File No: LTD/1804

March 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclohexanecarboxamide, N-[4-(cyanomethyl)phenyl]-5-methyl-2-(1-methylethyl)-

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1804	Procter & Gamble Australia Pty Ltd	Cyclohexanecarboxamide, *N*-[4-(cyanomethyl)phenyl]-5-methyl-2-(1-methylethyl)-	Yes	≤ 1 tonne per annum	Component of oral hygiene and personal care products

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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 2)	H411 - Toxic to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

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

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent 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 one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds 0.015% in toothpaste, 0.005% in mouthwash, 0.018 mg/inch of floss in dental floss or 7.5% in lubricating strips on razors.

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Procter & Gamble Australia Pty Ltd (91 008 396 245)
Level 4, 1 Innovation Road
MACQUARIE PARK NSW 2113

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Evercool 180

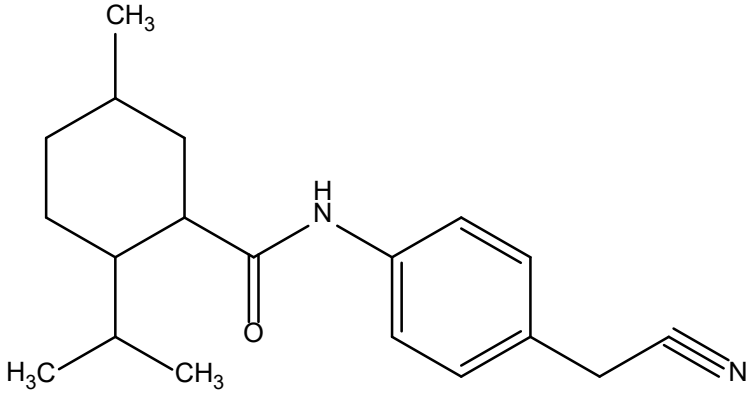
CAS NUMBER
852379-28-3

CHEMICAL NAME
Cyclohexanecarboxamide, *N*-[4-(cyanomethyl)phenyl]-5-methyl-2-(1-methylethyl)-

OTHER NAME(S)
N-p-Benzeneacetonitrile-menthanecarboxamide
G180
GR-72-0180

MOLECULAR FORMULA
$C_{19}H_{26}N_2O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
298.4 Da

ANALYTICAL DATA
Reference UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 99 %

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: White to pale yellow solid

Property	Value	Data Source/Justification
Melting Point	148 °C	(M)SDS
Boiling Point	> 403 °C at 101.3 kPa	Measured
Density	1,120 kg/m^3 at 25 °C	Measured
Vapour Pressure	4 x 10^{-12} kPa at 25 °C	Measured
Water Solubility	1 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ > 1 year at 25 °C (pH 4 – 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.6	Measured
Surface Tension	66.5 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.7	Measured
Dissociation Constant	Not determined	Contains dissociable functionality. Therefore, the notified chemical is expected to be ionised under normal environmental conditions of pH 4 – 9.
Particle Size	Inhalable fraction (< 100 μm): < 92% Respirable fraction (< 10 μm):	Measured

	0%	
Flash Point	> 100 °C	(M)SDS
Flammability	Not flammable	Not expected to be highly flammable based on flash point.
Autoignition Temperature	> 400 °C	Measured.
Explosive Properties	Not determined	Not expected to be explosive based on chemical structure
Oxidising Properties	Not determined	Not expected to be oxidising based on chemical structure

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

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

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 be imported into Australia as a component of finished oral hygiene (toothpaste, mouthwash and dental floss) and personal care (lubricating strips on razors) products.

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

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

PORT OF ENTRY
Sydney by sea and air

IDENTITY OF MANUFACTURER/RECIPIENTS
Givaudan Fragrances (Shanghai) Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component in finished oral hygiene (toothpaste, mouthwash and dental floss) and personal care products in tubes/containers suitable for retail sale. These products will be transported in the same form in which they are imported.

USE
The notified chemical will be used as an ingredient in toothpaste (at a concentration ≤ 0.015%), mouthwash (at a concentration ≤ 0.005%), dental floss (at ≤ 0.018 mg/inch of floss) and in the lubricating strip of razors (at ≤ 7.5% per strip).

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as a component of finished oral hygiene products including toothpaste (at a concentration ≤ 0.015%), mouthwash (at a concentration ≤ 0.005%), dental floss (at ≤ 0.018 mg/inch of floss), and in the lubricating strip of razors (at ≤ 7.5% per strip) which will be sold to the public in the same form in which they are imported.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

EXPOSURE DETAILS
Transport, storage and retail workers may come into contact with the notified chemical only in the event of accidental rupture of packages.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of oral hygiene and personal care products (at concentrations ≤ 0.015% for toothpaste, ≤ 0.005% in mouthwash, ≤ 0.018 mg/inch on dental floss and ≤ 7.5% per lubricating strip on razors). The principal routes of exposure will be oral and dermal, while accidental ocular and ingestion exposure is also possible.

