gestation in females treated at 3500 mg/kg bw/day but the overall body weight gain over gestation was similar to controls. During lactation, there was a statistically significant increase in body weight gain in females treated at 3500 mg/kg bw/day, but this was likely due to slightly lower absolute body weights in this treatment group at the start of lactation. Feed consumption or food efficiency during premating was similar in treated and control females. There were statistically significant decreases in food consumption over the first two weeks of gestation in females treated at 3500 mg/kg bw/day but there were no decreases in food efficiency. Overall, there was little to no effect on body weights in females treated at 3500 mg/kg bw/day.

There were no treatment related changes on sperm motility, morphology, epididymal sperm or testicular spermatid numbers at any treatment level. There was a slight but statistically significant decrease in the percent of normal sperm in males treated at 500 and 3500 mg/kg bw/day. However, based on the small magnitude (↓1%) and given that the decreases were within the historical control range, this effect is not considered to be of toxicological concern. There were weight changes in some male reproductive organs in the 3500 mg/kg bw/day group. There was a statistically significant increase in relative testis weights (↑25%) but there was no change in absolute testis weights. There were statistically significant increases in relative epididymis (↑16%) and seminal vesicles (↑14%), but there were statistically significant decreases in absolute epididymis (↓10%) and seminal vesicles (↓11%). Absolute prostate weights were statistically decreased (↓18%) with no change in relative prostate weights.

There were no treatment related changes on the percent of days in estrus, diestrus or proestrus mean cycle length, or precoital interval at any treatment level.

There were no treatment related changes on mating and fertility indices, gestation length or the number of implantation sites. There was a statistically significant decrease in implantation efficiency (↓4%) but this change was not considered to be treatment related as it was within the historical control range.

Haematology and clinical chemistry parameters were measured on day 74. There were treatment related changes in red cell morphology (anisocytosis, microcytosis and hypochromasia) in males treated at 3500 mg/kg bw/day, with mildly increased red cell distribution width in males treated at 3500 mg/kg bw/day. There were statistically significant decreases in red blood cell concentration in all treated males, with statistically significant decreases in haemoglobin in males treated at 500 and 3500 mg/kg bw/day and haematocrit in males treated at 3500 mg/kg bw/day.

Measured at day 116, Plasma fluoride concentration was increased in males treated at 500 and 3500 mg/kg bw/day and urine fluoride was increased in all treatment levels in males. Fluoride levels were not measured in females.

There were statistically significant increases in relative liver and kidney weights in all treated males. Hepatocellular hypertrophy in the liver were observed in males treated at 500 and 3500 mg/kg bw/day but was considered to be an adaptive effect. Minimal chronic progressive nephropathy was observed in the kidney in males treated at 500 and 3500 mg/kg bw/day and was considered to be an adverse effect. In females, there were statistically significant increases in relative liver weights at 3500 mg/kg bw/day and relative kidney weights at 500 and 3500 mg/kg bw/day, but there were no associated histopathological findings and the organ weight increases were therefore not considered to be adverse. Other relative organ weight increases occurred mostly in animals treated at 3500 mg/kg bw/day and were likely a result of the decreased body weights at this treatment level.

Follicular hypertrophy was observed in the thyroid of males at all treatment level and in females treated at 500 and 3500 mg/kg bw/day. However, without corresponding thyroid hormone levels, the relevance of the hypertrophy is unknown.

Effects on 1st Filial Generation (F1)

There were no clinical observations in pups or in the F1 adults. Following weaning, one female from the 3500 mg/kg bw/day group was found dead and was weak and hunched over prior to death. This death was not considered to be treatment related due to the low incidence.

There were no treatment related changes in the number of pups born, born alive, or alive on days 4, 7, 14 or 21. Sex ratio and survival indices were similar in treated and control groups during lactation.

There were statistically significant decreases in absolute pup weights in the 3500 mg/kg bw/day group at each observation point during lactation. Following weaning, there were statistically significant decreases in absolute body weights at each weekly observation and a statistically significant decrease in body weight gain (↓11%) in 3500 mg/kg bw/day males, but females at this dose gained weight similar to controls. Males in the 3500 mg/kg bw/day group had an associated statistically significant decrease in food consumption but there was no decrease in food efficiency.

There were no statistically significant changes in preputial separation in males or vaginal patency in females.

There were statistically significant increases in relative liver weights in F1 males in the 3500 mg/kg bw/day group but there were no associated histopathological findings. There were statistically significant decreases in absolute testes (↓9%), epididymis (↓14%) and prostate (↓19%) weights in males treated at 3500 mg/kg bw/day but there were no changes in the relative weights of these organs.

Remarks – Results

There was no evidence of reproductive toxicity in this study. There was little evidence of maternal toxicity in the females treated at 3500 mg/kg bw/day but there was clear evidence of an effect on body weights in the F1 generation at this treatment level. Noting that the F1 generation were not treated directly with the test substance, this may indicate that the test substance is being administered to the offspring via lactation and that this caused a decrease in body weight gains in pups, with some recovery observed after weaning. Reproductive effects from exposure during this life stage are unknown.

CONCLUSION

The NOAEL for adult systemic toxicity was established as 75 mg/kg bw/day (equivalent to 15 mg analogue chemical 2/kg bw/day), based on increased kidney weights and chronic progressive nephropathy in males. The NOAEL for maternal toxicity was 3500 mg/kg bw/day (equivalent to 700 mg analogue chemical 2/kg bw/day), based on the lack of adverse effects in maternal animals. The NOAEL for reproductive toxicity was established as 3500 mg/kg bw/day (equivalent to 700 mg analogue chemical 2/kg bw/day), based on the absence of treatment related effects on reproductive parameters. The NOAEL for the F1 generation was established as 500 mg/kg bw/day (equivalent to 100 mg analogue chemical 2/kg bw/day), based on decreased body weight gains in males.