Data on typical use patterns of oral hygiene products in which the notified chemical is proposed to be used are shown in the following tables for young children (2-4 year olds) and adults, respectively. The use of toothpaste is separately estimated for young children, as they represent a more susceptible receptor group. For the purposes of the exposure assessment, Australian use patterns for the product categories are assumed to be similar to those in Europe. In addition, 100% systemic exposure has been conservatively assumed based on buccal and/or gastrointestinal absorption. Using these data, the total systemic exposure for oral care products is estimated to be 0.0128 mg/kg bw/day notified chemical for young children and 0.0091 mg/kg bw/day for adults.

The contribution to dermal exposure from the proposed product categories is considered negligible due to the low concentrations of the notified chemical in these products and has therefore not been included in the exposure calculations.

Children's exposure (2-4 year old)

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Toothpaste[1]	1720	0.015	0.62[2]	**0.0128**

C = concentration (%); RF = retention factor; assumed brushing twice daily
Daily systemic exposure = (Amount × C(%) × RF x oral absorption)/body weight (12.5 kg)

[1]RIVM (2006)
[2]Based on 75th percentile of amount orally ingested

Adults' exposure

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Toothpaste[1]	2780	0.015	0.058[3]	0.0004
Mouthwash[1]	40,000	0.005	0.10	0.0033
Dental floss[2]	36 (inch/day)	0.018 (mg/inch)	0.5	0.0054
Total				**0.0091**

C = concentration (%); RF = retention factor; assumed brushing and flossing twice daily and using mouthwash 4x/day
Daily systemic exposure = (Amount × C (%) × RF x oral absorption)/body weight (60 kg)

[1]RIVM (2006)
[2]Notifier exposure estimate
[3]Based on 75th percentile of amount orally ingested

The use pattern for the personal care product in which the notified chemical is proposed to be used is shown below. For the purposes of the exposure assessment, the use pattern for the personal care product is based on an unpublished report submitted by the Notifier. The estimation assumes 100% dermal absorption and the

retention factor is based on that recommended for shaving cream. Using these data, the systemic exposure from use in razor strips is estimated to be 2.75 x 10^{-6} mg/kg bw/day for adults.

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Lubricating strip	0.22	7.5	0.01[1]	**2.75 x 10^{-6}**

C = concentration (%); RF = retention factor (as for shaving cream); assumes single use/day
Daily systemic exposure = (Amount × C(%) × RF x dermal absorption)/body weight (60 kg)

[1] SCSS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 8th revision, EC. December 2012

The total systemic exposure for a user of all products containing the notified chemical is estimated as 0.009 mg/kg bw/day.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 5.17 mg/L/4 h; low toxicity
Skin irritation	non-irritating
Eye irritation	non-irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral (diet) toxicity – 90 days.	NOAEL = 1000 mg/kg bw/day
Genotoxicity – in vitro Mammalian Chromosome Aberration Test.	non-genotoxic
Genotoxicity – in vivo Mammalian Erythrocyte Micronucleus Test	non genotoxic

Toxicokinetics
No toxicokinetic data on the notified chemical were submitted. Absorption of the notified chemical through the skin and gastrointestinal tract is expected based on the partition coefficient (3.6), water solubility (1 mg/L) and low molecular weight (298.40 Da).

Acute toxicity.
The notified chemical is expected to have a low acute oral, dermal and inhalation toxicity based on studies conducted in rats.

Irritation and sensitisation.
The notified chemical is not irritating to the skin and eyes. The notified chemical is not expected to be a sensitiser based on the results of a Guinea Pig Maximisation test.

Repeated dose toxicity.
A NOAEL of 1000 mg/kg bw/day was established for the notified chemical in a 90-day repeated dose oral dietary toxicity test in rats based on no treatment-related adverse effects observed. While effects on serum chemistry, haematology and body weight gain were attributed to the presence of the notified chemical, these effects were not considered adverse because of the low magnitude of change, no clear dose-response relationship, and recovery in the absence of test substance.

Mutagenicity/Genotoxicity.
The notified chemical was negative in an in vitro mammalian chromosomal aberration test but the negative result in the presence of metabolic activation was not confirmed in a second test. However, the notified chemical has also been reported to be negative with and without metabolic activation in an additional chromosomal aberration study (ESFA, 2012). There was also no indication of genotoxicity in an *in vivo* micronucleus test with bone marrow cells of the mouse, however there was no indication from this study that the notified chemical was reaching the target organ bone marrow.

Overall, based on the available evidence, the notified chemical is not expected to be genotoxic.

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical will be imported in finished products at low concentrations without a need for repackaging. Only transport and storage workers may come into contact with the notified chemical in the event of accidental rupture of packages. Therefore, the risk to the health of workers is not considered to be unreasonable.

6.3.2. Public Health
The notified chemical is proposed for use at ≤ 0.015% concentration in toothpaste, at ≤ 0.005% concentration in mouthwash, ≤ 0.018 mg/inch on dental floss and ≤ 7.5% per lubricating strip on razors.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products for adults of 0.0091 mg/kg bw/day and toothpaste only for young children (2-4 year olds) of 0.0128 mg/kg bw/day, and the NOAEL of 1000 mg/kg bw/day, which was established in a 90 day repeated dose toxicity study on the notified chemical. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure. Using the abovementioned NOAEL, a MoE of 109,890 for adults and 78,125 for young children was estimated, which are considered to be acceptable.

Based on the available information, the risk to the public associated with the notified chemical in the use of oral hygiene products (at concentrations ≤ 0.015% for toothpaste, ≤ 0.005% in mouthwashes, ≤ 0.018 mg/inch on dental floss and ≤ 7.5% per lubricating strip on razors) is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a component of toothpaste, mouth rinse and floss waxed coating. As manufacturing and reformulation will take place overseas, no release of the notified chemical is expected to occur in Australia from these activities. Any spills during transport are expected to be contained, collected and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
Toothpaste, mouth rinse and floss waxed coating containing the notified chemical will be sold nationwide. The majority of the notified chemical in toothpaste and mouthwash is expected to be used undiluted and will usually be released directly to the sewer. The notified chemical associated with the floss waxed coating is expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL
Residues in empty product containers are expected to be disposed of to landfill.

7.1.2. Environmental Fate
The notified chemical is not expected to be readily biodegradable based on the environmental fate study provided. For the details of the environmental fate studies please refer to Appendix C. The majority of the notified chemical is expected to be released to sewer during use. During waste water treatment processes in sewage treatment plants (STPs), most of the notified chemical is expected to be removed from waste waters to sludge due to its potential cationicity and measured log Koc value of 3.7. The notified chemical that partitions and/or adsorbs to sludge will be removed with the sludge for disposal to landfill or used in soil remediation. Small amounts of the notified chemical remaining in the effluent from STP may be released to surface waters.

The notified chemical that is released to surface waters is expected to partition to suspended solids and disperse. Hence, it is not anticipated to be significantly bioavailable to aquatic organisms. Due to its potential cationicity, the notified chemical is not expected to cross the biological membranes and hence it is not considered to be bioaccumulative. In landfill, the notified chemical will be associated with the disposed article or sludge, and is unlikely to be mobile due to its tendency to bind to soil/sediments. Ultimately, the notified chemical is expected to degrade in soil or water via abiotic and biotic pathways to form water, oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

Since most of the notified chemical will be washed into the sewer, under a worst case scenario, with no removal of the notified chemical in the sewage treatment plant, the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.61	µg/L
PEC - Ocean:	0.06	µg/L

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 0.606 µg/L may potentially result in a soil concentration of approximately 4.03 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 20.19 µg/kg and 40.39 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LC50 > 0.823 mg/L	No effect at saturation to fish
Daphnia Toxicity (48 h)	EC50 = 1.02 mg/L	Toxic to aquatic invertebrates
Algal Toxicity (96 h)	E_rC50 > 1.03 mg/L	No effect at saturation to algae

Based on the above ecotoxicity endpoints, the notified chemical showed no effect at the level of saturation for fish and algae. However, the notified chemical is toxic to aquatic invertebrates. On the basis of the acute toxicity data of the notified chemical, it is expected to be toxic to aquatic organisms. Therefore, under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS; United Nations, 2009), the notified chemical is formally classified as Acute Category 2; Toxic to aquatic life. Based on the acute toxicity and lack of ready biodegradability of the notified chemical, the notified chemical has been formally classified under the GHS as Chronic Category 2; Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the most sensitive toxicity endpoint (daphnia; EC50) of the notified chemical. An assessment factor of 100 has been used as acute toxicity endpoints for three trophic levels were provided.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Daphnia).	1.02	mg/L
Assessment Factor	100	
PNEC:	10.2	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.61	10.2	0.059
Q - Ocean:	0.06	10.2	0.006