TEST FACILITY DuPont (2003c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test. EC Council Regulation L142 (2008) Part C.4-E (Closed Bottle Test). US EPA OPPTS 835.3110 (o) Ready biodegradability (1998).
Inoculum	Effluent from domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Dissolved oxygen concentration
Remarks - Method	The test was conducted in accordance with the guideline above. Tests were performed in duplicate in the dark at 20ºC ± 2ºC with an inoculum control, reference substance control (sodium benzoate) and toxicity test. Results are expressed as mg O_2 consumed per milligram of test substance divided by the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD). The $ThOD_{NH3}$ of solids in the test substance was calculated to be 1.67 O_2/mg.

RESULTS

		% Degradation	
Day	*Test substance*	*Sodium benzoate*	*Toxicity test*
5	0.89		
7	4.17		
11	6.06		
14	7.07	68.1	38.6
18	8.46		
21	9.35		
25	10.4		
28	11.5		

Remarks - Results

Oxygen consumption was 1.04 mg O_2/L. Degradation of the reference compound, sodium benzoate, exceeded the pass level of 60% degradation after 14 days. The validity criteria were satisfied. In the toxicity test, there was more than 25% degradation after 14 days, hence the test substance is not inhibitory to the inoculum at the tested concentration.

The test substance underwent 11.5% biodegradation after 28 days under the conditions of the test. The test substance did not reach the pass level for ready biodegradability of 60% of ThOD within the 10-day window. However, some primary degradation is expected based on the percentage degraded after 28 days.

CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Key Lab of Pesticide for Environmental Assessment and Pollution Control, MEP (2011)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II) (1981).

Inoculum	Activated sludge, surface soil and surface water sampled from 10 sites in Nanjing city (100 mg/L)
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Biological oxygen demand (BOD) and analysis of residual chemicals in BOD bottles.
Remarks - Method	The test was conducted in accordance with the guideline above. There were no significant deviations from the protocol. Tests were performed in the dark at 25ºC ± 1ºC with a blank control, sterile control and reference substance control (aniline). Results are based on the mean chemical oxygen demand (COD) of 0.0741 mg/mg. The theoretical oxygen demand ($ThOD_{NH3}$) of the test substance was calculated to be 0.0746 O_2/mg. There was no significant between the COD and the ThOD of the test substance.

RESULTS

Day	*% Degradation by BOD*		*% Degradation by Analysis*
	Test substance (30 mg/L)	*Aniline (100 mg/L)*	*Test substance (30 mg/L)*
1	0.56		
2	3.65		
3	6.18		
4	7.87		
5	9.55		
6	12.1		
7	12.9	79.3	
10	14.9		
14	16.3	87.7	
28	16.3		15.7

Remarks - Results	Degradation of the reference compound, aniline, exceeded the 40% degradation after 7 days and 65% degradation after 14 days. Recovery rate of the test substance in the abiotic control was greater than 10%. The validity criteria were satisfied. Based on the residue analysis of the test substance the test substance underwent 15.7% biodegradation after 28 days. Degradation products were not identified. The abiotic control showed less than 3% decrease in the test substance concentration over the duration of the test. The BOD results showed that the test substance underwent 16.3% biodegradation after 28 days under the conditions of the test. The test substance did not reach the pass level for inherent biodegradability of 20% of BOD during the 28-day period of the test. However, some primary degradation is expected based on the percentage degraded after 28 days.
CONCLUSION	The notified chemical is not inherently biodegradable.
TEST FACILITY	Key Lab of Pesticide for Environmental Assessment and Pollution Control, MEP (2010c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

a. Fathead minnow

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	In house. Similar to OECD TG 203 Fish, Acute Toxicity Test - Static.
Species	Fathead minnow (*Pimephales promelas*)

Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	100 to 140 mg $CaCO_3$/L
Analytical Monitoring	None
Remarks – Method	A definitive test was conducted by an in-house method that was similar to OCED TG 203 and was not a GLP study. The 96-hour test was conducted under static conditions using natural well water as the dilution medium. A blank control (with one replicate) was run in conjunction with 4 test concentrations (with one replicate for each test concentration and 5 animals per replicate) in a geometric series with a factor of 10. Based on visual observations, the dilution water control and 0.12 and 1.2 mg/L test concentrations were clear and colourless with no visible precipitate at test start. The 12 mg/L test concentration was clear and colourless with surface film at test start, and the 120 mg/L test concentration was clear and colourless with surface film and undissolved test material present at test start. Test conditions were: 21.6 °C to 22 °C; pH 7.8 to 8.4; 8.2 mg O_2/L to 9.3 mg O_2/L; 16 hour light and 8 hour dark photoperiod.
Statistical Analysis	None required as there was no observed mortality under the conditions of the test.

RESULTS

Concentration (mg/L)	*Number of Fish*	*Mortality*			
Nominal		*24 h*	*48 h*	*72 h*	*96 h*
Control	5	0	0	0	0
0.12	5	0	0	0	0
1.2	5	0	0	0	0
12	5	0	0	0	0
120	5	0	0	0	0

LC50	> 120 mg/L at 96 hours.
NOEC	120 mg/L at 96 hours (mortality).
Remarks – Results	The validity criteria of OECD TG 203 for dissolved oxygen and mortality in the control were met. However, there is no evidence that the test concentrations were maintained ≥ 80% of the nominal throughout the test. There were a number of deviations from the guideline, including: no analytical monitoring of the test substance; the definitive test geometric series separation factor exceeded 2.2; and, only 5 fish were tested at each test concentration. Further, the test substance was not fully soluble within the test medium at test concentrations of 12 mg/L and above. Therefore, the results are not considered reliable. No mortality or sub-lethal effects were seen in the test control group. The highest test concentration resulting in no-observed effects (NOEC) was 120 mg/L. The 96-hour median lethal concentration (LC50) was determined to be > 120 mg/L. Effects other than mortality were not reported for the test groups. The above results based on the nominal concentrations are not corrected for the purity of the notified chemical in the test substance (< 20% solids). The corrected LC50 and NOEC, based on total solids in the test substance, are > 24 mg/L and 24 mg/L, respectively.

CONCLUSION	The test study results indicate that the notified chemical is, at worst, harmful to fathead minnow. However, the results are not reliable as observations indicated that the test substance was not fully solubilised in the test medium and there was no measurement of the test substance concentrations over the duration of the test.
TEST FACILITY	DuPont (2009h)

b. Rare gudgeon

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static (1992). US EPA OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine (1996).
Species	Rare gudgeon (*Gobiocypris rarus*)
Exposure Period	96 hour
Auxiliary Solvent	None
Water Hardness	140 to 142 mg $CaCO_3$/L
Analytical Monitoring	UPLC with MS/MS detection
Remarks – Method	After a range finding test, a limit test was conducted in accordance with the guideline above and in compliance with GLP standards and principles. There were no significant deviations to the protocol. The 96-hour test was conducted under semi-static conditions, with a renewal period of 48 hours, using dechlorinated tap water as the dilution medium. A blank control was run in conjunction with the limit test (in triplicate with 7 fish per replicate) at nominal concentrations of 100 mg/L and 150 mg/L total solids of the test substance. Test conditions were: 22.8 °C to 23.2 °C; pH 7.19 to 7.57; 91.5% to 98.2% O_2 saturation; 16 hour light photoperiod.
Statistical Analysis	None required as there was no observed mortality under the conditions of the test.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*			
Nominal	*Measured*		*24 h*	*48 h*	*72 h*	*96 h*
Control	Non-detected	21	0	0	0	0
100	110	21	0	0	0	0
150	165	21	0	0	0	0

LC50	> 150 mg/L at 96 hours.
NOEC	150 mg/L at 96 hours.
Remarks – Results	The validity criteria were met. Constant conditions were maintained for the duration of the test and measured concentrations of the test substance were within ±20% of the nominal concentration of solids in the test solution. Therefore, the results are reported based on the nominal concentration of solids in the test solution. No mortality was observed in the test groups or control group. Therefore, the 96-hour median lethal concentration (LC50) was > 150 mg/L. Normal behaviours were observed for fish in the test groups and control group for the duration of the study. Therefore, the 96-hour no-observed effect concentration (NOEC) was 150 mg/L.
CONCLUSION	The notified chemical is not harmful to rare gudgeon.

TEST FACILITY	Key Lab of Pesticide for Environmental Assessment and Pollution Control, MEP (2010d)

c. Rainbow trout

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing approximately 20% solids)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Flow-through (1992). US EPA OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine (1996).
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	154 to 156 mg $CaCO_3$/L
Analytical Monitoring	LC with MS/MS detection
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guideline above and in compliance with GLP standards and principles. There were no significant deviations to the protocol. The 96-hour test was conducted under flow-through conditions (5.9 volume additions over a 24-hour period) using filtered natural well water, adjusted to 130-160 mg $CaCO_3$/L, as the dilution medium. A dilution water control was run in conjunction with 5 test concentrations (with one replicate for each test concentration and 7 animals per replicate) in a geometric series with a factor of 1.65.The control solution was clear and colourless with no visible precipitate throughout the test. All test substance solutions had particulate matter present throughout the test. Test conditions were: 10.7 °C to 12.9 °C; pH 8.1 to 9.0; 84% to 98% O_2 saturation; 16 hour light photoperiod with a 30 minute transition.
Statistical Analysis	Insufficient mortality was observed to establish a concentration –response relationship.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Measured*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	<LOD[a]	7	0	0	0	0	1
16	6.39	7	0	0	0	0	0
26	7.88	7	0	0	0	0	0
43	13.6	7	0	0	0	0	0
72	22.3	7	0	0	0	0	0
120	36.4	7	0	0	0	0	1

[a] Limit of detection (LOD) = 0.000365 mg/L

LC50	> 36.4 mg/L at 96 hours.
NOEC	22.3 mg/L at 96 hours (mortality).
Remarks – Results	The validation criteria were met. Measured concentrations of the centrifuged test solution samples ranged 28% to 49% of the nominal test concentrations on day 0 and 29% to 35% of the nominal test concentrations on day 4. As the measured concentrations were not within 20% of the nominal concentration, the biological response results are based on the geometric mean of the measured concentrations on day 0 and day 4. No sub-lethal effects were observed in the control or test groups. The 96-hour no-observed effect concentration (NOEC) based on sub-lethal effects

was 36.4 mg/L, the highest tested concentration. The highest test concentration resulting in no mortality after 96 hours was 22.3 mg/L. The 96-hour NOEC is therefore 22.3 mg/L. The 96-hour median lethal concentration (LC50) was determined to be > 36.4 mg/L.

CONCLUSION The notified chemical is, at worst, harmful to rainbow trout.

TEST FACILITY DuPont (2011c)

C.2.2. Chronic toxicity to fish

a. Rainbow Trout

TEST SUBSTANCE Analogue chemical 1 (up to 40% concentration)

METHOD OECD TG 210 Fish, Early-life Stage Toxicity Test – Flow-through.