The Risk Quotients (Q = PEC/PNEC) for a worst case discharge scenario have been calculated to be < 1 for the river and ocean compartments. The notified chemical is not expected to be readily biodegradable or bioaccumulative in the environment. It is not likely to be present in ecotoxicologically significant concentrations in the aquatic environment. Therefore, the notified chemical is not considered to pose an unreasonable risk to the environment on the basis of the assessed use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Boiling Point > 402.6 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
Remarks Siwoloboff method
Test Facility Givaudan Suisse (2008)

Density 1,120 kg/m^3 at 25 °C

Method OECD TG 109 Density of Liquids and Solids.
Remarks Pycnometer method
Test Facility Givaudan Suisse (2007)

Vapour Pressure 4 x 10^{-12} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
Remarks Effusion method: vapour pressure balance
Test Facility Huntingdon (2007a)

Water Solubility 1 x 10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Column Elution Method
Test Facility Huntingdon (2007a)

Hydrolysis as a Function of pH t½ > 1 year at 25 °C (pH 4 – 9)

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)*	*$t_{½}$ Years*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks A preliminary test was conducted on the notified chemical at 50 °C. The degradation of the notified chemical was found to be < 10% after 5 days at pH 4, 7 & 9. This is equivalent to a half life of > 1 year at 25 °C. Therefore, the notified chemical is hydrolytically stable under acidic (pH 4), neutral (pH 7) and basic (pH 9) conditions.
Test Facility Huntingdon (2007a)

Partition Coefficient (n-octanol/water) log Pow = 3.6

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method
Test Facility Huntingdon (2007a)

Surface Tension 66.5 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks Concentration: 90% saturated aqueous solution
Test Facility Huntingdon (2007a)

Adsorption/Desorption log K_{oc} = 3.7

Method OECD TG 121 Adsorption – Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks The HPLC method using soil-adsorption-reference data was applied for the determination of the adsorption coefficient (K_{oc}) of the test substance. The determined log K_{oc} value of 3.7 (K_{oc}> 5000) suggests that the test substance will be immobile in soil.

Test Facility Huntingdon (2007a)

Particle Size

Method Sieve analysis followed by image analysis.

Sieve analysis
Sample of notified chemical (10 g) was weighed into the coarsest of a nest of sieves of aperture sizes 10, 30, 75, 125 and 400 µm. The nest was shaken for 30 minutes with tapping and the proportion of notified chemical passing through each sieve determined gravimetrically. Test was performed in duplicate.

Image analysis
Six replicate samples of notified chemical (5 – 10 mg) were suspended by agitation and mounted on a microscope slide for image analysis at 10x and 40x magnification. The mean particle length and axial ratio were used to calculate particle volumes in each range which gave a volume/mass distribution when combined with particle number.

Sieve analysis		***Image analysis***	
Particle size (µm)	*% in range by weight* *Mean*	*Particle size (µm)*	*% mass* *Mean*
> 400	1.0	320 - 600	6.7
400 – 125	6.2	160 - 320	19.2
125 – 75	21.8	60 - 160	63.9
75 – 30	66.6	30 - 60	9.3
30 – 10	4.3	10.4 - 30	0.9
< 10	0.0	0.5 – 10.4	0.0

Remarks The results from both the sieve and image analysis indicate that the notified chemical does not contain particles in the respirable range (< 10 µm)

Test Facility Huntingdon Life Sciences (2007)

Autoignition Temperature > 400 °C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.

Remarks Notified chemical suspended in centre of furnace. Temperature recordings made of furnace and notified chemical as the temperature of the furnace was increased at a rate of 0.5 ^{0}C/min to 400 ^{0}C. No exothermic reaction of the notified chemical was observed when heated up to 400 °C

Test Facility Huntingdon (2007a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD:

Pre-test: Dose-range study:
Two groups of 1 M and 1 F were dosed with the notified chemical at 300 and 1000 mg/kg in corn oil, with a third group of 1 M and 1 F dosed with the notified chemical at 1000 mg/kg in 1% methylcellulose. Animals were dosed by oral gavage. No mortality was recorded in any of the three groups. No clinical signs were observed in any animal at 300 or 1000 mg/kg notified chemical in corn oil. Loose stool was observed in the male dosed with 1000 mg/kg notified chemical in 1% methylcellulose.

Main study:
Three groups of 5 M and 5 F animals were dosed with the notified chemical by oral gavage at 500, 1000 and 2000 mg/kg in 1% methylcellulose. Clinical observations were recorded post-dose at 0, 0.5, 1 and 4 hours and then daily through to Day 15. Body weights were recorded prior to dosing and on days 8 and 15. Gross necropsy was performed on all animals on Day 15.