Species *Oncorhynchus mykiss* (Rainbow trout)

Exposure Period 90 days

Auxiliary Solvent None

Water Hardness 119 to 130 mg $CaCO_3$/L

Analytical Monitoring HPLC

Remarks – Method The method was conducted according to test guidelines using good laboratory practice (GLP) with no significant deviations. Average measured concentrations were 0.64, 1.2, 2.5, 5 and 10 mg/L.

Statistical Analysis Two controls were used for each endpoint, water and isopropyl alcohol (IPA). The controls were compared using either the Mann-Whitney test or Fisher's Exact test. If there was no significant difference, the controls were combined for all further analyses. If significant differences were observed, only IPA was used for further analyses.

Trend tests (Cochran-Armitage or Jonckheere trend tests) were used to determine the NOEC for each response. Hatching, mortality and larval abnormality data were evaluated by the Cochran-Armitage test in a step-down manner with equally spaced concentration scores. Continuous or reproduction data were evaluated for normality using the Shapiro-Wilk test and homogeneity of variance was assessed by Levene's test. Normal and homogenous data were evaluated in the context of ANOVA (ANalysis Of VAriance).

RESULTS

Summary of hatching, survival, abnormalities and swim-up from hatching to thinning

Nominal Concentration (mg/L)	*First Hatching day*	*Last Hatching day*	*Eggs Hatched*	*Survival*	*Abnormalities*	*First day of Swim-up*
Water Control	26	28	93%	99%	0%	40
IPA[a] Control	26	27	86%	100%	0%	40
0.63	26	28	83%	100%	0%	40
1.3	25	28	88%	96%	0%	40
2.5	26	28	90%	100%	0%	40
5	25	28	69%	98%	0%	40
10	25	27	74%	97%	0%	40

[a] Isopropyl Alcohol

Summary of hatching, survival, abnormalities and swim-up from thinning to test end

Nominal Concentration (mg/L)	*Survival*	*Abnormalities*	*Mean standard length (cm; ± std. dev.)*	*Mean wet weight (g; ± std. dev.)*
Water Control	100%	0%	4.54 ± 0.34	1.3192 ± 0.3244

IPA[a] Control	100%	0%	4.42 ± 0.40	1.3130 ± 0.4117
0.63	100%	3%	4.34 ± 0.52	1.2147 ± 0.5190
1.3	100%	3%	4.26 ± 0.50	1.1766 ± 0.4388
2.5	100%	0%	4.26 ± 0.48	1.1830 ± 0.4769
5	100%	3%	4.36 ± 0.50	1.2419 ± 0.4235
10	100%	10%	4.17 ± 0.43	1.1358 ± 0.4320

[a] Isopropyl Alcohol

NOEC	2.5 mg/L at 90 days (dead eggs)
NOEC	10 mg/L at 90 days (larval survival)
NOEC	10 mg/L at 90 days (swim up)
NOEC	10 mg/L at 90 days (length)
Remarks – Results	All relevant test validity criteria were met.
CONCLUSION	The test substance, and by inference the notified chemical, is not harmful to fish.
TEST FACILITY	DuPont (2003d)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	In house. Similar to OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	100 to 140 mg $CaCO_3$/L
Analytical Monitoring	Test substance concentrations in the test solutions were not verified by analytical monitoring.
Remarks - Method	A definitive test was conducted by an in-house method that was similar to OCED TG 202 and was not a GLP study. The 48-hour test was conducted under static conditions using natural well water as the dilution medium. A blank control (with one replicate) was run in conjunction with 4 test concentrations (with one replicate for each test concentration of 10 animals per replicate) in a geometric series with a factor of 10. The control and test solutions were clear and colourless with no visible precipitate at the start of the test. Test conditions were: 19.5 °C to 20.1 °C; pH 7.9 to 8.2; 8.2 mg O_2/L to 8.5 mg O_2/L; 16 hour light and 8 hour dark photoperiod.
Statistical Analysis	Calculated based on nominal concentrations. The employed methodology was not detailed in the study report but the following references were cited: Armitage (1950); Armitage (1955); Cochran (1954); and, Selwyn (1988).

RESULTS

Concentration mg/L Nominal	*Number of Daphnia magna*	*Number Immobilised*	
		24 h	*48 h*
Control	10	0	0
0.12	10	0	0
1.2	10	1	1
12	10	1	3
120	10	10	10

LC50 (95% confidence interval)	16.2 mg/L (10.3 mg/L to 91.1 mg/L) at 48 hours
NOEC	0.12 mg/L at 48 hours
Remarks - Results	The validity criteria of OECD TG 202 were met. However, there were a number of deviations from the guideline, including: no analytical monitoring of the test substance; the definitive test geometric series separation factor exceeded 2.2 resulting in a wide confidence interval for the EC50 endpoint; and, only 10 animals were tested at each test concentration. Therefore, on the basis that the test substance concentrations were not verified and that the separation factor resulted in a wide confidence interval, the results are reliable with restrictions. No immobility or sub-lethal effects were seen in the test control group. The highest test concentration resulting in no immobilisation (NOEC) was 0.12 mg/L. The 48-hour median immobilisation concentration (EC50) was determined to be 16.2 mg/L with a 95% confidence interval of 10.3 mg/L to 91.1 mg/L. Effects other than immobilisation were not reported for the test groups. The above results based on the nominal concentrations are not corrected for the purity of the notified chemical in the test substance (< 20% solids). The corrected 48-hour EC50 and NOEC, based on total solids in the test substance, are 3.24 mg/L (95% confidence interval: 2.06 mg/L to 18.2 mg/L) and 0.024 mg/L, respectively.
CONCLUSION	The test study results indicate that the notified chemical is toxic to aquatic invertebrates. However, the results are reliable with restrictions as there was no measurement of the test substance concentrations for the duration of the test.
TEST FACILITY	DuPont (2009i)

C.2.4. Chronic toxicity to aquatic invertebrates

a. Test 1

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 211 *Daphnia magna* Reproduction Test (1998) – Semi-static. U.S. EPA OPPTS 850.1300 Daphnid Chronic Toxicity Test (1996).
Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	None
Water Hardness	147-169 mg $CaCO_3$/L
Analytical Monitoring	HPLC with LC/MS/MS detection (limit of detection: 0.1 µg/L; limit of quantitation: 1.8 µg/L).
Remarks – Method	Daphnia magna (10 replicates of a single daphnid per group) were exposed to the test substance at five nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/L for a period of 21 days under semi-static conditions. The dilution water was filtered natural well water adjusted to a hardness of 100 mg $CaCO_3$/L to 140 mg $CaCO_3$/L. Test conditions were: 16h/8h light dark cycle, 19.1-20.8 ºC, pH 7.6-8.5, ≥ 7.4 mg O_2/L (> 81% of saturation). A dilution water control was run in parallel. Test solutions were renewed three-times weekly for the test duration. Samples were taken to verify test solution concentrations on days 0, 2, 9, 16 and 19 (fresh test solution) and days 5, 12, 19 and 21 (old test solutions). The daphnia were fed with algal suspension and supplements, and each day the vessels were assessed for adult survival (immobilisation), sub-lethal effects and production of young (number of living and immobilised neonates produced).

RESULTS

Day 21					
Measured Concentration (mg/L)	*Percent Adult Survival (%)*	*Total Number of Living Offspring per Surviving Parent*	*Total Number of Immobile Offspring per Surviving Parent*	*Mean Body Length (mm)*	*Mean Dry Weight (mg)*
< 0.0001 (control)	90	165.6 (27.8)[a]	0.0 (0.0)	4.65 (0.35)	0.55 (0.12)
0.0467	90	143.1 (30.6)	8.2 (16.4)	4.62 (0.28)	0.72 (0.17)
0.0930	100	142.1 (19.0)	6.5 (8.9)	4.41 (0.35)	0.66 (0.14)
0.189	90	166.3 (29.1)	11.0 (17.2)	4.65 (0.39)	0.61 (0.12)
0.409	90	164.8 (59.6)	1.8 (2.5)	4.42 (0.42)	0.59 (0.14)
0.843	90	166.6 (24.6)	4.8 (4.6)	4.65 (0.27)	0.63 (0.17)

[a] Numbers in brackets indicate the standard deviation.

Determined Endpoints (mg/L)	*Percent Adult Survival (%)*	*Total Number of Living Offspring per Surviving Parent*	*Total Number of Immobile Offspring per Surviving Parent*	*Mean Body Length (mm)*	*Mean Dry Weight (mg)*
NOEC	0.843	0.843	0.409	0.843	0.843
MATC	> 0.843	> 0.843	0.587	> 0.843	> 0.843
LOEC	> 0.843	> 0.843	0.843	> 0.843	> 0.843
EC50	> 0.843	[a]	[a]	[a]	[a]
Statistical Analysis	Cochran-Armitage	Jonckheere-Terpstra	Dunn's	Jonckheere-Terpstra	Jonckheere-Terpstra

[a] Could not be adequately determined

Remarks - Results

Mean measured test substance concentrations ranged from 72% to 84% of the nominal test concentrations.

The validation criteria were met. However, OCED TG 211 states that the highest test concentration must be high enough so that fecundity at that concentration is significantly lower than the control if the purpose of the test is to obtain the lowest observed effect concentration or the no-observed effect concentration (LOEC/NOEC). The test results do not indicate a significant effect on reproduction (living offspring) over the tested range. Further, it is unclear if the test solution was solubilised in the test medium. No precipitate was present in the dilution water control but surface film was visible in all test concentration solutions. Therefore, on the basis that the test substance may not have been in solution and that significant effects were not obtained for fecundity (live offspring) at the tested concentrations, the results should be used with caution.

The OECD TG 211 does not require reporting of the NOEC for immobilised neonates but is required by the US EPA OPPTS. This is the only endpoint for which a statistically significant effect was determined under the conditions of the study. The mean number of immobile young per surviving female on day 21 did not appear to be related to the test substance concentration in a monotonic manner (for the control and 0.047, 0.093, 0.189, 0.409 and 0.843 mg/L test substance concentrations, the mean number of immobile neonates per surviving female on day 21 was 0, 8.2, 6.5, 11.0, 1.8, and 4.8 and the proportion of immobile neonates relative to total neonates on day 21 per surviving female was 0%, 5%, 4%, 6%, 1%, and 3%). The biological significance of the immobile neonates is questionable given that there was no difference between the control treatment and the highest test concentration in the mean number of total live young per surviving female (165.6 young per female for control versus 166.6 young per female for the highest test substance concentration). The number of immobile neonates per surviving female at the 0.409 mg/L test concentration was not significantly different relative to control using the most applicable statistical analysis and therefore this

	was reported as the overall study NOEC. It is unclear from the study report if there was a statistically significant effect for this endpoint at the lower test concentrations. Therefore, the 21-day NOEC for *Daphnia magna* is 0.409 mg/L. For length, dry weight, mean total live young per female on day 21, first day of reproduction, and adult survival on day 21, the NOEC exceeded the highest tested concentration of 0.843 mg/L of the test substance.
CONCLUSION	The test study results indicate that the notified chemical is toxic to aquatic invertebrates with long lasting effects. However, the results should be used with caution due to incomplete solubility of the test substance and lack of significant effects on number of live offspring.
TEST FACILITY	DuPont (2009j)