Species/Strain	Rat/ Sprague Dawley
Vehicle	1% methylcellulose
Remarks - Method	None

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M, 5 F	500	0/10
2	5 M, 5 F	1000	0/10
3	5 M, 5 F	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity	All animals except one animal showed gains in body weight over the course of the study. The exception was 1 M in the mid-dose group who showed a drop in weight at Day 8. This animal then gained weight by Day 15 increasing in weight above its starting weight (Day 0). Soft faeces were observed in 2 M in the low-dose group (up to 1 hour after exposure) and 1 M in the high-dose group (30 minutes after exposure).
Effects in Organs	No visible lesions observed.
Remarks - Results	None

CONCLUSION: The notified chemical of low toxicity via the oral route.

TEST FACILITY: Calvert (2004)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	1% w/v aqueous methylcellulose
Type of dressing	Occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M, 5 F	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Reactions were recorded from 48 hours after exposure. No oedema was observed in any of the rats through the duration of the study. Very slight erythema was observed in 2 M with both animals recovered by day 4. Bandage reactions were observed in 2 F on day 2. This reaction was resolved in 1 F by day 3, and in the second F by day 8. This female also exhibited very slight erythema from day 3 - day 8, as well as eschar/scab formation on day 7 (oedema was not scored for this female on this day with no explanation provided in the study report).
Signs of Toxicity - Systemic	Body weight loss was recorded for 2 F during day 1 to 8, with one of these females showing body weight loss for the duration of the study (days 1 to 15). This female also had longer bandage and erythema reactions than the other animals in the study (male or female). All other animals were considered to have achieved satisfactory body weight gains.
Effects in Organs	Pallor of the kidneys was observed in 1/10 animals (1 M). No abnormalities were observed in any other animals.
Remarks - Results	There were no unscheduled deaths and no systemic response to treatment was observed in any animal throughout the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Huntingdon (2007b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity – Limit Test. EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation) – Limit Test.
Species/Strain	Rat/RccHan™ : WIST
Vehicle	None
Method of Exposure	Nose only exposure.
Exposure Period	4 h
Physical Form	Solid aerosol (particulate).
Particle Size	MMAD 4 μm ; Inhalable fraction (< 4 μm) - 50%.

Remarks - Method	Sighting test performed on two animals (1 M, 1 F) at a mean achieved atmosphere concnetration of 2.25 mg/L. Animals were exposed for 4 h. Animals exhibited increased respiration and wet fur during the exposure period as well as hunched posture, piloerection and ataxia on termination of exposure and one-hour post-exposure

RESULTS

Group	*Number and Sex of Animals*	*Concentration Mg/L*		*Mortality*
		Nominal	*Actual*	
1	10 (5 M, 5 F)	16.3	5.17	0/10

LC50	> 5.17 mg/L/4 h
Signs of Toxicity	All animals exhibited increased respiratory rate and laboured respiration. These effects are considered to be due to the test substance rather than an effect of the restrain procedure (which is associated with the effects of wet fur, hunched posture and piloerection observed during and post-exposure. Laboured respiration was observed at one hour post-exposure and increased respiration rate was observed up to 8 days post-exposure (2/10 animals). While ataxia was observed in the sighting study, it was not observed in the main study. One animal also exhibited a limp on the hind left leg (day 3) which was not considered to be treatment related. All males and one female exhibited a loss in body weight on the first day post-exposure. All males subsequently made acceptable body weight gains during the recovery period. The female exhibiting a body weight loss on day 1 gained weight on day 3 and then failed to exhibit any further body weight gains. Two other females showed a loss in body weight on day 3 but continued to gain weight over the duration of the study. Another female showed a loss in body weight on day 7 but then gained weight over the duration of the study. Only one female showed continued body weight gain over the duration of the study.
Effects in Organs	No abnormalities were recorded.
Remarks - Results	The authors noted that the geometric standard deviation was outside the generally acceptable target range. This deviation was very slight (+ 0.21) and was considered to be due to the physical characteristics of the test item. The aerosol concentration achieved was at the technical limit with a respirable particle size. As such the authors determined that the deviation was not considered to affect the purpose or validity of the study.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY Harlan (2013)

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Vehicle	None (test sites were moistened with water prior to application of test substance)
Observation Period	72 h
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	No dermal irritation was observed in any animal. No clinical signs of toxicity or ill health were observed during the observation period. Yellow staining at test sites was observed for all animals that did not interfere with assessment of irritation.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Huntingdon (2007c)

B.5. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	72 h
Remarks - Method	No significant protocol deviations

RESULTS

Remarks - Results	No clinical signs of toxicity or ill health were observed during the observation period. No ocular irritation was observed at 24, 48 and 72 h. All animals exhibited some hyperaemic blood vessels 1 h after exposure with the effect lasting < 24 h.
CONCLUSION	The notified chemical is non-irritating to the eye.
TEST FACILITY	Huntingdon (2007d)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/Ca
Vehicle	Dimethylformamide
Remarks - Method	All test animals were female. Positive control: Hexyl cinnamic aldehyde [in acetone: olive oil (4:1, v/v)] Negative control: Dimethylformamide Positive control was run concurrently with the study.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	206.33	n/a
10	155.93	0.8
25	183.18	0.9
50	278.03	1.3
Positive Control		
50	5990.08	29.0

Remarks - Results

There were no mortalities.