b. Test 2

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing approximately 20% solids)
METHOD	OECD TG 211 *Daphnia magna* Reproduction Test (2008) – Flow-through. U.S. EPA OPPTS 850.1300 Daphnid Chronic Toxicity Test (1996).
Species	*Daphnia magna*
Exposure Period	21 day
Auxiliary Solvent	None
Water Hardness	142-148 mg $CaCO_3$/L
Analytical Monitoring	HPLC with LC/MS/MS detection (limit of detection: 0.365 ng/L; limit of quantitation: 1.22 ng/L).
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guideline above and in compliance with GLP standards and principles. There were no significant deviations to the protocol. The 21-day test was conducted under flow-through conditions (5.7 volume additions over a 24-hour period) using filtered natural well water, adjusted to 130-160 mg $CaCO_3$/L, as the dilution medium. A dilution water control was run in conjunction with 6 test concentrations (with 4 replicates for each test concentration and 10 daphnia per replicate) in a geometric series with a factor of 2.0. The daphnia were fed three times per day with algal suspension and supplements. Test substance concentrations were verified on Days 0, 7, 15 and 21. Test conditions were: 19.7 °C to 21.2 °C; pH 8.3 to 8.5; 7.7 mg O_2/L to 8.4 mg O_2/L; 16 hour light photoperiod with 30 minute transition periods. The control and test substance solutions were clear and colourless with no visible precipitate, surface film, or undissolved test substance throughout the test.

RESULTS

Day 21					
Measured Concentration (mg/L)	*Adult Survival (%)*	*Mean Day of First Brood*	*Total Number of Living Offspring per Parent at Test Initiation*	*Mean Body Length (mm)*	*Mean Dry Weight (mg)*
< LOD[a] (control)	85	9	66	4.0	0.48
0.0467	88	9	63	3.9	0.55[e]
0.105	70	9	35[c]	3.8[c]	0.45[e]
0.232	63	11	36[c]	3.8[c]	0.53[e]
0.591	18[b]	14[d]	5[d]	3.7[d]	0.51[d]
0.820	58[b]	10[d]	25[d]	3.7[d]	0.53[d]
1.50	25[b]	12[d]	9[d]	3.7[d]	0.58[d]

[a] Limit of detection (LOD) = 0.365 ng/L; [b] Statistically significant reduction in survival as compared to the control (Fisher's Test with Hochbergs's family-wise adjustment for significance $p < 0.02$); [c] Statistically significant reduction compared to the control (Dunnett's Test; $p < 0.05$); [d] Treatment excluded from statistical analysis due to significant survival reduction compared to the control survival; [e] There was no statistically significant weight reduction when compared to the control (Dunnett's test; $p \geq 0.05$).

Determined Endpoints (mg/L)	*Adult Survival*	*Mean Day of First Brood*	*Total Number of Living Offspring per Parent at Test Initiation*	*Mean Body Length (mm)*	*Mean Dry Weight (mg)*
NOEC at 21 days	0.232	0.105	0.0467	0.0467	0.232
LOEC at 21 days	0.591	0.232	0.105	0.105	> 0.232
MATC at 21 days	0.370	0.156	0.0700	0.0700	
EC50 at 7 days			0.811 (0.608 – 1.08)[a]		
14 days			0.540 (0.389 – 0.750)[a]		
21 days			0.549 (0.390 – 0.772)[a]		

[a] Values within brackets are the 95% confidence limits.

Remarks - Results	The validity criteria were met. Mean measured test substance concentrations ranged from 36% to 59% of the nominal test concentrations. The biological endpoints above are calculated based on the mean measured concentration of the test substance and are not corrected for the purity of the notified chemical in the test substance (20% solids). The 21-day no-observed effect concentration (NOEC) for reproduction is 0.0467 mg/L.
CONCLUSION	The notified chemical is very toxic to aquatic invertebrates with long-lasting effects.
TEST FACILITY	DuPont (2012)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	In house. Similar to OECD TG 201 Alga, Growth Inhibition Test – Static.
Species	Green algae (*Pseudokirchneriella subcapitata*)
Exposure Period	72 hours
Concentration Range	Nominal: Control, 0.12, 1.2, 12 and 120 mg/L
Auxiliary Solvent	None
Water Hardness	Not specified
Analytical Monitoring	Test substance concentrations in the test solutions were not verified by analytical monitoring.
Remarks - Method	A definitive test was conducted by an in-house method that was similar to OCED TG 201 and was not a GLP study.

The test was conducted under static conditions with a synthetic algal-assay procedure (AAP) nutrient media. A blank control (in triplicate) was run in conjunction with 4 test concentrations (in duplicate) in a geometric series with a factor of 10. The control and test solutions were clear and colourless with no visible precipitate at the start of the test. The test substance appeared stable under the conditions of the study; no evidence of instability was observed.

Test conditions were: 23.7 °C ± 2 °C; pH 7.54 to 8.11; 24 hour light photoperiod; mean light intensity 7364 lux; initial cell density of 10 000 cells/mL; shaking speed 102 rpm.

Statistical Analysis

Conducted using SAS Version 8.02 and based on the nominal concentrations. The data for healthy cell count and growth rate were determined to be normally distributed using the Shapiro-Wilk test (Shapiro & Wilk, 1965) with equal variance (Levene's test; Box, 1953). Therefore, the Jonckheere-Terpstra trend test (Lehmann, 1975) was used to determine the NOEC and LOEC values. The E_rC50 and E_bC50 were determined by the Bruce-Versteeg regression model (Draper & Smith, 1981). All statistical tests were calculated at a significance level of p = 0.05.

RESULTS

Biomass		*Growth*	
E_bc50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 120	120	> 120	120

Remarks - Results

The results met the OECD TG 201 validity criteria for exponential growth (specific growth rate > 0.92 per day) and variability of the average specific growth rate (%CV < 7%) of the controls, but insufficient data was reported to demonstrate that section-by-section (day 0-1, day 1-2 and day 2-3) specific growth rates were less than 35%. There were a number of other deviations from the OECD TG 201, including: no analytical monitoring of the test substance; the definitive test geometric series factor exceeded 3.2; and, each test concentration was performed in duplicate not triplicate. Therefore, on the basis that test substance concentrations were not verified during the test, the results are reliable with restrictions.

Negative inhibition compared to the control was observed at test concentrations of 12 mg/L and less. The no-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were 120 mg/L and > 120 mg/L, respectively. The median effect concentration based on growth rate and nominal concentrations (E_rC50) was > 120 mg/L.

The above results based on the nominal concentrations are not corrected for the purity of the notified chemical in the test substance (< 20% solids). The corrected 96-hour E_rC50 and NOEC, based on the total solids in the test substance, are > 24 mg/L and 24 mg/L, respectively.

CONCLUSION

The test study results indicate that the notified chemical is, at worst, harmful to algae. However, the results are reliable with restrictions as there was no confirmation of the test substance concentrations for the duration of the test.

TEST FACILITY

DuPont (2009k)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test (1984).
Inoculum	Activated sludge, municipal sewage
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320 and 1000 mg/L solids content of the test substance
Statistical Analysis	Based on the nominal concentrations.
Reference Substance	3,5-dichlorophenol at nominal concentrations of 3.2, 10 and 32 mg/L.
Remarks – Method	There were no significant deviations from the protocol.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The study reports states that under the conditions of the test there was no significant activated sludge respiration inhibition (less than 15% inhibition) at up to 1000 mg/L solids content of the test substance compared to the positive controls. Therefore, the median inhibition concentration (IC50) could not be calculated. It is noted that the study report does not specify the statistical method used to determine that the effects were not significant. However, the provided results do not exhibit a concentration-response relationship over the tested concentrations. Therefore, although endpoints are not provided in the study report, the three-hour IC50 is taken to be greater than 1000 mg/L and the no-observed effect concentration (NOEC) is taken to be 1000 mg/L.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration at up to 1000 mg/L.
TEST FACILITY	DuPont (2009l)

C.2.7. Acute toxicity to earthworms

TEST SUBSTANCE	Notified chemical (aqueous dispersion containing up to 20% solids)
METHOD	OECD TG 207 Earthworm, Acute Toxicity Tests (1984). ISO-Guideline 11268-1 Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: Determination of acute toxicity using artificial soil substrate (1993).
Species	Earthworms (*Eisenia foetida*); adult – 9 to 12 months, with clitellum
Exposure Period	14 days
Vehicle	None
Remarks – Method	Following a range finding test, earthworms were exposed to the test substance that was evenly incorporated into an artificial soil. The artificial soil was composed of 74.7% fine quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% calcium carbonate and had a moisture content of 22.2% to 24.3%. A medium control was run in conjunction with 5 test concentration (with 4 replicates per treatment group with 10 earthworms per replicate) in a geometric series with a separation factor of 2. Test conditions were: 18 °C to 22 °C; pH 5.8 to 6.2; continuous photoperiod at 400 lux to 800 lux. Mortality and behavioural effects after 7 and 14 days were recorded. Total and mean body weight of live earthworms were determined at test start (day 0) and day 14.
Statistical Analysis	Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$). The median lethal effect concentration (LC50)

and its 95% confidence interval could not be determined by a statistical analysis as no mortality higher than 50% was observed.

Weight change data were tested for normal distribution and homogeneity using Shapiro-Wilk's test and Levene's test (α = 0.05). Further evaluation for the determination of the no-observed effect concentration was performed using Dunnett's t-test (multiple comparison, α = 0.05, two sided).

RESULTS

Nominal Concentration (mg/kg dry soil)	*Mortality at day 7 (%*	*Mortality at day 14 (%)*	*Mean Weight at day 0 (mg/worm)*	*Mean Weight at day 14 (mg/worm)*	*Weight change by day 14 (%)*
Control	0	0	428.0	416.7	-2.4
62.5	0	0	431.3	410.4	-4.7
125	0	0	418.5	403.5	-3.5
250	0	0	425.0	384.2	-9.5[b]
500	0	0	436.1	358.5	-17.8[b]
1000	5[a]	5[a]	424.6	338.9	-20.1[b]

[a] Not statistically different compared to the control (Fisher's exact test, α = 0.05); [b] Significantly different compared to the control (Dunnett's t-test, α = 0.05).

LC50	> 1000 mg/kg dry soil at 14 days
NOEC	125 mg/kg dry soil at 14 days
Remarks – Results	There were no significant deviations to the test protocol. Results for a reference substance, chloroacetamide, were provided and were acceptable. The validity criteria were met. Behavioural effects were observed at 1000 mg/L dry soil where worms were tensed and stiff after 14 days. No adverse behavioural effects were observed in any other treatment group. The acute 14-day LC50 for earthworms based on mortality and nominal concentrations of the test substance was greater than 1000 mg/kg dry soil, the highest test concentration. The 14-day no-observed adverse effect concentration (NOEC) for earthworms based on body weight change and nominal concentrations of the test substance was 125 mg/kg dry soil. The 14-day LC50 and NOEC, when corrected for the solids content of the test substance of 20%, are > 200 mg/kg dry soil and 25 mg/kg dry soil, respectively.
CONCLUSION	The notified chemical is, at worst, slightly toxic to earthworms on an acute basis.
TEST FACILITY	DuPont (2010b)

APPENDIX D: TOXICOLOGY OF PERFLUOROHEXANOIC ACID (PFHXA)

The following conclusions can be drawn from the data on PFHxA to assess health effects:

1. Absorption of PFHxA in mice and rats was rapid, with C_{max} achieved within 1 hour. Systemic exposure (AUC) was higher in males than in females in both mice and rats, probably as a result of the more rapid clearance in females than in males. Low levels of PFHxA were found in various rat tissues; these decreased rapidly and could not be detected in most tissues by 24 hours. Excretion of unchanged PFHxA was rapid and was largely via the urine. Most of the PFHxA was excreted via the urine within 24 hours, indicating almost 100% bioavailability. There was no evidence of bioaccumulation following repeat exposure in rats. Similar kinetics were observed in monkeys, with rapid absorption, similar exposure for males and females, and rapid and comprehensive urinary excretion of unchanged PFHxA. The volume of distribution in rats and monkeys indicates distribution mainly to extracellular fluid. The serum half-lives were 2.4/5.3 hours (male/female) in monkeys and 1/0.42 hours (male/female) in rats (Chengelis, 2009a; Gannon, 2011).
2. In a study comparing the toxicokinetics of PFHxA to PFOA following repeated oral exposure for 10 days, results indicate that the AUC was 9 times lower for PFHxA, which is attributed to the more rapid excretion of PFHxA. The half-life for PFHxA was 3 times lower than PFOA and persistence in the liver was much lower for PFHxA than PFOA (DuPont, 2003e).
3. The acute toxicity of PFHxA was low, with an LD_{50} value of >1750 mg/kg bw and <5000 mg/kg bw in female rats. Males are expected to be more sensitive to PFHxA based on higher exposure (AUC) and an expected lower LD_{50} for males (Loveless, 2009). No information was available to assess acute dermal toxicity or acute inhalation toxicity.
4. In repeat dose oral toxicity studies in rats (14 days, 90 days), there was evidence of effects on the liver and decreased haematological parameters at 500 mg/kg bw/day, with liver effects in males at 100 mg/kg bw/day. Nasal lesions (degeneration and atrophy of the olfactory epithelium) were observed at 100 mg/kg bw/day and above in the 90-day study and the NOAEL was 20 mg/kg bw/day in both sexes (DuPont, 2006a; DuPont, 2007a, Chengelis, 2009b).
5. In a 2-year chronic toxicity/carcinogenicity study in rats, there were treatment-related systemic effects (increased incidence of struggling, and papillary necrosis and tubular degeneration of the kidneys) at 100/200 mg/kg bw/day (male/female). The NOAEL for non-neoplastic effects was 15/30 mg/kg bw/day (male/female). There was no evidence of carcinogenicity in either male or female rats (AGC Chemicals, 2010).
6. NaPFHx showed no effect on fertility parameters in a one-generation reproduction study in rats. The NOAEL for maternal systemic toxicity in the P1 animals was 100 mg/kg bw/day based on excessive body weight gain during lactation. There were no biologically significant adverse effects on pups (DuPont, 2007a).
7. In a developmental toxicity study with NaPFHx in rats, there was evidence of maternal (reduced body weight and body weight gain) and foetal toxicity (reduced neonatal bodyweight) at 500 mg/kg bw/day (DuPont, 2007b). In a second developmental toxicity study in mice with ammonium PFHx, foetal toxicity (increased incidence of still births, perinatal death, and microphthalmia and corneal opacity) was noted at 175 mg/kg bw/day in the absence of maternal toxicity. There was no toxicity in pups post-weaning. The NOAEL was 35 mg/kg bw/day (Daikin Industries, 2011).
8. No evidence of genotoxicity was observed in an *in vitro* mutagenicity assay in bacteria (DuPont, 2006b) or in a test for chromosome aberrations in human peripheral blood lymphocytes (DuPont 2006c).

The toxicology of PFOA has been characterised previously (Environment Canada, 2012; Chemical Safety Report, 2009). Comparative analysis of the toxicokinetics of PFHxA and PFOA indicated the following:

- Bioavailability of PFHxA and PFOA after oral administration was high.
- In repeat oral exposure studies, PFHxA showed no evidence of bioaccumulation, whereas PFOA showed some evidence of bioaccumulation.
- Excretion of PFHxA via the urine was rapid and virtually complete over 24 hours, whereas excretion of PFOA was slower, with only 20% excreted over 24 hours.

- Half-lives of excretion of PFHxA after oral exposure were 2–3 hours, whereas the excretion half-life of PFOA was 4.8 days.

Comparative analysis of the toxicity of PFHxA and PFOA indicated the following:

- The acute toxicities of PFHxA and PFOA were low.
- No data were available to compare eye and skin irritation or sensitisation.
- In 90-day repeat dose studies in rats, the LOAEL for PFHxA (100 mg/kg bw/day) occurred at higher doses than for PFOA (0.64 mg/kg bw/day).
- In chronic toxicity studies in rats, the LOAEL for PFHxA (100/200 mg/kg bw/day [m/f]) was higher than for PFOA (14.2/16.1 mg/kg bw/day [m/f]).
- Reproduction studies with PFHxA produced no effect on reproductive parameters with a NOAEL of 500 mg/kg bw/day, whereas PFOA produced increased mortality, decreased bodyweight and delayed sexual maturity in the F1 generation with a NOAEL of 10 mg/kg bw/day in females.
- The LOAEL was 175 mg/kg bw/day for developmental effects in a rat study with ammonium PFHx. The NOEL for developmental effects for PFOA was 150 mg/kg bw/day in a rat study.
- There was no evidence of genotoxicity for PFHxA or PFOA.

A carcinogenicity study in rats with PFHxA produced no evidence of a treatment-related increase in tumours, whereas a study in rats with PFOA produced an increased tumour incidence in males. The US EPA considers PFOA is “likely to be carcinogenic to humans” (US EPA, 2012).

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