Post-exposure, slight greasy fur was recorded on the cranial region of 3/5 animals in the low-dose group (1/5 on day 2 and 2/5 on day 3), 1/5 animals in the mid-dose group (day 2 only) and 1/5 animals in the high-dose group (day 2).

Post-exposure, slight pale yellow particles were observed in all animals in the mid-dose group (days 2 and 3) and all animals in the high-dose group (days 1, 2 and 3). It was not recorded if these particles occurred in the same location as the administered dose.

Slight to moderate greasy fur was recorded post-exposure on the cranial region of all animals in the positive control group from day 1 and persisting for the duration of the study. Slight wet fur on the cranial region was also observed in 2/5 animals of the positive control group post-exposure (day 1 only).

No signs of ill health or toxicity were observed. No signs of irritation were observed. All animals gained body weight over the duration of the study

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

Huntingdon (2007e)

B.7. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain: Rats/Crl:CD(SD)

Route of Administration: Oral –diet

Exposure Information: Total exposure days: 91 days
Dose regimen: 7 days per week
Post-exposure observation period: 28 days

Vehicle: PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002

Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw/day*		*Mortality*
		Nominal	*Actual*	
control	10 F, 10 M	0	0	0/20
low dose	10 F, 10 M	100	102 F / 103 M	0/20
mid dose	10 F, 10 M	300	304 F/ 311 M	0/20
high dose	10 F, 10 M	1000	1009 F/ 1028 M	0/20
control recovery	5 F. 5 M	0	0	0/10
high dose recovery	5 F. 5 M	1000	1009 F/ 1028 M	0/10

Mortality and Time to Death
No animals died prior to scheduled euthanasia.

Clinical Observations
No test substance related clinical observations or effects on food consumption were observed.

Females in the mid- and high-dose groups showed slightly lower (occasionally statistically significant) mean body weight gains in the first five weeks of the study resulting in lower cumulative mean body weight gains and mean body weights throughout the dosing period of the study. Mean body weight gains and cumulative body weight gains in females in the high-dose recovery group were similar to or slightly higher than those of the control group. Any additional body weight changes observed in test substance groups during the dosing period were limited to a single interval and were not dose related.

There was no direct relationship between lower food consumption and lower body weight gains.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No test substance related observations on urinalysis parameters were recorded.

A number of effects on serum chemistry and haematology were observed at the end of the dosing period (week 13). Males in the mid- and high-dose groups showed minimally higher levels of cholesterol and minimally lower levels of potassium. Females showed lower alanine aminotransferase (all dose groups), aspartate aminotransferase (low- and high-dose groups), and triglyceride levels (mid- and high-dose groups). A minimally higher methemoglobin value was also observed in females in the high-dose group. Complete recovery was observed by the end of the recovery period (week 17).

These effects on haematology and serum chemistry parameters are not considered adverse because of the low magnitude of change, no clear dose-response relationship, and recovery in the absence of test substance (recovery period).

Effects in Organs
No test substance related macroscopic observations were made on necropsy.

Liver weight (relative to final body weight) was higher in males and females in the mid- and high-dose groups at the end of the 90-day study period. These differences form the control group did not show a clear dose-response effect and there was no associated morphologic change. The authors suggest that these findings were adaptive in nature (Williams and Iatropoulos 2002).

At the end of the dosing period, females in the low-dose group exhibited higher absolute brain weight. At the end of the recovery period, males in the high-dose group exhibited higher heart weight relative to body weight. Females in this group exhibited lower absolute and relative heart weights (to body and brain) and thymus (to body or brain) weights and lower values for absolute ovary weight. No clear dose –response was observed and there was no clear morphologic association. The authors considered these organ weight changes to be of no toxicological significance.

No test-substance related histological changes were observed.

Remarks – Results

No direct dose-response relationships were shown for the effects recorded. In addition, organ weight changes were observed which did not a have a morphologic correlate. Recovery of animals in the absence of test substance was shown for serum chemistry and haematology effects as well as body weight gain. Any effects observed cannot be attributed solely to the presence of the notified chemical.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on no treatment related adverse effects being observed.

TEST FACILITY WIL Research (2007)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	DMSO
Remarks - Method	Vehicle and positive controls (mitomycin C without metabolic activation and cyclophosphamide (monohydrate) with metabolic activation) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	23.31, 46.63, 93.26, 186.52, 373.04, 746.08*, 1492.15*, 2984.3*	3 h	21 h
Test 2	23.31, 46.63*, 93.26, 186.52*, 373.04, 746.08, 1492.15*, 2984.3	21 h	21 h
Present			
Test 1	23.31, 46.63, 93.26, 186.52, 373.04*, 746.08, 1492.15*, 2984.3*	3 h	21 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	> 2984.3	> 2984.3	negative
Test 2	-	≥ 746.08	> 2984.3	negative
Present				
Test 1	-	> 2984.3	> 2984.3	negative

Remarks - Results

In the absence of S9 mix, the notified chemical caused a reduction in the mitotic index to 65% in Test 1 at the highest concentration and to 45% in Test 2 at 1492.15 µg/mL. In the presence of S9 mix, the mitotic index was reduced to 58% of the negative control at the highest concentration tested.

In Test 2 (absence of S9 mix), a statistically significant increase in the proportion of cells with chromosomal aberrations at 186.52 µg/mL was

observed when gap-type aberrations were included. Gaps are generally not included in the total aberration frequency.

Excluding gaps, increases in aberrations at 186.52 µg/mL was not statistically significant but the increased incidence was outside the historical control range. However the increases were not reproducible between the replicate cultures and there was no evidence of a dose-related response.

No significant increases in polyploid metaphases were observed in either test.

The positive control gave a satisfactory response confirming the validity of the test system.

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

TEST FACILITY Huntingdon (2007f)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain Mouse/CD1
Route of Administration Oral – gavage
Vehicle 1% w/v aqueous methylcellulose
Remarks - Method No significant protocol deviations.

A preliminary toxicity test of 2000 mg/kg bw/day (based on LD_{50} result in rats) was performed on 4 animals (2 F, 2 M) with no observable toxic effects after two separate doses (dosed approximately 24 hours apart).

Male animals were chosen for main test as no substantial differences in toxicity was observed between the sexes.

Animals were dosed on Day 1 and Day 2 of the test. Animals were sacrificed 24 hours after the second dose.

Positive control (at a concentration of 0.6 mg/mL) animals were dosed once, approximately 24 h prior to termination.

Bone marrow was used to assess the presence of micronuclei.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7	0	48
II (low dose)	7	500	48
III (mid dose)	7	1000	48
IV (high dose)	7	2000	48
V (positive control, M)	5	12	24

M=mitomycin C

RESULTS
Doses Producing Toxicity Signs of toxicity were not observed at any dose level.
Genotoxic Effects No statistically significant increase in the number of micronucleated polychromatic erythrocytes was observed.
Remarks - Results No clinical signs or reduction in body weight observed. No mortalities were observed.

	No substantial increase in the incidence of micronucleated normochromatic erythrocytes or significant decrease in the proportion of polychromatic erythrocytes was observed. All individual and group mean values were within the ranges determined from laboratory historical data. The positive control gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo mouse micronucleus assay.
TEST FACILITY	(Huntingdon 2007g)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F: Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sewage sludge
Exposure Period	67 days
Auxiliary Solvent	Not reported
Analytical Monitoring	A Respirometer, SAPROMAT D 12, was used for measurement of the consumption of oxygen.
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1	7	58
14	1	14	93
28	2	28	95
35	0	35	94
67	-1	67	94

Remarks - Results

All validity criteria for the test were satisfied. The reference compound, sodium benzoate, achieved 85% degradation after 7 days and 93% after 14 days, and therefore the test is considered valid for this criterion.

The biological Oxygen Demand (BOD) curve for the toxicity control shows no toxic effect of the test substance to the microorganisms at the test concentration. The percentage of degradations has not been reported.

The test substance achieved 0% degradation after 67 days under the test conditions and, therefore it is not considered to be readily biodegradable.

CONCLUSION The notified chemical is not readily biodegradable

TEST FACILITY Givaudan (2006)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302 C: Manometric Respirometry Test
Inoculum	Activated sewage sludge
Exposure Period	31 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC) was measured for determination of biodegradability.
Remarks – Method	The test was conducted following the test guideline and good laboratory practice (GLP) principles.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	-8	7	75
28	-6	14	80

Remarks – Results: No information regarding test validity criteria is available. The reference compound, aniline, reached greater than 60% pass level by day 7 indicating the suitability of the inoculum. No toxicity control was performed according to the study. It is unclear if the no biodegradation degree outcome is due to the test substance's toxicity to bacteria. The notified chemical may be not inherently biodegradable based on the above test outcome.

CONCLUSION: The notified chemical may not be inherently biodegradable

TEST FACILITY: Givaudan (2007)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi-static Test

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Exposure Period: 96 hours

Auxiliary Solvent: dimethylformamide (DMF)

Water Hardness: 174 mg $CaCO_3$/L

Analytical Monitoring: HPLC Analysis

Remarks – Method: The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

The stock solution for the fish toxicity test was prepared by dissolving the test substance in dimethylformamide (DMF) before an aliquot was dispersed in the dilution medium. This aqueous mixture was treated by ultrasound and stirred in the dark overnight before being filtered and diluted to prepare the required number of treatment concentrations. A blank control and a solvent control group were included in the toxicity test. All the exposure treatments were observed to be clear and colourless.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Cumulative Mortality (%)*				
Nominal	*Geometric mean measured*		*2 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
Solvent control	Solvent control	7	0	0	0	0	0
0.427	0.0408	7	0	0	0	0	0
0.939	0.0921	7	0	0	0	0	0
2.07	0.197	7	0	0	0	0	0
4.54	0.403	7	0	0	0	0	0
10	0.823	7	0	0	0	0	0

LC50	> 0.823 mg/L(mean measured concentration) at 96 hours
NOEC	0.0921 (mean measured concentration) mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance in treatment solutions were measured every 24 hours within the 96-h test period. The treatment solutions were renewed every 24 hours during the test. The 96-hour LC50 was calculated using the SAS statistical analysis. NOEC was derived by visual observation for lethal and treatment-related-effects. An incidence rate of more than one affected fish out of seven was considered to be significant. No mortality of fish occurred at any of the treatment concentrations at the end of the 96-hour test. The highest treatment was considered to approximate the limit of aqueous solubility of notified chemical under the test conditions.
CONCLUSION	The notified chemical exhibited no effect at saturation to fish
TEST FACILITY	Huntingdon (2008h)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	dimethylformamide (DMF)
Water Hardness	224 mg $CaCO_3$/L
Analytical Monitoring	HPLC Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. The stock solution for the daphnia toxicity test was prepared by dissolving the test substance in dimethylformamide (DMF) before an aliquot was dispersed in the dilution medium. This aqueous mixture was treated by ultrasound and stirred in the dark overnight before being filtered and diluted to prepare the required number of treatment concentrations. A blank control and a solvent control group were included in the toxicity test. All the exposure treatments were observed to be clear and colourless.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Cumulative % Immobilised*	
Nominal	*Geometric mean measured*	*48 h*	*24 h*	*48 h*
Control	Control	20	0	0
Solvent control	Solvent control	20	0	0
0.625	0.0735	20	0	0
1.25	0.15	20	0	0
2.5	0.304	20	0	0
5	0.588	20	0	0
10	1.2	20	15	70

EC50	1.02 (0.94 – 1.21) mg/L at 48 hours
NOEC	0.588 mg/L at 48 hours

Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance in treatment solutions were measured at the beginning and end of the test. The EC50 value (48 h) was calculated using the SAS statistical analysis.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	Huntingdon (2008i)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 10 mg/L Mean measured: 1.03 mg/L
Auxiliary Solvent	dimethylformamide (DMF)
Water Hardness	Not reported
Analytical Monitoring	HPLC Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. The stock solution for the algae ecotoxicity was prepared by dissolving the test substance in dimethylformamide (DMF) before an aliquot was dispersed in the OECD medium. This aqueous mixture was treated by ultrasound and stirred in the dark overnight before being filtered and diluted to prepare the required number of treatment concentrations. A blank control and a solvent control group were included in the toxicity test. All the exposure treatments were observed to be clear and colourless.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_bC50 (mg/L)	*NOE_bC (mg/L)*	*E_rC50 (mg/L)*	*NOE_rC (mg/L)*
> 1.03	1.03	> 1.03	1.03

Remarks - Results	All validity criteria for the test were satisfied. The algae test was conducted as a limit test. The actual concentrations of the treatments were measured at the beginning and end of the test. The data were compiled in an excel spreadsheet and analysed using SAS statistical analysis. The E_rC50 and E_bC50 values could not be calculated as insufficient inhibition of growth was observed at the concentration tested, which was considered to approximate the limit of aqueous solubility of notified chemical under the test conditions.
CONCLUSION	The notified chemical exhibited no effect at saturation to algae
TEST FACILITY	Huntingdon (2008j)

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